High-Carbohydrate, Ketogenic Diets, Exogenous Ketones: Performance and Health Effects in Endurance Athletes

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Declaration:

I declare that this PhD dissertation is entirely my own work, other than the counsel of my supervisors Dr. Lorna Doyle of the Department of Sport and Exercise Science at Waterford Institute of Technology and Prof. Jeff S. Volek of the Kinesiology Department at The Ohio State University. This work has not been submitted for award at this or any other institution.

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Fionn T. M^{c}Swiney September 2018

I dedicate this dissertation to the loving memory of my Dad, Joe.

A man who embodied the term family man.

Hopefully, you're "18 again ♫"

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To my wonderful mother, Tess. The most honest, generous and hardworking person I know. Thank you for unwavering love and support. I think I speak for all of us when I say we feel privileged to have come from such a tight-knit loving family. Our morals and strong relationships are a testament to how you and Dad reared us. Thank you.

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Fionn Tomás Mc Swiney

High-Carbohydrate, Ketogenic Diets, Exogenous Ketones: Performance and Health Effects in Endurance Athletes

Abstract:

Despite conventional wisdom advocating a high-carbohydrate (HC) diet, low-carbohydrate ketogenic diets (LCKD) have grown in popularity among endurance athletes in recent years. In addition, the use of exogenous ketone esters to optimise performance is an emerging area of research within sports science. Little is known about the performance and health implications of a LCKD within endurance athletes, nor an exogenous ketone esters impact on performance within well-trained runners. A non-randomised 12 week dietary and training intervention was designed to assess performance, body composition and health responses to a traditional HC and LCKD within endurance athletes. In addition, a double blind randomised crossover designed investigation was carried out to determine a ketone esters impact on exercise metabolism and endurance performance when co-ingested with carbohydrates versus a group consuming equal carbohydrates (1.2 $g \cdot min^{-1}$). A LCKD maintained 100 km time trial (TT) performance, improved body composition and relative power outputs. The LCKD caused nutritional ketosis (0.5 ± 0.4 mM beta-hydroxybutyrate (βHB)), maintained homeostatic measures of wellness, including blood lipids, glycaemic control, inflammation and oxidative stress. Nutrient analysis demonstrated HC diet was reportedly deficient in fat soluble vitamins due to restricting fat to <20% of total energy, while the LCKD was reportedly deficient in fibre (19.2 ± 4.9 g/d) and high in saturated fat (29.5 ± 9.1 %/kcal). Ketone ester ingestion caused acute nutritional ketosis (1.2 ± 0.2 mM βHB) and homogenously improved (2.9%) simulated 10 km TT running performance in well-trained athletes; despite not measurably altering metabolic or peripheral responses to submaximal exercise. In conclusion, a LCKD appears proficient at maintaining submaximal exercise performance and homeostatic measures of wellness within an endurance trained population across a 12 week intervention, while ketone ester ingestion within well-trained runners' appears to benefit endurance performance, however, additional work is needed to determine the mechanism for improved performance.

Conference Proceedings and Publications

Journal Article. Published in 'Metabolism Clinical and Experimental' in November 2017 (see appendices J).
Fionn T. McSwiney, Bruce Wardrop, Parker Hyde, Richard Lafountain, Jeff S. Volek and Lorna Doyle.
"Keto-adaptation enhances exercise performance and body composition responses to training in endurance athletes"

Journal Article. Letter to the Editor. Published in 'Metabolism Clinical and Experimental' in November 2017 (see appendices K).
Fionn T. McSwiney, Bruce Wardrop, Parker Hyde, Richard Lafountain, Jeff S Volek and Lorna Doyle.
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Conference Proceeding. The Nutrition Society, University of Ulster (2017) (see appendices H).
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Fionn T. McSwiney and Lorna Doyle. "A comparison of the effect of a high-fat ketogenic diet versus a high-carbohydrate diet in endurance athletes"

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List of Abbreviations

3-hydroxybutyrase dehydrogenase	BDH
Acetoacetate	AcAc
Adenosine diphosphate	ADP
Adenosine monophosphate	AMP
Adenosine tri phosphate	ATP
Average	Avg
Beats per minute	bpm
Body mass index	BMI
Calorie	kcal
Calorie per gram	kcal/g
Carbohydrate	CHO
Carbohydrate electrolyte solution	CES
Carbon dioxide	CO_2
Chemokine ligand 5	RANTES/CCL5
CoA transferase	OXCT
Control trial	CT
C-reactive protein	CRP
Critical power test	CPT
Daily reference value	DRV
Dual-energy X-ray absorptiometry	DXA
D-βeta-hydroxybutyrate	D-βHB
Effect size	ES
E-selectin	E-SEL
Free radical theory of aging	FRTA
Glycaemic load	GL
Gram	g
Gram per day	g/d
Gram per hour	g/h
Gram per kilogram body mass	g·kg
Gram per kilogram body mass per hour	g·kg/h
Gram per minute	$g \cdot min^{-1}$

High-carbohydrate	HC
High-density lipoprotein	HDL
High-intensity interval training	HIIT
Histone deacetylase	HDAC
Hormone-sensitive lipase	HSL
Hour	h
Insulin resistance	IR
Interleukin – 6	IL-6
Interleukin - 8	IL-8
Intercellular adhesion molecule 1	ICAM1
Joule	J
Ketone ester condition	KET
Kilogram	kg
Kilogram per meter square	$kg \cdot m^2$
Kilojoule	kJ
Kilojoule per kilogram	$kJ \cdot kg^{-1}$
Kilojoule time trial	KJ TT
Kilometre	Km
Kilometre per hour	Km/h
Low density lipoprotein	LDL
Low-carbohydrate high-fat	LCHF
Low-carbohydrate ketogenic diet	LCKD
L-βeta-hydroxybutyrate	LβHB
Macrophage colony-stimulating factor	M-CSF
Matrix metalloproteinase-2	MMP2
Maximal oxygen consumption	VO_{2peak}
Maximum velocity	S^{-1}/Vmax
Mean corpuscular haemoglobin	MCH
Mean corpuscular haemoglobin concentration	MCHC
Mean corpuscular volume	MCV
Mean platelet volume	MPV
Medium chain triglycerides	MCT

Meter	m
Microgram	µg
Microgram daily folate equivalent	µg DFE
Microgram per day	µg/d
Milgram per mega joule	mg/MJ
Milliequivalent per day	$mEq \cdot day^{-1}$
Milligram	mg
Milligram per day	mg/d
Milligram per decilitre	mg/dL
Milligram per kilogram body mass	$mg \cdot kg^{-1}$ BM
Millilitre per minute	$mL \cdot min^{-1}$
Millilitres per kilogram body mass per minute	$mL \cdot kg^{-1} min^{-1}$
Millimoles per kilogram body mass	$mmol \cdot kg^{-1}$
Millimoles per litre	mM
Minute	Min
Mitochondrial free radical theory of aging	MFRTA
Moderate carbohydrate	MC
Monocarboxylate transporters	MCT_2
Net endogenous acid production	NEAP
Non-significant	NS
Not applicable	n/a
Nuclear factor kappa-light-chain-enhancer of activated B cells	NF-kB
One repetition maximum	1RM
Ounces	Oz
Oxygen	O_2
Peak watt(s)	W_{max}
Percentage calories	%/kcal
Periodized carbohydrate	PC
Placebo	PLA
Potential renal acid load	PRAL
Pyruvate dehydrogenase	PDH
Random control trial	RCT

Rated perceived exertion	RPE
Reactive oxygen species	ROS
Recommended daily allowance	RDA
Red blood cell	RBC
Red blood cell distribution width	RDW
Respiratory exchange ratio	RER
Respiratory quotient	RQ
Revs per minute	rpm
Standard deviation	SD
Second	Sec
Six Second sprint	SS Sprint
Tablespoon	Tbsp.
Teaspoon	Tsp
Thiobarbituric acid reactive substances	TBARS
Time to exhaustion	TTE
Time trial	TT
Tumour necrosis factor alpha	TNF-α
Very low density lipoprotein	VLDL
Watt	W
Watt(s) per kilogram body mass	W/kg
White blood cells	WBC
βeta-hydroxybutyrate	βHB

Chapter 1

Introduction

The ability to compete in an endurance event is primarily dependant on the aerobic and anaerobic capacity of an individual and the availability of two fuel sources, carbohydrate and fat. Carbohydrate and fat are utilised simultaneously during exercise, however the ratio of carbohydrate to fat utilization is dependent on the intensity and duration of an activity, as well as the physical fitness of an individual. Carbohydrates are predominantly utilised during short, high-intensity bouts of exercise, whereas the oxidation of free fatty acids are better suited to more prolonged bouts of exercise at lower intensities (Romijn *et al.,* 1993).

In long distance endurance events athletes aim to maintain as high a power output as possible during a >2–8 hour race, during which, muscle glycogen and the supply of blood glucose are considered two of the most important substrates necessary for contracting muscle (Romijn *et al.,* 1993). One of the primary causes of fatigue and drop out during an endurance event in well-trained athletes is the depletion of muscle glycogen (Jeukendrup, 2004). Traditional recommendations advocate a high-carbohydrate diet and to supplement with carbohydrate and/or glucose during exercise to maintain carbohydrate availability/stores (Jeukendrup, 2004; Burke et al., 2011). However, a number of alternative methods for fuelling endurance performance have emerged in an attempt to improve upon this fuelling strategy. Popular methods include the consumption of a low-carbohydrate high-fat (LCHF) diet (O'Keeffe *et al.,* 1989; Lambert *et al.,* 1994; Goedecke *et al.,* 1999; Rowlands and Hopkins, 2002; Vogt *et al.,* 2003) or the consumption of a LCHF diet followed by a period of carbohydrate restoration/loading (Burke *et al.,* 2000; Carey *et al.,* 2001; Lambert *et al.,* 2001; Burke *et*

al., 2002; Rowlands and Hopkins, 2002; Havemann *et al.,* 2006). A LCHF diet encourages reduced carbohydrates (<130 grams per day (g/d)) and increased energy contribution from dietary fats and protein (Feinman *et al.,* 2015; Noakes and Windt, 2016). Aforementioned experimental investigations attempted to increase energy contribution from fatty acids during exercise to slow the utilisation of finite and valuable glycogen stores, ultimately delaying glycogen depletion. Following the consumption of a LCHF diet there is an increase in fat oxidation (O' Keeffe *et al.,* 1989; Lambert *et al.,* 1994; Goedecke *et al.,* 1999; Rowlands and Hopkins, 2002; Vogt *et al.,* 2003), therefore, glycogen sparing appeared to be taking place. Notably however, only two investigations in well-trained individuals noted improvements in performance (Lambert *et al.,* 1994; Goedecke *et al.,* 1999; Lambert *et al.,* 2001), with one investigation in trained individuals noted a decrease in performance following acute consumption of a LCHF diet (7 days) (O' Keeffe *et al.,* 1989).

After a decade of 'failed attempts to harness fat adaptation as an ergogenic aid' (Burke, 2015), a new trend within endurance sports (re)emerged; low-carbohydrate ketogenic diets (LCKD) (Phinney, 2004; Brukner, 2013; Noakes, Volek and Phinney, 2014; Burke, 2015; Volek, Noakes and Phinney, 2015; Burke *et al.,* 2017; Zinn *et al.,* 2017), with one expert questioning will it be 'third time lucky for LCHF diets?' (Burke, 2017). Unlike the broad definition of a LCHF diet (i.e., <130 g/d of carbohydrates), guidelines for a LCKD are precise, with common guidelines recommending small amounts carbohydrate (<50 g/d), moderate protein (1.76-2.2 grams per kilogram body mass (g·kg)) and considerable energy from fat (>75-80 percentage energy (%/kcal)) (Volek and Phinney, 2012). What sets a LCHF and a LCKD apart is the consumption of a LCKD upregulates ketogenesis, an evolutionary adaptive response designed to provide substrate for the brain in the

relative lack of glucose, as it's unable to utilise free fatty acids as fuel (Evans, Cogan and Egan, 2016). This dietary approach is believed to provide athletes with access to a much greater fuel tank (i.e., 30,000 kcal in adipose tissue) versus traditional methods (i.e., 2000-2200 kcal in glycogen stores) (Volek and Phinney, 2012; Volek, Noakes and Phinney, 2015). Additionally, it's thought to reduce inflammation and accelerate recovery within ultra-endurance athletes (Volek *et al.,* 2016). Importantly however, there is no experimental evidence to support such claims, particularly in well-trained individuals. Current investigations involving LCKDs and endurance trained individuals illustrate aerobic performance can be sustained (Phinney *et al.,* 1983; Burke *et al.,* 2017), body composition improved (Zajac *et al.,* 2014; Zinn *et al.,* 2017), while performance (Zajac *et al.,* 2014; Burke *et al.,* 2017) and exercise economy become compromised at higher intensities (Burke *et al.,* 2017) following 21–28 day adaptation periods. Contrasting aforementioned short term studies, two cross sectional studies by Webster *et al.,* (2016) and Volek *et al.,* (2016) involving self-reported keto-adapted athletes habituated to a LCKD for >8 months showed no signs of decreased efficiency or increased ratings of perceived of exertion (RPE) during 2-3 hour submaximal exercise. Low-carbohydrate advocates believe an elongated adaptation period is paramount for an athlete to become proficient on a LCKD (Volek, Noakes and Phinney, 2015). However, conflicting experts note that exercise intensities implemented within these cross sectional studies (Volek *et al.,* 2016, Webster *et al.,* 2016) do not reflect the metabolic demands placed on endurance athletes (Leckey and Hawley, 2013; Burke *et al.,* 2017).

Over the past couple of million years' the human brain has been exclusively fuelled by glucose during times of plenty (i.e., carbohydrate availability) or via a mixture of endogenous ketones produced by the hepatic tissues (Cahill and Aoki, 1980) when food

and/or carbohydrates were not freely available (i.e., fasting, famine or animal fat/meat based diet). However, recent advances in molecular biology have allowed for circulating ketones to become elevated in the absence of carbohydrate restriction, by consuming exogenous ketone bodies in the form of ketone salts and esters. In response to exogenous ketones becoming commercially available and a key publication by Cox *et al.,* (2016), which found ketone esters to have ergogenic effects in endurance athletes, a growing body of evidence has developed to examine their impact on exercise performance. Current performance responses to ketone esters in cyclists are mixed (Cox *et al.,* 2016; Leckey *et al.,* 2017), with the (R)-3-hydroxybutyl (R)-3-hydroxybutyrate ketone monoester showing ergogenic effects (Cox *et al.,* 2016) and the D,L-1,3-butanediol acetoacetate diester showing ergolytic effects (Leckey *et al.,* 2017) in well-trained endurance athletes. An additional investigation in field athletes found the R)-3-hydroxybutyl (R)-3-hydroxybutyrate ketone monoester to have no impact on intermittent running or 15 m running performance. Other noteworthy findings were a lactate lower effect during exercise (Cox *et al.,* 2016; Evans and Egan, 2018). However, more work is needed to determine if these alterations in exercise metabolism can yield benefits to real world athletes. Current evidence within an endurance trained population is limited to cyclists (Cox *et al.,* 2016; Leckey *et al.,* 2017), therefore, their impact and suitability to other exercise modalities, such as swimming or running remains unexplored.

Early work by Mc Clellan and Du Bois (1930) demonstrated that 2 males could consume a diet high in animal fat and meat and void of fruit and vegetables (i.e., modern day “carnivore diet”) for 12 months without presenting any sign of ill health or malnutrition. More recent longitudinal work in overweight patients with type-2 diabetes mellitus

demonstrated that LCKD is safe and caused improvements in participants' HBA1c scores following a 12 month period of keto-adaption. Despite additional work in mice and overweight patients demonstrating a LCKD can have positive impacts (decreases) on triglycerides (Volek *et al.,* 2009; Brehm *et al.,* 2003; Tay *et al.,* 2008; Volek *et al.,* 2009), HDL/total cholesterol (Bazzano *et al.,* 2015; Yancy *et al.,* 2004; Tay *et al.,* 2008), mitochondrial oxidative stress (Calabrese *et al.,* 2001; Dröse and Brandt, 2012), inflammation (Kim, Davis and Sullivan, 2007; Forsythe *et al.,* 2008; Rhuy and Cho, 2014) and longevity (Edwards *et al.,* 2014; Scheibye-Knudsen *et al.,* 2014; Wallace *et al.,* 2016), it's long term impact and suitability to an endurance trained population remains relatively unexplored.

Given the growing popularity of LCKDs among endurance athletes (Burke, 2015; Volek, Noakes and Phinney, 2015) and oftentimes polarising debate (Noakes and Windt, 2016) regarding its suitability to the general population and endurance sports (Volek *et al.,* 2016; Burke *et al.,* 2017), there is a need for an investigation to examine this dietary paradigm whilst taking previous learnings into consideration, namely 1) short adaptation periods (i.e., <28 days) may provide inadequate time to adapt, for example, a potential marker of adaptation beyond increases in circulating beta-hydroxybutyrate (βHB) and fat oxidation may be the re-maintenance of fasting blood glucose and blood glucose concentrations during exercise. This feat has only been observed in athletes who've habituated towards a LCKD for a number of months (i.e., >8 months) (Volek *et al.,* 2016; Webster *et al.,* 2016), not weeks (i.e., <4 weeks) (Phinney *et al.,* 1983; Burke *et al.,* 2017) and 2) some exercise trials (namely, time to exhaustion trials and steady state exercise trials) do not reflect the metabolic demands placed on elite athletes during endurance events (70-90% of maximal oxygen consumption (VO_{2peak})) (Hawley

and Leckey, 2015), which hinders their transferability to real world practice. Notwithstanding the ergogenic potential of the diet, more information regarding a LCKD impact on biomarkers of health in trained individuals is warranted, as currently there is no experimental evidence of this nature within an endurance trained population.

With these gaps within the literature in mind, a 12 week dietary and training intervention was designed to monitor changes in performance and health relevant to an endurance athlete population, such as endurance and high-intensity exercise performance, body composition, blood lipids, glycaemic control, inflammation and oxidative stress. Further, as a LCKD is restrictive by nature (<50 g/d carbohydrates), micronutrient analysis will be carried out to assess the nutrient density of both a high-carbohydrate and LCKD in non-highly controlled setting. In addition, with current evidence being in its infancy and mixed (Cox *et al.,* 2016; Leckey *et al.,* 2017; Evans and Egan, 2018), an additional focus of this PhD will be to examine an exogenous ketone esters impact on exercise metabolism and simulated running performance within a well-trained endurance population using a double blind crossover designed investigation.

Research Hypothesis

1. Consumption of a LCKD by endurance athletes for a long enough to account for initial decreases in training intensity and allow for re-maintenance of blood glucose (12 weeks), with subsequent examination of performance will result in maintenance or improved endurance performance and impairment of high-intensity exercise performance versus a traditional high-carbohydrate diet.
2. Intake of a LCKD for 12 weeks by endurance athletes will result in reduced levels of oxidative stress and inflammation. In addition, total cholesterol and HDL-cholesterol will increase, while decreases in triglycerides will be observed within the LCKD group.
3. Consumption of a LCKD versus a high-carbohydrate diet will result in markedly different macro- and micronutrient intake. The LCKD will have reduced intakes of fibre (due to reduced fruit, vegetable and wholegrain intake), Vitamin C (due to reduced fruit intake), thiamine (due to reduced wholegrain consumption) and calcium (due to reduced dairy product intake) in comparison to a high-carbohydrate diet, which should meet current dietary guidelines.
4. Acute ingestion of (R)-3-hydroxybutyl (R)-3-hydroxybutyrate ketone monoester will enhance endurance running performance by reducing glycolytic flux (decreased blood glucose/lactate) during submaximal exercise.

Chapter 2

Literature Review

2.1 Fuel Utilisation and Exercise Metabolism

Carbohydrate and fat are predominant substrates utilised under aerobic conditions to fuel adenosine tri-phosphate (ATP) synthesis (i.e., energy production). The rate at which carbohydrates and fats are utilised can vary immensely, with one of the key determining factors for changes in relative energy contributions being exercise intensity (van Loon *et al.,* 2001). Krough and Lindhard (1920) demonstrated using indirect calorimetry that free fatty acids provide the majority of energy at submaximal intensities (25-65% of maximal oxygen consumption (VO_{2peak}), with their contribution declining at higher intensities (>85% VO_{2peak}), as carbohydrates became the primary substrate. Indirect calorimetry analyses exhaled air and determines the rate of fuel utilisation by comparing the rate of oxygen (O_2) uptake and carbon dioxide (CO_2) produced, providing a respiratory exchange ratio (RER) value (also known/described as respiratory quotient (RQ)). A non-protein RER value of 0.70 demonstrates 100% of energy production is derived from fatty acids, whereas an RER value of >1.0 demonstrates 100% of energy is derived from carbohydrate sources, with a midpoint value of 0.85 (RER) indicating 50% energy contribution from fatty acids and 50% energy contribution from carbohydrate sources (Ferrannini, 1988).

Energy contribution from carbohydrates are derived from a number of sources, namely, glycogenolysis, which is the breakdown of liver and muscle glycogen (i.e., carbohydrate stores) (Webster *et al.,* 2017), circulating blood glucose (i.e., ingested carbohydrates) and gluconeogenesis, which is the production of glucose from non-carbohydrate substrates by the liver, such as, lactate, glycerol and some amino acids (Glew, 2010;

Webster *et al.,* 2017). In addition, energy contribution from fatty acids are comprised of the mobilisation and oxidation of adipose tissue, intramuscular triglycerides and blood borne free fatty acids and triglycerides.

Using stable isotope tracer technology and muscle biopsies, Romijn *et al.,* (1993) noted increased utilisation of muscle glycogen and plasma glucose at 40-, 55- and 75% VO_{2peak} and observed increases in fat oxidation until 55% VO_{2peak}, but declines in their utilisation at 75% VO_{2peak}. In accordance with Romijn et al.'s (1993) earlier work, van Loon *et al.,* (2001) noted increases in carbohydrate and fat utilisation up until 55% VO_{2peak}, with increases in muscle glycogen and plasma glucose and decreases in free fatty acids and triglycerides, thereafter. More recently, there is now consensus that the utilisation of carbohydrate (i.e., muscle and liver glycogen; blood glucose from ingested carbohydrates and gluconeogenesis) and fat (i.e., adipose and intramuscular triglycerides; blood borne free fatty acids and triglycerides) are relative to changes in exercise intensity (percentage VO_{2peak}) and can be impacted upon by diet composition in the preceding days, i.e., low, medium or high-carbohydrate availability (Bergström, Hermansen and Hultman, 1967; Hawley, Maughan and Hargreaves, 2015).

Beyond dietary intake, training status (i.e., untrained versus highly-trained) and biological sex can impact upon fuel utilisation, with highly-trained persons obtaining a greater capacity to utilise carbohydrates and maintain high power outputs (Hawley and Leckey, 2015), while female athletes have higher rates of fat oxidation versus males (Randell *et al.,* 2017). Training status and gender aside, the energy demands of an event/race are primarily dictated by two key factors, namely, the duration an event and the relative exercise intensity an athlete completes it (Hawley and Leckey, 2015; Egan, Hawley and Zierath, 2016). For example, an Olympian completing a 100 m sprint would

rely almost exclusively on the ATP phosphocreatine system. The ATP phosphocreatine system powers exercise anaerobically for 8-10 seconds, regenerating ATP from adenosine diphosphate (ADP) and phosphate molecules catalysed by an interaction with phosphocreatine stored within the muscle (Egan, Hawley and Zierath, 2016). For longer duration sprints, such as a 400-800 m sprint, the ATP phosphocreatine system is insufficient (~10 seconds) to fuel a 2-3 minute bout of high-intensity exercise, necessitating energy contribution from glycolysis (i.e., breakdown of muscle glycogen). Similar to the ATP phosphocreatine system, glycolysis works anaerobically, producing ATP from the conversion of muscle glycogen to lactate (Egan and Zierath, 2013). In contrast, endurance sports typically range from 1-3 hours in length, requiring the need for aerobic metabolism. Similar to a sprint, the goal of an endurance event is to maintain high power outputs/speeds (Hawley and Leckey, 2015), ultimately leading to a competitive time to completion. Fernandez-Garcia *et al.,* (2002) demonstrated 93 minutes out of 123 minute race were completed >70% VO_{2peak}, with 16.8% completed under anaerobic conditions. In such a scenario, optimal athlete performance relies on efficient interaction of the ATP phosphocreatine system and anaerobic and aerobic metabolism (Egan and Zierath, 2013). Therefore, the presence of carbohydrate/glycogen or 'carbohydrate availability' (Burke et al., 2011) for athletes is an important characteristic of optimal athletic performance (Hawley and Leckey, 2015). In addition to carbohydrates providing athletes with an ability to perform glycolic modes of exercise (i.e., >75% VO_{2peak}) more effectively (Romijn *et al.,* 1993; van Loon *et al.,* 2001), even at moderate/aerobic intensities (60% VO_{2peak}), there is greater ATP produced per litre of oxygen consumed when carbohydrates are the primary substrate, versus fat (Cole *et al.,* 2014), therefore, greater efficiency at submaximal and maximal

intensities (Hawley and Leckey, 2015). For these reasons, a high-carbohydrate diet is described as the “evidence based choice for elite athletes” (Helge, 2017).

As discussed within Section 2.2, stores of carbohydrate are limited and require supplementation during events >60-90 minutes in length to delay the onset of fatigue. Fatigue is defined by a depletion of liver glycogen stores (Gonzalez *et al.*, 2016). However, glycogen is much more than a fuel source for exercise, it’s acts as a regulator of many cell signalling pathways, such as the oxidative phenotype, insulin sensitivity and contractile function (Hearris *et al.*, 2018), necessitating the need for carbohydrate availability (via glycogen stores, orally ingested and/or endogenously produced i.e., gluconeogenesis) to maintained function and performance (Gonzalez *et al.*, 2016). Additionally, one of the more interesting and emerging areas of research within sports nutrition is the ergogenic effect of nutrients which do not need to be ingested, i.e., mouth rinses (Burke and Maughan, 2015). Preliminary work by Chambers, Bridge and Jones (2009) demonstrated that rinsing the mouth with a glucose or maltodextrin solution resulted in increased pacing and power outputs. It’s thought exposing oral cavities to carbohydrate based food/fluids positively impacts on the central nervous system, ultimately increasing pacing and reducing perceived exertion at a relative intensity (Burke and Maughan, 2015). Mouth rinsing or “mouth swilling” appears to have practical implications, as it may provide athletes with an opportunity to train low (i.e., low-carbohydrate availability) to enhance cellular responses (Burke *et al.,* 2011) while causing enhanced enjoyment (i.e., decreased exertion) and/or enhance performance while not putting additional load on the gut for persons who are susceptible to gastrointestinal stress/discomfort (Burke and Maughan, 2015).

2.2 High-Carbohydrate Diet

General Population

A carbohydrate based diet derives the majority of energy from carbohydrates, such as fruit, vegetables, grains, cereals, pastas, bread and pastries. This dietary approach is recommended to the general public in Ireland to optimise health and wellbeing as it provides a variety of fruits and vegetables (Safe Food, 2017; Health Service Executive, 2016) and freedom to include a variety of other foods, such as dairy, meats, fish, nuts, seeds and eggs. This all-inclusive dietary approach is common practice among other developed nations (World Health Organisation, 2015), with just one country (Sweden) opting for a low-carbohydrate approach as their national dietary guidelines (Shilhavy, 2017)(Shilhavy, 2017). On a well-balanced carbohydrate based diet, daily guidelines recommend 5–7 servings of fruit and vegetables, 3-5 servings a day of wholemeal cereals, breads and potatoes, 2 servings of meat, fish, eggs, beans and nuts and finally, fats, spreads and oils in very small amounts (Safe Food, 2017). The Food Safety Authority of Ireland (2011) recently updated it's 'food pyramid' guidelines (Safe Food, 2017), putting less emphasis on servings of breads, grains and pasta's and greater emphasis on servings of fibrous vegetables and fruit.

Athlete Specific Guidelines

Similar to the general population, endurance athletes are recommended to consume a carbohydrate based diet (Burke *et al.,* 2011; Jeukendrup, 2011; Potgieter, 2013). However, within an athletic population, there are specific macronutrient guidelines to optimise carbohydrate availability and therefore, performance. For example, an

endurance athlete who completes 1–3 hours a day of moderate to high intensity training is recommended to consume 6–10 g·kg (grams per kilogram bodyweight) of carbohydrate, whereas an athlete who complete >4-5 hours a day is recommended to consume 8–12 g·kg of carbohydrate per day (Burke *et al.,* 2011; Jeukendrup, 2014). These guidelines are put in place to supply sufficient energy to fuel training sessions (carbohydrate availability) and to restore glycogen stores post exercise (Burke *et al.,* 2011). Burke (2015) stresses that daily consumption of carbohydrates is not static, rather carbohydrate consumption is based on the energy requirements of the activity at hand, thus high-carbohydrate expenditure means high-carbohydrate requirement.

An average person can store up to 400 g of glycogen in their active muscle and 100 g in their liver; which equates to roughly 2,000 kcal (Prevost, 1999). An elite endurance athlete oxidises >18-20 calories per minute ($kcal \cdot min^{-1}$) during exercise (Mauder, Kidling and Plews, 2018), necessitating the need for exogenous fuel for exercise lasting >90-100 minutes in length. To circumvent glycogen stores becoming depleted, athletes are recommended to consume 30–60 g/h of carbohydrates for exercise ranging from 1-3 hours in duration and 90 g/h of carbohydrate for exercise exceeding 2.5 hours (Burke *et al.,* 2011; Jeukendrup, 2014). In addition, in order to improve absorption, athletes are recommended to use multi-transportable source of carbohydrates (i.e., contains glucose and fructose) (Jeukendrup, 2010).

2.3 Low-Carbohydrate Diets

2.3.1 Definitions of Low-carbohydrate Diets

In essence, a low-carbohydrate diet encourages reduced energy from grains, starchy vegetables and fruits, with increased energy from nuts, seeds, animal fat, oils, meat, fish and poultry (Noakes and Windt, 2016). There are various names given to low-carbohydrate diets, e.g. low-carbohydrate high fat diet (LCHF), Atkins diet, Eco–Atkins (vegan), zero-carbohydrate diet or low-carbohydrate Mediterranean diet (Gunnars, 2017)(Gunnars, 2017). These diets all have two things in common, they are lower in carbohydrates than a traditional diet (<40–60% of total calories) and generally higher in fat (>30% of total calories) (Erlenbusch *et al.,* 2005). As a result, the implementation of a low-carbohydrate diet is very much open to interpretation, due to opposing scientific and non-scientific definitions as to what proportion of each macronutrient should make up a low-carbohydrate diet.

Though many definitions exist, the following is a commonly used three-tiered system describing various levels of carbohydrate restriction (Feinman *et al.,* 2015; Noakes & Windt, 2016).

Tier 1: Moderate carbohydrate diet (26-45% of daily calories)

Tier 2: Low-carbohydrate high-fat (LCHF) (<130g of carbohydrate (CHO)/day)

Tier 3: Low-carbohydrate, ketogenic (LCKD) (>75% of total energy from fat, <50 g/d of CHO)

In addition, two reviews examining a low-carbohydrate diets impact on performance defined a LCHF diet as a diet where >30% (Erlenbusch *et al.,* 2005) and >50% (Burke,

2015) of total calories are derived from dietary fat. For the purpose of the current investigation, LCHF diets are defined as containing >50% of total energy from dietary fat.

Although low-carbohydrate diets go by many different names, the metabolic principles behind each are quite similar. By reducing dietary carbohydrate and increasing dietary fat and protein intake, a minor shift in metabolism takes place, reliance on glycogen for energy is reduced and there's a subsequent increase in energy contribution from fat (Bergström and Hultman, 1967). This premise has been demonstrated in athletes via reduced RER values (Romijn *et al.*, 1993). Increases in fat oxidation are associated with improved satiety (McClernon *et al.,* 2007), hence low-carbohydrate diets are an increasingly popular weight loss tool. Some clinical studies have shown LCHF diets are more effective than a low fat diet for achieving weight loss (Brehm *et al.,* 2003; Volek *et al.,* 2004), however there is contradictory evidence to these findings (Hall *et al.,* 2015). This dietary strategy for achieving weight loss is breaking new ground in terms of popularity, Joe Wick's 'Lean in 15' cookbook, which encourages a low-carbohydrate diet surpassed all previous weight loss cook books sales (The Guardian, 2017). This popularity has most likely stemmed from the ease at which people appear to lose weight when carbohydrates are restricted to <100 g/d (Smith *et al.,* 2017).

2.4. Low-Carbohydrate High Fat Diets and Performance in Untrained Individuals

Early research demonstrated that <7 days of carbohydrate restriction caused decreases in endurance performance (Krogh and Lindhard, 1920; Murlin *et al.,* 1928; Kark, Johnson and Lewis, 1945; Bergström and Hultman, 1967). In the 1980's, Phinney *et al.,* proposed that an adaptation to a low-carbohydrate diet may need >7 days. Phinney *et al.,* (1980) demonstrated that moderate intensity exercise on a treadmill could be improved in untrained following consumption of a LCKD containing <20 g/d of carbohydrate for 5 weeks (Table 2.1). However, Phinney *et al.'s* (1980) investigation has a number of limitations, namely, there was a large decrease in body mass, therefore it may have positively impacted on performance despite a back pack containing weight lost (kg) during the intervention period being worn by participants to try and account for its impact as a confounding variable, and finally, the lack of a control group and presence of some outliers which appears to have skewed the mean values of improvement.

Helge, Richter and Kiens, (1996) carried out a randomized control trial in untrained males the effects severe carbohydrate restriction had on performance versus a high-carbohydrate diet was largely unknown. During the investigation (Table 2.1), untrained participants consumed a high-carbohydrate diet (*n*= 10, %carbohydrate:fat:protein = 65:20:15) or a LCHF diet (*n* = 10, 21:62:17) for 7 weeks and completed a training programme, consisting of 3–4 sessions a week lasting 60–75 minutes at moderate to high intensity (50–85% VO_{2peak}). In week 7 all participants repeated baseline measurements; VO_{2peak} and time to exhaustion (TTE) at 81% VO_{2peak} on a cycle ergometer. Time to exhaustion increased by +191% in the high-carbohydrate and +86% in the LCHF group ($P < 0.05$). Although the LCHF participants experienced an increase in

fat oxidation from baseline, their cumulative increase in performance, was less than the high-carbohydrate groups. Thus, the consumption of carbohydrates was once again associated with reduced performance in untrained males in this investigation.

Fleming *et al.,* (2003) carried out an investigation to assess a LCHF diets impact on high intensity exercise performance. This randomized control trial (RCT) incorporated untrained males and a 6 week adaptation period to a high-carbohydrate diet (*n* = 10, %carbohydrate:fat:protein = 59:25:15) or LCHF diet (*n* = 10, 8:61:30) (Table 2.1). Similar to Helge, Richter and Kiens (1996) investigation, a training plan was provided, including 2–4 sessions per week of walking, running, cycling and cross-training. Blood beta-hydroxybutyrate (βHB) increased from 0.08 to 0.29 mM, indicating good dietary adherence in the LCHF group. Although the LCHF group lost 2.2 kg, there was no significant change to either group's maximal oxygen consumption during exercise (VO_{2peak}). Peak and mean power outputs during the Wingate decreased in the LCHF group, whereas performance remained unchanged in the high-carbohydrate condition (*P* = 0.25). Fleming *et al.,* (2003) suggests that acute consumption of a LCHF diet does not improve performance in 'non-highly trained individuals' and that its implementation can be detrimental to performance nearing maximal intensities when compared to similar individuals consuming a high-carbohydrate diet. These findings support previous work by Helge, Richter and Kiens, (1996) illustrating a high-carbohydrate diet to be superior to a LCHF diet for improving acute performance responses in un-trained individuals.

Table 2.1. Low-carbohydrate high fat diets (>50% of total calories from fat) and performance investigations in untrained individuals

Author	Population Sample & Study Design	Adaptation Period & LCHF & HC Diets	Performance Tests	Performance Nutrition	Performance Results
Phinney *et al.,* (1980)	*n* = 6 moderately overweight & untrained participants (5 males, 1 female) (<44 mL·kg^{-1}min^{-1}) Non-random CT	6 weeks HC (45% CHO, 40% fat, 15% protein) LCHF (5% CHO, 80% fat, 15% protein)	TTE @ 75% VO_{2peak} in week 1 & 6	12 hour fast No CHO during exercise	LCHF group lost 10.6 kg (P < 0.05) LCHF TTE decreased to 80% of baseline after week 1, but increased to 155% in week 6 (P < 0.05)
Helge *et al.,* (1996)	*n* = 20 untrained males (<44 mL·kg^{-1}min^{-1}*)* RCT	7 weeks HC (65% CHO, 20% fat, 15% protein) LCHF (21% CHO, 62% fat, 17% protein) +1 week HC (65% CHO, 20% fat, 15% protein)	VO_{2peak} and TTE @ 81% VO_{2peak}	Pre-exercise meal not specified No CHO during exercise	TTE improved by 191% in HC (P < 0.05) and 68% in LCHF in week 7 (P < 0.05) TTE improved a further 18% in LCHF group in week 8 (P < 0.05), 26% shorter than HC improvement (P < 0.05)

Abbreviations: CHO = carbohydrate; CT = control trial; HC = high-carbohydrate; kcal = calorie; kg = kilogram; LCHF = low-carbohydrate high fat; min = minute; *n* = number; mL·kg^{-1}min^{-1} = millilitres per kilogram per minute; RCT = random control trial; TTE = time to exhaustion; VO_{2peak} = maximal oxygen consumption

Table 2.1. Contd. Low-carbohydrate high fat diets (>50% of total calories from fat) and performance investigations in untrained individuals

Author	Population Sample & Study Design	Adaptation Period & LCHF & HC Diets	Performance Tests	Performance Nutrition	Performance Results
Fleming *et al.,* (2003)	*n* = 20 untrained males 19–56 years (<45 $mL \cdot kg^{-1}min^{-1}$) RCT	6 weeks HC (59% CHO, 25% fat, 15% protein) LCHF (8% CHO, 61% fat, 30% protein)	VO_{2peak}, 30 sec Wingate, 45 min timed run	361 kcal (10% CHO, 37% fat, 53% protein) prior to VO_{2peak} 221 kcal (5% CHO, 55% fat, 40% protein) prior to Wingate and 45 min TT	30 sec Wingate performance decreased LCHF group ($P < 0.05$) Work output during 45 min ride 18% lower in LCHF group ($P < 0.05$)

Abbreviations: CHO = carbohydrate; HC = high-carbohydrate; kcal = calorie; LCHF = low-carbohydrate high fat; min = minute; *n* = number; $mL \cdot kg^{-1}min^{-1}$ = millilitres per kilogram per minute; RCT = random control trial; sec; second; TT = time trial; VO_{2peak} = maximal oxygen consumption

2.4.1 Low-Carbohydrate High Fat Diets and Performance in Athletes

As previously outlined, over the past number of years low-carbohydrate diets have grown in popularity amongst endurance athletes. This is despite "15 years of failed attempts to harness adaptations to a high-fat diet as an ergogenic strategy for sports performance in well-trained competitors" (Burke, 2015). Previous research trials involving a high-carbohydrate diet versus a LCHF diet scenario in athletes, have involved 1–5 week intervention periods and exercise trials following an overnight fast (O' Keeffe *et al.,* 1989; Lambert *et al.,* 1994; Goedecke, 1999; Rowlands and Hopkins, 2002; Vogt *et al.,* 2003) or with limited carbohydrate feeding (Goedecke, 1999; Rowlands and Hopkins, 2002; Vogt *et al.,* 2003). These investigations found that acute ingestion (>5-days) of a LCHF diet caused significant increases in whole body fat oxidation (O' Keeffe *et al.,* 1989; Goedecke *et al.,* 1999), with reduced reliance on carbohdyrates (O' Keeffe *et al.,* 1989; Lambert *et al.,* 1994; Goedecke, 1999, Rowlands and Hopkins, 2002; Vogt *et al.,* 2003). Further, evidence suggests that in some cases, time to exhaustion (Lambert *et al.,* 1994) and ultra-endurance performance (Rowland and Hopkins, 2002) can be improved following a 2–5 week period of consuming a LCHF diet, relative to a high-carbohydrate condition. On the back of these arguably promising findings, leading authors and dietary prescription authorities have taken a hard-nosed view towards LCHF diet as a dietary approach and disregard it as beneficial ergogenic aid and proclaimed a high-carbohydrate diet as the evidence based choice for endurance athletes (International Olympic Committee, 2010; Burke *et al.,* 2011; Jeukendrup, 2014; Helge, 2017). This is despite a review by Erlenbusch *et al.,* (2005) summarising that although a high-carbohydrate diet appears to improve performance more so than a LCHF diet in un-

trained individuals (Table 2.1), this may not be true in well-trained individuals and that "a conclusive endorsement of a high-carbohydrate diet based on the literature is difficult to make".

The subsequent sections includes investigations carried out comparing high-carbohdyrate and LCHF diets in athletes. These investigations are summarised within Table 2.2.

Table 2.2. Low-carbohydrate high fat diets (>50% of total calories from fat) and performance investigations in athletes

Author	Population Sample & Study Design	Adaptation Period & LCHF & HC Diets	Performance Tests	Performance Nutrition	Performance Results
O'Keeffe *et al.,* (1989)	*n* = 7 trained female cyclists (5 h/week) Crossover design	7 days MC (54% CHO, 25% fat, 21% protein) HC (72% CHO, 12% fat, 15% protein) LCHF (13% CHO, 59% fat, 28% protein)	VO_{2peak} and TTE @ 80% VO_{2peak}	Pre-exercise meal not specified No CHO during exercise	Time to exhaustion reduced by 47% with LCHF diet ($P < 0.05$) Improved from LCHF to MC (+38 min) and LCHF to HC (+53 min) ($P < 0.05$)
Lambert *et al.,* (1994)	*n* = 5 well trained male cyclists (100-120 km/week) Crossover design	14 days HC (74% CHO, 12% fat, 14% protein) LCHF (7% CHO, 70% fat, 23% protein)	30 sec Wingate, TTE @ 90% VO_{2peak}, TTE @ 60% VO_{2peak}	Overnight fast No CHO during exercise	Wingate performance (+58 W LCHF group) ($P > 0.05$) TTE @90% VO_{2peak} -4.2 min (33.6%) LCHF diet ($P > 0.05$) TTE @60% VO_{2peak} +37.2 min (87%) LCHF diet ($P < 0.001$)

Abbreviations: CHO = carbohydrate; CT = control trial; h = hour; HC = high-carbohydrate; km = kilometre; LCHF = low-carbohydrate high fat; min = minute; *n* = number; $mL \cdot kg^{-1}min^{-1}$ = millilitres per kilogram per minute; RCT = random control trial; sec = second; TTE = time to exhaustion; VO_{2peak} = maximal oxygen consumption; W = watt

Table 2.2. Contd. Low-carbohydrate high fat diets (>50% of total calories from fat) and performance investigations in athletes

Author	Population Sample & Study Design	Adaptation Period & LCHF & HC Diets	Performance Tests	Performance Nutrition	Performance Results
Goedecke *et al.,* (1999)	*n* = 16 well trained male cyclists (>61 VO_{2peak}) Parallel group design	15 days HC (53% CHO, 30% fat, 13% protein) LCHF (19% CHO, 69% fat, 10% protein)	150 min @ 63% VO_{2peak} and 40km TT, on day 0, 5, 10 and 15	Overnight fast, 14 g of MCT 90 min prior (0.3 g·kg BW MCT) 0.3 g·kg/h MCT & 0.8 g·kg/h CHO during	40 km TT day 0 to day 15 LCHF group (-5.9min) ($P < 0.05$), HC group (-4.3min) ($P < 0.001$) 40km TT between group ($P > 0.05$)
Rowlands and Hopkins, (2002)	*n* = 7 well trained male cyclists (nationally competitive cyclists) Crossover design	14 days HC (70% CHO, 15% fat, 15% protein) LCHF (15% CHO, 70% fat, 15% protein)	5 hour exercise trial including an incremental power test, 15 min TT & 100km TT	3 pre-exercise meals (each 42 KJ) 1.5 h prior 58 g/h CHO during (0.8 g·kg/h CHO)	Non-significant 2% increase in power outputs during final 5 km of 100 km within LCHF group ($P > 0.05$)

Abbreviations: CHO = carbohydrate; CT = control trial; g·kg/h = gram per kilogram body weight per hour; g/h = gram per hour; h = hour; HC = high-carbohydrate; kJ = kilojoule; LCHF = low-carbohydrate high fat; MCT = medium chain triglyceride; min = minute; *n* = number; $mL \cdot kg^{-1}min^{-1}$ = millilitres per kilogram per minute; RCT = random control trial; sec = second; TT = time trial; VO_{2peak} = maximal oxygen consumption

Table 2.2. Contd. Low-carbohydrate high fat diets (>50% of total calories from fat) and performance investigations in athletes

Author	Population Sample	Adaptation Period & LCHF & HC Diets	Performance Tests	Performance Nutrition	Performance Results
Vogt *et al.,* (2003)	*n* = 11 well trained male duathletes (nationally competitive cyclists) Crossover design	5 weeks HC (68.2% CHO, 16.5% fat, 14.3% protein) LCHF (31% CHO, 52.9% fat, 14.4% protein)	40 min incremental test, 20 min TT @ 89% VO_{2peak}, running outdoor 21 km TT	400-500 kcal LCHF or CHO meal Pre and mid race nutrition not stated	No difference in 20 min TT (HC 298 W, LCHF 297 W) or 21 km run times (80:12 min HC, 80:24 min LCHF) ($P > 0.05$)

Abbreviations: CHO = carbohydrate; CT = control trial; h = hour; HC = high-carbohydrate; kcal = calorie; km = kilometre; LCHF = low-carbohydrate high fat; min = minute; *n* = number; $mL \cdot kg^{-1}min^{-1}$ = millilitres per kilogram per minute; sec = second; TT = time trial; VO_{2peak} = maximal oxygen consumption; W = watt

Three decades ago O'Keeffe *et al.,* (1989) examined the acute impact of the LCHF diet in 7 moderately trained female cyclists (Table 2.2). Interestingly, there were three dietary components to this investigation, a LCHF diet (%carbohydrate:fat:protein = 13:59:13), a moderate carbohydrate diet (54:25:21) and a high-carbohydrate diet (72:12:15). Following completion of baseline measurements (VO_{2peak}), participants were randomly assigned to one of the three dietary protocols for 7-days, during which participants were asked to maintain their current training volume and intensity. On the 8th day of each dietary protocol participants arrived fasted and completed an exercise TTE trial on a cycle ergometer at 80% of their predetermined VO_{2peak}. Mean TTE was 60 minutes following the LCHF diet, 98 minutes following the moderate carbohydrate diet and 110 minutes on high-carbohydrate diet ($P < 0.05$), suggesting chronic adaptation (7-days) to a low-carbohydrate diet (<13% of calories from carbohydrates) can be detrimental to performance at 80% VO_{2peak} in moderately trained female cyclists. Unlike a number of studies, individual TTE times were presented in this article, which gives an insight into how each participant reacted to each of the three dietary protocols. Each of the 7 participants performed better when carbohydrate consumption was increased from LCHF to moderate, however just 5 of the 7 participants increased their TTE when carbohydrate consumption was increased from moderate to high. The high-carbohydrate groups RER curve at rest and during exercise was higher than the LCHF and moderate carbohydrate groups ($P < 0.05$), indicating greater levels of carbohydrates were being oxidised. Perhaps, the 2 outlier's TTE increased on the moderate carbohydrate diet due to their decreased reliance on carbohydrates and newly developed ability to oxidise fatty acids at 80% VO_{2peak}. A large study by Randell *et al.,* (2017) involving 1121 athletes from different sporting backgrounds illustrated that peak rates of fat oxidation occur at 23–89% of an athletes VO_{2peak}. This large degree of

variance among athletes gives value to the idea of individual outliers to dietary prescription suggest that some athletes may be better suited or naturally equipped to perform on a low-carbohydrate diet. Further, it adds value to Erlenbusch *et al.,* (2005) synopsis that a comprehensive endorsement of a high-carbohydrate cannot be made.

Lambert *et al.,* (1994) examined a similar high-carbohydrate diet (%carbohydrate:fat:protein = 74:12:14) and LCHF diet (7:70:23) to O' Keeffe *et al.,* (1989) although, participant type, length of the adaptation period and exercise protocol implemented were different. This investigation involved well trained male cyclists (*n* = 5) with a 14 day adaptation period implemented. Unlike O' Keeffe *et al.,* (1989) who tested time to exhaustion at 80% VO_{2peak}, Lambert *et al.,* (1994) tested TTE at 90% VO_{2peak} and 60% VO_{2peak} consecutively on the same day, both whilst being in a fasted state. During the course of the intervention participants were required to maintain current levels of activity. At the midpoint of this cross over study there was a 2 week washout period where participants were instructed to maintain training volume and eat *ad libitum*. On the 14th day of each dietary protocol, participants returned to the exercise laboratory fasted and completed a 30 second Wingate and a TTE trial on a cycle ergometer at 90% VO_{2peak}. Following 20 minutes rest, participants then completed a second TTE trial at 60% VO_{2peak}. RER was recorded at 5 minute intervals during each TTE trials, while βHB, lactate and glucose were measured intravenously throughout. LCHF participants demonstrated lower levels of pre-exercise muscle glycogen (120.6 to 68.1 $mmol \cdot kg^{-1}$), increased maximum velocity ($+25° \cdot S^{-1}$/Vmax) and a non-significant increase in maximal power output (+58 W). Decreased glycogen availability appears to have affected 90% VO_{2peak} performance, as performance was visibly, yet non-significantly compromised (TTE = 12.5 min HC versus 8.5 min LCHF). Spriet *et al.,* (2014) outlined that

exercise performance at higher intensities decreases when there is decreased glycogen availability, as the mitochondria cannot as rapidly convert fat to ATP, due to greater ATP produced/oxygen consumed ratio. Despite TTE at 90% VO_{2peak} not being significantly different between groups, TTE at 60% VO_{2peak} trial increased (79.7 min LCHF versus 42.5 min HC) ($P < 0.01$). RER values were lower at 60% VO_{2peak} (0.87 RER LCHF versus 0.92 RER HC, $P < 0.05$) and fat oxidation was almost two fold higher following the LCHF diet (60 $g \cdot min^{-1}$ LCHF versus 32 $g \cdot min^{-1}$ HC, $P < 0.05$). It's apparent that this increase in moderate intensity (60% VO_{2peak}) performance was due to participants increased ability to oxidise fat. It should be noted, that despite significant increases in fat oxidation, βHB readings remained unchanged throughout, indicating that a LCHF does not stimulate ketogenesis. This investigation by Lambert *et al.,* (1994) showed promise for fat adaptation as an ergogenic aid for endurance performance, which undoubtedly inspired further research.

Further research by Goedecke *et al.,* (1999) implemented a similar dietary intervention with an additional pre-exercise meal (14 g of medium chain triglyceride (MCT) oil 90-min prior) and some feeding during (0.3 g·kg/h MCT oil and 0.8 g·kg/h carbohydrate (CHO)). Well-trained male cyclists ($n = 16$) who habitually consumed a carbohydrate based diet were randomly assigned to a high-carbohydrate group ($n = 8$, %carbohydrate:fat:protein = 53:30:13) or a LCHF group ($n = 8$, 19:69:10) for 15 days in a parallel group designed investigation. Unlike previously investigations, changes in fuel utilisation and exercise performance were monitored every 5-days, thus, on day 0, 5, 10 and 15, participants arrived fasted, consumed their pre-exercise meal (14 g MCT oil) and completed a 2.5 hour constant load ride at 63% VO_{2peak} and a 40 km TT. Each groups 40 km TT performance improved from baseline measurements (HC, day 0: 69.9 min, day 15: 65.6

min, LCHF, day 0: 69.3 min, day 15: 63.4 min), but no significant difference between group was evident. Burke (2015) believes that each group's increase in performance were due to the training effect. However, this acute increase in performance is unlikely, as participants were already well-trained (>61% VO_{2peak}), thus, increases are more likely due to improved race strategies i.e., familiarisation with the exercise protocol. The unique and most important finding from this investigation is that by day 5, LCHF participants experienced increases in fat oxidation (0.67 to 0.91 $g \cdot min^{-1}$), whilst the high-carbohydrate groups re-maintained unchanged (0.60 $g \cdot min^{-1}$). These rates of fat oxidation were sustained in day 10 and day 15. This investigation demonstrates that acute consumption (5-days) of a LCHF diet can increase fat oxidation and spare muscle glycogen i.e., carbohydrate oxidation decreased from 2.5 $g \cdot min^{-1}$ to 2.0 $g \cdot min^{-1}$ during a 2.5 hour ride at 70% VO_{2peak}.

Up until this point, investigations involving LCHF diets incorporated exercise protocols ranging from 2–3 hours in length. Rowlands and Hopkins, (2002) were the first to assess the suitability of fat adaptation towards an ultra-endurance styled exercise protocol. Well-trained male cyclists (*n* = 7) consumed a high-carbohydrate diet (%carbohydrate:fat:protein = 70:15:15) or LCHF diet (15:70:15) for 14-days, with a 14-day washout period in-between (Crossover). Similar to O' Keeffe *et al.,* (1989) and Lambert *et al.,* (1994), participants were asked to maintain their current training volume and intensity during the course of the investigation. VO_{2peak} and peak power output were recorded on a cycle ergometer on day 11, however the ultra-endurance style exercise trial took place on day 14. Participants arrived to the laboratory fasted and received 3 pre-exercise meals (HC or LCHF) 60–90 minutes prior to beginning exercise, meals provided 42 kJ of energy and 8 ml of water per kilogram bodyweight. Upon completion

of a 15 minute warm up at 35% VO_{2peak}, participants completed a 15 minute TT, followed by a 45 minute steady state ride at 50% VO_{2peak} and an incremental test designed to measure fuel utilisation at different intensities (ranging from 37.5–82.5% peak power). Finally, participants completed a 100 km TT, which included a 5 km sprint finish. Respiratory samples were taken for 5–7 minutes at 10, 30, 70 and 90 km mark during the 100 km TT.

During the 5 hours of data collection, participants in each group consumed a carbohydrate (56 g/h) (glucose and fructose) and an electrolyte solution. Peak rates of fat oxidation were 2.9 times higher in the LCHF group, fat oxidation was 0.93 $g \cdot min^{-1}$ versus 0.32 $g \cdot min^{-1}$ in the HC group ($P < 0.001$). Mean 100 km TT time non-significantly decreased 2.5 minutes in the LCHF group, while non-significantly increased 1.6 minutes in the high-carbohydrate group, indicating a 4% improvement in performance in the LCHF trial versus the high-carbohydrate trial. Power output during the final 5 km sprint finish non-significantly decreased 4.5% in high-carbohydrate and non-significantly increased 6.3% in LCHF. Although statistically there was no difference between groups performance, increases in fat oxidation did tend to positively impact on ultra-endurance performance, relative to the high-carbohydrate group (Table 2.2).

Finally, Vogt *et al.,* (2003) examined the acute impact of consuming a LCHF diet on exercise metabolism and performance in well trained duathletes ($n = 11$) in a crossover designed study. The dietary intervention was unique as it was 5 weeks versus previous LCHF investigations being <15-days. Baseline measurements were taken 7 days prior to the start of the first dietary protocol and repeated at the end of each dietary protocol (Table 2.2). Prior to exercise, participants consumed a meal containing 400–500 kcal (HC or LCHF). The exercise protocol consisted of a submaximal test at 4 separate intensities

on a cycle ergometer, followed immediately by a 20 minute TT and a half marathon <48 hours after preceding tests. Carbohydrate consumption was 246 g/d in the LCHF group and 475 g/d in the high-carbohydrate group ($P < 0.05$). Interestingly, the reduction in carbohydrate consumption did not reduce pre-exercise muscle glycogen in the LCHF group (487.8 mmol·kg^{-1}) versus in high-carbohydrate group (534.4 mmol·kg^{-1}) ($P = 0.24$), but was associated with increases in fat oxidation (HC = RER range: 0.89 – 1.06, LCHF = RER range: 0.78 – 1.03; $P < 0.05$). The LCHF did impact on performance, with maximal power output (375 W HC Vs 374 LCHF), VO_{2peak} (63.9 mL·kg^{-1}min^{-1} HC, 63.6 mL·kg^{-1}min^{-1} LCHF), 20 minute TT performance or half marathon performance (80 min 24 sec HC, 80 min 12 sec LCHF) remaining unchanged (*all* $P > 0.05$). Thus, this investigation illustrated that well-trained male duathletes can maintain pre-exercise muscle glycogen stores and performance at moderate and high intensity, following a 5 week adaptation.

Performance literature involving LCHF diets containing >50% of total energy from fat have illustrated that well-trained male athletes can maintain endurance (Lambert *et al.,* 1994; Goedecke *et al.,* 1999; Vogt *et al.,* 2003) and ultra-endurance performance (Rowland and Hopkins, 2002). One of the most important observations from LCHF research to date is that well-trained endurance athletes can increase rates of whole body fat oxidation after as little as 5-days of consuming a LCHF diet (0.67 to 0.91 g·min^{-1}) (Goedecke *et al.,* 1999) and that these rates of fat oxidation remained uninterrupted despite some participants consuming up to 58 g/h of carbohydrate during exercise (Rowlands and Hopkins, 2002). Decreasing the body's reliance on carbohydrates as a primary fuel source may have its benefits to some endurance athletes, particularly those who compete in events lasting >2.5 hours or ultra-endurance/ironman events lasting 8–10+ hours. Gastrointestinal distress symptoms such as, nausea, vomiting, abdominal

cramping and diarrhoea effect 37–89% of endurance athletes (runners) in races lasting >2.5 hours (Baska, Moses and Graeber, 1990; Hoffman and Fogard, 2011; Stuempfle, Hoffman and Hew-Butler, 2013). The impact exercise has on gastrointestinal function is very complex, thus, identifying individual causes of gastrointestinal distress can be difficult to make (de Oliveira, Carlos Burini and Jeukendrup, 2014). Nutrition, particularly highly concentrated carbohydrates are known to cause gastrointestinal distress (de Oliveira, Carlos Burini and Jeukendrup 2014). An investigation by Wallis *et al.,* (2007) demonstrated that female participants experienced less gastrointestinal distress on a low-carbohydrate intake (0 – 0.5 $g \cdot min^{-1}$) during exercise versus those on a high-carbohydrate intake (>1.0 – 1.5 $g \cdot min^{-1}$). Endurance athletes who commonly suffer from gastrointestinal distress and have eliminated other potential factors such as, excessive fibre or dehydration (Stuempfle, Hoffman and Hew-Butler, 2013), could potentially benefit from becoming fat adapted. The resultant increases in fat oxidation would reduce their need to consume 6–12 g·kg carbohydrate for 2–3 days prior to an endurance event (Burke *et al.,* 2011; Jeukendrup, 2014) and potentially, reduce their carbohydrate requirement during exercise while maintaining performance.

Undoubtedly, fat adaptation has its downsides. Lambert *et al.,* (1994) investigation observed that submaximal performance (60% VO_{2peak}) was improved following 14-days of a LCHF diet while participant's performance was visibly compromised at 90% VO_{2peak}. The LCHF diet implemented by Lambert *et al.,* (1994) and Rowlands and Hopkins (2002) were similar (Table 2.2), both investigations were performed in 'well-trained cyclists' and the adaptation periods were both 14-days, yet the performance outcomes at higher intensities were different. Lambert *et al.,* (1994) participants performed the exercise trial fasted, whereas Rowlands and Hopkins (2002) participants consumed a pre-

exercise meal and 58 g/h of carbohydrate during exercise. Rowlands and Hopkins (2002) participants maintained increase in fat oxidation and improved 5 km sprint performance relative to the high-carbohydrate condition. Thus, perhaps the consumption of carbohydrate is necessary during exercise on a LCHF diet to maintain performance at higher intensities in well trained endurance athletes.

Future research should focus on optimally fuelling each dietary protocol (HC or LCHF) to improve accurate comparability and transferability to real world endurance and ultra-endurance athletes. This holistic approach could bring clarity to an argumentative topic within endurance sports nutrition (Burke, 2015; Volek, Phinney and Noakes, 2015). A LCHF approach may not be beneficial or preferable to the majority of the athletic population, but for some athletes it could prove beneficial and potentially provide an alternative to athletes who have carbohydrate intolerances and are currently struggling to optimally fuel their performance on a high-carbohydrate diet. Thus, further research is needed and a more holistic and individual approach to athletes dietary prescription is required.

2.4.2 Low-carbohydrate High Fat Diets with Carbohydrate Restoration and Athlete Performance

As mentioned previously high levels of pre-exercise muscle glycogen and prevention of glycogen depletion during exercise have long been considered a determining factor in successful or elite performance (Bergström, Hermansen and Hultman, 1967) so methods of maximising pre-exercise muscle glycogen stores or slowing the oxidation of glycogen are highly sought after by sports scientists, coaches and athletes alike. Guidelines by recommend the consumption of a high-carbohydrate diet (6-10 g·kg) for 24-36 hours prior to an endurance event (Jeukendrup 2014; Burke *et al.,* 2011) to ensure a state of high-carbohydrate availability (i.e., full glycogen stores). However, there are alternative theories and methods in practice. One common method is implementing a period of carbohydrate restriction (<11.5 days), followed by a period of carbohydrate loading (<3 days). This practice is referred to as 'super-compensation protocol' and is widely thought to increase pre-exercise muscle stores more so than a traditional high-carbohydrate loading phase (Jeukendrup 2014; Burke *et al.,* 2011). This theory has been extensively researched in laboratory studies in athletes, these investigations are summarised in Table 2.3.

Table 2.3. Low-carbohydrate high fat diets (>60% of total calories from fat), followed by a period of carbohydrate restoration in athletes

Author	Population Sample	Adaptation Period & LCHF & HC Diets	Performance Test	CHO Restoration & Performance Nutrition	Performance Results
Burke *et al.,* (2000)	*n* = 8 well trained male cyclists / triathletes (>64 $mL \cdot kg^{-1}min^{-1}$) Crossover design	5 days HC (74% CHO, 15% fat, 11% protein) LCHF (18% CHO, 68% fat, 14% protein)	Cycling 120 min @ 70% VO_{2peak}, 30 min TT (time to complete 7 J/kg BW)	1 day 10 g·kg CHO Fasted during	NS performance change between groups: HC 34.17 min, LCHF 30.73 min TT performance improved 8% in LCHF trial versus HC group (*P* = 0.21)
Burke *et al.,* (2002)	*n* = 8 well trained male cyclists / triathletes (>68 $mL \cdot kg^{-1}min^{-1}$) Crossover design	5 days HC (74% CHO, 15% fat, 11% protein) LCHF (18% CHO, 68% fat, 14% protein)	Cycling 120 min @ 70% VO_{2peak}, 30 min TT (7 J/kg BW)	1 day 10 g·kg CHO 2g·kg CHO 2 h prior to and 0.8g·kg/h CHO during exercise	TT 25.45 min HC, 25.53 min LCHF (*P* > 0.05)

Abbreviations: CHO = carbohydrate; h = hour; g·kg = gram per kilogram body weight; J/kg BW = joule per kilogram bodyweight; HC = high-carbohydrate; kcal = calorie; km = kilometre; LCHF = low-carbohydrate high fat; min = minute; *n* = number; NS = non-significant; $mL \cdot kg^{-1}min^{-1}$ = millilitres per kilogram per minute; sec = second; TT = time trial; VO_{2peak} = maximal oxygen consumption

Table 2.3 Contd. Low-carbohydrate high fat diets (>60% of total calories from fat), followed by a period of carbohydrate restoration in athletes

Author	Population Sample	Adaptation Period & LCHF & HC Diets	Performance Test	CHO Restoration & Performance Nutrition	Performance Results
Carey *et al.,* (2001)	*n* = 7 highly trained male cyclists/triathletes (>74 $mL \cdot kg^{-1}min^{-1}$) Crossover design	6 days HC (68% CHO, 16% fat, 16% protein) LCHF (16% CHO, 68% fat, 16% protein)	Cycling 240 min @ 65% VO_{2peak}, 60 minute TT	1 day 11 g·kg CHO CHO 3 g·kg 1 h prior 1.3 g·kg/h CHO during exercise (mean 100g/h)	Power output during 60min TT 11% higher LCHF trial (*P* = 0.11) and 60min TT performance NS improved (0.02km or 0.1%) (*P* = 0.98
Lambert *et al.,* (2001)	*n* = 5 trained male cyclists/triathletes Crossover design	10 days HC (65% CHO, 15% fat, 20% protein) LCHF (15% CHO, 65% fat, 19% protein)	Cycling 150 min @ 70% VO_{2peak}, 20 km TT	3 days 7 g·kg CHO 0.3 g·kg MCT 1 h prior (14g MCT) 0.8g·kg CHO during	20km TT LCHF 29.35min, 30.68min HC, 4% enhancement between groups (*P* < 0.05)

Abbreviations: CHO = carbohydrate; g·kg = gram per kilogram body weight; g·kg/ = gram per kilogram bodyweight per hour; h = hour; J/kg BW = joule per kilogram bodyweight; HC = high-carbohydrate; kcal = calorie; MCT = medium chain triglyceride; km = kilometre; LCHF = low-carbohydrate high fat; min = minute; *n* = number; NS = non-significant; $mL \cdot kg^{-1}min^{-1}$ = millilitres per kilogram per minute; sec = second; TT = time trial; VO_{2peak} = maximal oxygen consumption

Table 2.3 Contd. Low-carbohydrate high fat diets (>60% of total calories from fat), followed by a period of carbohydrate restoration in athletes

Author	Population Sample	Adaptation Period & LCHF & HC Diets	Performance Test	CHO Restoration & Performance Nutrition	Performance Results
Rowlands and Hopkins, (2002)	*n* = 7 well trained male cyclists/triathletes (nationally competitive) Crossover design	11.5 days HC (70% CHO, 16% fat, 14% protein) LCHF (15% CHO, 66% fat, 19% protein)	Cycling 45 min @ 50% VO_{2peak}, incremental test, 100 km TT (5 h total)	2.5 days 6.8 g·kg CHO 3 pre-exercise meals (each 42 KJ) 1.5 h prior 58 g/h CHO during	LCHF trial reduced 100 km TT 3.3% (*P* = 0.22), increased power output 8.4% (*P* = 0.24) relative to HC
Haveman *et al.,* (2006)	*n* = 8 well trained male cyclists (>51 $mL{\cdot}kg^{-1}min^{-1}$) Crossover design	6 days HC (68% CHO, 17% fat, 15% protein) LCHF (17% CHO, 68% fat, 15% protein)	100 km TT with 4 x 4 km sprints, 4 x 1 km sprints	1 day 8-10 g·kg CHO 200 ml every 20 min of a 10% glucose polymer	100 km TT times; 156.54 min LCHF, 153.10 min HC (*P* = 0.23) Power output during 1 km sprint @90% VO_{2peak} decreased in LCHF compared to HC (*P* < 0.05)

Abbreviations: CHO = carbohydrate; g·kg = gram per kilogram body weight; h = hour; HC = high-carbohydrate; kcal = calorie; km = kilometre; kJ = kilojoule; LCHF = low-carbohydrate high fat; min = minute; ml = millilitre; *n* = number; NS = non-significant; $mL{\cdot}kg^{-1}min^{-1}$ = millilitres per kilogram per minute; sec = second; TT = time trial; VO_{2peak} = maximal oxygen consumption

This nutritional strategy was intensively researched in the early 2000's, Burke *et al.,* (2000) first examined it in well trained male cyclists and triathletes (n = 8) in a randomised crossover designed study. Prior to each dietary intervention, participants completed an incremental trial to exhaustion to determine baseline VO_{2peak} and peak power output (W). Participants then consumed a high-carbohydrate (%carbohydrate:fat:protein=74:15:11) or LCHF diet (18:68:14) for 5 days, followed by 1 day of carbohydrate loading (day 6, 10 g·kg of carbohydrate). Participants also completed 20 minute ride at 70% VO_{2peak} and received a muscle biopsy to determine fuel utilisation and muscle glycogen levels. On the 7th day participants completed 120 minute steady state ride on a cycle ergometer at 70% VO_{2peak}, followed by a 7 kilojoules per kilogram ($kJ \cdot kg^{-1}$) body mass TT. Following the LCHF diet participants muscle glycogen had decreased (day 1 = 451 $mmol \cdot kg^{-1}$ dry weight, day 6 = 255 $mmol \cdot kg^{-1}$ dry weight), however, following carbohydrate restoration, pre-exercise muscle glycogen increased to 554 $mmol \cdot kg^{-1}$ dry weight. These rates of pre-exercise muscle glycogen were non-significantly lower than rates observed following the high-carbohydrate trial (608 $mmol \cdot kg^{-1}$ dry weight). Despite carbohydrate restoration, RER values were lower than the high-carbohydrate groups across all time points. Muscle biopsies illustrated that increases in fat oxidation were mirrored by a non-significant decrease in glycogen oxidation during the steady state ride at 70% VO_{2peak}; glycogen utilisation was 360 $mmol \cdot kg^{-1}$ dry weight during the high-carbohydrate trial and 260 $mmol \cdot kg^{-1}$ dry weight during the LCHF trial (P = 0.06). Time to complete 7 $kJ \cdot kg^{-1}$ of work was 8% faster following the LCHF trial (HC = 34.17 min, LCHF = 30.73 min, $P < 0.05$). Notably, two high-carbohydrate participants experienced severe symptoms of fatigue during the 7 $kJ \cdot kg^{-1}$ TT, with mean TT performance for the other 5 participants being 31.98 min. As participants in each group performed the exercise trials fasted, it was postulated that

the high-carbohydrate participants experienced symptoms of hypoglycaemia (Burke *et al.,* 2000). To address these concerns, Burke *et al.,* (2002) replicated this study design two years later. The only notable change to the exercise protocol was that each group consumed 2 g·kg of carbohydrate prior and 0.8 g·kg/h of carbohydrate during exercise, to preserve plasma glucose levels and preclude potential symptoms of hypoglycaemia. Unlike Burke *et al.,* (2000), a muscle biopsy was not performed, so levels of muscle glycogen were not obtained. Nevertheless energy contribution from carbohydrate during exercise was calculated using exhaled air (Frayn, 1983) and was lower following the LCHF trial during the steady state ride at 70% VO_{2peak} (HC = 419 kJ, LCHF = 354 kJ). Glycogen sparing did not transcend into increased performance in this instance with differences between groups being negligible (HC = 25.53 min versus LCHF 25.45 min; $P > 0.05$).

Subsequent to Burke *et al.,* (2000) promising findings Carey *et al.,* (2001) examined this fuelling strategies efficiency for ultra-endurance performance in well trained cyclists ($n = 7$). Each groups glycogen stores were first standardised by consuming a carbohydrate based diet providing 9 g·kg of carbohydrate for 1 day, followed by 7 days of a high-carbohydrate diet (%carbohydrate:fat:protein = 68:16:16) or a LCHF diet (16:68:16). This was followed by 1 day of carbohydrate loading (11 g·kg of carbohydrate) (Table 2.3). Prior to this ultra-endurance exercise trial, participants from each group consumed a breakfast supplying 3 g·kg of carbohydrate and rested for 60 minutes. Participants then completed a 4 hour steady state ride at 65% VO_{2peak} on a cycle ergometer. During the 4 hour steady state ride participants in each group consumed 100 g/h of carbohydrate, in the form of a glucose solution. The 5 hour exercise protocols came to a close with a 60 minute maximal distance TT; during which participants in each group consumed sports

drinks and water *ad libitum*. During each dietary protocol, a 20 minute sub maximal ride took place on day 2, 5 and 8 to monitor changes in fuel utilisation. Fat oxidation increased following 5–8 days of consuming a LCHF diet (RER decreased from 0.85 to 0.78–0.79). These metabolic adaptations were suppressed during the first 15 minutes of the 4 hour steady state ride at 65% VO_{2peak}, as RER values were restored to 0.88 subsequent to 24 hours of carbohydrate restoration. RER values increased in the high-carbohydrate group from 0.85 to 0.89, indicating increased carbohydrate availability following 8 days of a high-carbohydrate diet. During the 4 hour performance trial, energy contribution from carbohydrates decreased from 594 g in the high-carbohydrate group to 475 g in the LCHF group. Accordingly, energy contribution from fat increased from 119 g to 171 g during the steady state ride. Distance covered during the 60 minute TT after the 4 hour steady state ride at 65% VO_{2peak} was not statistically different between groups (HC = 42.1 km, LCHF = 42.25 km), however it should be noted that average power output was 11% higher following the LCHF trial. Carey *et al.,* (2001) concluded that despite sparing of muscle glycogen, the study failed to detect a statistical benefit to performance. Noakes (2004) belatedly wrote a letter to Carey *et al.,* (2001), questioning their evaluation of the investigation. Although improvements were not statistically significant, a 4% improvement in time during a 42 km ultra-marathon would tally a 6:36 minute improvement, a substantial effect (Carey *et al.,* 2001; Noakes, 2004). Noakes (2004) believes the correct conclusion that should have been drawn from Carey *et al.,* (2001) investigation was that statistically significant improvements were hampered by a small sample size, with 5 out of the 7 participant's performance improving on the LCHF trial. Thus, statistical significance may have been observed with a larger sample size (Noakes, 2004). Louise Burke replied to Noakes (2004) on Carey *et al.,* (2001) behalf and stated that both outcomes were adequately discussed and the

statistical type II error was accounted for. Further, Louise Burke recommended that it is in the best interest of every athlete to explore multiple dietary strategies to enhance training and competition performance.

Lambert *et al.,* (2001) further assessed this dietary approach in well-trained male cyclists and triathletes (*n* = 5) in a crossover design. Participants consumed a high-carbohydrate diet (%carbohydrate:fat:protein = 65:15:20) or a LCHF diet (15:65:19) for 10 days, followed by 3 days of carbohydrate restoration (7 g·kg of carbohydrate). The volume of carbohydrate consumed during this carbohydrate restoration period was modest in comparison to previous investigations (10–11 g·kg of carbohydrate) (Burke *et al.,* 2000; Burke *et al.,* 2002; Carey *et al.,* 2001). Participants from each group consumed 0.3 g·kg of MCT prior to exercise and 0.8 g·kg of carbohydrate during exercise. The exercise trial consisted of 150 minute ride on a cycle ergometer at 70% VO_{2peak} and a 20 km TT. Ten days of carbohydrate restriction and 3 days of carbohydrate loading resulted in a decrease in carbohydrate oxidation (HC = 1.7 g·min^{-1}, LCHF = 1.1 g·min^{-1}). Similar to Carey *et al.,* (2001) investigation, reduced reliance on carbohydrates positively impacted on performance in Lambert *et al.,* (2001) investigation, as 20 km TT performance improved in the LCHF group (HC = 30.68 min, LCHF = 29.35 min; $P < 0.05$) Although in Carey *et al.,* (2001) case, the improvements in performance were not statistically significant, Lambert *et al.,* (1994) investigation signifies that endurance performance can be improved in well-trained athletes when the bodies' reliance on carbohydrates is reduced. However, neither Carey *et al.,* (2001) nor Lambert *et al.,* (2001) assessed sprint performance, this would have been a valuable observation to assess if improvements in fat oxidation downregulated glycolytic flux (Burke, 2015). Further, this additional assessment would have illustrated how differences in author's carbohydrate restoration

strategies may have impacted on participants high intensity exercise performance. Carey *et al.,* (2001) incorporated an aggressive refeeding strategy (11 g·kg carbohydrate for 1 day) and 1.3 g·kg of carbohydrate during exercise, whereas Lambert *et al.,* (2001) participants practised a less severe refeeding strategy, by consuming 7 g·kg of carbohydrate for 3 days prior and 0.8 g·kg of carbohydrate during.

Rowlands and Hopkins (2002) investigation incorporated three dietary interventions; two of which were discussed earlier in the literature review (Table 2.2). The final dietary intervention within Rowland and Hopkins (2002) investigation is relevant to this section of the review; LCHF diet, followed by carbohydrate restoration (Table 2.3). LCHF participants consumed the same LCHF diet (*n* = 7, %carbohydrate:fat:protein = 15:66:19) but included 2.5 days of carbohydrate restoration (6.8 g·kg of carbohydrate). Peak rates of fat oxidation were 2.5 fold higher in the LCHF + HC group, relative to the high-carbohydrate group (HC 1.7 $g \cdot min^{-1}$, 3.6 $g \cdot min^{-1}$, $P = 0.001$). During the 100 km TT power output decreased in the high-carbohydrate group (-5.2%), whereas this decline was not apparent in the LCHF + HC (-0.8%) or LCHF group (-1.3%). Decreases in power output did not transcend into differences in 100 km TT performance (HC = +1.6%, LCHF + HC = -1.8%, $P = 0.22$). Lastly, power output during the final 5 km sprint was enhanced by a factor of 1.3 (1.1 to 1.6, $P = 0.04$) and 1.2 (1.0 to 1.5, $P = 0.02$) relative to the high-carbohydrate condition. Further, dietary fat was positively correlated with increases in 100 km TT performance, additional analysis carried out by Rowlands and Hopkins (2002) demonstrated that for every 10% increase in energy from dietary fat, there was a subsequent 2% improvement in power output during the 100 km TT (LCHF & LCHF + HC versus HC), a finding that neared statistical significance ($P = 0.06$).

Havemann *et al.,* (2006) designed a unique investigation, in the sense that it attempted to mimic race situations during its endurance protocol. Similar to each trial in this section (Table 2.3), a crossover design was implemented. Participants were randomised into a high-carbohydrate group (*n* = 7, %carbohydrate:fat:protein = 68:15:17) or LCHF group (*n* = 7, 17:68:15) for 6 days, followed by 1 day of carbohydrate restoration (8–10 g·kg of carbohydrate). Following successful completion of each nutritional intervention, participants arrived fasted to complete a 100 km TT, which incorporated 8 sprints; 4 x 4 km sprints and 4 x 1 km sprints. During data collection participants in each group consumed 200 ml of a 10% glucose polymer solution every 20 minutes. Participants mean RER values during exercise were lower following the LCHF carbohydrate restoration trial (HC = 0.93 RER, LCHF = 0.87 RER). Performance of the 100 km TT was not significantly different between groups (HC = 153:10 min, LCHF = 156:54 min). Mean power output during the 4 km sprints decreased in each group ($P < 0.01$), while 1 km sprint performance declined in the LCHF group along ($P < 0.05$). Havemann *et al.,* (2006) findings illustrate that 4 km sprint performance can be sustained following a LCHF + HC approach; which is in line with Rowlands and Hopkins (2002) findings surrounding 5 km sprint performance. However, power output during the more glycolytic (Egan and Zierath, 2013) 1 km sprint declined (Table 2.3).

Stellingwerff *et al.,* (2005) carried out an investigation in well-trained endurance trained male cyclists to assess the effects of consuming a LCHF diet (*n* = 7, %carbohydrate:fat:protein, 18:67:15) for 5 days, followed by 1 day of carbohydrate restoration (71:15:14) on the regulation of key enzymes involved in skeletal muscle fat and carbohydrate metabolism versus a high-carbohydrate diet (*n* = 7, 70:15:15). Pyruvate dehydrogenase (PDH) and hormone-sensitive lipase (HSL) activities were

assessed during 20 minute cycling at 70% VO_{2peak} and 1 minute sprinting at 150% peak power output. PDH is a multi-enzyme complex that catalyses the conversion of pyruvate to Acetyl-CoA; higher levels of PDH activity would be indicative of carbohydrate oxidation via the citric acid cycle and the electron transport chain (Peters *et al.,* 2001), whereas increased HSL indicates greater amounts of lipolysis. Further, muscle glycogenolysis was estimated through glycogen phosphorylase upon commencement of the 20 minute cycle at 70% VO_{2peak} and during the 1 minute sprint. The LCHF + HC diet was associated with a reduction in glycolysis and in the active form of PDH at rest (-56%, P = 0.09) and during submaximal exercise (-29%) and during the 1 minute of sprint cycling (-35%). Although two of PDH values were not statistically significant between groups, it's clear there was a large alteration in PDH activity between the high-carbohydrate and LCHF + HC groups. Further, decreases in concentrations of adenosine monophosphate (AMP) and adenosine diphosphate (ADP) and an increase of PDH kinase (PDK) activity previously reported in the literature in response to a LCHF diet (Peters *et al.,* 2004), were also observed during Stellingwerff *et al.,* (2005). Downregulation of important enzymes responsible for linking the glycolic pathway to the citric acid cycle appears to be a potential limiting factor for fat adaptation and is why Burke (2015) concluded that Stellingwerff *et al.,* (2005) investigation provided evidence that adaptation to a LCHF diet can cause "glycogen impairing, rather than glycogen sparing".

The consumption of a high-carbohydrate diet and 24–36 hours of carbohydrate loading are considered best practice in preparation for endurance events lasting >2 – 3 hours (Helge, 2017). However, this undisputed ideology is difficult to attain from the literature. The consumption of a LCHF diet followed by a period of carbohydrate restoration increases fat oxidation (Burke *et al.,* 2000; Burke *et al.,* 2002; Carey *et al.,* 2001; Lambert

et al., 2001; Rowlands and Hopkins, 2002; Haveman *et al.,* 2006) and decrease an athlete's reliance of glycogen (Burke *et al.,* 2000; Burke *et al.,* 2002; Carey *et al.,* 2001; Lambert *et al.,* 2001; Rowlands and Hopkins, 2002; Haveman *et al.,* 2006). Further, this dietary approach has been shown to preserve or non-significantly improve endurance performance and ultra-endurance performance in well-trained athletes (Burke *et al.,* 2000; Burke *et al.,* 2002; Carey *et al.,* 2001; Lambert *et al.,* 1994; Rowlands and Hopkins, 2002; Haveman *et al.,* 2006). Despite a restoration phase and being in a state of 'carbohydrate availability', an acute adaptation to a LCHF diet downregulates key enzymes responsible for linking the glycolytic pathway with the citric acid cycle (Peters *et al.,* 2001; Haveman *et al.,* 2006; Stellingwerff *et al.,* 2006), therefore, limiting ATP synthesis via glycoloytic flux.

In conclusion, this dietary approach may be of benefit to some athletes (Burke, 2015), particularly those who are at an increased risk of glycogen depletion (i.e., ultra-endurance athletes >5 hours) (Noakes, 2004). However, athletes who require an ability to produce and sustain high power outputs are better suited to a traditional high-carbohydrate approach. Further, this dietary approach should not be adopted lightly, even in ultra-endurance athletes who may not require an ability to perform at maximal intensities, as participants in Burke *et al.,* (2000), Burke *et al.,* (2002) and Havemann *et al.,* (2004) all noted decreases in performance and increases in RPE and heart rate during their carbohydrate depletion phase. To date, research on this dietary paradigm has involved endurance and ultra-endurance rides at set intensities, future work should incorporate self-selected intensity ultra-endurance styled exercise trials, to further improve translation to real world athletes.

2.5 Low-Carbohydrate Ketogenic Diet (LCKD) and Ketosis

Definition of a Low-carbohydrate Ketogenic Diet

Unlike the broad definition of a low-carbohydrate diet, the definition of a LCKD is precise. A popular definition of a LCKD is >75-80% kcal fat, moderate protein (1.32–2.2 grams per kilogram lean mass) and <50 g/d of carbohydrates (Robinson and Williamson, 1980; Laffel, 1990; Volek and Phinney, 2011; Volek and Phinney, 2012).

LCHF diets and LCKDs are similar in the sense that both restrict carbohydrate, but metabolically they are quite different. The aim of a low-carbohydrate diet is to restrict carbohydrates from sugary and starchy sources and to increase energy contribution from fats and protein. In contrast, the primary objective of a LCKD is to induce and sustain a metabolic state referred to as nutritional ketosis (Robinson and Williamson, 1980; Laffel, 1990; Volek and Phinney, 2011; Volek and Phinney, 2012).

Nutritional Ketosis

In a state of nutritional ketosis, a fundamental shift in metabolism takes place due to low-glucose availability, resulting from low-carbohydrate content and moderate protein intake. Carbohydrate restriction and moderate protein intake progressively lowers glycogen stores, previously discussed. In addition, due to the lack of glucose within the diet, insulin remains supressed in postprandial states and there are considerable increases in fat oxidation (Cahill and Aoki, 1980). Nutritional ketosis is defined by an increase in circulating ketone bodies to >0.5–5.0 mM (Volek and Phinney, 2011; Volek and Phinney, 2012). In order to achieve these increases in circulating ketone bodies in a fed state, insulin suppression must remain uninterrupted for 2–3 weeks (Volek and

Phinney, 2012). Once keto-adapted, the brain relies predominately on ketone bodies produced within hepatic tissues and glucose (30%) produced via gluconeogenesis, while skeletal muscle relies predominantly on free fatty acid oxidation and ketone bodies (Cahill and Aoki, 1980; Evans, Cogan and Egan, 2016; Webster *et al.,* 2016).

Ketone Bodies

A ketone is an organic acid which was first discovered in the 1900's in the urine of type 1 diabetics (Vanitallie and Nufert, 2003). 'Ketone bodies' describes the ketone bodies, acetoacetate (AcAc), acetone and βHB (beta-hydroxybutyrate). Although strictly speaking, βHB is not technically a ketone because the ketone moiety has been reduced to a hydroxyl group (Evans, Egan and Cogan, 2016). In healthy individuals who consume a traditional carbohydrate based diet (>40 – 60% of total calories), blood ketones are present in small quantities (<0.1 mM) (Volek and Phinney, 2012). However, concentrations rise to >0.5 mM after 2–3 weeks of consuming a 'well formulated ketogenic diet' (Volek and Phinney, 2012) or after periods of fasting or starvation where energy contribution from ketones can increase from 2–6% to 30–40% following 3 days of fasting (Laffel, 1990). Increases in plasma ketone bodies reflect a balance between hepatic production (ketogenesis) and peripheral breakdown and utilisation (ketolysis).

Ketogenesis

Under 'normal' consumption of a carbohydrate based diet, ATP can be produced via an intermediate of the citric acid cycle using acetyl CoA derived from β-oxidation and oxaloacetate derived from pyruvate (Laffel, 1990). However, during periods of fasting when not enough glucose is consumed, oxaloacetate is utilised during gluconeogenesis to maintain blood glucose concentrations (Evans, Cogan and Egan, 2017). In this

instance, ATP cannot be produced via the citric acid cycle as oxaloacetate is otherwise occupied.

Keto-Adaptation

During this intermediary stage when an individual is neither consuming sufficient glucose, nor efficient at relying on fat as their primary fuel source, fatigue, reduced performance and tiredness are common complaints (Volek and Phinney, 2012). These initial stages of a LCKD are referred to as keto-adaptation and short term (7 – 10 days) negative side effects are commonly referred to as 'keto or Atkins flu' (Irvin, 2017). Once in a keto-adapted state, free fatty acids are converted to fatty acyl CoA; which in turn undergo β-oxidation to produce acetyl–CoA. Ketogenesis from free fatty acids then involves a number of sequential reactions involving acetyl CoA and acetoacetyl CoA to form AcAc; which is central to further ketone body production (Evans, Cogan and Egan, 2016). The majority of AcAc ketone bodies are transformed into βHB and acetone; acetone's contribution to energy production is negligible, with the majority exhaled (Laffel, 1990). The exhalation of acetone is a sign of high rates of ketogenesis, which can lead to bad breath (Yellen, 2008). Once AcAc and βHB are produced, they are ready to enter the peripheral tissues to be further oxidised. These two ketones bodies produced by the liver are water soluble, thus transported via the blood to the peripheral tissues where they can be utilised as energy (Laffel, 1990). AcAc is catalysed by CoA transferase (OXCT) to re-produce acetyl CoA, whilst βHB must first be re-oxidised back into AcAc via 3-hydroxybutyrase dehydrogenase (BDH) to produce 2X acetyl CoA molecules; and in doing so it converts an NADH molecule to a NADH + H^+ molecule (Evans, Cogan and Egan, 2017). Each form of AcAc is then involved in a covalent reaction and Co^2 is catalysed by succinyl-CoA:3-oxoacid CoA transferase; resulting in AcAc-CoA. Two acetyl CoA

molecules are then released via hydrolysis and the acetyl CoA molecule enters the citric acid cycle to produce energy (Evans, Cogan and Egan, 2016). Further, when βHB was transformed into AcAc, it produced a NADH + H^+ molecule; which can enter the electron transport chain which further increases the energy potential within the cell. Resultantly, the energy potential from βHB ketone bodies is greater than AcAc ketone bodies that enter the peripheral tissues (Cox and Clarke, 2014). In addition, βHB are more effective at entering muscle mitochondria, due to their ability to pass through monocarboxylate transporters (Evans, Cogan and Egan, 2016).

History and Popularity of Ketogenic Diet

Ketone bodies were once believed to be dangerous by-products of metabolism (i.e., ketoacidosis), but in the last 100 years a much different picture is unfolding; ketone bodies are no longer considered to be the "ugly duckling" of metabolism, instead they are "emerging as an incipient swan" (Vanitallie and Nufert, 2003). The LCKD was referred to as one of the most popular trends in 2016 in an online blog (Verma, 2017), it reportedly aiding, hunger, insulin resistance, low blood pressure, low cholesterol, inflammation and improves gastrointestinal health. The 'keto' food industry is developing fast in response to this trend, an Amazon search displays numerous 'ketogenic cook books' ("Amazon.com: ketogenic cookbooks," 2017), while Quest; a large supplement company in the US is now offering a full range of 'keto meals' (Quest Nutrition, 2017).

A LCKD is best known for its treatment of epilepsy in children (Vanitallie and Nufert, 2003), whilst in adults it's commonly used to treat autoimmune diseases (Choi *et al.,* 2016), metabolic syndrome (Forsythe *et al.,* 2008) and recently athletes have adopted a

LCKD to aid performance (Burke, 2015; Volek, Noakes and Phinney, 2015). The perceived benefits of keto-adaptation which have attracted athletes are its positive effect on body composition (Volek *et al.,* 2004), improved recovery (Shimazu *et al.,* 2013; Volek, Noakes and Phinney 2015), slowed aging (Roberts *et al.,* 2017) and access to a much greater fuel source (Volek and Phinney, 2012; Volek, Noakes and Phinney, 2015).

Athletes and Ketogenic Diet

Increased fuel availability is one of the biggest selling points of a LCKD to athletes. By adopting a LCKD athletes are said to free themselves from limited carbohydrate stores (<2000 kcal) and 'aggressive' carbohydrate feeding strategies during exercise (Burke *et al.,* 2011), by allowing access to >30,000 kcal in adipose tissue and endogenous fuel for the brain (Volek *et al.,* 2012; Cahill and Aoki, 1980). However, it's important to bear in mind that these benefits are largely anecdotal, as they have not been extensively researched within athletes. However, a LCKD as an ergogenic aid has received endorsements from athletes who've made the metabolic switch and claimed to remain competitive (Brunker, 2015; Olsen, 2014).

2.6 Low-Carbohydrate Ketogenic Diets and Performance

Following on from previous work in overweight patients (Phinney et al., 1983), Phinney *et al.*. (1983) carried out a non-randomised crossover investigation examining performance a traditional high-carbohydrate and LCKD in 5 well-trained cyclists (Table 2.4). During the LCKD trial participants consumed a carbohydrate rich diet (57% carbohydrate) for 1 week, followed by 4 weeks of a LCKD (85% fat, 1.2 g·kg of protein, <20 g/d carbohydrates). Current training volume was maintained by 4 participants during the course of the intervention while 1 slightly decreased. Training volume was noted during the investigation and dietary adherence was monitored daily by tracking bodyweight and recording urinary ketones. The LCKD implemented in this trial was successful in achieving nutritional ketosis (Volek and Phinney, 2012), resting plasma βHB rose from 0.04 mM at baseline to 1.28 mM at the end of week 5 in the LCKD group. Performance was measured following an overnight fast at baseline and at the end of week 2 and week 5. Participants completed a 2 hour cycle on a stationary cycle ergometer at 60–65% their VO_{2peak}. Following the steady state ride, participants were asked to maintain current speed (>60 rpm) until exhaustion, during which participants were not made aware of time or distance completed, to avoid setting artificial time goals, nor were they allowed to consume an external source of energy. Blood samples were taken intravenously prior to and 30 minutes post exercise, while four blood samples and five breathe samples were taken at the later stage of the steady state ride (>90–120 minutes) and exhaled air was analysed every 2 out of 10 minutes using respiratory gas analyser. Following completion of each dietary protocol, TTE was 151 minutes in the high-carbohydrate group and 147 minutes in the LCKD ($P > 0.05$). These results suggest that the high-carbohydrate and LCKD were well matched, however there

was *n* = 1 outlier which may have skewed results, with time to exhaustion improving by 63.7% (84 minutes) on the LCKD trial. Two additional LCKD participants improved their time to exhaustion by 1.6% (3 minutes) and 30% (30 minutes), respectably, while the two remaining participants' performed better on the high-carbohydrate trial (28.4% or 21 minutes and 36.4% or 51 minutes).

Ketogenic diets have grown in popularity amongst athletes who are required to make competitive weight, such as taekwondo (Rhyu and Cho, 2014), mixed martial arts and boxing. Paoli *et al.,* (2012) investigated the effect of LCKD in 8 elite gymnasts to examine what impact 30-days of keto-adaptation had on strength performance and body composition. The 'elite gymnasts' (20.9 years) completed a range of strength tests before consuming a 'modified ketogenic diet' (%carbohydrate:fat:protein = 4:55:41) (Paoli *et al.,* 2012) (Table 2.4). Consuming a modified ketogenic diet resulted in favourable changes in body composition, body weight (-1.6 kg) and body fat (1.9%) decreased, lean mass non-significantly increased (0.3 kg) and strength performance remained unaffected. Despite this diet being referred to 'ketogenic', it's unlikely that nutritional ketosis was achieved. It's well established 21-28 days of keto-adaptation is sufficient to increase blood ketones to >0.5 mM (Burke *et al.,* 2017; Phinney *et al.,* 1980; Phinney *et al.,* 1983), but the 'modified ketogenic diet' implemented by Paoli *et al.,* (2012) was not 'ketogenic' (Volek and Phinney, 2012). Protein consumption throughout this investigation was 3.1 g·kg lean mass, whereas, protein consumption guidelines for achieving nutritional ketosis are 1.32–2.2 g·kg lean mass (Volek and Phinney, 2011; Volek and Phinney, 2012). Changes in blood ketones were not measured, even so a significant increase would have been unlikely, as excessive protein can have the same impact on keto-adaptation as excess dietary carbohydrates, as half of the amino acids

contained within protein are metabolised as glucose (Gannon and Nuttall, 2010) and when glucose is readily available, ketogenesis is supressed (Phinney, 2004). While this investigation should not be categorised as 'ketogenic', it did indicate that a short period (<30 days) of carbohydrate restriction (<22 g/d) can cause favourable changes in body composition, while having no impact on strength performance in elite gymnasts.

Table 2.4. Low-carbohydrate ketogenic diets (LCKD) and investigation in athletes

Author	Population Sample & Study Design	Adaptation Period, HC and LCKDs, & Performance Nutrition	Performance Test	Nutritional Ketosis (Ketones >0.5 mM)	Results
Phinney *et al.,* (1983)	*n* = 5 well trained male cyclists (>65 $mL \cdot kg^{-1}min^{-1}$) Crossover design	28 days (4 weeks) HC (57% CHO, 29% fat, 14% protein) LCKD (<20gCHO, 85% fat, 15% protein) Overnight fast, no CHO during	TTE at 60% VO_{2peak}	Yes βHB 1.16-2.44 mM	TTE 151 min LCKD vs 147 min HC) ($P > 0.05$)
Paoli *et al.,* (2012)	*n* = 8 elite male artistic gymnasts Crossover design	30 days (4.2 weeks) HC (47% CHO, 39% fat, 15% protein) LCKD (<25g CHO, 55% fat, 41% protein) Not stated	Strength exercises: squat jumps, countermovement jumps, push-ups, reverse grip chin test, legs closed barrier test	Not measured	No change to strength performance in each trial Bodyweight (-1.6 kg) ($P < 0.05$) and body fat percentage (-1.9%) ($P < 0.001$) decreased in LCKD group.

Abbreviations: CHO = carbohydrate; g·kg = gram per kilogram body weight; HC = high-carbohydrate; kcal = calorie; kg = kilogram; LCKD = low-carbohydrate ketogenic diet; mM = millimolar; min = minute; *n* = number; $mL \cdot kg^{-1}min^{-1}$ = millilitres per kilogram per minute; TTE = time to exhaustion; VO_{2peak} = maximal oxygen consumption; βHB = beta-hydroxybutyrate

Table 2.4. Contd. Low-carbohydrate ketogenic diets (LCKD) and investigation in athletes

Author	Population Sample & Study Design	Adaptation Period, HC and LCKDs, & Performance Nutrition	Performance Test	Nutritional Ketosis (Ketones >0.5 mM)	Results
Rhyu and Cho. (2014)	*n* = 10 taekwondo athletes RCT	21 days (3 weeks) HC (40% CHO, 30% fat, 30% protein) LCKD (4% CHO, 55% fat, 41% protein)	2000 m sprint, Wingate, grip test, sit-up test, 100 m sprint, broad jump	Not measured	Time to complete 2000 m sprint improved 6.2% in LCKD versus 0.2% in HC ($P < 0.05$)
Burke *et al.,* (2017)	*n* = 9 HC, *n* = 8 PC *n* = 10 LCKD, elite race walkers Non-random parallel group design	21 days (3 weeks) HC (65% CHO, 20% fat, 15% protein) PC (65% CHO, 20% fat, 15% protein) LCKD (<50g CHO, 78% fat, 15% protein) HC & PC – 2 g·kg CHO prior, 60 g/h CHO during. LCKD energy equivalent LCHF snack	VO_{2peak} and economy testing, 10 km race, 25 km walk	Yes BHB >1.0 mM	HC 10 km time improved 124 – 190 sec ($P < 0.01$), LCKD performance 23sec slower ($P > 0.05$), NS between groups ($P > 0.05$) Diet vs HR & RPE interaction ($P < 0.001$); decreased exercise economy and increased RPE in LCKD. LCKD, RER decreased during 25 km walk ($P < 0.05$)

Abbreviations: CHO = carbohydrate; g·kg = gram per kilogram body weight; g/h = grams per hour; kg; kilogram; HC = high-carbohydrate; HR = heart rate; LCHF = low-carbohydrate high fat; LCKD = low-carbohydrate ketogenic diet; m = meter; mM = millimolar; min = minute; *n* = number; $mL \cdot kg^{-1}min^{-1}$ = millilitres per kilogram per minute; NS = non-significant; sec = second; PC = periodized carbohydrate; RCT = random control trial; RER = respiratory exchange ratio; RPE = rating of perceived exertion; TTE = time to exhaustion; VO_{2peak} = maximal oxygen consumption; βHB = beta-hydroxybutyrate

Table 2.4. Contd. Low-carbohydrate ketogenic diets (LCKD) and investigation in athletes

Author	Population Sample & Study Design	Adaptation Period, HC and LCKDs, & Performance Nutrition	Performance Test	Nutritional Ketosis (Ketones >0.5mM)	Results
Zinn *et al.,* (2017)	*n* = 5 recreationally active endurance athletes (4 female, 1 male) Case study	70 days (10 weeks) LCKD (<50 g CHO, 75% fat, 15% protein) Not stated	VO_{2peak} (incremental test)	Yes Blood BHB >0.5-1.9 mM	TTE -2mins (ES: 0.53) Body mass -4 kg (*P* = 0.046, ES: 0.62) Peak watts -18 W (*P* > 0.05). Enhanced well-being, recovery, skin condition and self-reported reduced inflammation
Heatherly *et al.,* (2017)	*n* = 8 Case study	21 days (3 weeks) LCKD (7% CHO, 64% fat, 29% protein) Fasted	5 km TT (running)	Yes Blood βHB ~0.7 mM (finger stick)	Body mass loss (~2.5 kg *P* < 0.001) Non-sig improvement in 5 km TT time (-0.47 min)

Abbreviations: CHO = carbohydrate; g·kg = gram per kilogram body weight; HC = high-carbohydrate; kg = kilogram; LCKD = low-carbohydrate ketogenic diet; m = meter; mM = millimolar; min = minute; *n* = number; $mL \cdot kg^{-1}min^{-1}$ = millilitres per kilogram per minute; sec = second; TT = time trial; VO_{2peak} = maximal oxygen consumption; βHB = beta-hydroxybutyrate

Table 4 Contd. Low-carbohydrate ketogenic diets (LCKD) and investigation in athletes

Author	Population Sample & Study Design	Adaptation Period, HC and LCKDs, & Performance Nutrition	Performance Test	Nutritional Ketosis (Ketones >0.5mM)	Results
Kephart *et al.,* (2018)	*n* = 5 HC (1 female, 4 males), *n* = 7 LCKD (2 females, 5 males) CrossFit trainees Non-random control trial	90 days (12 weeks) HC (n/a) LCKD (6% CHO, 77% fat, 17% protein) Pre-exercise meal(s) not controlled	VO_{2peak} (incremental test), 1RM back squat, 1RM bench press, max push-ups test, 400 m run	Yes Blood βHB ~1.0 mM (finger stick)	Body fat -12.4% in the LCKD group (*P* = 0.053). No significant changes to either groups performance
Cipryan, *et al.,* (2018)	*n* = 8 HC, *n* = 9 LCKD moderately trained males RCT	28 days (4 weeks) HC (48% CHO, 35% fat, 17% protein) PC (65% CHO, 20% fat, 15% protein) LCKD (<50 g CHO, 63% fat, 29% protein) HC & PC – 2 g·kg CHO prior, 60 g/h CHO during. LCKD energy equivalent LCHF snack	VO_{2peak} (incremental test), 5 x 3 minute interval sprints (@100% VO_{2peak})	No Blood βHB ~0.4 mM (finger stick)	Sprint performance maintained, no difference between groups

Abbreviations: CHO = carbohydrate; g = gram; g·kg = gram per kilogram body weight; g/h = gram per hour; HC = high-carbohydrate; LCKD = low-carbohydrate ketogenic diet; m = meter; mM = millimolar; min = minute; *n* = number; $mL \cdot kg^{-1}min^{-1}$ = millilitres per kilogram per minute; sec = second; TT = time trial; VO_{2peak} = maximal oxygen consumption; βHB = beta-hydroxybutyrate; 1RM = one repetition maximum

Similar to Paoli *et al.,* (2012), Rhyu and Cho (2014) assessed the suitability of a LCKD to competitive athletes (taekwondo) attempting to make weight prior to event. Participants were randomly assigned to the high-carbohydrate group (*n* = 10, %carbohydrate:fat:protein = 40:30:30) or LCKD group (*n* = 10, 4:55:41) for 21-days. Changes in body composition, strength performance, aerobic and anaerobic performance were monitored (Table 2.4). At a glance, one would assume the LCKD group were more successful in cutting weight as LCKD participants lost 3.77 kg and the high-carbohydrate group lost 2.53 kg ($P > 0.05$), however skin calliper measures indicated that the LCKD group lost 2.18 kg lean mass whereas the high-carbohydrate group lost 1.39 kg lean mass. Power output (watts per kilogram (w/kg)) decreased in each group as bodyweight decreased, however aerobic capacity improved in the LCKD group, as time to complete 2000 m on a cycle ergometer decreased (-32 seconds, 6.2%) versus the high-carbohydrate group (-1.2 seconds, -0.2%). Changes in blood ketone concentrations were not monitored during this investigation, thus it's unknown if nutritional ketosis was achieved. Since participants lost weight, they were in calorie deficit over the study duration, however their total calorie intake was not stated, thus, their carbohydrate (4 %/kcal) and protein intake (41 %/kcal) cannot be quantified as grams of carbohydrate and/or grams per kilogram lean mass for protein. Granted carbohydrates were sufficiently restricted and LCKD participants protein consumption was <2.2 g·kg lean mass, it's likely that blood ketones were >0.5 mM at post-intervention testing, but really, this is impossible to tell.

Subsequent to Phinney and colleagues arguably promising findings in the 1980's, the following investigation which set out to measure endurance performance in a keto-adapted state in athletes didn't take place for over 3-decades. Zajac *et al.,* (2014)

examined a LCKDs impact on exercise metabolism and performance in well-trained off-road cyclists (*n* = 8). In this crossover designed study participants were randomly assigned to a mixed diet (%carbohydrate:fat:protein = 50:30:20) or a LCKD (15:70:15) for 4 weeks (Table 2.4). Participants exercise training was prescribed throughout the intervention, it consisted of high volume and moderate intensity work. At the end of week 4, participants completed post-intervention, which consisted of a VO_{2peak} test, a lactate threshold test and an incremental trial to exhaustion on a cycle ergometer. Upon completion, participants had 1 week of recovery with no dietary restrictions prior to beginning the ensuing dietary intervention. Data collection was spaced out over 3-days, prior to testing on each occasion, participants consumed a meal predominately high in carbohydrates or fat containing 600 kcal (HC or LCKD). Participant's plasma βHB increased from 0.04 mM at baseline to 0.15 mM following LCKD trial. Therefore, desite the statistical increase, participants were unsuccessful at achieving nutritional ketosis, as blood ketones were <0.5 mM (Volek and Phinney, 2011; Volek and Phinney, 2012). Failure to achieve nutritional ketosis was either due to poor dietary prescription or dietary adherence. Grams of carbohydrate per day were not specified by Zajac *et al.,* (2014), instead it stated that 15% of total calories (3865 kcal) came from carbohydrate, which is approximately 145 g/d of carbohydrate. This is significantly higher than <50 g/d guideline for achieving nutritional ketosis (Volek and Phinney, 2011; Volek and Phinney, 2012). Nevertheless, LCKD participants exhibited an increase in VO_{2peak} between groups; which is at least partially due to reduction in body mass by LCKD participants. Despite increases in VO_{2peak} and increases in fat oxidation (RER), max work load (W) and work load during the lactate threshold test both decreased in the LCKD group, indicating LCKD participants struggled to maintain performance at higher intensities.

Burke (2015) identified that LCKDs were a growing trend within endurance sports and was dismissive of the current body of scientific literature, identifying there was just one performance trial to date where nutritional ketosis was achieved in athletes (Phinney *et al.,* 1983). Burke *et al.,* (2017) followed on and designed an investigation to examine the impact a LCKD had on exercise performance and efficiency following 21-days of keto-adaptation involving 'world class' endurance athletes. Data collection took place during two separate training camps, during which food was cooked and provided to participants and training load and volume was controlled. Three different diets were implemented, a high-carbohydrate availability diet (*n* = 9, %carbohydrate:fat:protein = 65:20:15), periodized carbohydrate (PC) availability diet (*n* = 8, %carbohydrate:fat:protein = 65:20:15) and a LCKD (*n* = 10, %carbohydrate:fat:protein = 7:78:15). The macronutrient profile of the two high-carbohydrate diets were the same, however the timing of carbohydrates differed, high-carbohydrate availability consumed carbohydrates prior to exercise, whereas the PC diet incorporated periods of fasting and practised carbohydrate back loading. Pre-intervention was broken up into 3-days, day 1 was a fasted graded economy test and VO_2 peak on a treadmill, day 2 was a 10 km race walk and day 3 was a 25 km standardised long walk. Subsequent to pre-intervention, participants began an intensive training camp, incorporating race walking, resistance training and cross training and subsequently completed post-intervention testing. Following 21-days of keto-adaptation, LCKD participants achieved significant increases in whole body fat oxidation. Fat oxidation rates were greater than peak figures observed by Webster *et al.,* (2016) (1.2 g·min^{-1}) and Volek *et al.,* (2016) (1.57 g·min^{-1}). Both Webster and Volek's investigations were cross sectional studies on self-reported keto-adapted athletes, who had consumed a LCKD for >6 months (6–36 months). Peak rates of fat oxidation were observed at 72% VO_{2peak} (Webster *et al.,* 2016) and 70.3% VO_{2peak}

(Volek *et al.,* 2016), during a 2 hour steady state cycle and during a 3 hour run on a treadmill respectfully. Whereas, peak rates of fat oxidation observed by Burke *et al.,* (2017) were achieved during the later stages of 2 hours of exercise at 80% VO_{2peak} (50 km race pace). This 21 day metabolic shift in substrate metabolism came at a cost to LCKD participants, exercise economy decreased from baseline, as there was a significant diet versus test interaction effect on heart rate and RPE, both of which increased following the LCKD trial. Similarly, the O_2 cost of exercise increased during the later stages of the 10 km and 25 km walking trials. Whereas, the O_2 cost of exercise, heart rate and RPE remained consistent in both groups consuming carbohydrate based diets. These markers indicate that LCKD participants were exercising at a higher metabolic cost while experiencing increased exertion following 21-days of consuming a LCKD. Prior to the 10 km race (day 2), LCKD participants were not in nutritional ketosis (0.3 mM), as blood ketones were <0.5 mM. However, post exercise mean concentrations of βHB increased to 0.73 mM. Prior to 25 km race (day 3), LCKD participants blood ketones were >0.5mM (>0.78 mM), indicating participants were in nutritional ketosis. Decreases in exercise economy and increased perceived exertion did not negate 10 km and 25 km performance, however it must be noted that each of the high-carbohydrate groups improved upon times following 21-days of intensive training. Burke *et al.,* (2017) investigation illustrates that well-trained endurance athletes can achieve incredibly high rates of fat oxidation following just 21-days of keto-adaptation. However, its methodology failed to address the elephant in the room (Burke, 2015; Volek, Noakes and Phinney, 2015), 'the long term performance implications of keto-adaptation in athletes'. Phinney *et al.,* (1983) had previously illustrated that well-trained endurance athletes could increase fat oxidation (1.5 $g \cdot min^{-1}$) and become keto-adapted (ketones >0.5 mM) following 28-days of a LCKD, with similar impacts on endurance performance

observed by Burke *et al.,* (2017). By further extending the dietary intervention, Burke *et al.,* (2017) would have further accounted for LCKD participant's decreases in performance, which are associated with the initial stages of keto-adaptation (Phinney *et al.,* 1983), as described already. Additional time may have allowed for better optimisation of low-carbohydrate metabolic pathways during training, perhaps leading to greater exercise efficiency during post-intervention testing. Specifically, the re-maintenance of fasting blood glucose and blood during exercise was not achieved by Burke *et al.,* (2017), this feat has only been observed within athletes following a LCKD for >6 months (Volek *et al.,* 2016; Webster *et al.,* 2016). In addition, Noakes (2004) stressed the importance of having an exercise protocol with a sufficient duration to induce near total glycogen depletion. The 10-25 km races implemented by Burke *et al.,* (2017) would not have been long enough to do so (<50 minutes). A cross sectional study by Webster *et al.,* (2016) involving well-trained keto-adapted athletes (>6 months consuming LCKD) illustrated that percentage VO_{2peak}, heart rate and RPE were similar during a 2 hour submaximal test (72% VO_{2peak}) versus well-trained high-carbohydrate athletes. Similarly, Volek *et al.,* (2016) illustrated that average percent of maximal oxygen consumption, RPE and total energy expenditure were not different between keto-adapted athletes (>9 months consuming LCKD) and well-trained high-carbohydrate athletes throughout a 3 hour submaximal test (64% VO_{2peak}). Therefore, perhaps the poor training adaptations and decreases in exercise economy observed by Burke *et al.,* (2017) are a result of premature adaptation to a LCKD. Burke *et al.,* (2017) investigation may not have addressed a burning question within sports nutrition (Burke, 2015; Volek, Noakes and Phinney, 2015), however it did illustrate that an acute adaptation (<3 weeks) to a LCKD can be detrimental to an endurance athletes training and performance in the lead up to an event ranging in 10–25 km in length, in comparison to high-carbohydrate

approach. This inability to adapt and perform at such intensities following an acute adaptation period is an important observation by Burke *et al.,* (2017).

In line with Paoli et al.'s (2012) earlier work in elite gymnasts, Wilson and colleagues (2017) assessed resistance trained males body composition responses to a LCKD. The investigation also assessed strength, power, blood biomarkers of health and anabolic status. The investigation had a number of novel aspects, it was the first to carry out an investigation with an adaptation period beyond 30-days (10 weeks) and to include a period of carbohydrate restoration (1 week). The dietary intervention lasted 11 weeks in total (HC %carbohydrate:fat:protein = 50:30:20, LCKD = 5:75:20), however the training intervention didn't commence until the start of week 3, once nutritional ketosis was confirmed (βHB > 0.5 mM). This delayed start to the training intervention was implemented to measure training adaptations that occur when 'keto-adapted' and to diligently account for the LCKD groups initial decreases in performance, which are associated with the early stages of keto-adaptation, as described previously (Phinney *et al.,* 1983). Baseline measurements taken prior to the dietary intervention included body composition, muscle thickness, one repetition maximum (1RM) bench press, 1RM squat and a Wingate test. The training intervention consisted of a three day split, incorporating hypertrophy and strength training at varying intensities. At the end of week 10, body mass had decreased -2.6 kg in the LCKD group and increased +0.6 kg in the high-carbohydrate group. Lean body mass increased from week 0–10 in each group (HC +4.4%, LCKD +2.4%, $P < 0.0001$), while fat mass decreased (HC -13%, LCKD -22.4%, $P < 0.0001$). Max bench press (1RM) increased in each group (+5.5% HC, +3.3% LCKD), as did 1RM back squat (+10% HC, +5.4% LCKD). Peak power increased by +75.3 W (+9.08%) in the HC group during the Wingate and decreased by -15.38 W in LCKD group (-1.8%

watts), indicating some decrement in the glycolytic energy systems. Subsequent to these tests, the LCKD group increased their carbohydrate consumption for 1 week (%carbohydrate:fat:protein = 20:40:20) and repeated measures of performance/body composition for a final time, while the high-carbohydrate group's diet remained very consistent during this 1 week period (49:30:21). The LCKD group gained +4.9 kg in week 10-11 and experienced large increases in 1RM bench press (+4.5%) and back squat (+4%). Similarly, participants experienced a re-bound effect on Wingate power, as peak power increased by +67.2 w (+8%) from week 10 or by +51.82 w (+6%) since week 0. In contrast, the high-carbohydrate groups numbers remained relatively consistent (bench: +0.6%, squat: +2%, Wingate: +0.5%).

The results reported by Wilson *et al.,* (2017) in week 10-11 in the LCKD group attracted a lot of negative attention (SCI-FIT, 2017), with the main criticism being the extraordinary gains in body mass, lean body mass, strength and power from week 10-11. Carbohydrate consumption is known to effect DXA reliability i.e., increase water retention and glycogen stores, so can cause large increases in perceived lean body mass (Rouillier *et al.,* 2015; Bone *et al.,* 2017). Therefore, if training responses were only examined in week 0 and week 11, each group's strength, power and body composition responses were quite similar. Lean body mass was presented as % change, but estimated gains in lean body mass are +2.4 kg in the high-carbohydrate group and +4.5 kg in the LCKD group, which are considerable gains considering the participants were well-trained and were in a calorie deficit. Previously, Paoli *et al.,* (2012) noted a non-significant decrease in lean body mass (~-1.1 kg) and an increase in percentage lean body mass (~2.6%), due to weight lost during the intervention period. Participants in Paoli *et al.,*'s (2012) investigation consumed ~3.3 g·kg of protein, whereas participants in Wilson *et*

al., (2017) consumed a moderate ~1.72 g·kg of protein. Given that higher protein intakes (2.3-3.1 g·kg) are required to maximise muscle retention during hypocaloric conditions (Argon *et al.,* 2017), it's difficult to comprehend how each group gained ~2.4-4.5 kg of lean body mass during Wilson *et al.,* (2017) investigation. Although the investigations are not directly comparable, as Paoli *et al.,* (2012) participants were not enrolled in a training protocol designed to cause muscle hypertrophy. It has been suggested that ketone bodies are protective against muscle protein catabolism during hypocaloric conditions (Nair *et al.,* 1988; Manninen, 2006), however, this does not explain anabolism observed by Wilson and colleagues (2017). Therefore, additional work is necessary to examine the mechanisms behind such muscle hypertrophy in well-trained males.

Zinn and colleagues (2017) were the first to extend a period of keto-adaptation to >28 days (10 weeks) and endurance performance. Although this investigation was a case study and in recreationally-active individuals, it appears a good blue print for longitudinal changes that may occur in well-trained athletes. Besides performance, the case study was health orientated and included focus groups to evaluate participant's experiences, wellbeing and inflammation. Prior to undergoing the dietary intervention, participants completed an incremental cycle test to exhaustion for familiarization. The incremental test started at 30-watts and increased by 30-watts every 3 minutes until exhaustion, during which, oxygen uptake (VO_2) and heart rate were recorded continuously. Participants arrived back 1 week later and repeated the exercise trial (baseline) and again at the end of week 10 (post-intervention). Data collection at all time points was completed following a 12 hour overnight fast. The diet implemented by Zinn *et al.,* (2017) (*n* = 8, %carbohydrate:fat:protein = 6:75:19) was successful in achieving nutritional ketosis, blood ketones were monitored daily using 'FreeStyle' ketone meters

and strips and ranged from 0.5-1.9 mM. Interestingly, nutritional ketosis (βHB >0.5 mM) was achieved following ~14-days of consuming the aforementioned LCKD. Despite elevation in blood ketones, participants noted "it was embarrassing to the point where I just got to the point where you just don't have any energy" and "I got too tired and I got to the point where I might have had some big runs, 4 or 5 h runs and wake in the night before hand, worried about it. I'd think, how am I going to do that tomorrow…it's going to be hard", during the first 1-5 weeks of their dietary intervention. This suggests that being 'keto-adapted' is not defined exclusively by an elevation in blood ketones (βHB), mitochondrial adaptations within the muscle may also be necessary prior to an athlete feeling 'adapted'. This process appears to take ~5 weeks in recreationally-active males and females (Zinn *et al.,* 2017), but may not be an accurate reflection of how well-trained/elite athletes adapt to a LCKD. The 10 week adaptation period caused a decrease in TTE on a cycle ergometer (-2 minutes), despite an increase in fat oxidation. In accordance with Zajac *et al.,* (2014) and Burke *et al.,* (2017) investigations, the LCKD group in the current investigation appeared to struggle as exercise intensity increased, max work load decreased by -18 watts ($P > 0.05$). Beyond performance measures, LCKD participants experienced a decrease in body weight (-4 kg $P = 0.046$, ES: 0.62) and improvements in skin fold measurements (-25.9 mm $P = 0.001$, ES: 1.27). Further, participants experienced increased recovery, improvements in skin conditions and "decreased inflammation".

In what was seen as a landmark year for LCKD research, Heatherly and colleagues (2017) also published an investigation examining LCKD and endurance performance. This recent flurry of publications is representative of the interest in 'ketogenic diets' and endurance performance in recent years (Burke *et al.,* 2015; Volek *et al.,* 2015). Heatherly

et al., (2017) investigation examined the effects an *ad libitum* LCKD had on measures of performance in recreationally active male runners. Similar to Zinn *et al.,* (2017), this investigation put some emphasis on measures of health, as well as performance. At baseline each participant (*n* = 8) had their training history, dietary history and VO_{2peak} assessed and received dietary education. Participants also familiarised themselves with the testing protocol (5 km TT). Participants continued to consume a high-carbohydrate diet (%carbohydrate:fat:protein = 43:38:17) (~98% - typographical error in the paper) for 7-days. Prior to starting the dietary intervention, participants completed 2-days of testing, which were separated by 24 hours. All tests were completed fasted. On Day-1 of testing, participants received body composition (via skinfold), hydration, ketones (whole blood finger stick), glucose (whole blood finger stick), metabolic, thermoregulatory and perceptual assessments. The first of the exercise trials was a 50 minute run in the heat, metabolic, thermoregulatory and perceptual assessments took place during and after the run. Following the 50 minute run participants ketones and glucose were assessed and they were allocated 20 minutes rest. After 20 minutes rest, participants completed a 5 km TT run on a road course. Following successful completion of Day 1, participants returned to complete Day 2 of pre-intervention testing. Day-2 assessments were once again completed fasted, participants had body composition analysed and their hydration, ketones and glucose assessed. Participants then received further dietary education on a LCKD and received a copy of 'The Art and Science of Low-carbohydrate Performance' (Volek and Phinney, 2012). For the following 21-days, participants consumed a LCKD (%carbohydrate:fat:protein = 7:64:29), subsequent to which they repeated assessments completed in Day-1 and Day-2 (post-intervention testing). The 21 day adaptation to a LCKD caused weight loss (~2.5 kg, $P < 0.01$) and an increase in fat oxidation during exercise ($P < 0.01$). The intervention had no impact on

performance (*P* = 0.25), however, mean performance was numerically improved following the LCKD protocol (HC = 23.92 ± 2.57 min versus LCKD = 23.45 ± 2.25 min). The authors conceded that because this investigation was not a parallel arm design, there was a potential for an order effect, which may explain the mean performance improvement. *Ad libitum* weight loss may suggest an ergogenic aid (improved power to weight ratio) to LCKD consumption, however, weight lost may have impacted on the investigations findings. Unlike cycling on a cycle ergometer, running is very much a weight bearing exercise. Thus, weight lost during the intervention period means that following the LCKD, participants were completing less work. Unlike Burke *et al*.'s (2017) 21 day keto-adaptation investigation, participants O_2 and heart rate remained unchanged. Previously, Phinney *et al.,* (1980) got participants to wear a back pack containing weight lost (kg) during the intervention period to try and control for this as a confounding variable. Additionally the exercise protocol within the Healtherly *et al.,* (2017) research consisted of a 50 minute run, a 20 minute rest and a 5 km TT. This long rest period does not reflect real world practices, which may limit the transferability of these findings to field settings.

Early in 2018, Kephart *et al.,* (2018) published a pilot study examining the effects of a LCKD on body composition, blood parameters and performance in CrossFit trainees. Twelve participants were recruited, each participant had >3 months training at a Crossfit gym and were aged 19-45 years. Participants were required to display a 1RM back squat strength to mass ratio of at least 1.00 and self-selected into a LCKD group or control group. Pre-intervention data collection was broken up into 2-days, on 'day-1': participants had body composition analysed via DXA (following a 4 hour fast, with hydration monitored to improve reliability), received metabolic testing (resting energy

expenditure), gave a whole blood sample and completed an incremental test on a treadmill to determine maximal oxygen consumption (VO_{2peak}). Approximately 48-96 hours following 'day 1' of testing, participants completed anaerobic performance tests including a 1RM back squat and power clean. Following a brief rest period (10 mins), participant's completed a maximal repetition push-up test and a 400 m run/sprint. Unlike 'day 1', participants were not required to complete 'day 2' fasted so could choose or avoid a pre-exercise meal, which allowed the potential for 'the pre-exercise meal' to become a confounding variable. At the end of 'day 2' participants in the LCKD group began their dietary intervention (*n* = 7, 1,948 kcal %carbohydrate:fat:protein 6:77:17), whilst the high-carbohydrate/control group maintained their habitual diet. Dietary analysis took place using an open sourced dietary software called 'MyFitnessPal' (www.myfitnessspal.com), which is not recognised as a highly accurate means of dietary analysis (Teixeira *et al.,* 2017). Nevertheless, LCKD participants daily βHB ('CardioChek' finger sticks) readings were consistently >1.0 mM during the intervention period, indicating good dietary adherence. Participants repeated 'day 2' of testing after 2.5 weeks with post-intervention at 12 weeks consisting of the same 2 day pre-intervention testing protocol.

Unfortunately, this investigation did not present mean ± SD changes or percentage changes in body composition or measures of performance. The body composition figures provided suggest the LCKD group lost ~ -7.5 kg body mass (*P* = 0.002) and ~ -2.5kg fat mass (*P* = 0.053). There were no time*group effect observed in performance measurements, indicating a LCKD was proficient at maintaining performance. The paper stated training records were presented in 'Table 1', however, table: 1 contains participant's baseline characteristics. Thus, it is unknown what type of training each

group partook in. Following contact with the corresponding author, an Excel file was provided which outlined classes the participants regularly partook in. As it was a CrossFit gym, there was a large emphasis on metabolic conditioning classes, strength training and hypertrophy styled training. Kephart *et al.,* (2018) outlined the LCKD group completed 27 ± 3 workouts, while the high-carbohydrate/control group completed 20 ± 5 workouts (P = 0.245). Although not statistically significant, the high-carbohydrate/control group did complete -25.9% less training.

The most recent LCKD trial in athletes by Cipryan *et al.,* (2018) assessed the impact a 4 week LCKD had on sprint performance in moderately trained males (~53.85 VO_{2peak}). Based on previous knowledge, the chances of a LCKD having a positive impact on sprint performance were unlikely, as sprint performance is highly glycolytic (Egan and Zierath, 2013) and previous investigations utilising a LCKD and measures of high-intensity/sprint performance have noted decreases in performance (Zajac *et al.,* 2014; Burke *et al.,* 2017). Prior to the dietary intervention, baseline performance measures included an incremental exercise trial on a treadmill to determine participants VO_{2peak}. Maximal oxygen consumption values were subsequently used to determine exercise intensities during the main performance measure; 5x high-intensity sprints for 3 minutes (@100% VO_{2peak}), separated by 1.5 minutes of active recovery. At the outset participants were randomised into a high-carbohydrate (% carbohydrate:fat:protein = 48:35:17) or LCKD (8:63:29) group. At baseline, each group's anthropometric measurements, training history and dietary habits were similar (P > 0.05). During the intervention period participants were asked to perform 3-5 sessions per week of non-supervised endurance-based training. Despite each group completing a similar training volume (P > 0.05), high-carbohydrate participant's energy intake was greater, which was reflected in changes in

body composition across the intervention period (LCKD group -4.7 kg (-5.6%) body mass, high-carbohydrate group -0.8 kg (-0.95%). At the midway point capillary blood βHB had increased from <0.1 mM to ~0.7 mM, however, by post-intervention this value had decreased to ~0.4 mM. The authors did not discuss why this decrease occurred, however, it's most likely from an increase in dietary carbohydrate and/or protein within LCKD participants'. The authors did discuss how the ~0.4 mM βHB is considerably lower than previous feeding (>1.0 mM) (Phinney *et al.,* 1983; Burke *et al.,* 2017) and non-feeding LCKD studies (~0.5 mM) (McSwiney *et al.,* 2018) and attributed the lower βHB to greater amounts of dietary protein within their own investigation. Interestingly, the LCKD caused maintenance of high-intensity sprint performance. The authors discussed mechanisms for change and highlighted that the additional 1 week adaptation period versus Burke *et al.,* (2017) 3 week investigation allowed for participants' to become more 'accustomed' to their new diet. Considering that recreationally active individuals contained within Zinn *et al.,* (2017) investigation noted decreases in performance and fatigue up until week 5, it's likely the additional 1 week to adapt during Cipryan *et al.,* (2018) versus Burke *et al.,* (2017) investigation had additional benefits. An additional mechanism for maintenance of sprint performance which was not discussed within the manuscript is, given that it was a treadmill based test and running is a weight dependant sport and LCKD participants lost -4.7 kg (-5.6%) of body mass, decreases in body mass may have masked decreases in exercise economy and increases in RPE, previously observed by Burke *et al.,* (2017) who controlled for weight loss as a confounding variable by feeding maintenance calories throughout the 21-day adaptation period in elite race walkers.

2.7 Exogenous Ketones and Performance

As previously outlined, many strategies exist within endurance sports to optimise energy provision during exercise, these include, but are not exclusive to: 1) a traditional high-carbohydrate diet with carbohydrate loading prior to exercise (Burke *et al.,* 2011), 2) an acute adaptation to a LCHF diet (Table 2.2) or 3) an adaptation to a LCHF diet followed by a period of carbohydrate restoration (Table 2.3) and 4) an adaptation to a LCKD (Table 2.4) or 5) an adaptation to a LCKD with carbohydrate supplementation during exercise (Webster *et al.,* 2016; Maunder, Kilding and Plews, 2018). However, more recently there is growing interest in exogenous ketones. An adaptation to a LCKD is unattractive to many endurance athletes due to the restrictive nature of the diet (<50 g/d carbohydrates) and given that consistently elevating ketones >0.5 mM can prove difficult in non-highly controlled settings (Zajac *et al.,* 2014), unless an athlete is well educated on how to formulate a LCKD and is prepared to follow it rigidly. Exogenous ketones present an opportunity for athletes to consume a traditional high-carbohydrate diet and elevate blood ketones to ~2-3 mM for a number of hours by consuming a supplement, in the form a ketone salt or ketone ester.

2.7.1 Types of Exogenous Ketones

2.7.1.1 Ketone Salts

Ketone salts are white powered substances comprised of βHB molecule bound together with a mineral salt, such as sodium, calcium, magnesium or potassium. The dose of βHB and mineral load vary among ketone salts, but acute ketone salt ingestion elevates serum βHB to <1.0 mM post-ingestion in athletes (Rodger *et al.,* 2017; O'Malley *et al.,* 2017; Waldman *et al.,* 2018; Evans *et al.,* 2018). Ingestion of large quantities of ketone salts can result in gastrointestinal distress and potentially additional undesirable consequences such as cation overload and acidosis (Veech, 2004; Evans, Cogan and Egan, 2016).

2.7.1.2 Ketone Esters

Unlike ketone salts, ketone esters are salt free liquids that primarily exist in monoester and diester form. Ketone bodies AcAc or βHB are synthesised with 1-3-butanediol (1,3 BD) or glycerol, 1-3-butanediol is an alcohol (Frye *et al.,* 1981), which once broken down can be converted to βHB. A number of ketone esters have appeared within the performance literature, namely the R,S (Kesl *et al.,* 2016) and D,L (Leckey *et al.,* 2017) 1,3-butanediol acetoacetate diester and the (R)-3-hydroxybutyl (R)-3-hydroxybutyrate ketone monoester (Clark *et al.,* 2013; Cox *et al.,* 2016; Evans and Egan, 2018). The R,S (Kesl *et al.,* 2016), D,L (Leckey *et al.,* 2017) and R (Clark *et al.,* 2013; Cox *et al.,* 2016; Evans and Egan, 2018) are synonymous and used interchangeably, but represent the isoforms used. Optical isoforms of βHB are not equitant, L-βHB is an intracellular metabolite, whereas D-βHB (also known as R) is released by the liver. Therefore, L-βHB

is not as readily available for oxidative metabolism as D-βHB (Webber and Edmond, 1977; Desrochers *et al.,* 1992), which for an athletic population means elevating L-βHB may not be as efficient at enhancing performance as D-βHB (B. Stubbs *et al.,* 2017). Early work in rats demonstrated that a racemic R (D-βHB) isomer caused substantially greater increases in plasma βHB (2.38 ± 0.4 mM versus 0.92 ± 0.2 mM) and plasma AcAc (0.80 ± 0.2 mM versus 0.49 ± 0.1 mM) versus the non-racemic L-βHB isomer (Gueldry and Bralet, 1995). Notably, despite similar doses of ketone bodies (Gueldry and Bralet, 1995), βHB supplementation was more proficient at causing nutritional ketosis. Furthermore, despite a lack of experimental evidence, the use of βHB ketone esters are more recommended for performance than AcAc ketone esters (Stubbs *et al.,* 2018), as βHB molecules are ready to enter the mitochondrial matrix prior to being converted back to AcAc via 3-hydroxybutyrate dehydrogenase (BDH) to form two Ac-CoA molecules which enter the citric acid cycle to form ATP, whereas prior to entering the mitochondrial matrix, AcAc must first be reduced to βHB (Evans, Cogan and Egan, 2016).

Acute ingestion of ketone esters in humans causes short-term (~0.5-6 hours) nutritional ketosis (i.e., serum βHB >1.0 mM) (Evans, Cogan and Egan, 2016), while ingestion of 573 $mg \cdot kg^{-1}$ BM of (R)-3-hydroxybutyl (R)-3-hydroxybutyrate ketone monoester in a fasted state increases serum βHB to ~6.0 mM 30 minutes post-ingestion and to ~2.0 mM when co-ingested with a carbohydrate solution in cyclists (Cox *et al.,* 2016). Whereas, acute ingestion of 500 $mg \cdot kg^{-1}$ BM of D,L-1,3-butanediol acetoacetate diester caused a modest ~1.3 mM increase in plasma βHB 123 minutes post-ingestion in a fed state (2 g·kg CHO) in highly trained cyclist. Therefore, there appears large variability in increases in serum βHB observed between ketone esters used (Cox *et al.,* 2016; Leckey *et al.,* 2017) and

whether ketone esters are consumed in a fasted state (Cox *et al.,* 2016), fed (Leckey *et al.,* 2017) or co-ingested with carbohydrates (Cox *et al.,* 2016) in endurance athletes.

2.7.2 Ketone Salts and Performance

A number of investigations have taken place using ketone salts in humans, however for the purpose of this review only investigation which assessed athletic performance have been included (Table 2.5). Rodger *et al.,'*s (2017) investigation involved 12 highly-trained male cyclists (~68.0 ± 6.7 $mL \cdot kg^{-1}min^{-1}$ VO_{2peak}) in a double-blind, placebo-controlled, randomised, crossover design study, where participants were required to visit the laboratory on 3 separate occasions. Participants were instructed to log their food intake prior to arriving on their first visit to the laboratory and have their last meal 2.5 hours prior to arrival. On future visits, participants were instructed to replicate their dietary preparations 48 hours prior to arrival, to ensure energy availability and glycogen stores were not a confounding variable. On their first visit to the laboratory, participants familiarised themselves with the exercise protocol and had peak VO_2 quantified using an incremental test on a cycle ergometer. On subsequent visits, participants consumed a ketone salt (βHB = ~11.7 g; 3 g sodium; 3 g calcium) (Ketoforce; Prototype Nutrition, IL, USA) or a placebo 20 minutes prior to completing a 90 minute cycle at 80% VO_{2peak}, followed by a 4 minute maximal performance test. During the 90 minute cycle, participants VO_2/VCO_2 were collected for 5-10 minutes on 3 occasions.

Table 2.5. Ketone salts and performance investigations in athletes

Author	Population Sample	Ketone Salt & Dose	Performance Test	Performance + Blood βHB Results	Gastrointestinal Discomfort
Rodger *et al.,* (2017)	*n* = 12 highly-trained males VO_{2peak}: 68.0 ± 6.7 $mL \cdot kg^{-1}min^{-1}$	23.4 βHB, 6 g sodium, 3 g calcium (Ketoforce®, USA) or PLA	90 minute steady state exercise + 4 minute sprint	No performance difference (*P* = 0.38) (mean power: βHB 364 ± 58 W; PLA 355 ± 46 W) βHB ~0.6 mM	Not reported
O'Malley *et al.,* (2017)	*n* = 10 healthy-adult males VO_{2peak}: 45 ± 10 $mL \cdot kg^{-1}min^{-1}$	24.9 βHB, 0.8g potassium, 0.8 g sodium (Ketoforce®, USA) or PLA	15 minute incremental protocol followed by 150 kJ TT.	Time to complete 150 kJ TT was ~46 seconds slower in KET condition (*P* = 0.03) and average power was ~7% less (*P* = 0.029) βHB ~0.8 mM	Not reported
Waldman *et al.,* (2018)	*n* = 15 healthy-college aged males	11.38 g βHB, 0.3 mg magnesium and 0.5 g calcium (PerfectKeto, USA) or PLA	4 x 15 second Wingate	No difference in total work completed (βHB = 10730.7 ± 1403.5 J; PLA = 10707.1 ± 1387.3 J; *P* > 0.05). βHB ~0.5 mM	Value not reported, but "significant gastrointestinal distress (i.e., abdominal pain, diarrhea, nausea)"

Abbreviations: g = gram; mg; milligram; kJ = kilojoule; mM = millimolar; *n* = number; $mL \cdot kg^{-1}min^{-1}$ = millilitres per kilogram per minute; PLA = placebo; TT = time trial; VO_{2peak} = maximal oxygen consumption; W = watt; βHB = beta-hydroxybutyrate

Forty-five minutes into the cycle, participants re-ingested an additional ketone salt (βHB = ~11.7 g; 3 g sodium; 3 g calcium) or placebo. Blood glucose and βHB were re-examined at the outset of exercise and at time points 30-, 60- and 90 minutes (during 90 minute cycle) and subsequent to the 4 minute sprint using a capillary blood sample. The ketone salt caused an increase in βHB, peaking at ~0.63 mM during exercise ($P < 0.01$). It's noteworthy that this investigation relied on capillary blood to assess concentrations of βHB, glucose and lactate. An investigation examining the reliability of devices that measure blood metabolites suggested that commercial handheld monitors often overestimate concentrations, such as βHB versus traditional laboratory methods (Guimont *et al.,* 2015). Therefore, it's possible that capillary βHB values presented may be less than ~0.63 mM. Nonetheless, ketone salt ingestion increased RER (ES = 0.54 P = 0.06), whilst there was little difference between groups glucose and lactate responses to submaximal and maximal bouts of exercise (*all* $P > 0.05$). Despite consuming a total of 23.4 g of βHB, ketone salt ingestion had no impact on 4 minute maximal performance test ($P = 0.38$). Despite not causing any notable impacts on blood glucose, lactate or performance, the change in RER is of interest, as the respiratory quotient (RQ) value for βHB is 0.89 (Frayn, 1983). Following ketone ingestion, RER values increased from 0.85 ± 0.03 to 0.87 ± 0.05. As previously outlined, Cox *et al.,* (2016) participants' found that ~16-18% of total energy came from ketone bodies when plasma βHB was >2.0 mM in well-trained cyclists at <75% W_{max}. Therefore, it's plausible that participants contained within Rodger *et al.,*'s (2017) investigation may have experienced a fraction of that 'glycogen sparing effect'. However, as hypothesised by Evans, Cogan and Egan (2016), potential ergogenic benefits of exogenous ketones are likely to negligible unless increases in βHB exceeds ~1.0 mM.

Shortly after Rodger *et al.,*'s (2017) investigation, O'Malley *et al.,* (2017) examined ketone salts impact on physical performance in healthy adult males (~45 ± 10 mL·kg^{-1}min^{-1} VO_{2peak}). This was another double-blind placebo-controlled crossover design, whereby participants were required to attend the laboratory on 4-occasions. On their initial visit to the laboratory, participants completed a ramp protocol on a cycle ergometer to quantify their VO_{2peak}. The following visit was a familiarization day, where participants ran through the exercise protocol without the use of any supplement/placebo. On subsequent visits, participants arrived fasted and consumed a ketone salt (0.3 g·kg βHB (~24.9 ± 3.9 g), 0.01 g·kg (~0.8 ± 0.1 g) potassium; 0.01 g·kg (~0.8 ± 0.1 g) sodium) (Ketoforce; Prototype Nutrition, Urbana, Ill., USA) or a placebo (0.01 g·kg (~0.8 ± 0.1 g) potassium; 0.01 g·kg (~0.8 ± 0.1 g) sodium). Thirty minutes post-ingestion, participants completed a 3-stage incremental exercise protocol; 5 minute cycle at 30%, 60% and 90% of VO_{2peak}. Following incremental exercise, participants completed a simulated 150 kJ TT. Gas samples were recorded throughout the steady state cycle, while blood βHB, glucose and lactate were measured via capillary blood prior to exercise, 30 minutes post-supplementation and at the end of the steady state and TT. Blood capillary βHB increased from resting values (~0.1 mM) to ~0.8 mM post-ingestion and during exercise. These healthy individuals experienced greater increases in blood capillary βHB following ketone salt ingestion than previously observed within highly trained individuals (Rodger *et al.,* 2017). Participants experienced a decrease in RER values from ~0.85 to 0.83 ($P < 0.05$), in contrast to Rodger *et al.,* (2017) who experienced an increase. This may have stemmed from larger dose of βHB being ingested in healthy individuals (~24.9 g βHB versus ~23.4 g βHB) (Table 2.5) or perhaps, because untrained individuals are less efficient at utilising ketones bodies in the blood for fuel. Evans, Cogan and Egan (2016) postulated that ketone bodies are most likely to be of benefit in trained

individuals, who have a greater capacity to take up and oxidise ketone bodies during exercise. Namely, monocarboxylate transporters (MCT) transport ketone bodies into skeletal muscle. Increase in MCT1 expression occur in response to training and occur in an intensity-dependant manner (Thomas *et al.,* 2012). Therefore, in this instance, untrained individuals (O'Malley *et al.,* 2017) may have been unable to transport ketone bodies into skeletal muscle, resulting in greater blood concentrations of βHB than well-trained individuals (Rodger *et al.,* 2017) consuming a similar dose of βHB (Table 2.5). Evans, Cogan and Egan (2016) postulated that increases in βHB are unlikely to be performance enhancing unless 1.0 mM threshold is met or exceeded. Given Cox *et al.,* (2016) found that a ~16-18% of total energy is contributed from ketone bodies when plasma βHB was >2.0 mM in well-trained cyclists at <75% W_{max}, it's not surprising that the modest increase βHB (~0.63 mM) had no impact on blood lactate. Nonetheless, time to complete the 150 kJ TT was ~46 seconds slower following ketone salt ingestion (711 ± 137 seconds), when compare to the placebo group (665 ± 120 seconds) (P = 0.03), while power output during the time-trial was ~7% lower following ketone salt supplementation (P = 0.029).

In another double-blinded, placebo (PLA) controlled, counter-balanced, randomized cross-over investigation, Waldman *et al.,* (2018) examined the impact ketone salt supplementation had on repeated 15 second sprint performance on a cycle ergometer, in 15 healthy college aged males. Following an initial familiarisation visit, participants attended the laboratory an additional two times. Participants arrived fasted and received resting measures, which included blood βHB, glucose and lactate using capillary sample. Subsequently participants consumed ketone salts (11.38 g βHB, 0.3 g magnesium and 0.5 g calcium) or an isocaloric placebo 30 minutes prior to the

commencement of exercise. Participants began cycling at 100 watts for 5 minutes and then immediately began 4 x 15-second Wingate's, with 4 minute active recovery between each sprint at 70 revolutions per minute. Following the Wingate test, participants continued to cycle at 70 revolutions per minute and had βHB, glucose and lactate recorded for 60-seconds. Ketone salt ingestion increased capillary blood βHB to 0.53 ± 0.19 mM at the commencement of exercise ($P < 0.05$) versus the placebo, but had no impact on blood glucose or lactate (*both* $P > 0.05$). Blood βHB remained elevated versus the placebo condition post-exercise (0.26 ± 0.06 versus 0.14 ± 0.05; $P < 0.05$). Despite fatigue index increasing by ~8.9% in the ketone salt condition, mean power output (βHB 715.4 ± 93.5 W; PLA 713.8 ± 92.5 W), peak power output (βHB 969.2 ± 157.3 W; PLA = 954.5 ± 150.5 W) and total work (βHB = 10730.7 ± 1403.5 J; PLA = 10707.1 ± 1387.3 J) during the Wingate's remained unaffected (*all* $P > 0.05$).

2.7.3 Ketone Ester Performance Literature

Interest in ketones esters grew following a key publication by Cox and colleagues (2016). The manuscript included a number of investigations on a ketone monoester ((R)-3-hydroxybutyl (R)-3-hydroxybutyrate; examining its impact on macronutrient exercise metabolism, fuel utilisation and exercise performance. One of these investigations was a randomised, single-blind, crossover design involving 10 participants (Table 2.5). Subsequent to an overnight fast, ingesting ~573 $mg \cdot kg^{-1}$ BM of ketone ester increased plasma βHB to ~3.5 mM within 10 minutes at rest and remained elevated during 60 minutes of exercise at 75% VO_{2peak} versus an isocaloric (515 kcal) carbohydrate placebo. Most notably, the elevation in βHB caused a ~50% reduction (~2-3 mM) in participants

blood lactate during exercise. Muscle biopsies were performed prior- to and post-exercise to examine intramuscular concentrations of βHB and concentrations of glycolytic intermediates; glyceraldehyde-3-phosphate, 2 and 3-phosphoglycerate and pyruvate. Following the bout of exercise, all measures of glycolytic intermediates were lower following ketone ester supplementation, indicating a down regulation of skeletal muscle glycolysis during exercise and perhaps explaining decreases in blood lactate during exercise. In a subsequent investigation within Cox *et al.*'s (2016) various studies it was described that an elevation in βHB (~2.2 mM) could co-exist with carbohydrate feeding and 'normal' glycogen stores and insulin concentrations. This was an entirely new phenomenon, as prior to this, elevating βHB had only occurred in the absence of carbohydrates, following prolonged periods of fasting (Féry and Balasse, 1988) or following an adaptation to a LCKD (Phinney *et al.,* 1983; Volek *et al.,* 2016; Webster *et al.,* 2016; Burke *et al.,* 2017).

In Cox and colleagues (2016) final investigation, performance was assessed. This investigation was carried out in 8 'highly-trained endurance athletes' (*n* = 6 males: 5.37 ± 0.3 mL·kg^{-1}min^{-1} VO_{2peak}; *n* = 2 females: 3.30 ± 0.1 mL·kg^{-1}min^{-1} VO_{2peak}) and once again incorporated a randomised, single-blind, crossover design. The investigation incorporated a 60 minute pre-load/steady-state at 75% VO_{2peak}, followed by a 30 minute blinded TT to measure performance. Experimental drinks were ketone ester (KE = ~515 kcal: 60% dextrose and 40% ketone) or carbohydrate placebo (PLA = ~515 kcal: 40% dextrose, 40% fructose and 20% maltodextrin). A total of 573 mg·kg^{-1} BM of ketone ester ((R)-3-hydroxybutyl (R)-3-hydroxybutyrate) was ingested in three boluses, one 30 minutes prior to exercise and the final two boluses consumed at time points 30- and 60 minutes during the 60 minute pre-load. Ketone supplementation increased serum βHB

to ~2 mM 20 minutes post KET ingestion, where it remained during steady state exercise after an initial dip (~1.75 mM) once exercise had commenced. This decrease in serum βHB at the commencement of exercise is a result of increased ketone body oxidation within skeletal muscle and elevated metabolic clearance rate (MCR) (Evans, Egan and Cogan, 2016), MCR being a measure of the tissues ability to remove ketone bodies from the blood (Féry and Balasse, 1983; Balasse and Féry, 1989). Blood glucose was ~1.0 mM lower during the KET versus PLA feeding trial at ~25 minutes, but similar at ~60 minutes. Distance covered during the 30 minute TT increased by ~2% (411 ± 162 m) following KET ingestion versus the PLA trial ($P < 0.05$). The exact mechanism which enabled this enhancement remains to be explored. In the discussion, the authors outlined that they believe a carbohydrate feeding + ketone ester approach provides athletes with an advantageous physiological state versus a traditional multi-transportable source of carbohydrate, but warned that such an approach may not be advantageous whereby successful completion of a sport or event relies almost exclusively on anaerobic glycolysis, such as sprinting, as discussed earlier in Chapter 2.

Table 2.6. Ketone esters and performance investigations in athletes

Author	Population Sample	Ketone Ester & Dose	Performance Test	Performance + Plasma βHB Results	Gastrointestinal Discomfort
Cox *et al.,* (2016)	*n* = 11 highly trained cyclists (*n* = 6 males; *n* = 2 females) VO_{2peak}: 5.37 ± 0.3 $mL \cdot kg^{-1}min^{-1}$ males; ~3.30 ± 0.1 $mL \cdot kg^{-1}min^{-1}$ females	~573 $mg \cdot kg^{-1}$ BM of (R)-3-hydroxybutyl (R)-3-hydroxybutyrate ketone monoester (Clark *et al.,* 2012) + CHO (~515 kcal total) or PLA (CHO) (~515 kcal from CHO)	Cycling 30 minute TT (max distance)	Performance improved by ~2% (411 ± 162 m) in ketone ester condition βHB ~2.0 mM	No
Leckey *et al.,* (2018)	*n* = 10 elite cyclists VO_{2peak}: 71.4 ± 5.6 $mL \cdot kg^{-1}min^{-1}$	2 g·kg CHO + 200 mg of caffeine ~500 $mg \cdot kg^{-1}$ BM of D,L-1,3-butanediol acetoacetate diester (D'Agostino *et al.,* 2012) or PLA 500 ml Gatorade during TT	Cycling 31.17 km TT	Time to completion increased +2 ± 1% (~58 seconds) following ketone ester condition βHB ~1.2 mM	Yes - 100%

Abbreviations: g = gram; J = joule; kcal = calorie; mg; milligram; $mg \cdot kg^{-1}$ BM = milligram per kilogram body weight; mM = millimolar; *n* = number; $mL \cdot kg^{-1}min^{-1}$ = millilitres per kilogram per minute; PLA = placebo; TT = time trial; VO_{2peak} = maximal oxygen consumption; βHB = beta-hydroxybutyrate

Table 2.6. Contd. Ketone esters and performance investigations in athletes

Author	Population Sample	Ketone Ester & Dose	Performance Test	Performance + Plasma βHB Results	Gastrointestinal Discomfort
Evans and Egan (2018)	*n* = 11 male intermittent field sport athletes VO_{2peak}: ~53.9 ± 2.2 $mL \cdot kg^{-1}min^{-1}$	~750 $mg \cdot kg^{-1}$ BM (R)-3-hydroxybutyl (R)-3-hydroxybutyrate ketone monoester (KetoneAid, USA) or PLA & ~1.2 $g \cdot min^{-1}$ CHO	15 minute run at variable intensity, followed by TTE at speeds of 55 and 95% VO_{2peak}	TTE unaffected (KET = 229 seconds versus PLA 267 seconds; *P* = 0.126) βHB ~1.5-2.5 mM	Yes – 82%

Abbreviations: g = gram; $g \cdot min^{-1}$ = gram per minute; mg; milligram; mM = millimolar; *n* = number; $mL \cdot kg^{-1}min^{-1}$ = millilitres per kilogram per minute; PLA = placebo; TT = time trial; VO_{2peak} = maximal oxygen consumption; W = watt; βHB = beta-hydroxybutyrate

Following publication, an interesting viewpoint of Cox *et al.,*'s (2016) findings arose in lay press (Aragon, 2016). During each arm of the trial (KET or PLA), participants consumed ~515 kcal, however, conditions differed in carbohydrate content (i.e., KET = 40% kcal; PLA 100% kcal) due to the presence of the ketone ester in the KET condition (~60% kcal). Aragon (2016) postulated that the KET condition may have enhanced their performance versus the carbohydrate trial as they had received a more optimal dose of carbohydrates and not necessarily due to the ketone ester or reduced accumulation of lactate. This viewpoint is based on an investigation by Smith *et al.,* (2013), who discovered that there was a dose-response relationship between carbohydrates consumed and performance in recreationally active endurance athletes (~59.1 ± 5.6 $ml \cdot kg^{-1}$ min^{-1} VO_{2peak}). The investigation examined doses of carbohydrate from 0 to 120 g (10 g increments). Smith *et al.,* (2013) discovered that the optimal dose is ~78 g/h of carbohydrates, after which point 20 km TT (subsequent to 2 hour cycle at intensity that elicits lactate of ~4.0 mM) performance began to decline. Aragon (2016) calculated that an average 75 kg athlete consuming a KET condition would have consumed 77 g/h of carbohydrates and 43 g/h of ketones (βHB), whereas a 75 kg athlete within the PLA condition would have consumed ~129 g/h. Notably however, mean body weight (kg) contained within 'study 5' was ~84.9 kg. Thus, although the aforementioned thought process is accurate, the figures contained within Aragon's (2016) postulation are incorrect. Average carbohydrate consumption in the KET condition was ~70.3 g and ~48.6 g of ketones (233 kcal). Thus, it appears neither group received the 'optimal dose of carbohydrates', as suggested by Smith *et al.,* (2013). Nonetheless, the thought process raises an important question, should carbohydrate consumed (g/h) in the exogenous ketone and control group be matched and the ketone ester act as

supplementary source of energy or as is the case within Cox *et al.,*'s (2016) investigation, energy should be matched (total calories) and carbohydrate consumption in the intervention group should be reduced to make a deficit for energy derived from the ketone ester.

Subsequently, Leckey and colleagues (2017) examined another ketone ester (Table 2.6). This investigation incorporated a D,L-1,3-butanediol acetoacetate diester and included 10-elite cyclists (~71.4 ±5.6 $mL \cdot kg^{-1}min^{-1}$ VO_{2peak}). The investigation had 2-experimental arms. Prior to each experimental trial participants consumed 2 g·kg of carbohydrate the evening prior and an additional 2 g·kg upon arrival on the morning of testing 115 minutes before exercise commencement and 200 mg of caffeine 15 minutes, thereafter. This investigation was critical of Cox *et al.,*'s (2016) investigation, stating that it did not reflect real world practice, as the exercise trial was performed fasted and blinded (distance + speed). Resultantly, you can observe 'real world practices' steeped throughout this investigation, by incorporating a moderate carbohydrate load the morning of the experimental trials, as well as opting for a more traditional TT. Following participant's pre-exercise meal and caffeine, participants ingested 250 $mg \cdot kg^{-1}$ BM of D,L-1,3-butanediol acetoacetate diester (KET) or a PLA (both mixed with flat diet cola) 30 minutes prior and immediately prior to completing an incremental warm-up for 20 minutes. Subsequently, participants completed a simulation of the 2017 Bergan World Championship TT course, which was 31.17 km in length on a cycle ergometer. At the 15.74 km mark, participants ingested 250 ml of Gatorade (6% carbohydrate drink). Blood samples were acquired prior to and during the warm-up and throughout the 31.17 km TT. Similarly, urine samples were collected prior to and after the warm-up and prior to,

immediately after and 60 minutes after the TT. The 500 mg·kg^{-1} BM dose of ketone diester caused a modest increase in serum βHB, increasing to ~1.0 mM during the 31.17 km TT and peaking post exercise at ~1.25 mM (~123 minutes post-ingestion). However, increases in βHB were accompanied by increased time to completion and a decrease in power output (W) during the 31.17 km TT. The small effect in TT performance represents a +2±1% (58 seconds) increase in time to completion ($P < 0.001$) and a -3.7% (339 ± 37 W) decrease in power output ($P < 0.001$). Perhaps more importantly, the ketone diester caused severe side effects in 1 participant, causing them to drop out of the investigation, whilst the remaining 10 participants all reported gastrointestinal discomfort following the consumption of the ketone diester (symptoms ranged from dry retching, to nausea). Further, gastrointestinal symptoms likely attributed to decreased performance, as participants' nominated their gastrointestinal symptoms' as a distraction or interference to performance (Leckey *et al.,* 2017). The investigation was later criticised by Stubbs *et al.,* (2017), pointing out that the D,L-1,3-butanediol acetoacetate diester had not been extensively tested in humans and the dosing strategy implemented by Leckey *et al.,* (2018) likely contributed to gastrointestinal symptoms experienced.

Evans and Egan (2018) examined a ketone esters impact on intermittent running performance in field athletes (Table 2.6). This investigation was unique and warranted, as prior work on ketone esters had focused on endurance athlete's performance responses. Field sports such as Gaelic games, soccer and rugby comprise a large segment of the athletic population and are susceptible to fatigue, just like distance athletes. Eleven male team sports athletes (~53.9 ± 2.2 mL·kg^{-1}min^{-1} VO_{2peak}) partook in the investigation. Participants were required to attend the laboratory on 3-separate

occasions during a 14-21 day period. During visit-1, participants had peak VO_2 quantified. Maximal oxygen consumption was carried out to determine participants running speeds at 55 and 95% of VO_{2peak}, which were later used during a multi-stage shuttle 'Yo-Yo test', which is a validated intermittent test carried out until volitional fatigue (Nicholas, Nuttall and Williams, 2010). Prior to subsequent tests (visit 2-3), which were performed in a double-blind, randomised crossover manner, diet (40 kcal·kg body mass carbohydrate:fat:protein = 60:20:20) was controlled for 36 hours prior to remove its potential impact as a confounding variable. Similarly, prior to arriving to complete the afternoon test, each participant consumed 2-meals containing 3 g·kg body mass of carbohydrate. Twenty minutes prior to the commencement of exercise, participants consumed either 375 mg·kg^{-1} BM or placebo (flavouring) and carbohydrate (Table 2.6). Participants then completed five 15 minute intermittent running blocks at variable intensity intermittent work, followed by 3 minutes rest and a time to exhaustion trial with intensity ranging from 55 or 95% VO_{2peak}. Immediately prior to exercise, participants ingested carbohydrate electrolyte solution and four additional drinks during exercise. Total carbohydrates consumed in each group provided ~1.2 g·min^{-1} during exercise. Two of the additional 4 boluses contained carbohydrate, as well as the placebo flavouring or the remainder of the ~750 mg·kg^{-1} BM dose of ketone ester (i.e., 2 x 187.5 mg·kg^{-1} BM). Intravenous blood samples were taken at rest, during rest periods and subsequent to the battery of tests to determine changes in βHB, blood glucose and lactate. Despite a higher incidence of gastrointestinal discomfort being reported in the ketone ester group (KET = 82% versus PLA = 36%), heart rate and RPE did not differ between trials. Ketone ester ingestion caused an increase in plasma βHB concentrations during exercise (~1.5-2.6 mM, $P < 0.001$). This increase was accompanied by a small-

moderate (effect sizes) decrease (~-11.9%/-0.48 mM) in plasma lactate. Despite these changes and additional energy contained within the ketone ester condition, time to exhaustion following 15 minutes of intermittent running (KET = 229 seconds versus PLA 267 seconds; *P* = 0.126) and 15 m sprint speeds times remained unaffected.

2.8 Low-Carbohydrate Ketogenic Diet and Health

2.8.1 Ketogenic Diet and Bone Health

Endurance athletes, such as swimmers and cyclists are at an increased risk of developing low bone mineral density and osteoporosis in comparison to other active individuals, due to the low impact nature of their chosen sports (Scofield and Hecht, 2012). A long held belief is long term adaptation to a low-carbohydrate diet could cause a calcium imbalance, due to decreased in gut calcium absorption and an increase in urinary calcium excretion (Barzel and Massey, 1998). Carter, Vasey and Valeriano (2006) designed an investigation to test this hypothesis in overweight patients. Participants consumed a low-carbohydrate diet (*n* = 15: <20 g/d of carbohydrate) or a control diet (*n* = 15: no dietary restriction) for 12 weeks. Despite urinary calcium excretion increasing in the low-carbohydrate group in week 3 and week 12, bone mineral density remained unchanged in each group. The rate of bone turnover ratio non-significantly increased in each group (*P* = 0.86), indicating that there no significant change in bone formation between groups. During the intervention, participants in the low-carbohydrate group lost significantly more weight in comparison to control group (control -1.05 kg, low-carbohydrate -6.39 kg), suggesting a low-carbohydrate diet is an effective tool for achieving weight loss in a healthy overweight population, without putting bone health at unnecessary risk. Conversely, 12 weeks may not long enough to detect significant changes in bone mineral density. Previously Barry and Kohrt (2008) assessed cyclists bone mineral density over the course of a cycling season (~1 year), notably, significant decreases in bone mineral density weren't observed until the 9th month, which indicates that Carter, Vasey and Valeriano (2006) investigation may not have been long enough

to detect significant changes. These observations are in line with a recent pilot study, which noted that observable changes in bone turnover did not take place until week 24-48 of the intervention period (Nakatoh, 2016).

There is also conflicting evidence in rodents. Bielohuby *et al.,* (2010) assessed the short term effects of consuming a LCKD on bone formation and resorption, mice consumed a traditional chow diet (%carbohydrate:fat:protein = 58:9:33), a LCHF-1 diet (1:66:33) or a LCHF-2 diet (1:95:4) for 4 weeks. Each diet was supplemented with vitamin and mineral premixes to meet demands of the national research council (D3, calcium, phosphorus). Subsequent to 4 weeks of each diet, changes in rat's bone markers and body composition were analysed. Consumption of both LCHF diets resulted in unfavourable changes to rats bone mineral density, bone growth and mechanical function. Bone formation marker, procollagen type 1 and propeptide decreased in the LCHF-1 group and in the LCHF-2 group, while no significant changes to groups bone resorption marker CrossLaps, were noted. The author believes that the reduction in bone formation as a result of consuming the LCHF diets could be explained by reduced number of mesenchymal cells differentiating into osteoblasts, as transcription factors influencing osteoblasts were down regulated by 70–80% (Runx2, osterix and C/EBRB) (Bielohuby *et al.,* 2010). Furthermore, rats that consumed the chow diet and LCHF-1 diet gained more weight than the rats who consumed the LCHF-2 diet and consumption of the LCHF-2 diet resulted in an increased long bone growth deficiency, which suggests that protein must be consumed in moderation to promote and sustain healthy bones and growth patterns in rats (Bielohuby *et al.,* 2010).

To date the majority of data which exists on the effect a LCKD on bone health in humans is primarily carried out in children with epilepsy, as the consumption of a LCKD is a commonly used treatment for childhood epilepsy (Vanitallie and Nufert, 2003; Groesbeck and Bluml, 2006; Bergqvist *et al.,* 2008). Longitudinal data by Groesbeck *et al.,* (2006) shows that some children who suffer with epilepsy and consume a LCKD can see up to a 90% reduction in seizure frequency, however children who consumed a LCKD for >3–6 years were at an increased risk of developing kidney stones and experiencing fractures. Along with being at an increased risk of injury and illness, half of the children were underdeveloped in comparison to healthy children consuming a traditional diet, however participants included in the study were below the 10th centile for height and weight prior to starting the LCKD, so it's open to interpretation whether growth and bone underdevelopment were due to genetic growth difficulties or as a result of consuming the LCKD. An additional longitudinal study was carried out on young children (<10 years) with epilepsy, Bergqvist *et al.,* (2008) monitored changes to 25 participants bone health over 15 months. Starting out, participants included in the investigation had sub-optimal bone health and growth status. Despite participants seeing a reduction in the frequency of seizures and an increase in serum vitamin D concentrations progressive loss of bone mineral content resulted in osteopenia and osteoporosis.

With endurance athletes being at an increased risk of low-bone mineral density (Scofield and Hecht, 2012) and current evidence regarding a low-carbohydrate diets suitability a maintaining bone health, there is a need for an investigation to examine changes in bone mineral density, in addition to bone formation and bone resorption over an extended

period of time, i.e., >10-12 months based on current evidence (Carter, Vasey and Valeriano, 2006; Nakatoh, 2016).

2.8.2 Reactive Oxygen Species (ROS) and Free Radical Theory

The mitochondria is commonly referred to as 'the power-house of the cell' (McBride, Neuspiel and Wasiak, 2006), as it plays a crucial role in nutritive energy production. It plays a similarly pivotal role in the aging process and in age related diseases (Ristow and Zarse, 2010). Reactive oxygen species (ROS) are a by-product of oxidative phosphorylation, with the mitochondria generating >90% of intramuscular ROS (Ristow and Schmeisser, 2011). Thus, being the main energy producer (ATP) and primary producer of ROS, its role within physiological and pathophysiological processes within the cell cannot be understated. Oxidative stress occurs when the level of reactive oxygen species (ROS) overwhelms the body's antioxidant defence mechanism and causes damage (Ziegler, 2003).

In 1956 Denham Harman introduced his Free Radical Theory of Aging (FRTA) (Harman, 1956). This concept postulated that increased ROS production causes damage within the cell, resulting in aging and age related impairment of the cell, attributing to cell death. In 1972 Harman updated his FRTA to the Mitochondrial Free Radical Theory of Aging (MFRTA) as respiratory enzymes were proposed to be the main generators of ROS during energy production (Harman, 1972). Since the 1970's, considerable research has taken place attempting to prove/disprove this theory, however, a review by Pérez *et al.,* (2009) highlighted that such efforts showed conflicting results. An important observation by

Lapointe and Hekimi (2010) highlighted that an increase in resting metabolic rate or 'metabolism' does not necessarily result in an increase in the ROS production. Similarly, growing evidence suggests that although oxidative phosphorylation produces ROS, the relationship between ROS production and oxidative stress/aging is not linear (Ristow and Schmeisser, 2014). In light of Denham Harman theories, supplements to reduce ROS production became commercially available for human consumption, i.e., anti-oxidants. Anti-oxidants were designed to 'scavenge ROS' and in turn attribute to reduced levels of oxidative stress. However, most investigations found a lack of effect in humans, with some noting hazardous effects (Ristow and Schmeisser, 2014).

Increased reliance on mitochondrial respiration causes an increased flow of electrons through the mitochondria, this increases the likelihood and prevalence of ROS being produced by the mitochondria. Once viewed as a negative consequence of metabolism, evidence now suggests that mitochondrial ROS is closely linked with cellular oxidative homeostasis and propagation of cellular signalling pathways (Hamanaka and Chandel, 2011). Thus, homeostatic levels of mitochondrial ROS are not viewed as a negative, in fact it's now seen to integrate cellular energy metabolism and have upstream effects on cellular stress signalling and cellular survival (Hamanaka and Chandel, 2011). This attractive cellular response which is used in the prevention and treatment of chronic disease is referred to as 'mitohormesis' (Tapia, 2006).

2.8.2.1 Oxidative Stress and the Ketogenic Diet

By adapting to a 'ketogenic' style of eating, athletes are altering their energy metabolism towards fatty acid oxidation and ketone bodies (Volek *et al.,* 2016; Burke *et al.,* 2017) due to decreased glucose availability (Evans, Cogan and Egan, 2016). Miller, Villamena

and Volek (2018) believe that such an adaptation would logically increases mitochondrial respiration and as a result, mitochondrial ROS. Although not extensively studied in larger mammals or humans, this is an area whereby a LCKD is being examined as a potential treatment to chronic disease (Miller, Villamena and Volek, 2018). Previously two investigations in C. elegans found that inhibition of glycolysis and increased fat oxidation and mitochondrial consumption, improved lifespan and antioxidant enzyme activity (Schulz *et al.,* 2007; Zarse *et al.,* 2012). An adaptation to a LCKD has long been known to downregulate ROS production (Sato *et al.,* 1995), however, the specific mechanism behind this reduction has remained relatively unknown for decades. As previously outlined, C. elegans were found to experience improved lifespan and increases in antioxidant enzyme activity following glucose inhibition. Notably, this caused an increase in mitochondrial ROS at 24 and 48 hours, however, mitochondrial ROS was lower at ~120 hours, indicating that increases in antioxidant enzyme activity decreased net ROS versus the control group, resulting in a two-fold increase in lifespan. Although speculative, this may be why 'keto-adapted' athletes anecdotally report accelerated recovery after adapting to a LCKD, athletes report returning to training within days, not weeks after an ultra-marathon (Volek, Noakes and Phinney, 2015; Noakes and Windt, 2016). Contrary to this postulation, an 8 month investigation in mice consuming a LCKD (%carbohydrate:fat:protein = 10:67:23) who achieved mean βHB of ~1.5 mM had ~40% greater mitochondrial ROS produced in their gastrocnemius (calf muscle) ($P = 0.007$) versus a standard chow diet (% = 58:18:24) and a chow diet (% = 58:18:24) with an exogenous ketone supplement (DL-3 sodium hydroxybutyric acid, 5.8 kcal/g, NNB Nutrition, Lewisville, TX, USA) at post-intervention (8 month follow up).

Current evidence in this area is contradictory, while research in humans and in an athletic population is lacking, further work is needed to examine the potential mechanisms, if any, that support improved recovery on a LCKD versus a high-carbohydrate diet in ultra-endurance athletes (Volek, Noakes and Phinney, 2015). If a LCKD did in fact contribute to a reduction in ROS being produced, it could have considerable knock on effects. Decreasing net ROS may not be of grave concern to the general population, however, in an athletic population whereby endurance and ultra–endurance athletes compete in 26-100+ mile races, excessive production of ROS over an extended period of time (months/years/decades) could manifest into excessive exposure to oxidative stress, which could have a profound impact on an athletes health and performance, potentially attributing to prolonged recovery and increased risk of injury/disease.

2.8.3. Low-Carbohydrate Diet and Inflammation

Traditionally 'high-fat' diets were considered to be associated with increased inflammation (Erhardt *et al.,* 1997; Djuric *et al.,* 2001). However, when discussing the body's inflammatory response to any diet, it's important to look at it in the right context. Research suggesting low-carbohydrate diets have an adverse effect on inflammatory response (Erhardt *et al.,* 1997; Djuric *et al.,* 2001) were not 'ketogenic' as they did not induce nutritional ketosis, as blood ketones were <0.5 mM. Volek, Noakes and Phinney (2015) who are undoubtedly big advocates of LCKD believe the association of dietary fat promoting a pro-inflammatory state is unfairly based on this misleading literature, at least in comparison to keto-adapted persons. A review by Herieka *et al.,* (2013) summarised 57-studies which examined the impact of consuming a high-fat meal

(containing at least 30g of dietary fat) on pro inflammatory markers in humans. Results illustrated that consumption of a high-fat meal didn't impact on cytokines or soluble adhesion molecules but increased pro-inflammatory leukocyte surface markers, mRNA and proteins. Volek, Noakes and Phinney (2015) believe this response should not be associated with risks associated with consuming a LCKD as participants selected for the Herieka *et al.,* (2013) review were not keto-adapted, nor were they accustomed to consuming large quantities of fat in one sitting, which resulted in participants experiencing an acute pro-inflammatory response. The following section will focus on inflammatory responses to carbohydrate restricted diets (LCHF and LCKD), containing >50% of energy from dietary fat and <150 g/d of carbohydrates.

Keogh *et al.,* (2008) carried out an 8 week investigation in overweight males and females with at least one additional risk factor for the metabolic syndrome. One of this investigations strongest points was the large population sample (n = 99). Participants were randomised into a high-carbohydrate (1,575 kcal %carbohydrate:fat:protein = 47:28:25) or LCKD (1579 kcal %6:59:35) for the duration of the investigation with each group instructed to restrict calories. There was a significant time*group effect for plasma ketones, as concentrations increased in the LCKD group (0.07 to 0.4 mM) and remained unchanged in the high-carbohydrate group (0.06 to 0.08 mM), indicating good adherence to the LCKD protocol. Weight loss occurred in each group (HC = -6.2 kg vs LCKD = -7.5 kg $P < 0.001$), however, LCKD participants tended to outperform the high-carbohydrate group (TxG $P < 0.01$). The investigation monitored changes in a number of inflammatory markers, namely, E-selectin, ICAM-1, VCAM-1, PAI-1 and CRP. Although there was a main effect of time ($P < 0.05$) on a number of measures, there was only a

time*group interaction for CRP ($P < 0.05$), with values decreasing to a greater extent within the high-carbohydrate group (HC = -28% vs LCKD = -9.3%). These findings are contrary to postulations by Volek, Noakes and Phinney (2015), who suggested carbohydrate restriction and elevating βHB would more positively impact on markers of inflammation than a carbohydrate rich diet.

Forsythe *et al.,* (2008) compared the effect of a low-fat (high-carbohydrate) diet and a LCKD on circulating fatty acid composition and markers of inflammation in overweight men and women with atherogenic dyslipidaemia. Participants were randomly prescribed a high-carbohydrate diet (HC 1,478 kcal, %carbohydrate:fat:protein = 56:24:20) or LCKD for 12 weeks (LCKD 1,504 kcal, %12:59:28). Despite similarities in reported caloric intake (Forsythe *et al.,* 2008), the LCKD group lost twofold the amount of body weight (HC = -5.2 kg, LCKD = 10.1 kg $P < 0.01$) (Volek *et al.,* 2009). The authors reported a high degree of compliance to the LCKD to achieve 'low-level ketosis', however blood ketones were notably less (~0.21 mM) than thresholds set out by the same author in lay press (>0.5 mM) (Volek and Phinney, 2012). Nonetheless, Forsythe *et al.,* (2008) LCKD participants demonstrated more favourable reductions in TNF –a (HC = -11.5% vs -32% $P = 0.017$), IL–8 (HC = +4.2% vs LCKD -58.5%, $P = 0.007$), MCP–1 (HC = -4.9% vs LCKD = -24.2% $P = 0.023$), E-SEL (HC = -13.7 vs LCKD -34.3% $P = 0.014$) and PAI-1 (HC = -8.0% vs LCKD -34.4% $P = 0.026$) compared to high-carbohydrate participants. Due to randomisation the groups were well matched (body composition/inflammatory markers), notably however, protein was not matched (HC = 71.5 g/d versus LCKD 104.8 g/d) and LCKD participants lost considerably more weight. Due to proteins impact on satiety (Aragon *et al.,* 2017) and considering weight loss is said to have a profound

impact markers of inflammation and health (Coimbra *et al.,* 2017), greater decreases in body mass need to be considered when critically analysing these results. One of the key limitations of the aforementioned investigations and a limitation which is frequently the case within the 'low-carbohydrate' literature is that dietary protein (g·kg) is not matched between groups. Dietary protein causes the greatest feeling of satiety (Stubbs and Whybrow, 2004; Aragon *et al.,* 2017) versus dietary carbohydrate and fat. Therefore, greater *ad libitum* weight loss observed within LCKD groups may be due to increased dietary protein, attributing to improved satiety, weight loss and perhaps, greater improvements in markers of 'health'.

Brinkworth *et al.,* (2009) randomised 122-overweight individuals (male and females) with at least one additional metabolic syndrome risk factor into 2-groups. Following the 1-year study, there was considerable drop out, however, 59% of participants completed all aspect of the trial and were included for analysis. Participants were initially divided into an isocaloric low-fat high-carbohydrate group (*n* = 36 %carbohydrate:fat:protein = 46:30:24) and an energy restricted low-carbohydrate diet (LCKD) (*n* = 33 % 4:61:35). The investigation monitored a number of measures of cardiometabolic health (which will be discussed later in text), as well as 1-inflammatory marker, CRP. In addition to significant decreases in body mass (HC = 11.5 kg, LCKD = -14.5 kg, both $P < 0.05$), each group demonstrated non-significant decreases in CRP (HC = -48.1% vs LCKD = -50%, TxG *P* = 903). Similar to previous investigation, dietary protein was greater in the low-carbohydrate group (HC = 24% versus LCKD = 35% of energy).

Rhyu *et al.,* (2014) examined changes in inflammatory markers within an athletic population, the investigation measured inflammatory responses in taekwondo athletes

attempting to make weight using a high-carbohydrate (*n* = 10, %carbohydrate:fat:protein = 40:30:30) or LCKD (*n* = 10, 4:55:41). Each groups IL-6, IFN-γ and TNF-α readings were monitored throughout the intervention period. No effects in IL-6 and IFN-γ were observed, notably however, there was an interaction between TNF-α and weight lost throughout the intervention ($P < 0.05$). Each group experienced a non-significant rise in TNF-α (HC = pre:post 6.07 pg/ml to 9.90 pg/ml (+38%), LCKD = 6.94 pg/ml to 8.35 pg/ml (+16%)). The authors concluded that although absolute changes to TNF-α cytokines were not statistically significant, the carbohydrate restricted diet (LCKD) was more effective at attenuating an inflammatory response to the aggressive weight making regime, thus, potentially attributing to increased performance during the 2,000 m ride, previously outlined (Table 2.4). Rhyu *et al.,* (2014) concluded that further research is needed to examine LCKD and inflammatory responses in athletes. Notably, dietary protein was ~11% greater in the LCKD group.

Bazzano *et al.,* (2014) carried out another 12 month randomized control trial in overweight otherwise healthy males and females aged 22-75 years. Participants were randomised into a high-carbohydrate (*n* = 73, 1,527 kcal %carbohydrate:fat:protein = 54:30:19) or a LCKD (*n* = 75, 1,448 kcal %34:41:24) for 12 months. Although interim measurements were taken (3-6 months), however, for the purpose of this review only longitudinal changes that occurred in each group are examined. Urinary ketones were said to be lower in the low-carbohydrate group, indicating good adherence to the protocol, however, measurements were not presented. However, even under hypocaloric conditions, it's difficult to foresee ketones reaching levels of nutritional ketosis (> 0.5 mM), as dietary carbohydrate were ~127 g/d in the LCKD group, which

would have resulted in considerable blood glucose and insulin responses in a postprandial state (Cahill and Aoki, 1980). There was a significant time*group interaction for CRP, as the high-carbohydrate groups increased by +16.2 nmol/L, whilst the LCKD group experienced a -4.8 nmol/L decrease.

As previously outlined in the LCKD performance chapter, Zinn and colleagues (2017) carried out a 10 week investigation to assess the suitability of a LCKD in recreationally active endurance athletes. As well as assessing performance, dietary satisfaction and inflammatory responses were also described. Despite 'reduced inflammation' being reported within the abstract and main test of Zinn *et al.'s* (2017) manuscript, inflammation was not quantitatively measured, rather participants anecdotal experiences. Thus, further conclusions cannot be drawn from this investigation, apart from participants qualitatively reporting improved recovery and inflammation. Unfortunately participants habitual diet was not described in detail, instead the manuscript stipulates that >45% of total energy was derived from carbohydrates. Thus, it would be unwise to associate an adaptation to a LCKD or 'ketosis' with improved recovery based on this assessment, without taking into consideration the fact that participants may have simply increased their dietary protein (g·kg), which has been shown to assist with the recovery process (Pasiakos, Lieberman and McLellan, 2014).

2.8.4 Low-Carbohydrate Diet and Cardio-metabolic Measures of Health

Although there is considerable research examining the relationship between dietary fat and cardio-metabolic health in overweight and athletic populations, only investigations

whereby a ‘low-carbohydrate diet’ contained >50% of energy from fat and <150 g/d of carbohydrates or if the words ‘ketogenic diet” is mentioned, will be included here.

While figures have steadily decreased since peaks in the 1960-70’s, globally, cardiovascular disease remains the world’s number one killer (World Health Organistion, 2011). Similarly, in high-income countries, such as, Ireland, it remains the number one cause of ill health (World Health Organistion, 2018). However, this positive downward trend is at risk of reversing itself, as the world experiences a greying population, increases in cardiovascular disease related deaths with incidence of type-2 diabetes are likely to increase over the next 2-3 decades (Allender *et al.,* 2008), by a staggering ~50% in the United States according to calculations (Vigen, Maddox and Allen, 2012). Thus, methods of assisting the general population, such as lifestyle interventions (diet and/or exercise) to help live disease free, are of huge interest to researchers and the population alike.

Beyond possessing an appropriate body composition (BMI/body fat etc.) (Després, 2012) and doing appropriate amounts of physical activity (Irish Heart Foundation, 2018), concentrations of triglycerides, glucose, insulin, cholesterol and a HBA_{1c} are important measures of cardio-metabolic health to be conscious of. Cholesterol is a fat like substance present in cell membranes, it travels through the blood as lipoproteins. Three major lipoproteins exist, (low density lipoprotein) LDL-cholesterol, (high density lipoprotein) HDL-cholesterol and very low-density lipoproteins (VLDL) (NCEP, 2002). LDL-cholesterol encompasses ~70% of total cholesterol found within serum, with ~30% coming from HDL-cholesterol (NCEP, 2002). LDL is secreted from the liver as a large VLDL particle, containing triglycerides and cholesterol, triglycerides are extracted by lean

body mass or stored in adipose tissue, leaving LDL-cholesterol and triglycerides (Goldstein and Brown, 2015). In a perfect scenario, LDL-cholesterol are efficiently removed from circulation by LDL receptors located in the liver, however if left in circulation due to insufficient LDL receptors, it builds up in plasma leading to atherosclerotic plaques (Goldstein and Brown, 2015). Long term exposure to elevated LDL-cholesterol is positively associated with risk of coronary heart disease (Ference *et al.,* 2012). In addition, LDL-cholesterol is broken up into two substrates, small dense LDL partials which attribute to atherogenic effects while the larger more buoyant LDL particles are considered less problematic (Superko, 2001). Despite 40-years of research, the relationship between HDL-cholesterol and cardio-metabolic health is not well understood, however the association between high levels of LDL-cholesterol and low levels of HDL-cholesterol and incidences of heart attacks is described as one of the most profound associations in medicine (Goldstein and Brown, 2015). Therefore, within the context of a normal total cholesterol count, having a higher HDL-cholesterol to total cholesterol ratio is a desirable feature (Lewington *et al.,* 2007). Consequently, a LCKD is discussed as cardio protective as it causes an increase in HDL-cholesterol (Noakes and Windt, 2016). However, if increases in HDL-cholesterol were to occur in accordance with increased total cholesterol, this would be seen as problematic on a population level, as a meta-analysis involving 61 prospective studies and 892,337 persons found a strong correlation between total plasma cholesterol and risk of ischaemic heart disease mortality (Lewington *et al.,* 2007).

Although guidelines vary, the American College of Sports Medicine recommends keeping total cholesterol <200 mg/dL for optimal health (Altena, 2016). The National

Cholesterol Education Program recommends keeping LDL-cholesterol, which is commonly referred to as 'bad cholesterol' for aforementioned reasons to <100 mg/dL, while 130-159 mg/dL is bordering high and >190 mg/dL is considered very high (NCEP, 2002). In contrast, HDL-cholesterol with its inverse relationship with cardiovascular disease is referred to as 'good cholesterol' and is recommended to be >60 mg/dL, whilst <40 mg/dL is considered low (NCEP, 2002). Elevated fasting triglycerides are a solitary risk factor for cardiovascular disease and as a result a key marker of health (Rosinger *et al.,* 2016). An average fit and healthy individual usually has a triglyceride count of <100 mg/dL (Heiss *et al.,* 1980), however, in obese individuals who may smoke and/or drink excessive alcohol, it's known to rise to 150-199 mg/dL (Denke, Sempos and Grundy, 1993). Management of elevated triglycerides can be achieved through commencement of exercise (i.e., increased oxidation) or by implementing a caloric deficit (Drakopoulou *et al.,* 2016). Under hypocaloric conditions, there is a reduction in fatty acids ingested (Frayn *et al.,* 2012) and therefore, stored as triglycerides and/or a reduced intake of carbohydrates, resulting in reduced conversation of carbohydrates to triglycerides for storage (i.e., de novo lipogenesis) (Donnelly *et al.,* 2005; Luukkonen *et al.,* 2018). Additionally, it's noteworthy that a low-carbohydrate diet is associated with reduced intakes glucose and fructose, there is a limited evidence in rodent models that fructose (Havel, 2005) consumed in moderate amounts can have delirious effects on triglycerides and other markers of the metabolic syndrome, however, an energy surplus in addition to a high intake of glucose and/or fructose is thought to more relevant and accurate concern/risk factor (Tappy *et al.,* 2010).

Preliminary work in the 1980's demonstrated that TTE could be maintained in overweight males (Phinney *et al.,* 1980) and well-trained male endurance athletes (Phinney *et al.,* 1983). Despite each investigations population sample differing significantly (overweight versus well-trained), participants glycaemic responses at rest were similar, with each group experiencing notable decreases in insulin (Phinney *et al.,* 1980: -4.3 uU/ml; Phinney *et al.,* 1983: -2.1 uU/ml), while the overweight population experienced a decrease in blood glucose (-14.5 mg/dL). Improvements in glycaemic control observed by Phinney *et al.,* (1980) are synonymous with decreases in body fatness (Rock *et al.,* 2014; Lean *et al.,* 2017), while the maintenance of blood glucose within the well-trained athletes despite carbohydrate restriction illustrated the generation of glucose via non-carbohydrate sources (gluconeogenesis), which was later noted by athletes who were habituated to a LCKD for a number of months (Volek *et al.,* 2016; Webster *et al.,* 2016).

Brehm *et al.,* (2003) carried out an investigation in 42 'healthy overweight women'. Participants were randomised into a hypocaloric high-carbohydrate group (*n* = 20, 1,302 kcal %carbohydrate:fat:protein = 53:18:29) or an *ad libitum* LCKD group (*n* = 22, 1,247 kcal % = 30:23:46) for 6 months. Decreases in body mass occurred in each group ($P < 0.001$) and there was a time*group interaction (HC = -3.9 kg and LCKD = -8.5 kg, TxG $P < 0.01$). The LCKD contained 24.0 g/d saturated fats, 22.8 g/d monounsaturated fats, 11.8 g/d polyunsaturated fats and contained 285.4 mg/d of cholesterol, while the high-carbohydrate diet contained 15.3 g/d kcal saturated fat, 10.1 g/d monounsaturated fat, 5.1 g/d polyunsaturated fat and 182.2 mg/d cholesterol. Despite differing dietary composition, total cholesterol, LDL-cholesterol and HDL-cholesterol were similar in each

group post-intervention (total cholesterol: HC = 182.8 mg/dL, LCKD = 184.4 mg/dL, $P > 0.05$; LDL-cholesterol: HC = 124.0 mg/dL, LCKD = 107.8 mg/dL, $P > 0.05$; HDL-cholesterol: HC = 58.8 mg/dL, LCKD = 58.7 mg/dL, $P > 0.05$), while triglycerides decreased in the LCKD group (HC = +1.7 mg/dL $P > 0.05$, LCKD = -34.8 mg/dL $P < 0.05$), most likely due to decreased carbohydrate consumption (Parks, 2001). In addition, despite 41 g/d of carbohydrates being reported by LCKD participants, plasma βHB was a modest ~0.2 mM at post-intervention, indicting difficulties with reporting of nutrients and/or poor adherence. This preliminary investigation demonstrated restricting carbohydrates and increasing dietary fat does not affect total cholesterol or LDL-cholesterol, but positively impacts upon triglycerides.

In what was a novel investigation, given the population sample in question, Sondike, Copperman and Jacobson (2003) examined a high-carbohydrate (n = 14 % carbohdyrate:fat:protein = 56:12:32) and LCKD (n = 11 % = 8:60:32) impact on weight loss and risk factors of cardiovascular disease in overweight adolescents aged 14-15 years of age. Weight loss was greater in the LCKD group (~-9.9 kg) versus the high-carbohydrate group (~-4.1 kg) at post-intervention testing ($P < 0.05$). Greater intakes of saturated fat (HC = 8.9 g/d, LCKD = 44.7 g/d, $P < 0.05$) and dietary cholesterol (HC = 164. 57 mg/d, LCKD = 667 mg/d $P < 0.05$) were associated with greater total blood cholesterol at post-intervention in the LCKD group (Post-intervention: HC = 165.7 mg/dL, LCKD = 193.2 mg/dL)), however as previously observed (Brehm *et al.,* 2004), the LCKD group experienced more favourable effects on post-intervention triglycerides (Post-intervention: HC = 104 mg/dL, LCKD = 71.0 mg/dL). Neither group saw improvements in HDL-cholesterol ($P > 0.05$), but the high-carbohydrate group experienced a decrease in

LDL-cholesterol (-25.1 mg/dL $P < 0.05$, while the LCKD group experienced a non-significant increase (+3.8 mg/dL $P > 0.05$). In summary, despite differing intakes of saturated fat, total cholesterol remained within a normal range (i.e., < 200 mg/dL) (Altena, 2016) in each group, while the LCKD groups LDL-cholesterol (137.1 mg/dL) was bordering high (130-159 mg/dL) (NCEP, 2002), but experienced positive reductions in triglycerides and therefore, lowered relative risk (Rosinger *et al.,* 2016).

In 2004, Yancy *et al.,* instructed participants who were randomised into the LCKD group to consume meat products *ad libitum*, 2 cups of green salad and to consume 1 cup vegetables each day (LCKD 1,461.0 kcal, %carbohydrate:fat:protein 8:68:26), whereas the high-carbohydrate group were instructed to consume <30% of energy from fat, < 300 mg/d of cholesterol and <10% of total energy from saturated fats (HC 1,502.0 kcal, % = 52:29:19). Weight loss was greater in the LCKD group (-12 kg) versus the high-carbohydrate group (-6.5 kg $P < 0.01$). A breakdown of type of fat consumed in each diet was not described, but following the aforementioned macronutrients, total cholesterol (HC = -13.7 mg/dL, $P = 0.008$, LCKD = -8.1 mg/dL, $P = 0.08$) and triglycerides (HC -27.9 mg/dL $P = 0.02$, LCKD -74.2 mg/dL, $P = 0.001$) decreased in each group. HDL-cholesterol increased in the LCKD group (5.5 mg/dL $P < 0.001$), but no effect was observed in the high-carbohydrate group (-0.04 mg/dL $P > 0.05$). However due to greater decreases in total cholesterol previously outlined, the high-carbohydrate group had a more favourable total cholesterol to HDL-cholesterol ratio post-intervention (HC = 4.4, LCKD = 4.1, $P > 0.05$). No impact on LDL-cholesterol was observed in either group (HC = 140.6 mg/dL, LCKD = 158.8 mg/dL, $P > 0.05$), but similar to previous investigations (Brehm *et al.,* 2003; Sondike, Copperman and Jacobson, 2003), the LCKD decreased triglycerides

(HC = 162.7 mg/dL, LCKD = 83.6 mg/dL) and in this case, increased HDL-cholesterol to desirable levels (HC = 52.5 mg/dL, LCKD = 60.9 mg/dL).

As previously outlined, Keogh *et al.,* (2008) carried out an 8 week investigation in overweight males and females with at least one additional risk factor for the metabolic syndrome. As well as measuring changes in inflammatory markers, the investigations primary objective was to observe what impact the high-carbohydrate (*n* = 47, %carbohydrate:fat:protein = 47:28:25) and LCKD (*n* = 52, %6:59:35) had on markers of cardiovascular disease. As previously outlined, weight loss occurred in each group (HC = -6.2 kg vs LCKD = -7.5 kg $P < 0.001$). Total cholesterol decreased in the high-carbohydrate group (HC = -19.3 mg/dL $P < 0.05$) and there was a time*group interaction for decreases in total cholesterol versus the LCKD group (-15.4 mg/dL TxG $P < 0.001$). HDL-cholesterol increased in the LCKD group (+3.8 mg/dL, $P < 0.05$), while additional time*group interactions were observed for LDL-cholesterol (HC = -15.4 mg/dL vs LCKD = -11.5 mg/dL, TxG $P < 0.001$) and triglycerides (HC = -11.6 mg/dL vs LCKD = -19.3 mg/dL TxG $P < 0.001$). Similar to previous investigations by Sondike, Copperman and Jacobson (2003), the diet lower in dietary cholesterol (HC = 140 mg/d, LCKD = 596 mg/d $P < 0.001$) and saturated fat (HC = 10.5 g/d, LCKD = 64.9 g/d $P < 0.001$) was associated with greater reductions in total cholesterol and LDL-cholesterol, but the LCKD was associated with more positive alterations in triglycerides and HDL-cholesterol.

As previously outlined, Forsythe *et al.,* (2008) carried out an investigation in 40-overweight males and females with atherogenic dyslipidaemia. An adjoining publication by the same research group (Volek *et al.,* 2009) gave more insight into Forsythe et al.'s (2008) investigation. Ketogenic diet participants increased dietary cholesterol (+251

mg/d) and saturated fat (+8.7 %/kcal) while the high-carbohydrate group decreased dietary cholesterol (-123 mg/d) and saturated fat (-36.4 g/d). No time*group interactions for total cholesterol (HC = -9.5 mg/dL vs LCKD = -11.5 mg/dL, TxG $P > 0.05$) were observed, however a time*group interaction for HDL-cholesterol (HC = -0.3 mg/dL vs LCKD = +4.6 mg/dL, $P = 0.000$) took place. Unlike previous investigations, no time or time*group interactions for LDL-cholesterol took place ($P > 0.05$). This investigation demonstrated that a LCKD implemented for 12 weeks did not adversely impact upon total cholesterol or LDL-cholesterol, while positive changes in HDL-cholesterol were observed.

As previously discussed within Chapter 2, Brinkworth *et al.,* (2009) randomised a 122-overweight male and female with at least one additional metabolic syndrome risk factor into 2 groups. To recap, participants consumed a high-carbohydrate ($n = 36$ %carbohydrate:fat:protein = 46:30:24) or a LCKD ($n = 33$, %4:61:35) for 1-year. Body-mass decreased in each group (HC = -11.5 kg vs LCKD = 14.5 kg, $P < 0.05$). Participants in each group experienced similar improvements in fasting insulin (HC = -22.9 pmol/L, LCKD = -23.6 pmol/L) and glucose (HC = -11.5 mg/dL, LCKD = -11.5 mg/dL), but no time*effect for each variable ($P > 0.05$). A time*group interaction for increases in total cholesterol (HC = +3.8 mg/dL vs LCKD +15.4 mg/dL, TxG $P = 0.004$), LDL-cholesterol (HC = +3.8 mg/dL vs LCKD +23.2 mg/dL, TxG $P = 0.001$) and HDL-cholesteorl (HC = +2.7 mg/dL vs LCKD +11.6 mg/dL, TxG $P = 0.018$) and decreases in triglycerides (HC = -8.5 mg/dL vs LCKD -22.4 mg/dL, TxG $P = 0.004$) was observed. βHB peaked in week 2 in the LCKD group at ~0.45 mM and remained above 0.3 mM until week 8, however, following week 8, mean βHB was consistely <0.2 mM in the LCKD group, indicating adherence may have been an

issue. In summary, each diet was effective at causing weight loss and improvments in glycemic control, but neither group observed notable improvements in cardio-metabolic health.

As previously discussed within the LCKD performance chapter, Zajac *et al.,* (2014) also examined changes in lipid and lipoprotein profiles in an athletic population following the consumption of a 'ketogenic'. Despite βHB remaining relatively low (~0.15 mM) in week 4, the investigation appears a good blueprint for changes that may occur within an athletic population when carbohydrates are somewhat restricted (~145 g/d) and dietary fat and protein are increased. The non-ketogenic LCKD high in monounsaturated (130 g/d) and saturated fatty acids (68 g/d) caused decreases in body mass (-3.8% BF), but increased total cholesterol by 27 mg/dL (P = 0.001) and HDL-cholesterol (+20.69 mg/dL, P = 0.002), while triglycerides decreased (-27.1 mg/dL, P = 0.002) and LDL-cholesterol remained unchanged (+5.46 mg/dL, P > 0.05). In isolation, the increase in HDL-cholesterol and decrease in triglycerides would be seen as a positive, however total cholesterol increased ~15.34 mg/dL above the recommended threshold, which would be potentially seen as a cause of concern.

Tay and colleagues (2015) carried out a one year investigation in 78-males and females with type-2 diabetes. This 56 week study randomised participants into a high-carbohydrate (%carbohydrate:fat:protein = 53:30:17) or a LCKD (14:58:28). Participants experienced similar improvements in body composition (HC = -7.9 kg, LCKD = -8.6 kg, TxG P = 0.09). There were no differences in glycated haemoglobin i.e., 'HBA_{1C}' (HC = -1.0%, LCKD = -1.0%, P = 0.65) between groups, a key measure of long-term blood sugar levels in diabetics. No impact on total cholesterol (HC = -3.8 mg/dL, LCKD = -3.8 mg/dL,

P > 0.05) or LDL-cholesterol was observed (HC = 7.6 mg/dL, LCKD = -3.8 mg/dL, *P* > 0.05), while the LCKD group experienced greater increases in HDL-cholesterol versus the LCKD group (HC = +2.3 mg/dL, LCKD = +3.8 mg/dL, *P* = 0.002) and more favourable changes in triglycerides (HC -0.3 mg/dL, LCKD = -15.4 mg/dL, *P* = 0.001). Despite differences reported, both diets were low in saturated fat (HC = 16.8 g/d, LCKD = 21.2 g/d), with the main composition effects between diets being monounsaturated fats (HC = 12.1 g/d, LCKD = 54.4 g/d, *P* < 0.05) and dietary cholesterol (HC = 151 ± 37 mg/d, LCKD = 270 ± 67 mg/d, *P* < 0.05). This long term investigation demonstrates that a LCKD high in monounsaturated fats does not cause adverse effects such as increases in total cholesterol or LDL-cholesterol versus a well-match group consuming a high-carbohydrate diet low in saturated fat.

Unfortunately, a number of subsequent investigations involving LCKDs and an athletic population did not include a cardio-metabolic panel in their analysis (Burke *et al.,* 2017; Zinn *et al.,* 2017; Gregory *et al.,* 2017). However, Wilson *et al.'s* (2017) investigation in (well-trained) resistance trained males did include a number of measures in their comprehensive analysis. The investigation included a 10 week adaptation to a LCKD (LCKD %carbohydrate:fat:protein = 5:75:20), followed by 1 week of carbohydrate loading/restoration (HC % = 50:30:20). Cardio-metabolic measures were taken in week 10 and following 1 week of carbohydrate loading/restoration. This elaborate study design gives athletes and researchers an indication into changes that occur following the consumption of a LCKD for 10 weeks and the acute impact carbohydrate loading/restoration has on the body in a keto-adapted state. The investigation found that 10 weeks on a LCKD in resistance trained males had no impact on blood glucose,

triglycerides, total cholesterol or HDL-cholesterol (all $P > 0.05$). Following 1 week of carbohydrate loading, the LCKD groups triglycerides increased from baseline (+29.3 mg/dL, $P < 0.05$). This investigation demonstrated that a LCKD primarily high in saturated fat (109.3 g/d) and monounsaturated fats (75 g/d) causes no adverse effects when carbohydrates are restricted (30.9 g/d) in resistance trained males, however, demonstrated that carbohydrate restoration (263 g/d) for one week can have acute adverse effects, such as increased circulating triglycerides.

Researchers at Virta Health in California carried out a 12 month randomized-control trial in overweight adults, with type 2 diabetes (Saslow *et al.,* 2017). The investigators randomised 34 participants into a moderate-carbohydrate low-fat diet (MCLF) (1,681.1kcal %carbohydrate:fat:protein = 36:41:17or a LCKD (% =19:62:26). Participants in the MCLF diet were required to consume ~50% of calories from carbohydrates, decrease energy contribution from fat and create a ~500 kcal/d deficit, whilst the LCKD group were instructed to restrict their carbohydrate intake to 20-50 g/d and had a goal of elevating blood βHB to >0.5-3 mM (measured twice weekly at home – βHB not reported). The investigation was successful in 'reversing' some individual's diabetes in the LCKD group. Six of 10-subjects consuming a LCKD discontinued their glucose-lowering medication at the 1-year mark. In contrast, none of the participants following the MCLF diet discontinued their medication. The success of the LCKD is attributed to the significant decreases in participants HBA_{1c} (HC = -2.8%, LCKD = -7.5%, $P = 0.007$). However, this elaborate study wasn't without its limitations. Not unlike a number of previous investigations, there was poor dietary control as body weight (kg) decreased by -1.7kgs (-1.7%) in the high-carbohydrate group and -7.9kgs (-7.9%) in the LCKD group,

causing a significant group effect ($P < 0.001$). Participants in the LCKD group experienced a -9.9 mg/dL decrease in triglycerides (MCLF = +14.5 mg/dL group interaction: $P = 0.022$) and triglyceride to HDL-cholesterol ratio (MCLF = 2.8%, LCKD = -22.7%, group interaction: $P = 0.022$), while LDL-remained unchanged in the LCKD group (+6.9 mg/dL) and decreases in the MCLF group (-3.1 mg/dL). Finally, similar to previous longitudinal studies, carbohydrate consumption was ~73.7 g/d at the ~1 year mark in the LCKD group, this was despite participants have access to dietitians, indicating difficulties in adhering to <50 g/d of carbohydrate longitudinally.

Finally, Kephart *et al.'s* (2018) 3 month investigation in CrossFit trainees examined changes in glucose, HDL-cholesterol, LDL-cholesterol and triglycerides. Participants enrolled in the control/high-carbohydrate group did not receive a dietary intervention, nor were diet logs retrieved. Nonetheless, low-carbohydrate group adhered to a LCKD (%carbohydrate:fat:protein 6:77:17) and experienced decreases in body mass (-7.5kg, $P = 0.002$). In contrast, the high-carbohydrate group's body composition remained consistent ($P > 0.05$). No changes in blood glucose, HDL-cholesterol or triglycerides were observed, however LDL-cholesterol increased in the LCKD group (+39.8 mg/dL $P < 0.05$). In contrast, despite the high-carbohydrate group not receiving any formal dietary intervention, LDL-cholesterol decreased by -9.4 mg/dL ($P < 0.05$). Total cholesterol was not reported (i.e., change and *P* value), but calculated total cholesterol increased from to 181.24 mg/dL to 218.38 mg/dL. Therefore, despite increases in capillary blood βHB being associated with positive decreases in body mass, the LCKD brought total cholesterol (<200 mg/dL) and LDL-cholesterol (<130-159 mg/dL – bordering high) to undesirable ranges (NCEP, 2002). These findings in CrossFit trainees are contrary to

earlier work by Wilson *et al.,* (2017) in resistance trained males. Wilson *et al.,* (2017) achieved increases in capillary blood βHB > 0.5 mM, while Kephart *et al.,* (2018) participants experienced similar increases in capillary blood βHB to levels representative of nutritional ketosis. Unfortunately, diet composition beyond macronutrients were not elaborated on by Kephart *et al.,* (2018) so it's not possible to read into that further. Assuming adherence to a LCKD and/or poor reporting of self-reported βHB wasn't an issue, well-trained (Wilson *et al.,* 2017) and moderately trained (Kephart *et al.,* 2018) individuals who partake in resistance training (body building and CrossFit) appear to experience different responses to a LCKD, namely increases in LDL-cholesterol in moderately trained individuals (Kephart *et al.,* 2018).

In conclusion, although the population samples described varied from overweight patients (Tay *et al.,* 2015; Saslow *et al.,* 2017) to resistance trained males (Wilson *et al.,* 2017), an adaptation to a low-carbohydrate diet containing <150 g/d of CHO is most commonly associated with decreases (Yancy *et al.,* 2003) or maintenance of total cholesterol (Brehm *et al.,* 2003; Sondike, Copperman and Jacobson, 2005; Keogh *et al.,* 2008; Forsythe *et al.,* 2008; Tay *et al.,* 2015; Wilson *et al.,* 2017) and LDL-cholesterol (Brehm *et al.,* 2003; Yancy *et al.,* 2004; Keogh *et al.,* 2008; Forsythe *et al.,* 2008; Zajac *et al.,* 2014; Tay *et al.,* 2015; Saslow *et al.,* 2017; Wilson *et al.,* 2017), however on some occasions, increases in total cholesterol (Brinkworth *et al.,* 2009; Zajac *et al.,* 2014; Kephart *et al.,* 2018) and LDL-cholesterol (Brinkworth *et al.,* 2009; Kephart *et al.,* 2018) are observed. In addition, an adaptation to a LCKD is associated with decreases in triglycerides (Brehm *et al.,* 2003; Sondike, Copperman and Jacobson, 2003; Yancy *et al.,* 2004; Keogh *et al.,* 2008; Brinkworth *et al.,* 2009; Zajac *et al.,* 2014; Tay *et al.,* 2015;

Saslow *et al.,* 2017) and increases in HDL-cholesterol (Yancy *et al.,* 2004; Keogh *et al.,* 2008; Brinkworth *et al.,* 2009; Zajac *et al.,* 2014; Tay *et al.,* 2015) in some instances. Of the studies carried out in well-trained individuals where nutritional ketosis (βHB >0.5 mM) was objectively achieved (Wilson *et al.,* 2017), no changes in total cholesterol, LDL-cholesterol, HDL-cholesterol and triglycerides were observed, suggesting that well-trained individuals with normal homeostatic lipoprotein concentrations do not experience undesirable increases in total cholesterol and LDL-cholesterol frequently observed in overweight individuals following a 10 week adaptation to a LCKD (<50 g/d CHO) whilst completing a training intervention, despite increased consumption of saturated fat.

2.9 Conclusion

Since the 1930's it has been widely accepted possessing high levels of pre-exercise muscle glycogen is a precursor for optimal athletic performance (Christensen and Hansen, 1939). However since then, many athletes and scientists have developed opposing theories to fuel endurance exercise (Phinney, 2014; Brunker, 2015; Olsen, 2015; Volek, Noakes and Phinney, 2015; Noakes and Windt, 2016). These alternative views can cause confusion and anger, but in reality they can only be good for the area of sports nutrition research, as they result in a more comprehensive understanding of how the body reacts to different metabolic stimuli, by embarking on further research.

Current knowledge suggests endurance performance (>2 hours) can be sustained on a cycle ergometer following ~28-days of keto-adaptation in well-trained male endurance athletes (Phinney *et al.,* 1983), however, acute (<21-days) ingestion of a LCKD is associated with poor training adaptations, decreases in exercise economy and increased RPE in elite race walkers completing <1 hour of work (Burke *et al.,* 2017). Although it's unwise to compare elite and non-elite athletes, given the dearth of investigations, it perhaps may be acceptable in this instance. Burke *et al.,* (2017) investigation may have further accounted for wavering effects previously reported (Phinney *et al.,* 1980; Phinney *et al.,* 1983) during the initial stages of keto-adaptation by allowing additional time to adapt, as it has been noted performance lags until week 5-6 in recreationally active individuals following an adaptation to a LCKD (Zinn *et al.,* 2017). This additional time to 'adapt' (>3 weeks) would account for initial decreases in training intensity and potentially, allow for better optimisation of low-carbohydrate metabolic pathways (i.e.,

gluconeogenic production of glucose), perhaps leading to greater exercise efficiency, as observed previously at <75% VO_{2peak} (Webster *et al.,* 2016; Volek *et al.,* 2016).

In contrast to the LCKD performance literature, the use of exogenous ketones does not require an adaptation period or carbohydrate restriction and can achieve markedly higher concentrations of nutritional ketosis within a matter of minutes (Table 2.5 and 2.6). Early work by Evans, Cogan and Egan (2016) examined the metabolism of ketone bodies and suggested circumstances where exogenous ketones would most likely be of benefit. Subsequent work using ketone salts re-established their viewpoints, as ketone salts were found to have negligible impacts on blood glucose, lactate or ergogenic benefits following modest elevations in blood βHB (<1.0 mM), particularly in an untrained population (O'Malley *et al.,* 2017) (Table 2.6). In contrast, the use of ketone esters has a greater potential to increase plasma βHB and potentially, enhance performance. However, the term ketone ester is a broad term which fails to acknowledge the nuances of ketone esters. By taking a holistic view of the ketone ester performance literature (Table 2.5), it becomes clear there are three investigations on two ketone esters, rather than three investigations on 'ketone esters'. The use of the (R)-3-hydroxybutyl (R)-3-hydroxybutyrate ketone monoester (~573 $mg \cdot kg^{-1}$) enhanced 30 minute TT performance (~2%) and contributed ~16-18% of total energy at 75% W_{MAX} in well-trained cyclists when βHB was >2.0 mM. In contrast, 500 $mg \cdot kg^{-1}$ BM of the D,L-1,3-butanediol acetoacetate diester increased βHB to ~1.2 mM during exercise and inhibited 31.17 km TT performance in elite cyclists, while ~750 $mg \cdot kg^{-1}$ BM of aforementioned (R)-3-hydroxybutyl (R)-3-hydroxybutyrate ketone monoester elevated

serum βHB to ~1.5-2.5 mM during exercise in field athletes, but ultimately had no impact on intermittent running or 15 m sprint performance.

Despite the fast emerging literature, a lot remains to be explored with regards dosing strategies to optimise increase in βHB whilst avoiding gastrointestinal symptoms. The co-ingestion of carbohydrates and 573 $mg \cdot kg^{-1}$ of (R)-3-hydroxybutyl (R)-3-hydroxybutyrate ketone monoester was well tolerated, however 3 g·kg feeding (CHO) 2 hours prior to co-ingestion of carbohydrates and 750 $mg \cdot kg^{-1}$ of (R)-3-hydroxybutyl (R)-3-hydroxybutyrate ketone monoester caused gastrointestinal symptoms. Similarly, co-ingestion of 500 $mg \cdot kg^{-1}$ of D,L-1,3-butanediol acetoacetate diester following 2 g·kg (CHO) feeding caused substantial distress. Thus, it remains to be seen was it the larger dose implemented by Evans and Egan (2018) or the ingestion of 500 $mg \cdot kg^{-1}$ of ketone ester within 30 minutes with flat diet cola, the contributing factor to gastrointestinal issues or perhaps, the presence of pre-exercise meal. Were it the presence of the pre-exercise meal, it would diminish the 'glycogen sparing' properties of exogenous ketones, previously observed (Cox *et al.,* 2016), due to less carbohydrates being ingested.

Notwithstanding proposed performance benefits or decrements associated with a LCKD, an athlete's health and wellbeing should be of the upmost importance. A LCKD appears effective at reducing body mass, inflammation and oxidative stress in overweight (Keogh *et al.,* 2008; Forsythe *et al.,* 2008; Brinkworth *et al.,* 2009; Bazzano *et al.,* 2014) and trained individuals (Rhyu and Cho, 2014). In addition, despite limited experimental evidence, a LCKD appears effective at sustaining normal lipoprotein concentrations despite increased saturated fat intake in well-trained resistance trained males (Wilson *et al.,* 2017). However, given the interest in LCKDs within an endurance athlete

population, more work is needed to determine its impact on aforementioned markers of health in this population. Finally, haven completed a comprehensive review of the LCKD performance and health literature, to the author's knowledge, no investigation has examined the nutrient density of a LCKD. This may be a concern, as a LCKD is restrictive by nature (<50 g/d CHO) and void of whole grains, citrus fruits and starchy vegetables, therefore, poor implementation may lead to nutrient deficiency such as fibre and iron, consequently, it's an area that warrants further exploration and discussion. Additionally the nutrient density of a high-carbohydrate diet consumed by athletes is also an unexplored area in terms of micro-nutrient adequacy, when carbohydrates are consumed to the 6 g·kg recommendation and so represent a very large component of the diet.

Chapter 3

Keto-adaptation enhances exercise performance and body composition responses to training in endurance athletes

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3.1 Abstract

Background

Low-carbohydrate diets have recently grown in popularity among endurance athletes, yet little is known about the long-term (>4 wk) performance implications of consuming a low-carbohydrate high fat ketogenic diet (LCKD) in well-trained athletes.

Methods

Twenty male endurance-trained athletes (age 33 ± 11 y, body mass 80 ± 11 kg; BMI 24.7 ± 3.1 kg/m^2) who habitually consumed a carbohydrate-based diet, self-selected into a high-carbohydrate (HC) group (*n* = 11, %carbohydrate:protein:fat = 65:14:20) or a LCKD group (*n* = 9, 6:17:77). Both groups performed the same training intervention (endurance, strength and high intensity interval training (HIIT)). Prior to and following successful completion of 12 weeks of diet and training, participants had their body

composition assessed and completed a 100 km time trial (TT), six second (SS) sprint and a critical power test (CPT). During post-intervention testing the high-carbohydrate group consumed 30–60 g/h carbohydrate, whereas the LCKD group consumed water and electrolytes.

Results

The LCKD group experienced a greater decrease in body mass (HC −0.8 kg, LCKD−5.9 kg; *P* = 0.006, effect size (ES): 0.338) and percentage body fat percentage (HC− 0.7%, LCKD −5.2%; *P* = 0.008, ES: 0.346). Fasting serum beta-hydroxybutyrate (βHB) increased from 0.1 at baseline to 0.5 mM in the LCKD group (*P* = 0.011, ES: 0.403) in week 12. There was no significant change in performance of the 100 km TT between groups (HC − 1.13 min, LCKD−4.07 min, *P* = 0.057, ES: 0.196). SS sprint peak power increased by 0.8 watts per kilogram bodyweight (w/kg) in the LCKD group versus a −0.1 w/kg reduction in the high-carbohydrate group (*P* = 0.025, ES: 0.263). CPT peak power decreased by −0.7 w/kg in the high-carbohydrate group and increased by 1.4 w/kg in the LCKD group (*P* = 0.047, ES: 0.212). Fat oxidation in the LCKD group was greater throughout the 100 km TT.

Conclusion

Compared to a high-carbohydrate comparison group, a 12 week period of keto-adaptation and exercise training, enhanced body composition, fat oxidation during exercise and specific measures of performance relevant to competitive endurance athletes.

Keywords: ketogenic, high-carbohydrate, fat, endurance, performance, body composition

3.2 Introduction

Traditional sports nutrition guidelines recommend consumption of high-carbohydrate diets for endurance performance (Rodriguez and DiMarco, 2009; Jeukendrup, 2014), yet a growing number of athletes have adopted a LCKD approach (Burke, 2015; Volek, Noakes and Phinney, 2015). Endurance performance is limited when endogenous carbohydrates are the dominant fuel (Volek and Phinney, 2012; Rapoport, 2010), necessitating provision of exogenous carbohydrate during exercise (Burke *et al.,* 2011). A LCKD increases oxidation of endogenous fat stores (Volek *et al.,* 2016) partially relieving an athlete's dependency on glucose (Volek, Noakes and Phinney, 2015). There is no universally agreed definition for a LCKD. The level of carbohydrate and protein restriction required to induce nutritional ketosis varies, however, some guidelines recommend consuming >75 % of energy from fat, moderate protein (1.76 – 2.2g per kg lean mass) and < 50g/d carbohydrate (Volek, Noakes and Phinney, 2015).

There is a scarcity of investigations examining the effects of a LCKD on performance (Phinney *et al.,* 1983; Paoli *et al.,* 2012; Zajac *et al.,* 2014; Burke *et al.,* 2017) with a greater number of investigations examining low-carbohydrate high fat (LCHF) diets and performance (O'Keeffe *et al.,* 1989; Lambert, Speechly and Dennis, 1994; Goedecke *et al.,* 1999; Rowlands and Hopkins, 2002; Vogt *et al.,* 2003). A recent review (Burke, 2015a) defined a LCHF diet to contain >60% energy from fat, with moderate levels of carbohydrate restriction (<25% energy). This definition of a LCHF diet is similar to a LCKD, both are higher in dietary fat than a traditional diet and restrict carbohydrates. However, a LCHF diet may not optimise metabolic adaptations associated with accelerated fat oxidation and ketone related metabolic and signalling effects (Volek, Noakes and

Phinney, 2015; Volek *et al.,* 2016). LCHF diet investigations have focused on short (7–14 days) (O'Keeffe *et al.,* 1989; Lambert, Speechly and Dennis, 1994; Goedecke *et al.,* 1999), to medium term adaptation periods (14–35 days) (Rowlands and Hopkins, 2002; Vogt *et al.,* 2003) in athletes. These investigations have reported consistent alterations in fuel utilization and exercise metabolism in fasted and carbohydrate depleted states, but fail to test the hypothesis surrounding long-term keto-adaptation and exercise performance (Volek *et al.,* 2016). When well-formulated ketogenic diets are implemented for a minimum of four weeks, enhanced fat oxidation rates are observed, with no decrement in aerobic capacity (Phinney *et al.,* 1983). What happens to exercise performance beyond 4 weeks of keto-adaptation remains unclear, but empirically several endurance athletes using this approach remain highly competitive (Volek *et al.,* 2016).

Changes in performance due to consumption of LCHF diets are mixed (O'Keeffe *et al.,* 1989; Lambert, Speechly and Dennis, 1994; Goedecke *et al.,* 1999). A recent cross sectional study examined the metabolic characteristics of keto-adapted ultra-endurance athletes who consumed a LCKD for 9–36 months (Volek *et al.,* 2016). Peak and sub-maximal fat oxidation rates during exercise in keto-adapted participants were more than two-fold higher compared to high-carbohydrate counterparts and 50% higher than peak rates previously reported (Venables, Achten and Jeukendrup, 2005). Two of the most notable differences between the LCKD investigation (Volek, Noakes and Phinney, 2015) and the current body of LCHF research are the level of carbohydrate restriction and the length of the adaptation period.

LCKD research on performance has focused on short to medium term adaptation periods (21–30 days) (Phinney *et al.,* 1983; Paoli *et al.,* 2012; Zajac *et al.,* 2014), possibly

due to challenges of long term dietary interventions. Two of these investigations should not be categorised as “ketogenic”, since protein (Zajac *et al.,* 2014) and carbohydrate (Paoli *et al.,* 2012), were not sufficiently restricted. Nonetheless, strength and time to exhaustion were not negatively affected (Phinney *et al.,* 1983; Paoli *et al.,* 2012; Zajac *et al.,* 2014; Burke *et al.,* 2017), however two trials reported a decreased ability to perform at higher intensities (Zajac *et al.,* 2014) and decreased exercise economy (Burke *et al.,* 2017). Despite a lack of experimental scientific literature advocating clear performance benefits of adapting to a LCKD, interest in this dietary paradigm has continued to gather traction (Burke, 2015; Volek, Noakes and Phinney, 2015; Noakes and Windt, 2016). Keto-adaptation is believed to unlock a much larger fuel tank versus a carbohydrate-based diet (Volek, Noakes and Phinney, 2015; Volek and Phinney, 2012); hence reducing an athlete's need for carbohydrate supplementation during exercise. Thus, unlike previous long term cross-sectional LCKD investigations where keto-adaptation had already taken place (Volek *et al.,* 2016; Webster *et al.,* 2016) we designed an experimental study to investigate the long-term (12 week) performance implications of consuming a LCKD on performance relevant to competitive endurance athletes and tested the hypothesis that a keto-adapted athlete can maintain/improve performance on a LCKD. This research also involved incorporation of a training programme to enhance mitochondrial biogenesis and hence fuel utilization, an aspect not incorporated within previous research.

3.3 Methods

3.3.1 Experimental Approach

This was a non-randomised control trial comparing long term performance implications of consuming a high-carbohydrate and LCKD, in male endurance trained athletes. A non-randomised approach was chosen due to the length of the adaptation period and to promote dietary adherence. Participants were informed of the purpose and any risks associated with taking part, prior to written consent being obtained (see appendices A and B). The investigation was approved by the research ethics committee at Waterford Institute of Technology, IE (see appendix C). At baseline participants completed a DXA scan, SS sprint, 100 km TT and CPT. Following baseline testing both groups began a 12 week dietary and training intervention (endurance, strength and HIIT). Participants returned at the end of week 12 and repeated the testing protocol.

3.3.2 Participants

Forty-seven male endurance trained athletes (18–40 years) were enrolled. Twenty participants completed all requirements associated with the current study. The reasons for dropout were: an injury or illness not related to the intervention (HC *n* = 7; LCKD *n* = 9), intervention too time consuming (HC *n = 1*; LCKD *n* = 1), dietary intervention too difficult to adhere to (LCKD *n* = 5), participants unable to complete post-intervention testing (LCKD *n* = 2), strength and HIIT training too difficult to incorporate into training week (HC *n* = 1) and technical difficulty at post-intervention testing (LCKD *n* = 1). Participants were recruited by contacting clubs and via social media; Cycling Ireland, Triathlon Ireland and Irish Triathlon. The following sports were represented: triathlon (*n*

= 6), cycling (*n* = 5), Ironman (*n* = 4), marathon runners (*n* = 3), ultra-marathon runner (*n* = 1) and adventure racer (*n* = 1).

3.3.3 Pre-Participation Screening

Screening took place to ensure all participants were; male, 18-40 years of age, endurance athletes for >2 years (>7 hours a week training for last two years) and currently consumed a carbohydrate based diet (>50% kcal). Exclusion criteria included; diseases or conditions known to affect performance, use of pharmaceuticals that may affect any measurements of performance and Illness or injury prior to start date.

3.3.4. Pre-Intervention testing

Participants avoided racing or training 48 h prior to testing and maintained habitual carbohydrate based diet. Participants reported to the Human Performance Laboratory at 09:00 following 12 h fast (Figure 3.1). Upon arrival, weight was recorded to the nearest 0.1 kg (SECA 711, Hamburg, Germany) and height was recorded to the nearest 0.1 cm (SECA 213, Hamburg, Germany). Body composition was measured by DXA (Norland XR-46) via whole-body scan set to a resolution of 4.5 × 9.0 mm and a scan speed 260 mm/s. Fasting blood samples were collected from an antecubital vein using a 21G BD Vacutainer blood collection set (BD Diagnostics). Blood samples were centrifuged and the resultant serum stored at − 80 °C for later analysis.

This initial phase of testing was completed by 09:30 and participants were allowed 2 h to “fuel up” prior to the exercise trial. “Fuelling up” included consumption of the individual's habitual breakfast or pre-exercise nutrition (%carbohydrate:protein:fat HC = 52:20:28; LCKD = 64:16:20). Each group was allowed to self-select their pre-exercise

carbohydrate based meal, to ensure habitual dietary practices and performance measurements were obtained. Participants returned to the Human Performance Laboratory at 11:30, set up their bike position (Wattbike Ltd., Nottingham, UK) and began a 10 min warm up. The Wattbike is reported to sufficiently track performance changes in trained and untrained athletes with a reliability coefficient of 2.2% in a trained population and 6.7% in an untrained population (Hopker, Jobson and Passfield, 2010). After warm up participants completed a SS sprint on the Wattbike to determine peak and average power output. During the SS sprint a relative load of 0.5 of air resistance was applied for every 5kgs of body weight. Participants were then connected to the MOXUS Metabolic System (AEI Technologies, Chicago, IL) via mouthpiece (2700 series (large) 2 way T-shape non-rebreathing valve with saliva collector Hans Rudolph, Shawnee, KS) for the determination of energy expenditure and began their 100 km TT. Participants were instructed to complete the 100 km TT as fast as possible. During the 100km TT, air resistance on the Wattbike was self-selected. VO_2 and VCO_2 were averaged every 15 s for the first 5 min of every 20 km and used to calculate oxygen uptake, minute ventilation and carbohydrate and fat oxidation; which were presented as a respiratory exchange ratio (RER) value (VCO_2/VO_2). During the final 30 s of these 5 min, a capillary blood sample was obtained and blood lactate concentrations analysed using a lactate analyser (Lactate Pro, Arkay, Shiga, Japan). Measurements were repeated at 20 km intervals up until 80 km (20 km, 40 km, 60 km& 80 km), the final measurement was the concluding 2500 m of the 100 km TT. Immediately after the 100 km TT, participants completed a CPT on a Wattbike, where peak VO_2, peak power (watts) and average power (watts) were recorded. Participants were instructed to maintain as high a power output as possible while remaining seated for 3-min, during which, the same

resistance applied during the SS sprint, was applied. Absolute power measurements were converted to relative power (RP) by dividing watts by body mass in kg. Following completion of the CPT, the mouthpiece was removed and the final lactate measurement was recorded. Participants then completed a gradual self-selected cool down.

3.3.5. Intervention

Diet

Food diaries were obtained at baseline using a 3 day weighed food diary (2 week days and 1 weekend day). These were analysed using Nutritic's dietary analysis software (Nutritic's Professional v3.09, Nutritic's, Dublin, Ireland). The dietary and training intervention began the day after pre-intervention testing. The macronutrient goals were: HC 65% CHO, 20% fat and 14%protein or LCKD >75%fat, 10–15%protein and <50 g/d CHO. High-carbohydrate participants were instructed to consume carbohydrates based on their daily energy requirements (Burke *et al.,* 2011), whereas LCKD participants were instructed to adhere to carbohydrate and protein guidelines and consume dietary fat *ad libitum*. Three day food diaries were also obtained at week 12 and analysed.

Nutritional Counselling

Subsequent to pre-intervention testing each participant received a detailed handout (see appendices D and E) and nutritional counselling from the researcher. The researcher contacted each participant each week and participants were required to submit a weighed food diary (2 weekdays and 1 weekend day) each week to ensure participants in each group were implementing and adhering to their respective guidelines. The high-carbohydrate group's nutritional handout included guidelines on

how to formulate a high-carbohydrate diet according to their daily energy requirements (Burke *et al.,* 2011). The LCKD group's handout included information on how to formulate a LCKD, a shopping list and example meal plans. To preclude orthostatic symptoms, LCKD participants were recommended to supplement salt to taste at meal times, consume electrolytes and water when exercising and supplement 1–2 g/d of sodium from bouillon cubes or homemade broth (Volek and Phinney, 2012; Phinney *et al.,* 1983).

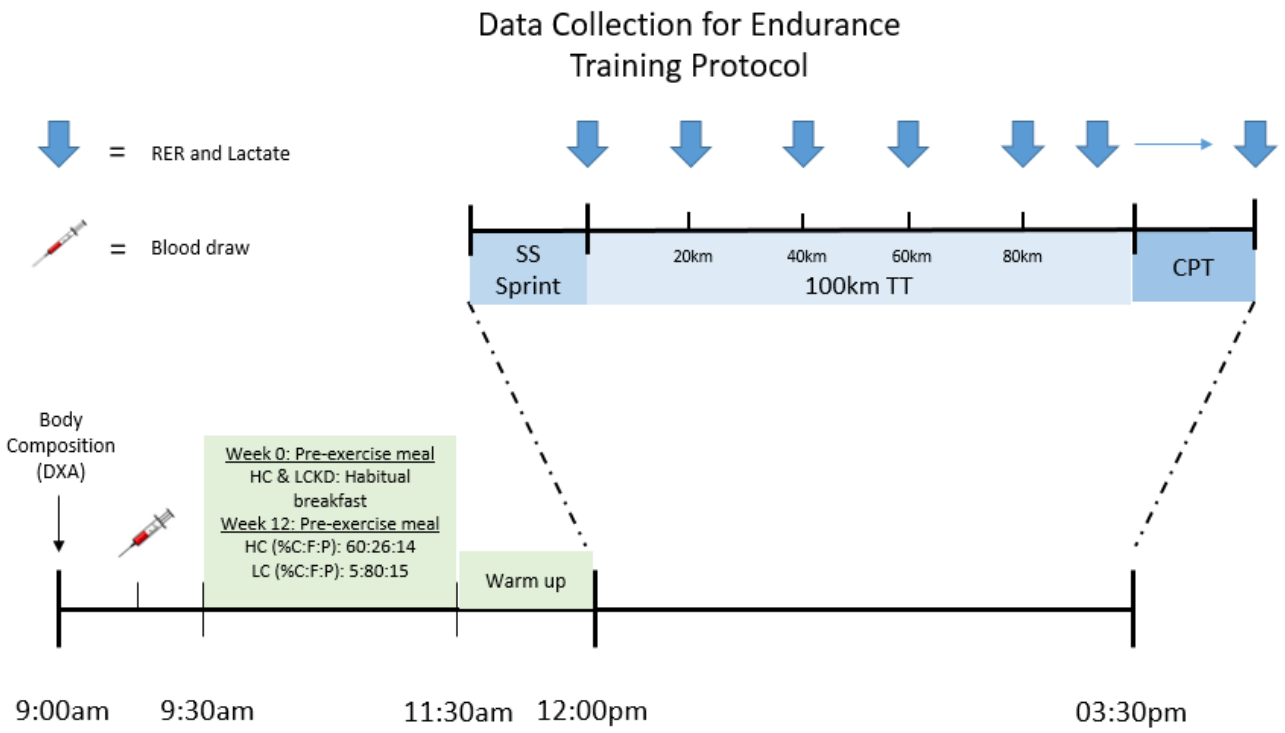

Figure 3.1. Experimental protocol implemented at pre- and post-intervention testing.

Training

Each group received the same training intervention (see appendices F), with endurance training (cycling and running), strength training and high intensity interval training (HIIT) to encourage mitochondrial biogenesis (Hood, Little and Tarnopolsky, 2011; Wang, Mascher and Psilander, 2011). Each participant completed 7+ h a week endurance training (moderate intensity 56–68% VO_{2peak}), 2 strength sessions; 6 sets of 8–10 reps on a leg press or free squat (70–80% of participants 1RM) and 2 HIIT sessions/week (10 sets of 1 min bouts at 70% peak power with 1 min recovery). During endurance training the LCKD group were instructed to restrict carbohydrate intake prior to training and avoid food consumption if possible during exercise to condition themselves for post-intervention testing.

3.3.6 Post-intervention Testing

Post-intervention testing was similar to pre-intervention testing with the exception of fuelling prior to and during the exercise trial. Following an overnight fast and preliminary tests, high-carbohydrate participants consumed a high-carbohydrate breakfast (%carbohydrate:fat:protein, 60:26:14) to meet dietary guidelines (Jeukendrup, 2014) and LCKD participants consumed a high fat breakfast (5:80:15) to maximize fat oxidation (Spriet, 2014).

Participants were again allocated 2 h rest. Once exercise had commenced, participants in the high-carbohydrate group consumed 30–60 g/h of carbohydrate (glucose, maltodextrin, sucrose and fructose), according to carbohydrate recommendations

(Jeukendrup, 2014), whereas LCKD participants consumed water and zero calorie electrolytes.

Blood Analysis

Fasting Beta-hydroxybutyrate (βHB) concentrations were determined at baseline and post-testing via colorimetric enzymatic assay (Sigma-Aldrich; St. Louis, MO). Intra-assay coefficient of variation was <10%. All samples were thawed one time prior to analysis.

3.3.7 Statistical Analysis

IBM Statistics SPSS 24 (Illinois, Chicago, USA) was used for statistical analysis. Data was tested for normality, with parametric tests used for normally distributed data or non-parametric, for data not normally distributed. Independent sample t-tests or Mann Whitney U test (if data was not normally distributed) were used to determine differences between high-carbohydrate and LCKD groups at baseline, with the alpha level for significance set at $P < 0.05$. Effects for each group were analysed using ANCOVA, with pre-intervention measures acting as a covariate. ANCOVA with baseline body fat (kg) as an additional covariate was carried out, due to a significant difference in body fat between high-carbohydrate and LCKD groups at baseline. As a measure of effect size, partial eta-squared (ηp^2) was used. Effect sizes were evaluated as: $\eta p^2 = 0.01$ (small effect), $\eta p^2 = 0.09$ (medium effect) and $\eta p^2 = 0.25$ (large effect) (Cohen, 1988). Paired samples t-tests or Wilcoxon signed ranks test (if data was not normally distributed) examined changes over time within each group, if ANCOVA *P* value was <0.05.

3.4 Results

3.4.1 Baseline Subject Characteristics, Diet and Performance Measurements

Body fat (kg) (P = 0.046) and carbohydrate intake (g) (P = 0.028) were different between the high-carbohydrate and LCKD group at baseline. All other physical characteristics (Table 3.1), dietary (Table 3.2), performance and anthropometric measurements (Table 3.3) were not statistically significant between groups (P >0.05).

3.4.2 Diet and Exercise Adherence

Mean duration of the intervention was 84 ± 2.8 days for the high-carbohydrate group and 81.2 ± 4.9 days for the LCKD group. Reported energy intake remained unchanged in each group (Table 3.2). Mean carbohydrate intake increased in the high-carbohydrate group (+85 g/d) and decreased (−414 g/d) in the LCKD group (Table 3.2). Fat intake decreased (−23 g/d) in the high-carbohydrate group and increased in the LCKD group (+195 g/d) (Table 3.2). Protein intake was greater post-intervention in LCKD group compared to the high-carbohydrate group (P = 0.010). There was no significant difference in high-carbohydrate and LCKD group's number of HITT (HC: 18.2 ± 2.0 versus LCKD: 19.7 ± 2.3), strength sessions (HC: 17.8 ± 2.1 versus LCKD: 18.3 ± 3.9) or hours endurance training (HC: 11.1 ± 1.7 versus LCKD: 13.0 ± 2.8) completed per week. βHB non-significantly decreased in the HC group (from 0.2 down to 0.1 mM) and increased in the LCKD group (from 0.1 up to 0.5 mM) (P = 0.021).

3.4.3 Body Composition

Body mass decreased in the LCKD group, with a loss of 5.9 kg compared to 0.8 kg in the high-carbohydrate group (Table 3.3). The significant change in body mass resulted from LCKD participants losing more body fat compared to the high-carbohydrate group (LCKD = −4.6 kg vs HC = −0.5 kg, P = 0.002). Despite significant loss in body mass, both groups maintained lean body mass (HC = +0.1 kg, LCKD = +0.3 kg). When baseline body fat was added as a covariate to body composition changes, similar levels of difference between high-carbohydrate and LCKD groups were obtained (body mass P = 0.009; lean mass P = 0.281).

3.4.4 Performance and Fuel Utilization

VO_{2peak} changed similarly in high-carbohydrate (+8.7%) and LCKD (+6.9%) groups (*P* = 0.968). Time required to complete the 100 km TT was not different between groups (*P* = 0.057, ES: 0.196) (Table 3.3), but change was numerically greater within the LCKD group (−4.07 min) compared to the high-carbohydrate group (−1.13 min) (Table 3.3). Participants individual 100 km TT times are shown in Fig. 2.2. Improvements in time were observed in 6 out of 9 LCKD participants and 7 out of 11 high-carbohydrate participants. When baseline body fat was added as a covariate to time trial performance, performance differences between group remained insignificant (*P* = 0.137).

There was a significant difference in SS peak power between groups (*P* = 0.025) with a significant increase in the LCKD group (Table 3.3), but no changes to participants average power observed (*P* = 0.336). Similar to the SS Sprint, peak power in the CPT was different between groups; decreasing in the high-carbohydrate group (−0.7 w/kg) and increasing

in the LCKD group (1.4 w/kg) (*P* =0.047) (Table 3.3), while average power during the CPT remained unchanged. Significant changes found in SS peak power (*P* = 0.024) and CPT peak power (*P* = 0.045) remained when baseline body fat was considered. Significant differences in RER were observed at 20 km (*P* = 0.000), 40 km (P = 0.000), 60 km (*P* = 0.000), 80 km (*P* = 0.000) and at 100 km (*P* = 0.040) (Figure 3.3). These differences were present due to significant changes within the LCKD group. No changes were found in blood lactate responses to exercise for the high-carbohydrate and LCKD groups (Figure 3.4). RER and blood lactate results remained similar when baseline fat was considered as a covariate.

Table 3.1. Subject characteristics					
	HC diet (*n* = 11)		LCKD (*n* = 9)		t-Test
	Mean ± SD	Range	Mean ± SD	Range	*P* Value
Age, years	32.1 ± 6.4	20.0-38.0	33.8 ± 6.9	19.0-40.0	0.566
Height, cm	181.2 ± 4.9	177.0-192.1	183.1 ± 5.5	175.5-191.6	0.408
BMI, kg/m^2	23.9 ± 2.9	20.0-30.5	25.6 ± 3.0	22.2-31.2	0.090

Abbreviations: cm = centimetre; kg/m^2 = kilogram per metre squared

Table 3.2. **Daily energy intake, macronutrient distribution and circulating ketones pre and post-intervention**

	HC diet (n = 11) [a]						LCKD (n = 9) [a]						ANCOVA		
	Pre			Post			Change	Pre			Post			Change			
	Mean	±	SD	Mean	±	SD	Mean	Mean	±	SD	Mean	±	SD	Mean	F – value [b]	P value	ES [c]: Np^2
Energy, kcal/d	2440.2	±	773.8	2643.6	±	358.0	+203.4	2843.8	±	558.4	3022.3	±	911.1	+178.5	(1,17)=0.646	0.433	0.037
Carbohydrate, g/d	315.6	±	107.5	400.3	±	102.7[d]	+84.7	454.8	±	152.0[a]	41.1	±	13.3[d]	-413.7	(1,17)=77.711	0.000*	0.821
Carbohydrate, g·kg BM	4.2	±	1.6	5.3	±	1.4	+1.1	5.2	±	1.4	0.5	±	0.2[d]	-4.7	(1,17)=95.937	0.000*	0.849
Fat, g/d	77.7	±	33.5	55.2	±	10.7[d]	-22.5	64.7	±	39.1	259.3	±	83.4[d]	+194.6	(1,17)=62.415	0.000*	0.786
Fat, g·kg BM	1.0	±	0.5	0.8	±	0.5	-0.2	0.7	±	0.1	3.2	±	0.9[d]	+2.5	(1,17)=86.678	0.000*	0.836
Protein, g/d	118.9	±	31.8	90.9	±	23.6	-28.0	110.3	±	25.5	130.7	±	35.8	+20.4	(1,17)=8.270	0.010*	0.327
Protein, g·kg BM	1.6	±	0.5	1.2	±	0.3	-0.4	1.2	±	0.3	1.6	±	0.4	+0.4	(1,17)=5.306	0.034*	0.238
βHB (mM)	0.2	±	0.3	0.1	±	0.0	-0.1	0.1	±	0.1	0.5	±	0.4[d]	+0.4	(1,17)=8.780	0.011*	0.403

[a] Original means and standard deviations, i.e., without adjustment for covariate (i.e., pre-treatment data).

[b] When assumption of sphericity is violated, degrees of freedom are corrected using Greenhouse–Geisser estimate. F- and p-values adjusted accordingly.

[c] ES = effect size. Np^2 = 0.01 (small effect), Np^2 = 0.09 (medium effect), Np^2 = 0.25 (large effect) (Cohen, 1988).

[d] Significant difference (P < 0.05) within group between pre and post-intervention.

* ANCOVA significant difference at P < 0.05.

Table 3.3. **Body composition and performance variables for pre and post-intervention**

	HC diet (n = 11) [a]							LCKD (n = 9) [a]							ANCOVA		
	Pre			Post			Change	Pre			Post			Change	*P* Value		
	Mean	±	SD	Mean	±	SD	Mean	Mean	±	SD	Mean	±	SD	Mean	F – value [b]	*P* value	ES [c]: Np^2
Body mass, kg	76.5	±	9.9	75.7	±	8.7	-0.8	86.3	±	14.3	80.4	±	13.4[d]	-5.9	(1,17)=8.682	0.006*	0.338
Lean mass, kg	63.6	±	5.4	63.7	±	5.0	+0.1	67.6	±	9.0	67.9	±	9.4	+0.3	(1,17)=1.239	0.167	0.068
Body fat, kg	10.6	±	6	10.1	±	5.6	-0.5	15.8	±	7.2	11.2	±	5.0 [d]	-4.6	(1,17)=13.058	0.002*	0.434
Body fat, %	12.8	±	5.1	12.1	±	4.7	-0.7	17.5	±	5.5	12.3	±	4.7[b]	-5.2	(1,17)=8.998	0.008*	0.346
Bone density (g/cm^2)	1.13	±	0.12	1.12	±	0.11	-0.01	1.16	±	0.11	1.14	±	0.11	-0.02	(1,17)=0.001	0.978	0.000
VO_{2peak}, $ml \cdot kg^{-1}min^{-1}$	52.6	±	6.4	57.2	±	6.1	+4.6	53.6	±	6.8	57.3	±	6.7	+3.7	(1,17)=0.002	0.968	0.000
TT, min.sec	169.57	±	9.36	168.44	±	9.14	-1.13	166.00	±	12.38	161.53	±	8.44	-4.07	(1,17)=4.152	0.057	0.196
SS peak RP (w/kg)	13.9	±	2.7	13.8	±	2.2	-0.1	13.7	±	1.4	14.5	±	1.1[d]	+0.8	(1,17)=6.064	0.025*	0.263
SS Av RP (w/kg)	12.2	±	1.5	12.5	±	1.7	+0.3	12.3	±	1.3	12.8	±	1.1	+0.5	(1,17)=0.982	0.336	0.055
CPT peak RP (w/kg)	9.1	±	2.6	8.4	±	2.2	-0.7	8.3	±	2.2	9.7	±	2.3[d]	+1.4	(1,17)=4.574	0.047*	0.212
CPT Av RP (w/kg)	4.5	±	2.2	4.6	±	2.2	+0.1	3.9	±	0.7	4.0	±	0.6	+0.1	(1,17)=0.362	0.555	0.021

[a] Original means and standard deviations, i.e., without adjustment for covariate (i.e., pre-treatment data).

[b] When assumption of sphericity is violated, degrees of freedom are corrected using Greenhouse–Geisser estimate. F- and p-values adjusted accordingly.

[c] ES = effect size. Np^2 = 0.01 (small effect), Np^2 = 0.09 (medium effect), Np^2 = 0.25 (large effect) (Cohen, 1988). * Significant difference at $P < 0.05$.

[d] Significant difference ($P < 0.05$) within group between pre and post-intervention.

*ANCOVA statistical significance $P < 0.05$

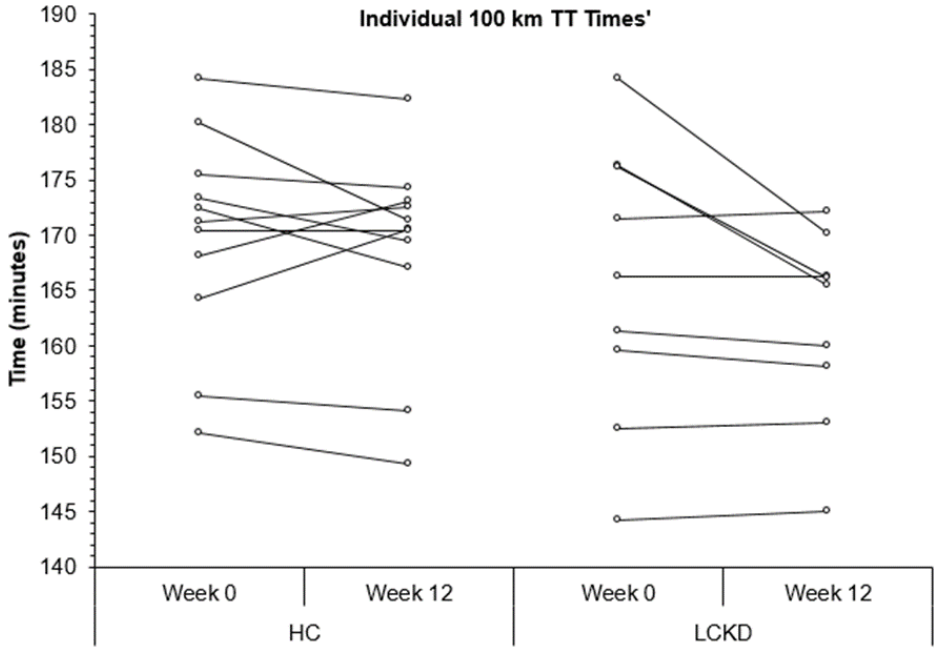

Figure 3.2. Individual 100 km TT times for high-carbohydrate (HC) and low-carbohydrate ketogenic diet (LCKD) groups at pre- and post-intervention testing

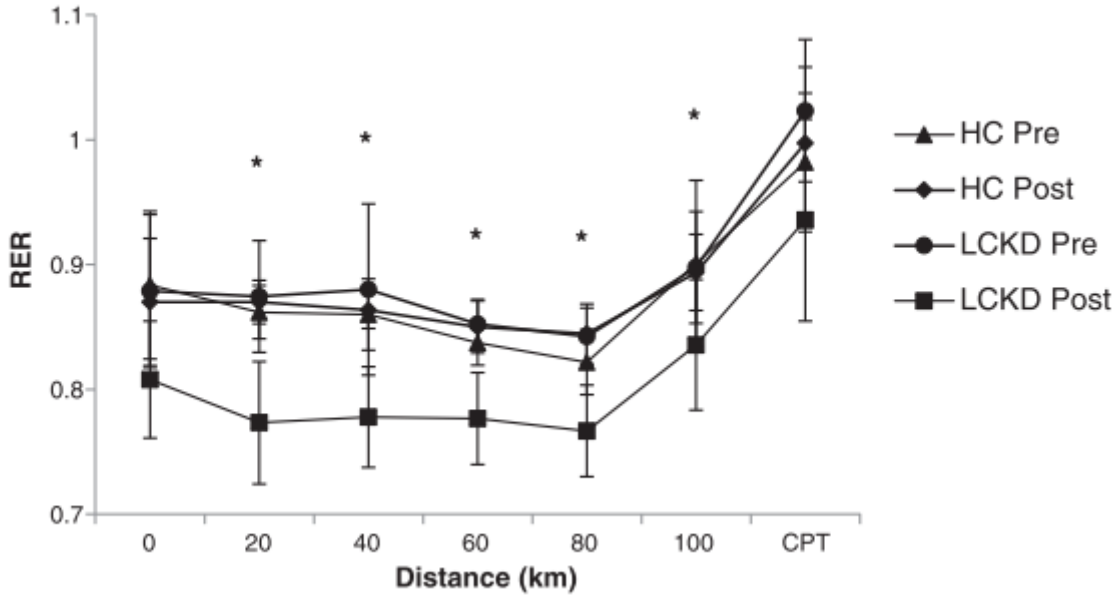

Figure 3.3. RER for High-carbohydrate (HC) and low-carbohydrate ketogenic diet (LCKD) groups at 0 km, 20 km, 40 km, 60 km, 80 km, 100 km and CPT data points, at pre- and post- intervention testing. *Indicates significant ($P < 0.05$) difference from ANCOVA, with changes within the LCKD group

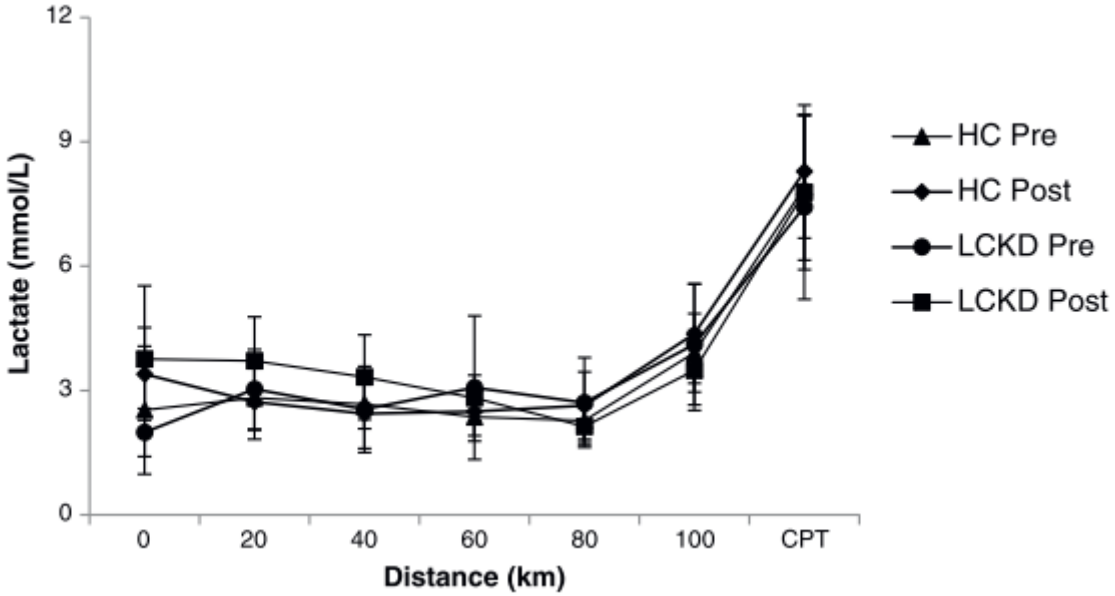

Figure 3.4. High-carbohydrate (HC) and low-carbohydrate ketogenic diet (LCKD) groups lactate responses at 0 km, 20 km, 40 km, 60 km, 80 km, 100 km and CPT data points, at pre- and post- intervention testing. $P < 0.05$ at all time-points from ANCOVA

3.5 Discussion

This study examined the implications of a consuming a 12 week LCKD versus a high-carbohydrate diet, while incorporating a training intervention, on exercise performance and body composition. We show compared to athletes consuming a high-carbohydrate diet, 12- weeks of keto-adaptation is associated with greater improvements in body composition, fat oxidation and peak power, with endurance performance being maintained in both groups. A 12 week period of keto-adaptation reduced total body mass and fat mass, while maintaining lean body mass and increased peak and average relative power during the SS sprint and CPT compared to high-carbohydrate participants. Performance of 100 km TT improved over the course of the intervention, but was not statistically different between groups.

Carbohydrate intake was greater within LCKD compared to high-carbohydrate participants at baseline (4.2 vs 5.2 g·kg). Increased fuel availability may have accounted for slightly better performance of LCKD participants at baseline, but this performance difference was not statistically significant. Due to high-carbohydrate intake within LCKD participants at baseline, effect of carbohydrate reduction on fuel utilization was extreme, resultantly; LCKD participants reported fatigue and tiredness during initial adaptation period. The high-carbohydrate groups increase in carbohydrate intake to meet current carbohydrate recommendations (Jeukendrup, 2014) was moderate but enabled high-carbohydrate participant's equal LCKD baseline intake. Even though the high-carbohydrate participants consumed 5.3 g·kg of carbohydrate for 12 weeks, they did not equal the LCKD participant's mean time trial performance post-intervention.

One of aims of this investigation was to determine if a high-carbohydrate athlete could improve/maintain endurance performance when keto-adapted. The findings here suggest endurance performance can be maintained and in some cases improved when compared to a high-carbohydrate diet. This is evident in 3 LCKD participants, as 100 km TT performance improved, with maintenance observed in 6 LCKD participants. LCKD participants with the greatest improvements possessed the slowest baseline times. High-carbohydrate participants with similar baseline times did not experience similar improvements in performance, despite similar VO_{2peak} gains. Strength training in cyclists alters muscle fibre type recruitment patterns and improves exercise economy by increasing maximal strength of type 1muscle fibres and postponing activation of less economical type 2 muscle fibres (Rønnestad and Mujika, 2014). Ability to maintain/increase performance may be due to favourable mitochondrial and oxidative enzyme adaptations occurring within muscle (Saunders *et al.,* 2004), due to diet and/or training effect within some LCKD participants. This challenges decades of conventional wisdom advocating a high-carbohydrate diet to optimise performance (Burke, 2015; Helge, 2017). Two important factors in this study likely to contribute to the positive responses to training are diet composition and prolonged adaptation period.

Diet Composition

Nutritional counselling in this investigation focused on the fundamentals of a well-formulated LCKD with carbohydrate restricted to <50 g/day, protein consumed in moderation and remaining energy derived from natural fat sources (Volek and Phinney, 2012). Dietary fat accounted for 81% of energy, protein 1.9 g·kg LBM and carbohydrate 41 g/day. Macronutrient profile is similar to previous research (Phinney *et al.,* 1983; Burke *et al.,* 2017), but inconsistent with others (Paoli *et al.,* 2012; Zajac *et al.,* 2014).

Studies where fat accounts for 55–70% of total energy (Paoli *et al.,* 2012; Zajac *et al.,* 2014), with higher protein intakes will fail to adequately induce nutritional ketosis and may hinder adaptation (Paoli *et al.,* 2012; Zajac *et al.,* 2014). Although βHB increased to 0.15 mM within Zajac *et al.,* (2014) investigation, it was below the threshold of nutritional ketosis (>0.5–3.0 mM) (Volek and Phinney, 2012), which suggest poor adherence and/or dietary prescription to a LCKD, which may explain poor performance responses. In contrast, βHB increased from 0.1 at baseline to 0.5 mM at week 12 in LCKD participants. Although nutritional ketosis was achieved (Volek and Phinney, 2012), concentrations of βHB were less than previously observed in experimental trials (>1.0 mM) (Phinney *et al.,* 1983; Burke *et al.,* 2017), however, these trials were feeding trials, with βHB concentrations monitored throughout intervention periods. Greater control and accuracy of dietary prescription may contribute to greater βHB concentrations. A LCHF diet that does not achieve nutritional ketosis provides endurance athletes with a dilemma. When carbohydrates are restricted and protein consumed to a point that induces nutritional ketosis, ketones supply the brain with energy (Volek and Phinney, 2012; Cahill and Aoki, 1980). Consuming a high fat non-ketogenic diet may increase fat oxidation, but the brain is unable to use long-chain fatty acids for fuel. An interruption or inadequate supply of glucose to the brain in the absence of nutritional ketosis is the metabolic basis for reduced performance and bonking/hitting the wall. Phinney and colleagues (Phinney *et al.,* 1983) identified time to exhaustion was maintained following 28 days of LCKD with βHB concentrations >1.0 mM, however, Burke *et al.,* (2017) recent investigation found performance adaptations were negated with βHB concentrations of >1.0 mM following 21 days of a LCKD. Burke *et al.,* (2017) investigation did not assess endurance performance, as the 10 km race implemented was not sufficient to deplete

muscle glycogen stores (Jeukendrup, 2014), with exercise times <46 min. It is important to highlight that the LCKD may not be suitable for everyone, 5 participants found the LCKD too difficult to adhere to and two participants were unable to complete post-intervention testing. Participants unable to complete post-intervention testing were subsequently found to have βHB levels <0.2 mM.

Length of Keto-Adaptation

Previous research incorporated 21–30 day adaptation periods (Phinney *et al.,* 1983; Paoli *et al.,* 2012; Zajac *et al.,* 2014; Burke *et al.,* 2017), with one investigation in 'recreational endurance athletes' adopting a 10 week dietary protocol (Zinn *et al.,* 2017). Previous LCKD investigations with 21–30 day adaptations noted decreased performance (Zinn *et al.,* 2017), decreases in exercise efficiency and increases in rates of perceived exertion (Burke *et al.,* 2017). Decreased performance was also reported in a 10 week LCKD protocol (Zinn *et al.,* 2017). However Zinn and colleagues (2017) did not incorporate additional training into their study protocol, which the current trial possessed. A significant decrement in peak power was also reported (Zinn *et al.,* 2017) but study participants lost − 4 ± 3.1 kg. The effect body mass loss had on power was not considered, decreased power reported by Zinn and colleagues (2017) cannot be compared to current results, which are presented as relative power due to body mass loss. During this investigation, LCKD participants noted a drop in energy levels and performance during the first 7–10 days and a "lag" in performance for the first 4–6 weeks. The collective lag in performance and that 5 LCKD participants dropped out because the dietary intervention was too difficult indicates a LCKD may not be for every athlete, should not be undertaken 4–6 weeks prior to an event or without consideration of individual's dietary preferences.

Phinney *et al.,* (1983) first coined the term keto-adaptation to describe the physiological adaptations an individual goes through following consumption of a LCKD. They showed performance decreased 20% after one week of consuming a LCKD despite significant increases in fat oxidation, but time to exhaustion increased 155% at week 6. The temporal pattern and full scope of the keto-adapted phenotype have not been rigorously studied. The available evidence indicates at least 4–6 weeks is necessary to return to performance and additional time may be necessary to observe consistent increases in performance. Thus, we examined performance after 12 weeks of keto-adaption.

Body Composition

LCKD participants had greater body fat (kg) pre-intervention which may have distorted fat loss findings. When differences in baseline data and body fat are considered, significance values remain similar. Despite LCKD participants losing 4.4 kg body fat, lean body mass increased 0.3 kg. Calorie intake in each group slightly increased during the trial, which contradicts how weight loss occurred. Weight loss is attributed to increased energy output, due to added training. Added weight loss within LCKD participants could be due to slightly greater volume of training undertaken each week. Loss of 4.4 kg body fat within LCKD participants and 0.7 kg body fat within high-carbohydrate participants resulted in both groups having very similar body fat post-intervention (HC 12.1%; LCKD 12.3%). Theses figure are similar to Ackland and colleagues (2012) finding that collegiate endurance athletes carry 5–11% body fat and resulted in current study participants possessing the ideal body fat range for endurance athletes (Jackson and Bartek, 2010). A LCKD is a useful tool for achieving weight loss in an untrained population (Gibson *et al.,* 2015), recreational athletes (Zinn *et al.,* 2017) and resistance trained males (Wilson

et al., 2017). These findings suggest a LCKD could be a valuable aid to endurance athletes who struggle to maintain race weight or athletes required to make competitive weight. Furthermore a recent cross-sectional study (Jurimae *et al.,* 2017) positively correlated fat mass with increases in inflammatory biomarkers in male endurance trained athletes (17.1 ± 5.1% body fat), therefore reduced body fat is desirable.

Performance

The 100 km TT is short enough to encourage participants to work at high work rates, yet long enough to challenge fuel availability. During exercise there was a significant shift in fuel utilization in the LCKD group, with rates of fat oxidation increasing throughout exercise. This pronounced shift in fuel utilization is a hallmark of keto-adaptation (Burke, 2015; Volek *et al.,* 2016; Phinney *et al.,* 1983; Erlenbusch, Haub and Munoz, 2005). In attempts to simulate actual race conditions high-carbohydrate group athletes were allowed to fuel following standard dietary recommendations (30–60 g CHO/h). The observed shift in fuel utilization allowed LCKD athletes utilize a greater amount of endogenous lipid stores (Volek, Noakes and Phinney, 2015). 100 km TT improved by 01:13 min (0.7%) in the high-carbohydrate group and 04:07 min (2.5%) in the LCKD group (ES: 0.196). A 01.13 and 04:07 min increase in performance in 12 weeks in well-trained endurance athletes is practically significant, considering the difference between winning and losing the Tour de France may be seconds (Cycling News, 2012). The training programme was designed to enhance mitochondrial biogenesis (Hood, Little and Tarnopolsky, 2011; Wang, Mascher and Psilander, 2011). Although mitochondrial adaptations were not measured during this trial, it is possible that improvements in participant's mitochondrial density enhanced participant's abilities to utilize oxygen, attributing to increases in VO_{2peak} and performance. Prolonged keto-adaptation may

result in an increased transcription and translation of lipid metabolism machinery. This may allow for an enhanced rate of lipolysis and subsequent energy production via oxidative pathways and potentially explain the performance enhancement observed.

The SS sprint was performed prior to 100 km TT to determine participants' exercise capacity at higher intensities, in a non-fatigued state. Relative peak power increased in LCKD participants as did relative average power, with no changes observed in the high-carbohydrate group. SS sprint, due to the very short duration, is primarily reliant on the phosphocreatine (PCr) energy system. Previous research indicates a carbohydrate restricted diet does not impair strength performance (Paoli *et al.,* 2012). Our findings show 12 weeks keto-adaptation is associated with improvement in all-out short-duration exercise capacity, implying no detrimental effects on the phosphagen energy system.

The CPT was performed directly following the 100 km TT to mimic a sprint finish at the end of an endurance event. Previous research examining the effect of a non-ketogenic low-carbohydrate diet for 2 weeks, demonstrated no effect on power (Rowlands and Hopkins, 2002), with other research indicating decreased performance at higher intensities (Zajac *et al.,* 2014). This investigation incorporated a strength training programme and resulted in a significant increase in relative peak power, with no change in the high-carbohydrate group. Incorporating endurance training with strength training has resulted in increased maximal power output (Rønnestad and Mujika, 2014).Improvements in power output observed in the LCKD group may be due to LCKD participants improved power to weight ratio. It is unlikely improvements in power output resulted from strength training, since high-carbohydrate participants had similar levels.

Increases in VO_{2peak} were partially due to decreases in bodyweight ($l \cdot kg^{-1}min^{-1}$) and potentially due to improvements in participant's aerobic capacity from the new training stimuli and/or volume of aerobic training. There is little evidence to suggest strength training is an effective mode of improving an athlete's VO_{2peak} (Rønnestad and Mujika, 2014), however investigations have positively correlated endurance training and HIIT with improvements in aerobic parameters (Obradović *et al.,* 2016; Astorino *et al.,* 2017; Keating, Johnson and Mielke, 2017). In a previous trial involving well trained keto-adapted athletes, muscle glycogen stores were not different to well-trained high-carbohydrate athletes after 180 minute sub-maximal exercise (Volek *et al.,* 2016). Both LC and high-carbohydrate athletes demonstrated similar glycolytic and glycogen synthesis rates during and after exercise (Volek *et al.,* 2016). Although muscle glycogen was not assessed in this study, similar lactate responses between groups suggest keto-adaptation did not impair glycolysis during high-intensity exercise.

3.6 Conclusion

In summary, a LCKD may benefit some athletes, particularity those who struggle with maintaining competitive race weight. Adaptation to a LCKD for 12 weeks did not negate measures of performance relevant to endurance athletes and caused more favourable adaptations to take place in some individuals. Thus, implementation or avoidance of this dietary protocol should be based on an individual's own dietary preference. Despite the concept of keto-adaption being over 30 years old (Phinney *et al.,* 1983), we are still in the early stages of understanding this dietary paradigm. The finding that 12 weeks of keto-adaptation improved aerobic and anaerobic exercise capacity, as well as body

composition, in endurance athletes most certainly implies there is potential for using LCKD to improve performance and metabolism. In striving for a more individualistic approach to dietary prescription, keto-adaptation is one approach worth considering.

Contributions of Authors

The study was designed by Fionn McSwiney, Lorna Doyle and Bruce Wardrop; data was collected by Fionn McSwiney, Lorna Doyle and Bruce Wardrop; data interpretation and manuscript preparation were undertaken by Fionn McSwiney, Lorna Doyle, Bruce Wardrop, Parker Hyde, Richard LaFountain and Jeff Volek. All aforementioned authors approved the final version of the article.

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Conflicts of Interest

Dr. Volek receives royalties from books on nutrition and exercise.

Chapter 4

12 Week Ketogenic Diet increases Total Cholesterol with increased Beta-hydroxybutyrate (βHB) levels associated with reduced resting measures of Inflammation and Oxidative Stress in Endurance Athletes

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Keywords Ketogenic diet, endurance athlete, cholesterol, inflammation, oxidative stress, ketones

4.1 Abstract

Prevalence of endurance athletes consuming low-carbohydrate ketogenic diets (LCKD) has increased steadily over the past decade. Despite a number of investigations examining its place as a treatment for many metabolic disorders, little is known about its health implications to the endurance athlete population. This investigation examined blood biomarkers, including blood glucose, insulin, total cholesterol, triglycerides and various inflammatory and oxidative stress markers within male endurance athletes. Participants' self-selected into an *ad libitum* high-carbohydrate (HC) diet (*n* = 7, %carbohydrate:fat:protein = 50:30:20) or *ad libitum* LCKD (*n* = 12, %carbohydrate:fat:protein = 5:77:18) for 12 weeks while completing a training

intervention; endurance training, high-intensity interval training (HIIT) and strength training. The intervention maintained body fat (%) in high-carbohydrate group (-0.2%, *P* > 0.05) and decreases in LCKD group (-4.7%, *P* = 0.002). Serum beta-hydroxybutyrate (βHB) (+0.4 mM, *P* = 0.011, ES: 0.273) and total cholesterol (+29.4 mg/dL, *P* = 0.002, ES: 0.502) increased in LCKD group. Post-intervention tumor necrosis factor alpha (TNF-α) was higher in the high-carbohydrate group compared to the LCKD group (HC +1.4 pg/ml, LCKD -0.2 pg/ml, P = 0.046). Relationship analysis demonstrated a greater of βHB to be associated with higher blood glucose (*P* = 0.045), reduced leptin (*P* = 0.004), interleukin 8 (IL-8) (*P* = 0.010), macrophage-colony stimulating factor (M-CSF) (*P* = 0.003) and protein carbonyl (P = 0.004). No effects were observed on DNA/RNA damage or thiobarbituric acid reactive substances (TBARS). A LCKD predominately high in monounsaturated fats increased total cholesterol, and when associated with large changes in βHB reduced oxidative stress and inflammation.

4.2 Introduction

Despite conventional wisdom advocating a high-carbohydrate (HC) diet for endurance sports (Burke *et al.,* 2011; Jeukendrup, 2014), low-carbohydrate ketogenic diets (LCKD) have experienced a renascence within the scientific literature (Zajac *et al.,* 2014; Burke, 2015; Volek *et al.,* 2016; Webster *et al.,* 2016; Zinn *et al.,* 2017; McSwiney *et al.,* 2018; Heatherly *et al.,* 2017; Cipryan *et al.,* 2018) subsequent to a notable gap since the 1980's (Phinney *et al.,* 1983). This is in light of growing interest among endurance and ultra-endurance athletes (Volek and Phinney, 2012; Volek, Noakes and Phinney, 2015; Burke, 2015). Habitual or cyclical consumption of a LCKD results in a greater than two-fold increase in fat oxidation, affording endurance athletes access to a much larger endogenous energy supply (>30,000 kcal in adipose tissue) (Volek, Noakes and Phinney, 2015), an approach currently in use within well-trained cyclists (Webster *et al.,* 2016), ultra-endurance runners (Volek *et al.,* 2016) and elite Ironman (Maunder, Kilding and Plews, 2018) as it's thought to reduce the need for aggressive feeding strategies during exercise (>60-90 g/h CHO) (Jeukendrup, 2004; Burke *et al.,* 2011).

Nutritional ketosis results in a dedicated increase in circulating ketone concentrations as a result of hepatic ketogenesis. While the ketone bodies acetoacetate (AcAc) and beta-hydroxybutyrate (βHB) are produced in roughly equal proportion in hepatic tissue, the predominant circulating ketone body is βHB (Robinson and Williamson, 1980; Laffel, 1990; Evans, Cogan and Egan, 2016). While once viewed as simply an intermediate metabolite of fatty acid oxidation, recent research has demonstrated an ability of βHB to serve as a signaling molecule (Shimazu *et al.,* 2013; Newman and Verdin, 2014; Rahman *et al.,* 2014). In a series of experiments, Shimazu and colleagues (2013)

demonstrated the ability of βHB to serve as a histone deacetylase (HDAC) inhibitor *in vitro,* conveying a decrease in oxidative stress. Additional *in vitro* work has demonstrated the ability of βHB to inhibit the activation of the NLRP3 inflammasome in innate immune cells, which are mediators of systemic inflammation (Youm *et al.,* 2015). While recent advances in molecular biology and ketone physiology have highlighted novel therapeutic effects of increased ketone concentration, use of LCKD as an effective treatment for children with drug-resistant epilepsy has been employed since the 1920's (Vanitallie and Nufert, 2003) and in more recent times have been examined as a potential treatment for other conditions, such as obesity (Bueno *et al.,* 2013; Paoli, 2014), heart disease (Paoli *et al.,* 2013; Cotter, Schugar and Crawford, 2013; Kosinski and Jornayvaz, 2017), breast cancer (Hyde *et al.,* 2017), neurodegenerative diseases (Branco *et al.,* 2016; Stafstrom and Rho, 2012; Hartman, 2012; Gasior, Rogawski and Hartman, 2006; Maalouf, Rho and Mattson, 2009; Paoli *et al.,* 2014) and Type 2 diabetes mellitus (McKenzie *et al.,* 2017; Saslow *et al.,* 2017).

Oxidative phosphorylation along the electron transport chain in mitochondria is accompanied with the generation of ROS. To meet the increased energy demands associated with the 26-100+ mile races commonly completed during endurance athletics, rates of oxidative phosphorylation would be elevated, potentially resulting in elevated oxidative stress. With LCKD associated with reduced oxidative burden, it is plausible to postulate that endurance athletes could find profound benefits to overall health and longevity of their career (Edwards, Copes and Bradshaw, 2015; Moreno and Mobbs, 2017). While anecdotal, keto-adapted athletes have previously attributed an increased ability to recover from 100+ mile races to a LCKD (Volek, Noakes and Phinney, 2015). Further, reducing the volume of ROS production across a lifetime could

potentially cease or delay the development of many chronic illnesses and diseases (Volek, Noakes and Phinney, 2015; Noakes and Windt, 2016), particularly among athletes who are insulin resistant/pre-diabetic (Reaven, 1988; Reaven, 2012; Brukner, 2013). Despite the growing popularity of LCKD among athletes, comprehensive research that examines its impact on health in an athletic population is absent. Thus, we designed an experimental study to investigate performance (McSwiney *et al.,* 2018) and biomarkers of heath (cholesterol, inflammation and oxidative stress) amongst male endurance athletes who adhered to a traditional high-carbohydrate or LCKD for 12 weeks.

4.3 Materials and Methods

4.3.1 Study Design and Participants

Details of this investigation have been described previously (McSwiney *et al.,* 2018). In brief, forty-seven male endurance athletes aged 19-40 years participated in a 12 week non-randomized control trial, comparing performance (McSwiney *et al.,* 2018) and blood responses to a high-carbohydrate diet and LCKD. A non-randomized approach was chosen due to the length of the adaptation period and to promote dietary adherence during the investigation. All participants' had previously competed in endurance events (>2 years) and had consumed a carbohydrate-based diet (>50% of total calories) prior to the intervention. Exclusion criteria included the use of pharmaceuticals that may affect any measurements of performance, illness or injury prior to the start date. Twenty participants' completed all aspects of the performance trial (McSwiney *et al.,* 2018). Due to an insufficient amount of sample being obtained from some participants (*n* = 1 participant felt faint and blood sample was stopped prematurely and *n* = 3 participants

had smaller volume of plasma), a blood chemistry, inflammatory and oxidative stress panel was completed on 16 participants' (HC *n* = 7, LCKD *n* = 9) and an additional 3 LCKD participants. Two additional LCKD participants' were unable to complete the exercise trial at post-intervention testing, while one LCKD participant experienced technical difficulty at post-intervention testing, as previously outlined (McSwiney *et al.,* 2018). All 12 LCKD participants' adhered to a LCKD for 12 weeks and therefore were included within this analysis. Whole blood was collected via venipuncture from the antecubital vein using a 21G BD Vacutainer blood collection set (BD Diagnostics). Blood samples were centrifuged at 1500 x g for 15 minute at 4°C, aliquoted and stored at -80°C. Samples were only thawed once before analysis. Pre- and post-intervention blood samples were obtained while participants were rested following a 48 hour break from training and a 12 hour overnight fast. All procedures were approved by the research ethics committee at Waterford Institute of Technology, IE (ref: 15/HSES/03) (see appendix C) and all participants' provided written informed consent (see appendix B).

4.3.2 Dietary Intervention

Dietary intake was recorded at baseline via 3 day weighed food diary (2 week days and 1 weekend day) and analyzed using Nutritic's dietary analysis software (Nutritic's Professional v3.09, Nutritic's, Dublin, Ireland). The dietary and training intervention began the day after baseline testing. The macronutrient goals were as follows: HC: 65% CHO, 20% fat and 14% protein (see appendix D) or LCKD: >75% fat, 10-15% protein and <50g/d CHO (see appendix E). Participants in each group received nutritional counselling throughout the 12 week intervention and 3 day food diaries were also obtained in week 12.

4.3.3 Training Intervention

The training intervention comprised of endurance training, high-intensity interval training (HIIT) and strength training (see appendix F), as described previously (McSwiney *et al.,* 2018). Hours of endurance training (HC: 11.3 ± 1.9 versus LCKD: 11.9 ± 1.9) completed per week did not differ between groups, nor did the number of HIIT (HC: 18.6 ± 2.1 versus LCKD: 19.1 ± 2.1) or strength sessions (HC: 18.1 ± 2.3 versus LCKD: 17.8 ± 3.2) completed.

4.3.4 Blood Analysis

Blood Chemistry Panel

All blood measurements were determined at baseline and after 12 weeks of dietary intervention. Fasting βHB concentrations were determined via colorimetric enzymatic assay (Sigma-Aldrich; St. Louis, MO). Intra-assay coefficient of variation was <10%. Cholesterol, glucose and triglycerides were analyzed using enzymatic colorimetric assays from Pointe Scientific (Michigan, USA). Intra-assay coefficient of variation for cholesterol, glucose and triglycerides were 0.7, 1.1 and 1.82% respectively. Ella™ multiplex assay by Protein Simple (Protein Simple Ltd, California, USA) was used to determine changes in insulin. Intra-assay coefficient of variation was 1.5%.

Hormonal Panel

An Ella^{TM} multiplex assay by Protein Simple (Protein Simple Ltd, California, USA) was used to determine changes in leptin. Intra-assay coefficient of variation was 1.1%.

Inflammatory Panel

An Ella ™ multiplex assay by Protein Simple (Protein Simple Ltd, California USA) was used to determine changes in the following serum cytokines and adhesion molecules: CCL 2 ((C-C motif) ligand 2), E-SEL (E-selectin), ICAM-1 (intercellular adhesion molecule 1), IL – 6 (interleukin-6), IL – 8 (interleukin-8), M-CSF (colony stimulating factor 1), MMP-2 (matrix metalloproteinase-2), RANTES/CCL5 (Chemokine ligand 5) and TNF-α (tumor necrosis factor alpha). In addition, C reactive protein (CRP) was determined via a Human C Reactive Protein 96 well ELISA Kit (Abcam, USA). All analytes coefficient of variation was <5%.

Oxidative Stress Panel

DNA/RNA damage, protein carbonyl and TBARS (thiobarbituric acid reactive substances) were assessed via 96 well colorimetric or immunoassay kits from Cayman Chemical, USA. Intra-assay coefficients of variation were 9.6, 4.7 and 5.5% respectively.

HOMA-IR

Insulin resistance index (HOMA-IR) was calculated according to the formula: fasting insulin (µU/L) x fasting glucose (nmol/L)/22.5 (Matthews *et al.,* 1985).

4.3.4 Statistical Analysis

IBM Statistics SPSS 24 (Illinois, Chicago, USA) was used for statistical analysis. All data were tested for normality, with parametric tests used for the normally distributed data. Independent sample t-tests or Mann Whitney U test (if not normally distributed) were used to determine differences between high-carbohydrate and LCKD groups at baseline, with the alpha level for significance set at $P < 0.05$, *a priori*. The effects for each group

were analyzed using an ANCOVA repeated measures design, with pre-intervention measures acting as a covariate. As a measure of effect size, partial eta-squared (Np^2) was used. Effect sizes were evaluated as follows: $Np^2 = 0.01$ (small effect), $Np^2 = 0.09$ (medium effect) and $Np^2 = 0.25$ (large effect) (Cohen, 1988). Paired samples t-tests or Wilcoxon signed ranks test (if data was not normally distributed) were used to examine changes over time (baseline to week 12) within each group, if ANCOVA *P* value was < 0.05. Pearson's moment correlation coefficient was carried to examine relationship among selected variables. The alpha level for significance was set at 0.05.

4.4 Results

4.4.1 Baseline Subject Characteristics, Diet and Blood Measures

There were no significant differences in baseline subject characteristics (Table 4.1) or body composition measurements (Table 4.2). Habitual dietary cholesterol was greater in the high-carbohydrate group (*P* = 0.011) (Table 4.3), while concentrations of IL-6 (*P* = 0.024) were greater in the high-carbohydrate group at baseline (Table 4.4).

4.4.2 Body Composition

LCKD participants' experienced a decreases in body mass (-5.7 kg, *P* = 0.000) (Table 4.2), decreases were largely comprised of body fat loss (-4.7 % body fat *P* = 0.002), with lean body mass remaining unchanged (-0.4 kg, *P* = 0.514) (Table 4.2). No changes in body mass, fat mass or lean mass occurred within high-carbohydrate participants.

Table 4.1. Subject characteristics					
	HC diet (*n* = 7)		LCKD (*n* = 12)		t-Test
	Mean ± SD	Range	Mean ± SD	Range	*P* Value
Age, years	32.4 ± 6.0	23.0-38.0	33.2 ± 6.5	19.0-40.0	0.794
Height, cm	180.0 ± 4.0	177.0-188.5	182.0 ± 5.6	175.4-191.6	0.845
BMI, kg/m^2	24.4 ± 3.2	21.3-30.5	25.8 ± 2.7	22.2-31.2	0.317

Abbreviations: cm = centimetre; $kg/^2$ = kilogram per metre squared

Table 4.2. Body composition measurements of high-carbohydrate (HC) and low-carbohydrate ketogenic diet (LCKD) groups																
Dependent variables	HC diet (n = 7) [a]							LCKD (n = 12) [a]							ANCOVA		
	Pre			Post			Change	Pre			Post			Change			
	Mean	±	SD	Mean	±	SD	Mean	Mean	±	SD	Mean	±	SD	Mean	F – value	P value	ES [b]: η_p^2
Body mass, kg	77.6	±	8.32	77.4	±	7.2	-0.2	84.6	±	12.8	78.9	±	12.3 [c]	-5.7	(1,16) = 11.126	0.004	0.410
Lean mass, kg	64.7	±	4.7	65.0	±	4.9	+0.3	67.1	±	8.1	66.7	±	8.7	-0.4	(1,16) = 0.658	0.429	0.039
Body fat, kg	9.9	±	4.5	9.5	±	3.4	-0.4	14.6	±	6.6	10.3	±	4.7 [c]	-4.3	(1,16) = 5.822	0.028	0.267
Body fat, %	12.2	±	3.5	12.0	±	2.9	-0.2	16.5	±	5.1	11.8	±	4.3 [c]	-4.7	(1,16) = 3.287	0.089	0.170

[a] Original means and standard deviations, i.e., without adjustment for covariate (i.e., pre-treatment data). [b] ES = effect size. η_p^2 = 0.01 (small effect), η_p^2 = 0.09 (medium effect), η_p^2 = 0.25 (large effect) [25]. * ANCOVA significant difference at $P < 0.05$. [c] Significant difference within group between pre- and post-intervention.

Changes in participants' nutrient intake are presented in Table 3. By design absolute carbohydrate (-392 g/d, *P* = 0.000), relative carbohydrate (-4.6 g·kg, *P* = 0.000) and fiber (-27.4 g/d, *P* = 0.003) decreased in the LCKD group. Decreases in carbohydrate consumption in the LCKD group were accompanied by increases in dietary fat (+175.5 g/d, *P* = 0.000), saturated fat (+64.0 g/d, *P* = 0.008), monounsaturated fat (+69.7 g/d, *P* = 0.005), polyunsaturated fat (+14.5 g/d, *P* = 0.000), omega-3 fatty acids (+0.4 g/d, *P* = 0.000), omega-6 fatty acids (+7 g/d, *P* = 0.002), cholesterol (+738.2 mg/d, *P* = 0.011) and trans fatty acids (+2.2 g/d, *P* = 0.044). In contrast, the high-carbohydrate group's diet remained relatively consistent, but there was a decrease in saturated fat (-20.9 g/d, *P* = 0.019) and cholesterol (-249.9 mg/d, *P* = 0.024).

4.4.4 Blood Measures

Blood responses to the intervention are presented in Table 4. Serum βHB increased from 0.1 to 0.4 mM in the LCKD group (*P* = 0.011) and remained unchanged in the high-carbohydrate group (*P* = 0.602) (Figure 4.1). In addition, total cholesterol increased in the LCKD group (+29.4 mg/dL, *P* = 0.002) and remained unchanged in the high-carbohydrate group (-8.6 mg/dL, *P* = 0. 127). ANCOVA demonstrated a significant difference for TNF-α (*P* = 0.046), at post-intervention between high-carbohydrate and LCKD groups (HC = 6.9 ± 2.9 pg/ml, LCKD = 5.0 ± 1.1 pg/ml) (Figure 4.2). The ANCOVA analysis found no other changes to be statistically significant (*P* > 0.05), however medium effects in ICAM-1 (HC = -6.1 ng/ml, LCKD = -33.5 ng/ml, ES: 0.186), IL-6 (HC = +0.5 pg/ml, LCKD = +0.1 pg/ml, ES: 0.116) and MCSF (HC = +41.2 pg/ml, LCKD = -52.8, ES: 0.097) were observed (Table 4.4). Further analysis to examine how changes in body mass or serum βHB were associated with changes in blood measures (Table 4.5) revealed loss

of body mass was associated with decrease in blood leptin (P = 0.027) (Figure 4.3), while increased serum βHB was correlated with increased blood glucose (P = 0.045) (Figure 4.4), reduced leptin (P = 0.004) (Figure 4.5), reduced IL-8 (P = 0.010) and reduced M-CSF (P = 0.003) (Table 4.5).

Table 4.3. Average nutrient intake of high-carbohydrate (HC) and low-carbohydrate ketogenic diet (LCKD) groups																
Dependent variable	HC diet (n = 7) [a]							LCKD (n = 12) [a]							ANCOVA		
	Pre			Post			Change	Pre			Post			Change			
	Mean	±	SD	Mean	±	SD	Mean	Mean	±	SD	Mean	±	SD	Mean	F – value	P value	ES [b]: Np^2
Energy, kcal/d	2546.7	±	436.7	2598.1	±	218.4	+51.4	2709.5	±	599.2	2768.2	±	912.2	+58.7	(1,16) = 0.031	0.863	0.002
Carbohydrate, g/d	319.5	±	111.6	421.0	±	92.8	+101.5	430.6	±	149.7	37.8	±	14.4 [d]	-392.8	(1,16) = 177.3	0.000	0.917
Carbohydrate, g·kg BM	4.2	±	1.5	5.4	±	1.1	+1.2	5.0	±	1.4	0.4	±	0.2 [d]	-4.6	(1,16) = 208.2	0.000	0.929
Fat, g/d	86.5	±	38.9	59.7	±	7.4	-26.8	60.2	±	34.7	235.7	±	83.8 [d]	+175.5	(1,16) = 28.837	0.000	0.643
Fat, g·kg BM	1.1	±	0.5	0.7	±	0.1	-0.4	0.7	±	0.4	2.9	±	0.9 [d]	+2.2	(1,16) = 40.573	0.000	0.717
Protein, g/d	122.4	±	35.4	94.4	±	19.4	-28.0	111.3	±	22.0	123.9	±	33.3	+12.6	(1,16) = 4.775	0.044	0.230
Protein, g·kg BM	1.6	±	0.5	1.2	±	0.2	-0.4	1.3	±	0.2	1.5	±	0.3	+0.2	(1,16) = 4.928	0.041	0.235
Saturated Fat, g	38.4	±	16.5	17.5	±	7.1 [d]	-20.9	24.5	±	14.1	88.5	±	24.1 [d]	+64.0	(1,16) = 43.945	0.000	0.733
Monounsaturated-Fat, g	34.9	±	19.8	20.2	±	8.4	-14.7	21.2	±	15.9	90.9	±	40.3 [d]	+69.7	(1,16) = 21.564	0.000	0.574
Polyunsaturated-Fat, g	10.3	±	4.5	10.9	±	4.3	-0.6	7.8	±	3.7	22.3	±	5.6 [d]	+14.5	(1,16) = 29.340	0.000	0.647
Omega 3, g	1.1	±	1.1	1.0	±	1.0	-0.1	0.4	±	0.3	4.4	±	0.8 [d]	+4.0	(1,16) = 127.0	0.000	0.888
Omega 6, g	4.0	±	2.3	4.8	±	3.2	+0.8	3.7	±	2.7	10.7	±	5.8 [d]	+7.0	(1,16) = 6.620	0.020	0.293
Cholesterol, mg	379.0	±	220.6	129.1	±	40.2 [d]	-249.9	167.7	±	102.3 [c]	905.9	±	387.9 [d]	+738.2	(1,16) = 26.434	0.000	0.623
Trans Fatty Acids, g	1.7	±	0.8	0.9	±	0.7 [d]	-0.8	1.1	±	0.7	3.3	±	1.1 [d]	+2.2	(1,16) = 18.057	0.001	0.546
Fibre, g	46.1	±	9.3	48.5	±	11.8	-2.4	47.7	±	12.8	20.3	±	7.7 [d]	-27.4	(1,16) = 51.430	0.000	0.763

[a] Original means and standard deviations, i.e., without adjustment for covariate (i.e., pre-treatment data). [b] ES = effect size. η_p^2 = 0.01 (small effect), η_p^2 = 0.09 (medium effect), η_p^2 = 0.25 (large effect) [25]. * ANCOVA significant difference at $P < 0.05$. [c] Significant difference between groups at baseline.

[d] Significant difference within group between pre- and post-intervention.

Table 4.4. Serum responses to a high-carbohydrate (HC) diet and low-carbohydrate ketogenic diet (LCKD)																
Dependent variable	HC diet (n = 7) [a]							LCKD (n = 12) [a]							ANCOVA		
	Pre			Post			Change	Pre			Post			Change			
	Mean	±	SD	Mean	±	SD	Mean	Mean	±	SD	Mean	±	SD	Mean	F – value	P value	ES[b]: Np^2
βHB, mM	0.0	±	0.1	0.0	±	0.0	-0.0	0.0	±	0.0	0.4	±	0.3 [d]	+0.4	(1,16) = 5.712	0.030*	0.263
Cholesterol, mg/dL	114.4	±	16.8	105.8	±	17.4	-8.6	128.1	±	19.0	157.5	±	29.0 [d]	+29.4	(1,16) = 13.590	0.002*	0.459
Glucose, mg/dL	80.7	±	8.0	77.2	±	9.4	-3.5	81.4	±	12.3	81.0	±	9.8	-0.4	(1,16) = 0.632	0.438	0.038
Triglycerides, mg/dL	40.8	±	13.8	44.4	±	19.4	+3.6	44.6	±	21.8	47.6	±	37.8	+2.9	(1,16) = 0.010	0.923	0.001
Insulin, pmol/dL	40.4	±	21.5	27.0	±	11.3	-13.4	29.7	±	6.6	29.8	±	10.5	+0.1	(1,16) = 0.061	0.808	0.004
HOMA-IR	1.1	±	0.5	0.7	±	0.3	-0.4	0.8	±	0.2	0.8	±	0.3	0.0	(1,16) = 0.128	0.725	0.008
Leptin, ng/ml	1.1	±	0.7	1.0	±	0.5	-0.1 [j]	1.9	±	0.8	1.0	±	0.4	-0.9 [e]	(1,15) = 0.400	0.537	0.026
CCL2, ng/dl	21.1	±	9.5	28.7	±	5.2	+7.6 [j]	29.1	±	6.0	29.1	±	3.6	-0.0	(1,15) = 0.082	0.779	0.005
CRP, mg/dL	3.7	±	2.9	3.3	±	2.2	-0.4	2.6	±	4.8	2.3	±	1.8	-0.3 [f]	(1,14) = 0.935	0.350	0.063
E-SEL, ng/ml	24.4	±	5.4	23.4	±	4.9	-1.0	22.1	±	6.6	20.4	±	7.2	-1.7	(1,16) = 0.356	0.559	0.022
ICAM-1, ng/ml	368.3	±	53.6	362.5	±	34.5	-5.8	373.0	±	139.2	339.5	±	82.6	-33.5 [e]	(1,15) = 3.731	0.073	0.199
IL-6, pg/ml	0.8	±	0.1	1.3	±	1.0	+0.5 [k]	0.5	±	0.1 [c]	0.6	±	0.2	+0.1 [h]	(1,10) = 1.309	0.279	0.116
IL-8, pg/ml	7.1	±	1.7	9.1	±	1.5	+2.0 [j]	14.0	±	15.6	9.6	±	2.2	-4.6	(1,15) = 0.100	0.756	0.007
M-CSF, pg/ml	450.5	±	71.0	491.7	±	169.1	+41.2	480.0	±	267.5	427.2	±	72.0	-52.8	(1,16) = 1.716	0.209	0.097
MMP-2, ng/ml	285.7	±	53.6	266.1	±	46.5	-19.6	280.2	±	74.9	281.1	±	86.0	+0.9 [e]	(1,15) = 0.652	0.432	0.042
CCL5, ng/ml	42.9	±	14.1	47.3	±	14.7	+4.4	47.3	±	22.7	57.3	±	26.6	+10.0 [e]	(1,15) = 0.583	0.457	0.037
PAI-1, ng/dl	94.5	±	27.1	98.6	±	23.4	+4.1	116.7	±	32.7	108.2	±	23.7	-8.5 [e]	(1,15) = 0.336	0.571	0.022
TNF-α, pg/ml	5.5	±	2.0	6.9	±	2.9	+1.4 [k]	5.1	±	1.0	5.0	±	1.1	-0.1 [g]	(1,12) = 4.954	0.046*	0.292
DNA/RNA, ng/ml	7.9	±	1.9	7.2	±	1.2	-0.7	6.2	±	1.3 [c]	6.8	±	1.9	+0.6 [g]	(1,13) = 0.130	0.724	0.010
Protein Carbonyl, nmol/L	4.2	±	3.2	4.4	±	1.9	+0.2 [k]	8.7	±	4.5 [c]	4.5	±	6.3	-4.2 [f]	(1,12) = 0.318	0.583	0.026
TBARS, µM	3.3	±	1.1	3.1	±	0.6	-0.2	2.7	±	0.6	2.9	±	0.9	+0.2 [i]	(1,11) = 0.000	0.989	0.000

[a] Original means and standard deviations, i.e., without adjustment for covariate (i.e., pre-treatment data). [b] ES = effect size. η_p^2 = 0.01 (small effect), η_p^2 = 0.09 (medium effect), η_p^2 = 0.25 (large effect) [25]. * ANCOVA significant difference at $P < 0.05$. [c] Significant difference between groups at baseline.

[d] Significant difference within group between pre- and post-intervention [e] n = 11, [f] n = 10, [g] n = 9, [h] n = 8, [i] n = 7, [j] n = 6, [k] n = 5

Table 4.5. Correlation between change in body mass or change in serum βHB and change in blood parameter measured				
	Body Mass (kg)		βHB (mM)	
	Correlation Coefficient	*P* value	Correlation Coefficient	*P* value
Cholesterol, mg/dL [a]	-0.300	0.212	0.370	0.119
Glucose, mg/dL [a]	0.052	0.833	0.464	0.045*
Triglycerides, mg/dL [a]	0.141	0.564	-0.097	0.694
Insulin, mg/dL [a]	0.038	0.876	-0.069	0.779
HOMA-IR [a]	0.055	0.824	0.073	0.767
Leptin, ng/ml [b]	0.535	0.027*	-0.666	0.004*
CCL2, ng/dl [c]	0.058	0.819	0.170	0.501
CRP, ng/dl [a]	-0.092	0.726	-0.215	0.408
E-SEL, mg/L [b]	0.221	0.364	-0.384	0.104
ICAM-1,ng/ml [b]	0.305	0.218	-0.042	0.869
IL-6, pg/ml [g]	0.321	0.284	-0.296	0.326
IL-8, pg/ml [b]	0.165	0.513	-0.587	0.010*
M-CSF, pg/ml [a]	0.198	0.416	-0.636	0.003*
MMP-2, ng/ml [b]	-0.002	0.993	0.076	0.765
CCL5, ng/ml [b]	-0.047	0.852	0.445	0.064
PAI-1, pg/ml [b]	0.447	0.063	-0.388	0.112
TNF-α [e]	0.393	0.148	-0.321	0.243
DNA/RNA Damage, ng/dl [d]	-0.250	0.350	0.176	0.514
Protein Carbonyl, nmol/L [e]	0.226	0.417	-0.693	0.004*
TBARS, µM [f]	0.080	0.786	-0.008	0.978

[a] n = 19, [b] n = 18, [c] n = 17, [d] n = 16, [e] n = 15, [f] n = 14, [g] n = 13

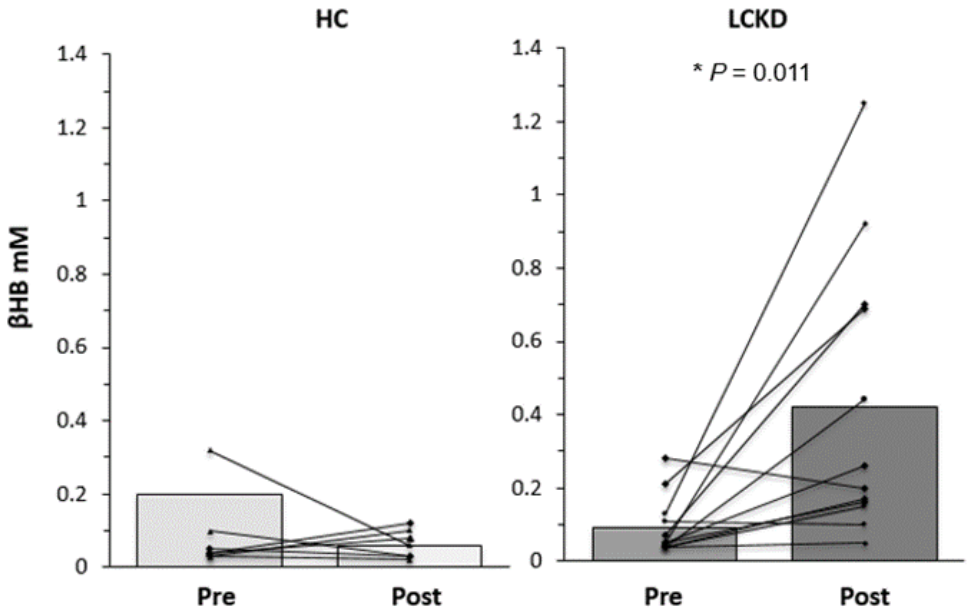

Figure 4.1. High-carbohydrate (HC) and low-carbohydrate ketogenic diet (LCKD) resting serum βHB at pre- and post-intervention testing.

***** Significant increase ($P < 0.05$) within the LCKD group

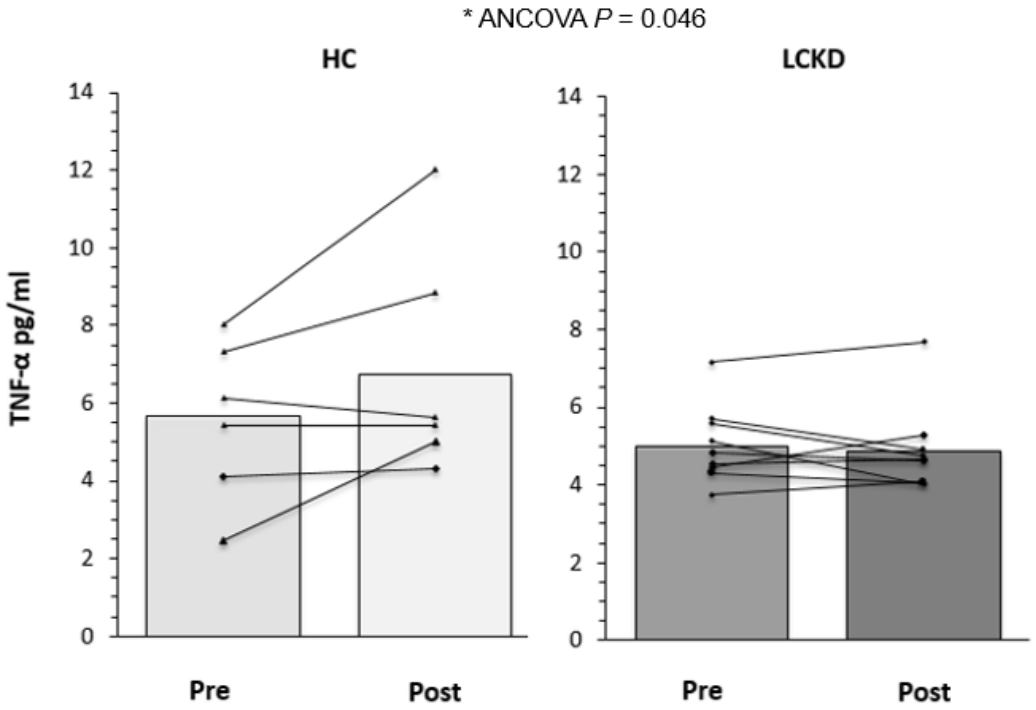

Figure 4.2. High-carbohydrate (HC) and low-carbohydrate ketogenic diet (LCKD) resting serum TNF-α at pre- and post-intervention testing.

***** ANCOVA $P < 0.05$

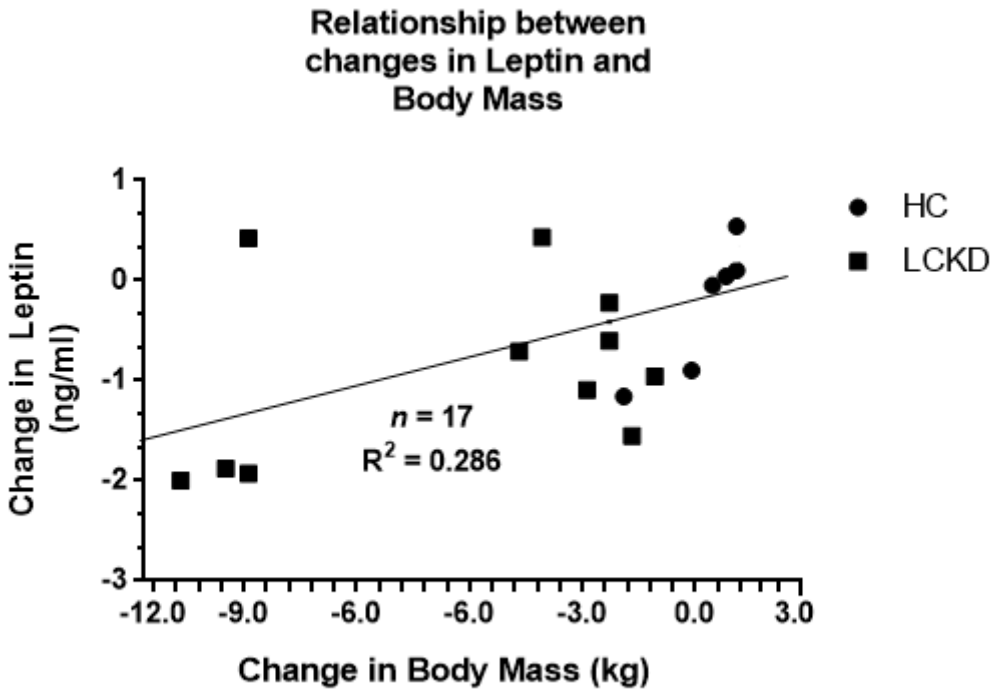

Figure 4.3. Relationship between change in leptin (ng/ml) and change in body mass (kg) (*P* = 0.027) in the high-carbohydrate (HC) and low-carbohydrate ketogenic diet (LCKD) groups

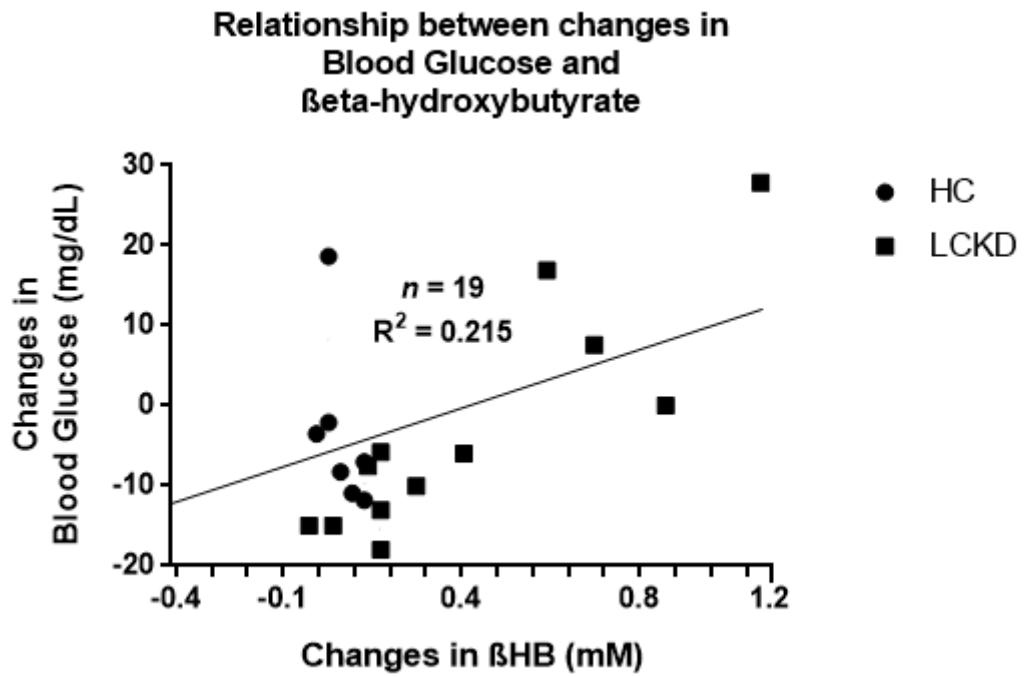

Figure 4.4. Relationship between change in blood glucose (mg/dL) and change in body mass (kg) (*P* = 0.045) at pre- and post-intervention testing in the high-carbohydrate (HC) and low-carbohydrate ketogenic diet (LCKD) groups

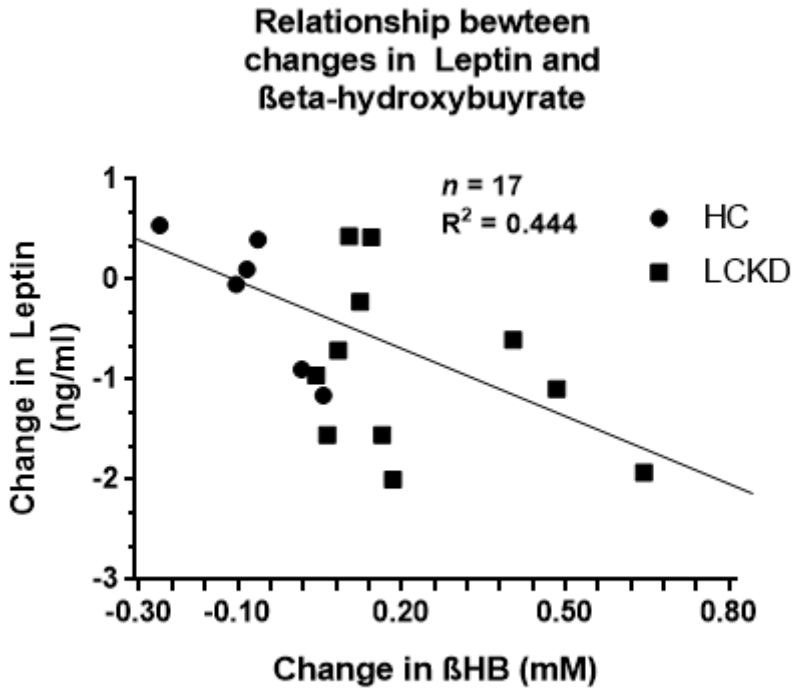

Figure 4.5. Relationship between change in leptin (ng/ml) and change in serum βHB (mM) (*P* = 0.004) at pre- and post-intervention testing in the high-carbohydrate (HC) and low-carbohydrate ketogenic diet (LCKD) groups

4.5 Discussion

The current investigation examined the implications of consuming a high-carbohydrate and LCKD for 12 weeks on various biomarkers of health in endurance trained males. Participants' in the LCKD group experienced an increase in serum βHB (~0.4 mM) concentration post-intervention, demonstrating good adherence to the protocol (Table 4.4). Total cholesterol increased in the LCKD group, while TNF-α was higher post intervention in the high-carbohydrate compared to the LCKD group (Table 4.4). Decreased body mass was associated with reduced leptin while increased serum βHB was associated with reduced leptin, IL-6, IL8 and protein carbonyl but increased blood glucose levels.

Despite no significant change in energy intake, loss of body mass occurred within LCKD participants (Table 4.2), as described previously (McSwiney *et al.,* 2018). Due to the free-living design of this investigation, a number of potential confounding variables were not stringently controlled, such as non-exercise activity thermogenesis and training intensity (heart rate/VO_2 etc.). Body mass lost during the intervention period is believed to be a product of increased training volume (energy output) and enhanced focus on dietary adherence throughout the intervention period. Although not statistically significant, the LCKD group had a numerically greater body fat percentage at baseline (P = 0.067). These unforeseen differences in anthropometric measurements may be attributed to lack of randomization through diet self-selection and should be considered when critically analyzing the results. There were no significant differences in the number of training sessions (strength, endurance and HIIT) completed between high-carbohydrate and LCKD participants', as described previously (McSwiney *et al.,* 2018). Therefore, changes

in blood measures presented in Table 4 may be consequent of dietary changes and/or body mass losses within the LCKD group.

The elevation in fasting serum βHB is less than previously observed in highly controlled short-term (<28 days) experimental feeding trials in endurance athletes (>1.0 mM) (Phinney *et al.,* 1983; Burke *et al.,* 2017). This is despite all investigations' reporting diets with similar macronutrient profiles, which points to difficulties in reporting of nutrient intake in non-highly controlled settings. Further, a recent investigation demonstrated that moderately trained males could elevate capillary blood βHB to 0.7 mM subsequent to 2 weeks of a LCKD containing <50 g/d carbohydrates, however βHB dropped to ~0.4 mM in week 4 (Cipryan *et al.,* 2018). This demonstrates that elevating βHB to desirable levels (>0.5-1.0 mM) Volek and Phinney, 2012) may be an issue in some individuals', perhaps due to poor dietary adherence. Nonetheless, βHB values are comparable to a cross-sectional study involving ultra-endurance athletes who habitually consumed a LCKD for >9 months (Volek *et al.,* 2016); keto-adapted athletes had resting βHB of ~0.5 mM, while βHB steadily increased as exercise intensity increased to ~1.0 mM.

Blood glucose and insulin responses to a LCKD are inconsistent between studies. Untrained overweight individuals experience decreases (Volek *et al.,* 2009; Saslow *et al.,* 2017), attributing to improvements in homeostatic model assessment (HOMA-IR) (Volek *et al.,* 2009) and $HBA1_c$ (Saslow *et al.,* 2017) scores. Improvements in HOMA-IR (Table 4.4) previously observed (Volek *et al.,* 2009) were unnecessary within the current population sample, as it involved healthy individuals. Trained individuals who adopt a LCKD for >28 days maintain pre-dietary intervention thresholds of blood glucose (Phinney *et al.,* 1983; Wilson *et al.,* 2017), while cross sectional investigations involving

endurance athletes demonstrated no difference in fasting blood glucose in high-carbohydrate and LCKD participants' habituated to their respective diets for >8 months (Webster *et al.,* 2016; Volek *et al.,* 2016). In contrast, elite athletes within Burke *et al.'s* (2017) 21 day investigation experienced decreases in fasting blood glucose and blood glucose throughout exercise (25 km walk). From an athletic standpoint, the maintenance of fasting blood glucose may be an important marker, as the length of time necessary to become proficient on a LCKD or 'keto-adapted' (Volek and Phinney, 2012) is often discussed (Volek, Noakes & Phinney, 2015; Burke 2015). A number of recent investigations' in trained individuals have highlighted that nutritional ketosis (i.e., βHB >0.5 mM) (Volek and Phinney, 2012) can be objectively achieved within 2 weeks (Wilson *et al.,* 2017; Zinn *et al.,* 2017; Cipryan *et al.,* 2018). However, reestablishing fasting blood glucose appears to take >3 weeks in elite athletes (Burke *et al.,* 2017), which may be as important a marker of adaptation as increases in βHB/ketones. The relationship between changes in blood glucose and βHB is outlined within Figure 4. Most notably, participants' who demonstrated the greatest dietary adherence, i.e., increases in βHB, experienced increases in blood glucose, while participants who experienced modest changes in βHB experienced decreases. Maintaining resting glycogen stores, despite dietary carbohydrate restriction (<50 g/d), from gluconeogenic production of glucose from non-carbohydrate substrates such as glycerol, lactate and glycogenic amino acids (Glew, 2010; Webster *et al.,* 2016) is a metabolic characteristic of keto-adapted athletes who've consumed a LCKD for a number of months (i.e., >9 months) (Volek *et al.,* 2016). However, the precise length of time necessary to achieve this characteristic remains unknown, but warrants further exploration. Finally, it must be noted, aforementioned studies that achieved nutritional ketosis within 2 weeks (Wilson *et al.,* 2017; Zinn *et al.,*

2017; Cipryan *et al.,* 2018) relied on capillary blood samples versus plasma/serum βHB to assess ketogenisis. It has been observed that commercial handheld monitors often overestimate concentrations, such as βHB versus traditional laboratory methods (Guimont *et al.,* 2015).

Leptin is secreted by white adipose tissue and is typically proportional to body fatness (Lee *et al.,* 2015). In accordance with previous knowledge, a relationship between changes in body mass and concentrations of leptin was observed (Figure 4.3). Leptin plays an important role in energy hemostasis (Lee *et al.,* 2015) and activates inflammatory cell responses and induces pro-inflammatory cytokine production (Conde *et al.,* 2014). Despite this, no relationship between decreases in body mass and measures of inflammation were observed (Table 4.5). In contrast, a relationship between change in serum βHB, leptin (Figure 4.5) and two inflammatory markers (IL-8 and M-CSF) was observed (Table 4.5), while a main effect for TNF-α was observed (Table 4.4). TNF-α is a central regulator of inflammation and activates NF-kB, a major transcription factor that regulates the expression of various chemokines, cytokines and adhesion molecules (Monaco and Paleolog, 2004; Winther, Kanters and Kraal, 2005). Greater decreases in body mass and leptin (-34.9%) were previously observed within patients following a carbohydrate restricted diet versus a group consuming a carbohydrate based diet (-14.8%, TxG $P = 0.013$) (Volek *et al.,* 2009). Additionally, these patients experienced greater reductions in TNF-α (-32%) and I-CAM (-16.9%), as well as IL-6 (-57.0%), IL–8 (-58.5%), MCP–1 (24.2%) and E-selectin (-34.3%) (Forsythe *et al.,* 2008). In an athletic population, Rhyu *et al.,* (2014) monitored changes in inflammatory markers IL-6 and TNF-α in taekwondo athletes attempting to make weight using high-carbohydrate and LCKD. Unlike the current investigation, each group achieved similar

weight loss, however the LCKD appeared better equipped at attenuating inflammatory responses in TNF-α (HC = +38%, LCKD = +16%) caused by the intensive training/cutting regime.

C-reactive protein is a commonly measured marker of wellness. Although not extensively researched in an athletic population, changes in CRP in response to a LCKD in an overweight population are mixed. Overweight women experienced a ~25% increase in CRP following 4 week adaptation to a LCKD (Rankin and Turpyn, 2007), while overweight individuals experienced greater decreases in CRP versus high-carbohydrate group (~29.8% versus ~2.7%)(P = 0.03) (Ruth *et al.,* 2013) or no effect (-16% P > 0.05) (Forsythe *et al.,* 2008) following 12 week adaptations to a LCKD. Dietary adherence was only assessed via ketone bodies within one of these investigations (~0.2 mM) (Forsythe *et al.,* 2008; Volek *et al.,* 2009), however our investigation is supportive of the literature by displaying a 12 week adaptation to a LCKD had no impact on resting measures of CRP (Table 4.4). Positive or neutral responses observed with longer adaptation periods may have resulted from greater increases in ketogenesis (i.e., increases in βHB). Cellular work in mice demonstrated that βHB within a range achievable during nutritional ketosis (βHB >1.0 mM) is a class I and II HDAC inhibitor. This feature of βHB allows for greater genetic expression of endogenous antioxidant defense that protect cells and tissues against oxidative damage (Shimazu *et al.,* 2013; Miller, Villamena and Volek, 2018). As previously outlined, increases in βHB within the current population reached a modest ~0.4 mM; Shimazu *et al.,* (2013) demonstrated that βHB works in a dose dependent manner. Therefore, the moderate βHB concentrations achieved in the present study may not have induced a strong enough signal to maximize the potential antioxidant effect of HDAC inhibition.

By adapting to a ketogenic style of eating, athletes are altering their energy metabolism towards fatty acid oxidation, generating ketones (Volek *et al.,* 2016; Burke *et al.,* 2017) to meet energy needs during decreased carbohydrate/glucose intake (Evans, Cogan and Egan, 2016). Miller, Villamena and Volek (2018) believe that such adaptation would increase mitochondrial respiration and as a result, increase mitochondrial ROS production, thereby stimulating an upregulation of endogenous antioxidant defense through mitohormesis. Although mitohormesis has not been extensively studied in humans, the prominence of oxidative stress in chronic disease warrants future research on the potential induction of mitohormesis by a LCKD as a preventative measure and possibly, even a form of treatment (Miller, Villamena and Volek, 2018). Two previous investigations using C. elegans found that increasing fat oxidation, by inhibiting glycolysis, resulted in increased mitochondrial oxygen consumption, while upregulating endogenous antioxidant expression and increasing longevity (Schulz *et al.,* 2007; Zarse *et al.,* 2012). An adaptation to a LCKD is thought to reduce oxidative burden (Sato *et al.,* 1995), however the specific mechanisms remain unclear. As previously outlined, C. elegans were found to experience improved lifespan and increases in antioxidant enzyme activity following glycolysis inhibition. Notably, this caused an increase in mitochondrial ROS at 24 and 48 hours, however, mitochondrial ROS was lower at ~120 hours, indicating that increases in antioxidant enzyme activity decreased net ROS, resulting in a two-fold increase in lifespan. Although speculative, protection from ROS-mediated tissue damage may be why keto-adapted athletes anecdotally report accelerated recovery after adapting to a LCKD. These athletes report returning to training within days, not weeks after an ultra-marathon (Volek, Noakes and Phinney, 2015; Noakes and Windt, 2016). Healthy untrained females improved their ROS defense

capacity by adopting a LCKD for 14-days, presumably from an elevation in oxidative metabolism (Nazarewicz *et al.,* 2007). Despite improved recovery times being anecdotally reported by some LCKD participants' during the current investigation, oxidative stress markers remained statistically unchanged in each group. Improved recovery may have stemmed from non-significant increases in protein (+0.2 g·kg, P = 0.055) within the LCKD group and not necessarily from increases in oxidative metabolism and/or βHB. However, each group consumed adequate protein (Thomas, Erdman and Burke, 2016) and there was no changes in protein over time or between groups (*both* $P > 0.05$). It is accepted that moderate increases in ROS are necessary for cell signaling and regulation of skeletal muscle tissue, however excessive elevations in ROS may prove hazardous (Miller, Villamena and Volek, 2018). TBARS (Polotow *et al.,* 2016) and protein carbonyl (Bloomer *et al.,* 2007) increase in response to overtraining, which highlights a limitation of the current investigation. Although there was no significant difference post-intervention in protein carbonyl (Table 4.3), a relationship a relationship between changes in βHB and protein carbonyl was observed (Table 4.4). Recently, rats consuming a LCKD for 8 months were found to have lower protein carbonyl than rats consuming a standard chow diet (HC diet) (Kephart *et al.,* 2017). Suggesting an adaptation to a LCKD may be associated with increases in oxidative stress defence, as previously observed via increased Nrf2 pathway redox signalling (Milder, Liang and Patel, 2010) and hypothesised (Miller, Villamena and Volek, 2018). Unfortunately, neither acute nor chronic changes in redox balance or antioxidant capacity were measured. Therefore, it is not known if the lack of change (Table 4.3) was a result of increased capacity to tolerate more exercise-induced oxidative stress or if the exercise intervention simply did not increase oxidative burden beyond that of the

athletes' habitual training. Nonetheless, the results of the intervention suggest that a LCKD does not alter oxidative damage in endurance-trained athletes following a 12 week adaptation period and demonstrated the relationship between βHB and protein carbonyl previously observed in rats (Kephart *et al.,* 2017) appears to be present in an endurance trained population following a 12 week adaption.

During the current investigation, one of the largest effects to occur was the change in total cholesterol (Table 4.4). In responses to changes in dietary cholesterol (HC = -217.7 mg, LCKD = +738.2 mg) (Table 4.3), total blood cholesterol increased in the LCKD group (+29.4 mg/dL, P = 0.002) and remained unchanged in the high-carbohydrate group (-8.6 mg/dL, P = 0.127). High intakes of dietary cholesterol appear common on a LCKD in trained individuals; keto-adapted athletes contained within Volek *et al.'s* (2016) cross sectional investigation consumed ~844 ± 351 mg/d which is in line with current intakes (Table 4.3). It must be noted, homeostatic and post-intervention concentrations of total cholesterol appear low (Table 4.4). Unfortunately, additional lipoprotein fractions such as, LDL-cholesterol and HDL-cholesterol were not examined to confirm the reliability of this assay. Post-intervention total cholesterol within the LCKD group is within ranges previously observed in non-athletic (165 ± 30.5 mg/dL) and athletic populations (156.6 ± 22.8 mg/dL) (Petridou and Mougios, 2005), however, the high-carbohydrate group and LCKD pre-intervention cholesterol concentrations are considerably low (Table 4.4). Total cholesterol as low as ~122 mg/dL (143 ± 21 mg/dL) has previously been observed in n = 20 long distance endurance runners (Cardoso *et al.,* 1994). Triglycerides are a solitary risk factor for cardiovascular disease and can be used to predict fluctuations in the type of cholesterol that exists within the blood (ACSM, 2016). Longitudinal data from 1999-2014 by Rosinger *et al.,* (2016) indicates that LDL cholesterol and triglycerides are closely

linked. However, no fluctuations in participants' triglycerides concentration took place during the current investigation (Table 4.4).

Assessment of long-term suitability and safety is critical prior to making any generalizable dietary recommendations, often requiring consideration of many prognostic biomarkers of wellness. Since the 1970s, the general population has been warned about the supposed cardiovascular risks of foods high in cholesterol (Keys *et al.,* 1986). As a result, the general population, including athletes, is preconditioned to be alarmed by increases in their total or LDL cholesterol. However, as previously outlined, it is important to examine multiple measures of health prior to drawing a conclusion on the suitability of any diet. Although long-term studies in an endurance trained population are absent, an investigation by Wilson *et al.,* (2017) demonstrated a 10 week adaptation to a LCKD in resistance trained males caused no effect on total cholesterol, LDL-cholesterol or triglycerides. While some clinical trials have demonstrated increases in LDL-cholesterol (Hernandez *et al.,* 2010), others have noted no effect (Forsythe *et al.,* 2008; Dashti *et al.,* 2004; Wilson *et al.,* 2017). Importantly however, these investigations (including the present research) fail to examine changes in particle sub-fraction distributions, namely the atherogenic effects of small, dense LDL particles and the contrastingly neutral effect of large, buoyant LDL particles (Superko, 2001). In an overweight population, the low-carbohydrate literature consistently demonstrates a reduced proportion of small, dense LDL particles and increases in non-atherogenic large LDL particles (Aude *et al.,* 2004; Volek, Sharman and Forsythe, 2005; Wood *et al.,* 2006; Keogh *et al.,* 2008; Forsythe *et al.,* 2008; Volek *et al.,* 2009). In addition, participants who adopt a LCKD during research investigations often tend to outperform high-carbohydrate counterparts in some facets, including positive increases in HDL-

cholesterol (Bazzano *et al.,* 2015; Yancy *et al.,* 2004; Tay *et al.,* 2008) and decreases in triglycerides (Volek *et al.,* 2009; Brehm *et al.,* 2003; Tay *et al.,* 2008; Volek *et al.,* 2009). Consequently, a LCKD is discussed as cardio protective (Noakes and Windt, 2016). However, it's important to highlight, if increases in HDL-cholesterol were to occur in accordance with increased total cholesterol (>200 mg/dL) (NCEP, 2002), this would be seen as problematic on a population level, as a meta-analysis involving 61 prospective studies and 892,337 persons found a strong correlation between total cholesterol and risk of ischaemic heart disease mortality (Lewington *et al.,* 2007).

4.6 Limitations and Conclusions

This investigation has a number of limitations, namely, 1) small population sample, 2) results are limited to the population sample at hand (i.e., male endurance athletes), 3) baseline differences in anthropometric measurements (i.e., body fat) occurred due to non-randomization, which complicated the comparison of weight loss between groups and 4) dietary protein was not precisely matched (g·kg), which may explain greater *ad libitum* weight loss in the LCKD group.

In conclusion, a LCKD high in monounsaturated and saturated fat was associated with an increase in total cholesterol and maintenance of other markers of wellness, including glycaemic control, triglycerides, inflammation and oxidative stress. Importantly, the increase in total cholesterol remained within a normal range (i.e., <200 mg/dL) (ACSM, 2016). In addition, the LCKD prevented a non-significant increase in TNF-α observed within the high-carbohydrate group, while increases in βHB were associated with reduced leptin, IL-8, M-CSF and protein carbonyl; all of which may have been at least partially due to weight lost throughout the intervention period. Redox responses to

exercise were not measured during this investigation. Therefore, future research should examine acute responses to exercise, particularly in well-trained athletes.

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Conflict of Interest

Dr. Volek receives royalties from books on nutrition and exercise.

Chapter 5

Comparison of Micronutrient Intake of a Low-Carbohydrate Ketogenic Diet versus, a High-Carbohydrate Diet in *ad libitum* non-highly controlled settings in Endurance Athletes

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5.1 Abstract

Background

Low-carbohydrate ketogenic diets (LCKD) have been used in clinical settings for the last century and recently among athletes. Conversely high-carbohydrate (HC) diets are also consumed by athletes to enhance performance. However, nutrient density of a self-selected high-carbohydrate or LCKD within athletes has not been assessed.

Methods

Using a non-randomised control trial, micronutrient density over 3 days, within endurance athletes following a self-selected high-carbohydrate (n = 11) or LCKD (n = 9) over a 12 week dietary and training intervention, was examined. Total blood count and serum ferritin was assessed pre- and post-intervention.

Results

LCKD participants consumed greater protein (+0.4 g·kg, P = 0.016) and saturated fat, with higher omega 3:6 ratio, reduced fibre, vitamin C and copper intake. High-

carbohydrate participants failed to meet carbohydrate (>6 g·kg) and dietary fat (>20 %/kcal) guidelines, with low intakes of selenium, iodine, vitamin A, D, E and B_{12} and riboflavin. Serum ferritin remained unchanged over 12 weeks, while mean corpuscular haemoglobin (MCH) (-1.61 pg, *P* = 0.004) and mean corpuscular haemoglobin concentration (MCHC) (-1.45 g/dl, *P* = 0.005) decreased in the LCKD group.

Conclusion

Irrespective of dietary approach (HC or LCKD), sufficient emphasis should be placed on variety and nutrient density in order to meet recommended micro-nutrient intakes and prevent deficiency in the long-term.

Keywords: nutrient density, high-carbohydrate, ketogenic, diet, endurance athletes

5.2 Introduction

Low-carbohydrate diets, such as Atkins and Paleo have been renowned diets for the past 3 decades (Atkins, 1998). More recently, a low-carbohydrate ketogenic diet (LCKD) has emerged as a popular diet for causing decreases in body mass in overweight patients (Saslow *et al.,* 2017) and improvements in body composition in an athletic population (Wilson *et al.,* 2017; McSwiney *et al.,* 2018; Kephart *et al.,* 2018). Ketogenic diets were initially used as a treatment for children with drug-resistant epilepsy (Woodyatt, 1921; Wilder, 1921), but its efficacy has also been examined in other clinical settings, such as neuromuscular and neurodegenerative diseases (Stafstrom and Rho, 2012; Paoli, Bianco, Damiani and Bosco, 2014), type-2 diabetes mellitus (McKenzie *et al.,* 2017; Saslow *et al.,* 2017), breast cancer (Hyde *et al.,* 2017) and within athletic circles (Phinney *et al.,* 1983; Paoli *et al.,* 2012; Rhyu and Cho, 2014; Zajac *et al.,* 2014; Burke *et al.,* 2017; McSwiney *et al.,* 2018; Kephart *et al.,* 2017; Cipryan *et al.,* 2018). Despite the broad range of research in this area, to our knowledge, no investigation has examined the micro-nutrient density of a LCKD or indeed a high-carbohydrate diet, when consumed by athletes.

A recent hypothetical case study by Zinn *et al.,* (2018) calculated that Australian males and females could exceed the minimum nutrient intake values for all micronutrients on a low-carbohydrate high-fat (LCHF) diet, containing ~80g/d of carbohydrates. Ketogenic diets and LCHF diets are similar in the sense that both restrict carbohydrates and increase dietary fat, however it may be more difficult for an individual following a LCKD to meet nutrient guidelines, such as fibre and thiamine. This is due to the greater level of carbohydrate restriction (<50 g/d) required to induce a metabolic state referred to as

'nutritional ketosis' (Volek and Phinney, 2012) versus a LCHF diet (~130 g/d carbohydrate) (Feinman *et al.,* 2015; Zinn *et al.,* 2018). Heikura *et al.,* (2017) recently carried out a pilot study in elite endurance athletes and discovered that there is disconnect between current sports nutrition guidelines and athletes dietary practices. With these pieces of information in mind, this investigation set out to compare reported nutrient intakes of endurance athletes following a high-carbohydrate (HC) diet or a LCKD during a 12 week dietary and training intervention (McSwiney *et al.,* 2018). This investigation was not a highly controlled short term feeding study, which has been the norm within LCKD endurance performance literature (Phinney *et al.,* 1983; Burke *et al.,* 2017), therefore it provides an opportunity to examine endurance athletes own interpretations of what constitutes a high-carbohydrate and LCKD in non-highly controlled settings. This analysis is based on a nutrient intake reported over 3 days, for each time-point. As is the case with all areas of nutrition science, judgments of deficiency and inadequacy cannot be made from such acute assessments, particularly when evidence is provided by dietary recall/food diaries (Burke *et al.,* 2001). Therefore this research investigates how micro-nutrient intake changes when a high-carbohydrate athlete either tries to increase carbohydrate intake or adhere to a LCKD. It additionally examines how these 12 week changes affect full blood count and iron status.

5.3 Materials and Methods

Details of this investigation have been described previously (McSwiney *et al.,* 2018). In brief, forty-seven male endurance athletes aged 19-40 years participated in a 12 week non-randomised control trial, comparing performance (McSwiney *et al.,* 2018). All participants competed in endurance events (>2 years), were 18-40 years of age and

habitually consumed a high-carbohydrate diet (~50% of total calories). Twenty participants completed all aspects of the dietary and training intervention (McSwiney *et al.,* 2018), whilst a total blood count was performed on 19 participants (HC *n*=10, LCKD *n*=9), all of which have been included within this manuscript for analysis. All procedures were approved by the research ethics committee at Waterford Institute of Technology, IE (ref: 15/HSES/03) and all participants' provided written informed consent.

5.3.1 Dietary Intervention

Food diaries were obtained at baseline using a 3-day weighed food diary (2 week days and 1 weekend day). These were analysed using Nutritic's dietary analysis software (Nutritic's Professional v3.09, Nutritic's, Dublin, Ireland). The macronutrient goals were: HC 65% CHO, 20% fat and 14% protein or LCKD >75% fat, 10-15% protein and <50g/d CHO. At the outset participants in each group received a nutritional handout, detailing how best to formulate their diet according to daily energy requirements. Participants in each group received nutritional counselling throughout the 12 week intervention, as previously described (McSwiney *et al.,* 2018). Example meal plans for the high-carbohydrate and LCKD groups are detailed in Table 1. Three day food diaries were also obtained in week 12 and analysed using the Nutritic's dietary analysis software. Average nutrient intake, including glycaemic load (GL) and potential renal acid load (PRAL) for 3 days pre- and post-dietary intervention were obtained from the dietary analysis report.

Table 5.1. Example meal plans for high-carbohydrate (HC) and low-carbohydrate ketogenic diet (LCKD) groups

	Breakfast	Snack	Lunch	Dinner	Other
HC	80 g granola, 135ml semi-skimmed milk, 1 med apple, 1 med orange, 1 med banana, 6oz Americano, 30ml semi-skimmed milk	2 tbsp natural yoghurt, 20 g almond butter, 1 med banana	2 cups raw baby spinach, ½ avg. avocado, 90 g chicken, 335 g sweet potato, 70 g cherry tomatoes, 35 g beetroot	80 g white fish, 405 g new potatoes, 2 tsp low-fat butter, 85 g broccoli boiled in unsalted water, 90 g steamed baby carrots	0-1 scoop whey protein (containing 20-25 g protein) and/or 0-4 energy gel(s) (containing 20-30 g of carbohydrate)
LCKD	4 avg eggs scrambled, with 1 tbsp full-fat butter, 1 tbsp full-fat cream, 2 cups of fried baby spinach, 1 cup of fried kale, 1 tsp chia seeds	6 oz Americano, 1 tbsp full-fat butter, 1 tbsp coconut oil	100g salmon, 1 avg avocado, 10.5 g blueberries, 40 g raspberries, 30 g mixed nuts, 1 tbsp coconut oil, 2 tbsp olive oil	120 g sirloin steak, 1.5 cups steamed broccoli, 2 cups steamed cauliflower, 2 tbsp full-fat butter, 2 tbsp olive oil	135 g Greek yoghurt, 1 tbsp cream, 2 avg squares 85-90% dark chocolate, 40 g raspberries and bouillon cubes or homemade broth

Abbreviations: Med = medium, tbsp = tablespoon, tsp = tea spoon, avg = average, oz = ounces.

5.3.2 Blood Analysis

Fasting blood samples were collected into an EDTA tube from an antecubital vein using a 21G needle (BD Diagnostics) at pre- and post-intervention testing, subsequent to a 12 hour overnight fast. Blood samples were put through a haematology analyser (Beckman Coulter AcT diff Analyzer) 20-30 minutes subsequent to collection. The haematology analyser measured participants' white blood cells, lymphocytes, monocytes, granulocytes, red blood cells, haematocrit, mean corpuscular volume (MCV), mean corpuscular width (MCW), red blood cell distribution width (RDW) and platelet content.

5.3.3 Net Endogenous Acid Production

Net endogenous acid production (NEAP) was estimated using protein and potassium intakes at pre- and post-intervention testing, using two algorithms from Frassetto, Todd, Morris and Sebastian (1998).

1. Estimated $NEAP_1$ ($mEq \cdot day^{-1}$) = [0.91 × protein ($g \cdot day^{-1}$] − [0.57 × potassium ($mEq \cdot day^{-1}$)] +21
2. Estimated $NEAP_2$ ($mEq \cdot day^{-1}$) = [54.5 × protein ($g \cdot day–^{1}$)/potassium ($mEq \cdot day^{-1}$)] − 10.2

5.3.4 Statistical Analysis

IBM Statistics SPSS 24 (Illinois, Chicago, USA) was used for statistical analysis. Data was tested for normality, with parametric tests used for normally distributed data or non-parametric, for data not normally distributed. Independent sample t-tests or Mann Whitney U test (if data was not normally distributed) were used to determine differences between high-carbohydrate and LCKD groups at baseline, with the alpha

level for significance set at $P < 0.05$. An ANCOVA was used for statistical analysis at post-intervention testing, with pre-intervention measures acting as a covariate. Paired samples t-tests or Wilcoxon signed ranks test (if data was not normally distributed) examined changes over time within each group, if ANCOVA *P* value was <0.05.

5.4 Results

5.4.1 Nutrient Intake

Table 2 presents mean nutrient intake for high-carbohydrate and LCKD groups, as well as recommended daily allowances (RDAs) for European citizens (European Food Safety Authority, 2017) and endurance athletes (Burke, Hawley, Wong and Jeukendrup, 2011; Thomas, Erdman and Burke, 2016).

No changes in intake of energy, potassium, calcium, zinc, manganese, iodine, vitamin E and K_1, riboflavin, niacin, vitamin B_6, folic acid, vitamin C or caffeine took place across the intervention period (*ANCOVA* $P > 0.05$). As expected carbohydrate consumption decreased (-4.7 g·kg, $P = 0.000$) and fat consumption increased (+56.8 %/kcal, $P = 0.000$) in the LCKD group, causing a reduction in GL (-210, $P = 0.000$). Post-intervention carbohydrate intake within the high-carbohydrate group failed to reach >6 g·kg body weight recommended for endurance athletes (Thomas *et al.,* 2016). Protein remained unchanged in the high-carbohydrate group ($P = 0.159$) and increased +0.4 g·kg in LCKD participants across the intervention period. Both carbohydrate ($P = 0.000$), fat ($P = 0.000$) and protein ($P = 0.016$) in g·kg differed between groups at post-intervention.

Table 5.2. Nutrient intake for high-carbohydrate (HC) and low-carbohydrate ketogenic diet (LCKD) groups with Recommended Intakes and Dietary Reference Values (DRV)

	HC diet (*n* = 10)		LCKD (*n* = 9)			
	Pre	Post	Pre	Post	ANCOVA	Recommended Intakes/DRV
Nutrient	Mean ± SD [a]	Mean ± SD	Mean ± SD [a]	Mean ± SD	*P* value	
Energy, kcal	2366 ± 774	2672 ± 363	2843 ± 558	3022 ± 911	0.695	NA
Carbohydrates, g·kg	3.9 ± 1.3	5.3 ± 1.4 [c]	5.2 ± 1.3[b]	0.5 ± 0.1[c]	0.000*	6-10[d]
Protein, g·kg	1.5 ± 0.4	1.2 ± 0.2	1.2 ± 0.2	1.6 ± 0.3[c]	0.031*	1.2–1.7[e]
Fat, %/kcal	29.2 ± 13.4	18.6 ± 3.7	20.4 ± 12.3	77.2 ± 24. 8[c]	0.000*	20-35%[e]
Saturated Fat, %/kcal	12.4 ± 6.2	5.6 ± 2.2[c]	7.8 ± 1.5	29.5 ± 9.1[c]	0.000*	ALAP[f]
Omega 6:3 ratio	5.1:1	6.0:1	10:1	2.7:1[c]	0.004*	NA[f]
Fibre, g	42.8 ± 10.1	56.0 ± 19.8	48.3 ± 9.0	19.2 ± 4.9[c]	0.000*	25[f]
Free sugars, %/kcal	2.5 ± 3.8	2.2 ± 1.7	2.2 ± 2.9	0.7 ± 0.3	0.021*	<10 [g]
Sodium, mg	2802 ± 1344	3714 ± 1352	3403 ± 1051	1713 ± 1100[c]	0.006*	<2,000[h]

Abbreviations: g = gram; kcal = calorie; mg = milligram; %/kcal = percentage energy

Table 5.2 Contd. Nutrient intake for high-carbohydrate (HC) and low-carbohydrate ketogenic diet (LCKD) groups with Recommended Intakes and Dietary Reference Values (DRV)

	HC diet (*n* = 10)		LCKD (*n* = 9)			
	Pre	Post	Pre	Post	ANCOVA	Recommended Intakes/DRV
Nutrient	Mean ± SD [a]	Mean ± SD	Mean ± SD [a]	Mean ± SD	*P* value	
Potassium, mg	4301 ± 1495	3650 ± 497	4674 ± 1373	4166 ± 729	0.491	3,500[f]
Chloride, mg	4697 ± 2519	7618 ± 3176	4983 ± 3047[b]	2181 ± 622[c]	0.001*	NA
Calcium, mg	1223 ± 424	1030 ± 278	1154 ± 352	928 ± 240	0.714	950[f]
Phosphorus, mg	2085 ± 565	2190 ± 460	2102 ± 422	1838 ± 379 [c]	0.040*	550[f]
Magnesium, mg	437 ± 121	513 ± 125	509 ± 124	380 ± 39[c]	0.017*	350[f]
Iron, mg	18.8 ± 6.9	18.2 ± 5.4	18.7 ± 4.2	12.0 ± 2.0[c]	0.012*	11 [f]
Zinc, mg	18.2 ± 13.1	13.4 ± 2.2	14.3 ± 4.2	14.4 ± 3.7	0.810	16.3[f]
Copper, mg	1.7 ± 0.7	1.9 ± 0.4	1.6 ± 0.3	1.3 ± 0.1 [c]	0.011*	1.6[f]
Manganese, mg	6.0 ± 2.2	10.1 ± 3.7 [c]	8.0 ± 2.5	2.3 ± 0.4 [c]	0.000*	3.0[f]

Abbreviations: mg = milligram

Table 5.2. Contd. Nutrient intake HC and LCKD groups with Recommended Intakes and Dietary Reference Values (DRV)

	HC diet (*n* = 10)		LCKD (*n* = 9)			
	Pre	Post	Pre	Post	ANCOVA	Recommended Intakes/DRV
Nutrient	Mean ± SD [a]	Mean ± SD	Mean ± SD [a]	Mean ± SD	*P* value	
Selenium, µg	63.9 ± 23.9	56.6 ± 15.0	39.3 ± 20.6[b]	136.3 ± 71.2[c]	0.017*	70[f]
Iodine, µg	218 ± 142	95 ± 35 [c]	80 ± 45	250 ± 108	0.130	150[f]
Vitamin A, µg	1565 ± 1335	581 ± 450 [c]	714 ± 637 [b]	1849 ± 558 [c]	0.014*	750[f]
Vitamin D, µg	5.4 ± 5.2	3.5 ± 5.2[c]	2.7 ± 2.4	17.6 ± 4.5[c]	0.000*	15[f]
Vitamin E, mg	11.1 ± 8.5	5.9 ± 2.3	8.7 ± 7.7	23.7 ± 4.7 [c]	0.156	13[f]
Vitamin K_1, µg	103.3 ± 100	101.4 ± 115	34.1 ± 62[b]	301.3 ± 174 [c]	0.017*	70[f]
Thiamine, mg/MJ	0.21 ± 0.06	0.22 ± 0.04	0.23 ± 0.05	0.11 ± 0.04 [c]	0.000*	0.1[f]
Riboflavin, mg	2.0 ± 0.9	1.2 ± 0.4	2.3 ± 0.6	2.8 ± 0.7	0.239	1.6[f]
Niacin, mg NE/MJ	5.7 ± 2.0	4.6 ± 1.4	4.9 ± 1.9	4.5 ± 0.7	0.517	1.6[f]
Pantothenic Acid, mg	8.1 ± 3.6	6.1 ± 1.2	8.9 ± 3.2	10.2 ± 1.7	0.002*	5.0[f]

Abbreviations: mg = milligram; µg = micro-gram; MJ = milligram per mega-joule

Table 5.2. Contd. Nutrient HC and LCKD groups with Recommended Intakes and Dietary Reference Values (DRV)

	HC diet (*n* = 10)		LCKD (*n* = 9)			
	Pre	Post	Pre	Post	ANCOVA	Recommended Intakes/DRV
Nutrient	Mean ± SD [a]	Mean ± SD	Mean ± SD [a]	Mean ± SD	*P* value	
Vitamin B_6, mg	2.7 ± 0.8	3.2 ± 0.3	3.2 ± 0.9	3.6 ± 0.8	0.229	1.7[f]
Folic Acid, µg DFE	379 ± 145	311 ± 75	318 ± 164	309 ± 68	0.644	330[f]
Vitamin B_{12}, µg	5.4 ± 2.5	2.9 ± 2.3 [c]	4.9 ± 2.3	13.5 ± 4.5 [c]	0.045*	4.0[f]
Biotin, µg	45.0 ± 16.1	46.5 ± 14.6	50.3 ± 24.1	70.4 ± 14.6 [c]	0.034*	40[f]
Vitamin C, mg	143 ± 85	123 ± 71	134 ± 84	86 ± 68	0.203	110[f]
GL	146 ± 50	200 ± 86	216 ± 62 [b]	6 ± 3 [c]	0.000*	NA[f]
PRAL	26.0 ± 32.9	35.7 ± 16.6	10.0 ± 18.0	22.9 ± 27.2	0.012*	NA
Caffeine, mg	111.6 ± 66.9	149.6 ± 65.8	113.3 ± 104.8	162.6 ± 113.0	0.682	NA

Abbreviations: MJ = mega-joule, NE = niacin equivalents, DFE = dietary folate equivalents, ALAP = as low as possible, NA = not applicable.
[a] Original means and standard deviations. *ANCOVA significant difference at $P < 0.05$. [b] significant difference between groups at baseline. [c] significant difference ($P < 0.05$) within group between pre- and post-intervention testing. [d] RDA from Burke *et al.,* (2011). [e] RDA from Thomas, Erdman and Burke (2016). [f] RDA from European Food Safety Authority (2017). [g] World Health Organization (2015). [h] World Health Organization (2015a).

Additional energy from dietary fat resulted in increases in energy contribution from saturated fat in the LCKD group (+21.7 %/kcal, *P* = 0.000), while the high-carbohydrate group reduced their intake by -6.8 %/kcal (*P* = 0.015). Omega 6:3 ratio remained unchanged within high-carbohydrate participants (*P* = 0.516), but reduced within LCKD participants (*P* = 0.004) with increased intake of omega 3 fatty acids. Intakes of fibre (-29.1 g/d, *P* = 0.041) and copper (-0.3 mg/d, *P* = 0.000) decreased within LCKD participants to below the recommended levels. Intakes of chloride (-5428.1 mg/d, *P* = 0.001), phosphorus (-263.7 mg/d, *P* = 0.039), magnesium (-129.4 mg/d, *P* = 0.011), iron (-6.7 mg/d, *P* = 0.002) and thiamine (-0.12 mg/MJ, *P* = 0.000) decreased within LCKD participants, but remained within the recommended levels. Intakes of selenium (+93.0 µg/d, *P* = 0.003), vitamin A (1135.5 µg/d, *P* = 0.009), D (14.9 µg/d, *P* =0.000) and B_{12} (8.6 µg/d, *P* = 0.001) and biotin (20.1 µg/d, *P* = 0.006) increased within LCKD participants, but remained unchanged within high-carbohydrate participants (*all P* > 0.05).

5.4.2 Net Endogenous Acid Production

Table 3 presents the NEAP values for high-carbohydrate and LCKD groups. There were no differences between groups $NEAP_1$ and $NEAP_2$ at baseline (*both P* > 0.05). Adaptation to a LCKD increased $NEAP_1$ (*P* = 0.038) and caused a difference between groups at post-intervention testing (*P* = 0.041). No changes in $NEAP_2$ were observed (*all P* > 0.05).

Table 5.3. Net endogenous acid production (NEAP) for high-carbohydrate (HC) and low-carbohydrate ketogenic diet (LCKD) groups using two algorithms by Frassetto *et al.,* (1998)

	HC group (*n* = 10)		LCKD group (*n* = 9)	
	Pre	Post	Pre	Post
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
$NEAP_1$, $mEq \cdot day^{-1}$	64.9 ± 27.3	52.8 ± 20.9	53.0 ± 16.7	79.0 ± 30.1*†
$NEAP_2$, $mEq \cdot day^{-1}$	52.7 ± 19.2	44.8 ± 14.8	42.3 ± 13.1	57.4 ± 16.9

* Significant difference ($P < 0.05$) within groups between pre and post-intervention testing.

† Significant difference ($P < 0.05$) between groups at post-intervention testing.

5.4.3 Blood Analysis

Table 4 presents changes in blood measures for high-carbohydrate and LCKD groups. Mean corpuscular haemoglobin (-1.61 pg, $P = 0.004$) and MCHC (-1.45 g/dl, $P = 0.005$) both decreased in the LCKD group. No other significant changes were observed (ANCOVA $P > 0.05$), but a medium effect in RDW (HC = -0.13 µm, $P = 0.417$; LCKD = +0.94 µm, $P = 0.219$) occurred.

Table 5.4. Haematology results for high-carbohydrate (HC) and low-carbohydrate ketogenic diet (LCKD) groups

Blood Measures	HC group (n = 10) [a]							LCKD group (n = 9) [a]							ANCOVA		
	Pre			Post			Change	Pre			Post			Change			
	Mean	±	SD	Mean	±	SD	Mean	Mean	±	SD	Mean	±	SD	Mean	F – value	*P* value	ES [b]: Np^2
RBC, 10^{-6} µl	4.59	±	0.33	4.30	±	0.63	-0.29	4.67	±	0.42	4.44	±	0.61	-0.23	(1,17) = 0.074	0.788	0.00
WBC, 10^{-3} µl	5.7	±	1.0	5.5	±	1.0	-0.2	6.0	±	1.9	6.5	±	2.8	+0.5	(1,17) = 0.875	0.363	0.049
Haemoglobin, g/dl	14.15	±	1.25	13.36	±	1.87	-0.79	14.29	±	0.99	12.95	±	1.62	1.34	(1,17) = 0.414	0.529	0.025
Haematocrit, %	0.41	±	0.03	0.38	±	0.05	+0.03	0.41	±	0.03	0.39	±	0.05	-0.02	(1,17) = 0.140	0.752	0.006
MCV, fL	90.47	±	4.52	89.35	±	4.78	-1.12	89.19	±	3.14	88.31	±	2.40	-0.88	(1,17) = 0.226	0.641	0.014
MCH, pg	30.81	±	1.78	31.14	±	2.00	+0.33	30.88	±	1.75	29.27	±	1.63 [c]	-1.61	(1,17) = 7.190	0.016*	0.310
MCHC, g/dl	33.66	±	1.95	34.85	±	1.52	+1.19	34.59	±	0.87	33.14	±	1.33 [c]	-1.45	(1,17) = 5.426	0.033*	0.250
RDW, µm	12.65	±	0.70	12.52	±	0.69	-0.13	12.89	±	0.44	13.83	±	2.34	+0.94	(1,17) = 1.897	0.187	0.106
Platelets, 10^{-6}µl	204.6	±	36.2	202.9	±	40.4	-1.7	194.5	±	49.1	208.5	±	56.6	+14.0	(1,17) = 0.957	0.342	0.053
Lymphocytes, % WBC	36.3	±	7.2	34.5	±	6.5	+2.2	34.8	±	7.1	36.2	±	8.1	+1.4	(1,17) = 1.023	0.326	0.057
Monocytes, % WBC	5.7	±	1.4	7.0	±	2.5	+1.3	5.7	±	1.5	6.0	±	1.2	+0.3	(1,17) = 1.344	0.262	0.073
Granulocytes, % WBC	57.9	±	7.2	58.1	±	6.1	+0.2	59.4	±	7.5	57.7	±	8.4	-1.7	(1,17) = 0.204	0.657	0.012
MPV, fL	8.1	±	0.6	7.9	±	0.8	-0.2	8.4	±	1.2	7.9	±	0.9	-0.5	(1,17) = 0.254	0.621	0.015
Serum Ferritin, ug/mL	117.6	±	107.0	149.2	±	103.6	+31.6	235.7	±	90.3	249.9	±	113.6	+14.2	(1,11) = 0.004	0.951	0.000

[a] Original means and standard deviations, i.e., without adjustment for covariate. [b] ES = effect size. [c] significant difference within group between pre- and post-intervention testing. η_p^2 = 0.01 (small effect), η_p^2 = 0.09 (medium effect), η_p^2 = 0.25 (large effect) [25]. * ANCOVA significant difference at $P < 0.05$. [c] significant difference within group between pre and post-intervention. Abbreviations: RBC = red blood cells, WBC = white blood cells, MCV = mean corpuscular volume, MCH = mean corpuscular haemoglobin, MCHC = mean corpuscular haemoglobin concentration, RDW = red blood cell distribution width, % WBC = relative to total white blood cell count; MPV = mean platelet volume.

5.5 Discussion

Examination of nutrient density within endurance athletes who adhered to a high-carbohydrate or LCKD revealed consumption of a high-carbohydrate diet resulted in greater reported intakes of fibre, sugar, sodium, chloride, phosphorus, magnesium, iron, copper, manganese and thiamine, with higher GL and greater PRAL, compared to LCKD consumption. Conversely, free-living consumption of a LCKD resulted in greater intake of saturated fat, protein, a higher omega 3:6 ratio and greater intakes of selenium, vitamins A, D and Vitamin K_1, pantothenic acid, Vitamin B_{12}, biotin and higher NEAP. Blood analysis revealed consumption of a LCKD for 12 weeks reduced MCH and MCHC amongst LCKD participants, but no change in iron stores were evident.

The American College of Sports Medicine advocates a nutrient dense high-carbohydrate diet with appropriate energy intake for optimal sports performance and health amongst endurance athletes (Thomas *et al.,* 2016). However, metabolic adaptations such as decreased reliance on carbohydrates (Phinney *et al.,* 1983; Volek *et al.,* 2016; McSwiney *et al.,* 2018; Maunder, Kilding and Plews, 2018) , improvements in power to weight ratio (Zajac *et al.,* 2014; Zinn *et al.,* 2017; McSwiney *et al.,* 2018) and emerging epidemiological evidence (Saslow *et al.,* 2017; Newman *et al.,* 2017) have made a LCKD a desirable approach for some endurance athletes.

Carbohydrate intake in the high-carbohydrate group was marginally below the 6-12 g·kg recommended range for endurance athletes exercising 1-3 hours a day (Burke *et al.,* 2011). This is contrary to a review which found male athletes generally achieve their carbohydrate recommendations (Burke *et al.,* 2001). Notably however, Burke *et al.,* (2001) included competition dietary intakes where energy and carbohydrate

requirements would be greater. In contrast, participants' food diaries within the current investigation were obtained during the final week of a training intervention, when participants' completed 11.7 ± 1.7 hours of endurance training (~1.6 hours' a day) (McSwiney *et al.,* 2018). Another factor which likely contributed to sub-optimal carbohydrate intake was the prioritisation of whole foods, resulting in a high fibre intake (Table 5.2). Gastrointestinal limits to bulky, high fibre foods such as whole grains products and potatoes are reported to impede optimal carbohydrate intakes (Burke *et al.,* 2001). By design, carbohydrate intake within the LCKD group was beneath this threshold (0.5 g·kg) (Table 5.2) in order to induce a state of nutritional ketosis (i.e., elevate blood ketones to >0.5 mmol/L) (Volek and Phinney, 2012). When adopting a LCKD, individuals exclude many foods high in fibre, such as cereals and wholegrain products. That being said, fibre is not exempt on a well-formulated LCKD (Volek and Phinney, 2012), green leafy fibrous vegetables, nuts, seeds and low-carbohydrate fruits such as avocados are nutritious sources of fibre encouraged on a LCKD. On a high-carbohydrate diet, a high fibre intake is associated with reduced risk of various cancers (Kritchevsky, 1986) and thought to positively impact on gut microbiome diversity (Menni *et al.,* 2017). Fibre undergoes bacterial fermentation in the gut to form short-chain fatty acids, principally butyrate (Kanauchi *et al.,* 1999). Butyrate is an important substrate for epithelial cells within the intestinal tract and thought to improve gut lining integrity (Hague, Singh and Paraskeva, 1997). In addition, butyrate has been shown to inhibit NF-kB (McNeil, Cummings and James, 1978), a major transcription factor that regulates the expression of various chemokines, cytokines and adhesion molecules (Monaco and Paleolog, 2004; Winther, Kanters and Kraal, 2005). Conversely, a reduced fibre intake on a LCKD is not thought to be as problematic. Lower fibre content cause a reduction in

bacterial fermentation from butyrate, however, the very nature of a LCKD is to increase endogenous production of ketone bodies, namely βeta-hydroxybutyrate (βHB) (Evans, Cogan and Egan, 2016). In addition to butyrate being produced endogenously by hepatic tissues, butyric acid can be exogenously sourced from foods that are otherwise restricted on a high-carbohydrate diet, such as dietary fat and cholesterol. For example, a tablespoon of full-fat butter contains 14 g of fat and 560 mg of butyric acid. Therefore, for persons following a LCKD (70-80 %/kcal fat), consuming in excess of 1000 mg/d of butyric acid from natural sources is easily attainable (Cavaleri and Bashar, 2018). Although not extensively studied in larger mammals, mice consuming a LCKD with trace carbohydrates (% carbohydrate:fat:protein = 1:89:10) and circulating βHB of ~0.8 mM experienced an 8.8% increase in median lifespan and a reduced incidence of tumors at the time of death (Roberts *et al.,* 2017) versus a low-carbohydrate high-fat diet (% 10:70:20) and control diet (% = 65:17:18).

Post-intervention protein intake differed between groups (P = 0.016) (Table 5.2), however each group's intake was within the 1.2-2.0 g·kg range recommended for endurance athletes (Thomas *et al.,* 2016). Additionally, the fact neither group experienced loss of lean body mass (lean body mass: HC = +0.1 kg and LCKD +0.3 kg, P > 0.05, ES: 0.068) (McSwiney *et al.,* 2018) is a good indication each group were consuming adequate energy and protein across the intervention period. Had the high-carbohydrate group increased protein consumption (g·kg) it may have helped with satiety and contributed to improvements in body composition (Aragon *et al.,* 2017a), however this may have further impeded participants ability to meet carbohydrate guidelines (6-12 g·kg) (Burke *et al.,* 2011; Thomas *et al.,* 2016).

Dietary fat increased to 77.2% energy intake within LCKD participants but non-significantly (P = 0.059) decreased to 18.6% energy intake within high-carbohydrate participants (Table 5.2). Following popular publications in lay press (Volek and Phinney, 2011; Volek and Phinney, 2012) this investigation encouraged consumption of foods containing monounsaturated (e.g. nuts, avocados, olive oil) and saturated fats (e.g. fatty animal meat, butter, coconut oil) and less so of foods containing polyunsaturated fats (e.g. soy and corn oil). This was reflected within participants' reported diets, as monounsaturated (34.2% total energy) and saturated fat (29.5% total energy) comprised ~63.7% of total energy. Saturated fat has received a lot of attention in recent years', in light of two systematic reviews concluding there was no association between saturated fat intake and risk of cardiovascular disease (CVD) (Chowdhury *et al.,* 2014; de Souza *et al.,* 2015). These publications along with growing interest in low-carbohydrate diets resulted in Time Magazine making 'we were wrong about saturated fat/butter' a cover story in 2016 (Time, 2016). Importantly however, the aforementioned investigations (Chowdhury *et al.,* 2014; de Souza *et al.,* 2015) relied on observational studies which examined individual fatty acids and their association with CVD. In contrast, a prospective cohort study found greater intakes of polyunsaturated fatty acids and whole grains were associated with reduced risk of CVD (P < 0.001), while carbohydrates from refined sources containing added sugars, were positively associated with risk of CVD (P = 0.04) (Li *et al.,* 2016). In addition, a meta-analysis containing 15 randomised control trials calculated for every 5% decrease in calories from saturated fats was replaced with polyunsaturated fats such as, fish, nuts and seeds, there would be a 10% reduced risk of CVD (Mozaffarian, Micha and Wallace, 2010). High-carbohydrate participants' within the current investigation reduced their saturated fat intake by -7%

to 5.9% of total energy (Table 5.2), but this was accompanied by a +10.9% increase in carbohydrates and not polyunsaturated fats, thus a homogenous improvement in cardiovascular risk cannot be assumed. Conversely, energy contribution from free sugar (Table 5.2) remained unchanged and importantly below the recommended range set out by the World Health Organisation (2015), therefore increased energy contribution from carbohydrates was primarily sourced from whole foods, such as wholegrain/brown bread, starchy vegetables and wholegrains. In contrast, the 29.5% of energy derived from saturated fat within LCKD participants' is considerably more than <10% threshold set out within nutritional guidelines (European Food Safety Authority, 2017). Increased saturated fat was achieved by increasing fatty animal meat consumption (i.e., choosing 70% lean versus 95% lean cuts), opting for full fat dairy products such butter and cream and by using coconut oil when cooking. An epidemiological investigation by Forsythe *et al.,* (2008) incorporating a LCKD containing ~21.7% of energy from saturated fat in addition with significant weight loss, improved circulating fatty acid composition and markers of inflammation, suggesting higher intakes of saturated fat may not be problematic when combined with carbohydrate restriction. Increases in saturated fat occurred in the presence of increased polyunsaturated fatty acid intake within LCKD participants with improved omega 3:6 ratio (Table 5.2). A high omega 6:3 ratio is pro-inflammatory and thought to accelerate pathogenesis of chronic diseases, such as CVD, cancer and autoimmune diseases (Kang, 2011; Simopoulos, 2008). Subsequent work demonstrated a high saturated fat intake (84 g/d / 29.6% total energy) did not accumulate in plasma lipid fractions when carbohydrates were restricted (~47 g/d) and that incrementally decreasing saturated fat intake to ~11.4% total energy and increasing dietary carbohydrates to 345 g/d did not cause a consistent decrease in plasma

saturated fat (Volk *et al.,* 2014). The Volk *et al.,* (2014) investigation had a number of strengths, namely it was a feeding study so it possessed considerable dietary control, throughout each 3 week incremental feeding phase (6 in total) dietary protein was matched (1.8 g·kg) and caloric intake remained the same, invariably removing changes in body composition as a confounding variable. The investigation also emphasized wholegrain foods and low glycaemic sources of carbohydrates. However, when participants' consumed the diet highest in carbohydrate (345 g/d), saturated fat (11.4% total energy) was the primary source of fat, with small amounts of cardio-protective polyunsaturated fatty acids (~5.0% total energy) (Endo and Arita, 2016) and monounsaturated (~8.6% total energy) fats. Thus, although the diet was low in saturated fat, the overall composition of the diet did not match dietary characteristics previously described by Li *et al.,* (2016) or nutritional guidelines (European Food Safety Authority, 2017). Other investigations involving LCKDs have noted positive increases in HDL-cholesterol (Bazzano *et al.,* 2015; Yancy *et al.,* 2004; Tay *et al.,* 2008), decreases in triglycerides (Volek *et al.,* 2009; Brehm *et al.,* 2003; Tay *et al.,* 2008; Volek *et al.,* 2009) and a reduced proportion of small, dense LDL particles and increases in non atherogenic large LDL particles (Aude *et al.,* 2004; Volek, Sharman and Forsythe, 2005; Wood *et al.,* 2006; Keogh *et al.,* 2008; Forsythe *et al.,* 2008; Volek *et al.,* 2009). Based on epidemiological evidence and knowledge within lipidology, athletes following a high-carbohydrate diet should be advised to keep saturated fat intake low (<10% of total energy) and to prioritise polyunsaturated and monounsaturated fats (+10-20% of total energy), while persons adhering to a LCKD appear to tolerate higher amounts of saturated fat (20-30% of total energy) once carbohydrates are restricted (i.e., <50 g/d)

(Volk *et al.,* 2014). However, more longitudinal investigations are required, particularly in an athletic population.

With the exclusion of wholegrain foods, a decrease in reported thiamine consumption was observed within the LCKD group. This was not a surprising, as wholegrain foods are the predominant sources in modern culture. Thiamine can be sourced from organ meats derived from pork, poultry and beef products, although organ meat may not be a dietary preference for everyone. However, as outlined by Zinn *et al.,* (2018) in the context of a low-carbohydrate high-fat diet containing ~80 g/d of carbohydrates, one must question if an individual's thiamine requirements are reduced on LCKD, as one of the key functions of thiamine is in the metabolism of carbohydrates (Manzetti, Zhang and Van Der Spoel, 2014).

During our assessment, LCKD participants had greater intakes of Vitamins A, D, K_1, pantothenic acid, biotin and vitamin B_{12} compared to high-carbohydrate participant. Vitamin D insufficiency is common in an athletic population and dietary interventions are often unsuccessful at remedying deficiencies (Larson-Meyer and Kentz, 2010), therefore its recommended athletes supplement with vitamin D above their RDA (Thomas *et al.,* 2016). Previously, it was observed that doses of vitamin D ranging from 1,000-1,500 µg/d caused no adverse effects on health (McCullough and Amend, 2017). Therefore, although LCKD participant's intake increased, its unlikely ~17.6 µg/d would cause adverse effects, such as hypercalcemia. Average intakes of vitamin B_{12} range from 4.2-8.6 µg/d in adults, however intakes in males are higher in a number of European countries (European Food Safety Authority, 2017), but not considered harmful (Carmel, 2008). Although only a snapshot of high-carbohydrate participant's diets, vitamin A and

B_{12} consumption within the current group was below recommended levels for European males (Table 5.2). Individuals following a high-carbohydrate diet should be advised to keep dietary fat ~20-30% of total energy to ensure a diverse range of fat soluble vitamins. A recent position stand from the American College of Sports Medicine stated that there is no performance benefit to consuming <20% of total energy from fat (Thomas *et al.,* 2016). Increases in fat soluble vitamins such as vitamin D can be achieved by incorporating more fatty fish (i.e., salmon, tuna or mackerel), cheese and eggs.

Electrolyte balance is an important consideration when working with any athlete, however when following a LCKD its paramount athletes' take additional measures to ensure adequate hydration, as the absorption of glucose across the intestinal wall is very much dependent on sodium, with the involvement of a sodium/glucose co-transporter (Crane, 1960). Low-carbohydrate experts recommend consuming ~5000 mg/d of sodium on a LCKD to preclude orthostatic symptoms (Volek and Phinney, 2012). Therefore, LCKD participants' were recommended to supplement sodium at meal times (i.e., table salt), consume electrolytes when exercising and consume 1-2 g/d of sodium from bouillon cubes or homemade broth (McSwiney *et al.,* 2018). Additionally, LCKD participants' were advised to savage nutrient dense meat drippings when cooking to garnish dishes and recommended to grill meat and steam vegetables, as magnesium and other nutrients can be spoiled through boiling/evaporation (Volek and Phinney, 2012). Despite these measures, LCKD participants' reported sodium intake was sub optimal even by general dietary guideline standards (<2000 mg), while they consumed adequate potassium and magnesium (Table 5.2). Orthostatic symptoms were not reported to the researcher subsequent to the first 0-2 weeks of the intervention, when participants'

were still developing new dietary routines and physiologically adapting to their new lifestyle. Thus, we must assume that the figure presented in Table 2 is an accurate representation of LCKD participants' daily intake and sufficient to preclude orthostatic symptoms in trained individuals. Conversely, LCKD participants' may have poorly reported their added salt (sodium) at meal times. Appropriate sodium, potassium and magnesium intakes are necessary to achieve water balance and maintain nerve and muscle function (Volek and Phinney, 2012). Phosphorus, magnesium, iron and copper were consumed in higher amounts by high-carbohydrate participants with lower amounts consumed by the LCKD group.

Iron deficiencies, even in the absence of anaemia can impair muscle function (Lukaski, 2004; Driskell and Wolinsky, 2006), which in an athletic population can attribute to poor work capacity and poor training adaptations (Thomas *et al.,* 2016). Endurance athletes are at an elevated risk, along with persons who follow a vegan/vegetarian style of eating and/or donate blood frequently (Driskell and Wolinsky, 2006). Males at an increased risk are recommended to be screened regularly by a doctor and consume ~8 mg/d (Thomas *et al.,* 2016) above their recommended daily allowance (~11 mg/d) (European Food Safety Authority, 2017). Therefore, iron intakes met the requirements for the general population in each group (Table 5.2), but decreases observed within the LCKD meant their intake was below the RDA specific for endurance athletes (~19 mg/d) (Thomas *et al.,* 2016). A well-formulated ketogenic diet should include a variety of foods, including organ meat, red meat, eggs and green leafy vegetables which should provide sufficient iron intake, hence the participants within the current investigation may not have consumed a sufficient variety of these advised foods. Of additional concern, the LCKD

observed decreases in copper (Table 5.2). A recent article highlighted the development of copper deficiency anaemia in a child transitioning from a formula based LCKD to a pureed food-based LCKD, with the deficiency being rectified by copper supplementation (Chin, 2018). Similar to iron, reduced variety in food intake may have resulted in reduced copper intake within LCKD participants, as such it is paramount this aspect is emphasized to anyone adopting a LCKD. Organ meat, nuts, soy-products, beans and sea-food are all high in copper, so when included within a LCKD should provide sufficient intake. Iron is primarily found within haemoglobin in the blood, a key molecule responsible for transporting oxygen (Otto, Montgomery and Richards, 2013). Post-intervention, the high-carbohydrate groups' haemoglobin count was >13.0 g/dl (Table 5.4), a recommended threshold set for healthy males (World Health Organization, 2011). In contrast, post-intervention haemoglobin was below ~13 mg/dL threshold (12.95 ± 1.62 mg/dL) within the LCKD group, placing LCKD participants 'marginally above mild anaemia threshold (i.e., 11-12.9 g/dl) (World Health Organization, 2011). In addition, LCKD participants' experienced decreases in MCH and MCHC (Table 5.4). MCHC (33-36 g/dl) and MCH (27-31 pg) remained within the normal range in each group, however haematocrit concentrations in each group were low (normal values: 42-52% or 0.42-0.52). These findings are not uncommon among trained individuals, Sharpe *et al.,* (2002) found that 85% of females and 22% of males in a cohort of >1000 people had haematocrit of <0.42. Additionally, vitamin C intake within the LCKD group was 21.8% below recommended daily intake (Table 5.2), as a LCKD is limited in fruit intake. Ascorbic acid has a dose-dependent enhancing effect on iron absorption in humans (Lynch and Cook, 1980). Thus, in light of low haemoglobin/haematocrit count and decreased MCH and MCHC within the LCKD (31-36 g/dl) (Table 5.4), the researchers examined if

observed decreases in iron, copper and vitamin C (Table 5.2) had an impact serum ferritin concentrations. There is no agreed upon definition as to the concentration of serum ferritin which constitutes a problematic level of iron depletion, but expert opinions range from <10 to <35 ng/mL (Peeling, Dawson, Goodman, Landers, & Trinder, 2008; Thomas *et al.,* 2016). Despite aforementioned concerns, serum ferritin concentrations remained unchanged within the LCKD group (Table 5.4), with concentrations of serum ferritin in each group being considerably greater than deficiency thresholds, previously outlined (Peeling, Dawson, Goodman, Landers, & Trinder, 2008; Thomas *et al.,* 2016). Zinn *et al.,* (2018) highlighted that iron bioavailability is reduced by phytates, given a LCKD is free of foods high in phytates, such as wholegrains, it's not inconceivable to postulate that persons' following a LCKD could have increased iron bioavailability. This thought process may explain maintenance of serum ferritin despite low intake of iron, copper and vitamin C (Table 5.2), however it does not explain low concentrations of haemoglobin and decreased MCHC (Table 5.4), thus a more controlled investigation is required.

Higher intakes of vitamins A, E and selenium were observed within the LCKD group, while decreases in intakes Vitamin C, manganese, zinc and copper, as previously outlined (Table 5.2). Although our brief assessment of a LCKD suggests participants intakes of Vitamin C, manganese, zinc and copper were low, empirical evidence would suggest it's unlikely they would transcend into nutrient deficiencies. Previously, Stefansson (1921) noted an ability to survive on an Inuit diet (85% fat, 15% protein) without signs of deficiency, while an experimental investigation carried out in the early 1930's by Mc Clellan and Du Bois's (1930) demonstrated that two participants could

consume a diet containing only fatty animal meat and organs for one year. At the end of the study, participants were mentally and physically well and showed no signs of vitamin deficiencies. This suggests that the body can extract essential vitamins contained within fatty animal meat and avoid delirious side effects associated with a low-fruit/vegetable diet, such as scurvy (Talarico *et al.,* 2014). Additionally, as dietary assessment was carried out over 3 days using a food diary, this assessment lacks sufficient time and accuracy to determine if inadequacy or deficiency occurred, as previously outlined (Burke *et al.,* 2001).

Interest in PRAL and NEAP calculations experienced a renaissance when NEAP calculations were included in a recent manuscript in elite endurance athletes. Similar to Carr *et al.'*s (2018) investigation, $NEAP_1$ increased following a period of keto-adaptation (+26.0 $mEq \cdot day^{-1}$, P = 0.038) and was greater than the high-carbohydrate groups estimated $NEAP_1$ score at post-intervention testing (P = 0.041) (Table 5.3). Unlike Carr et al.'s (2018) investigation, blood bicarbonate and pH were not measured, but participants' contained within the current investigation had similar PRAL scores at post-intervention testing (P = 0.256) and previously experienced similar blood lactate responses to the high-carbohydrate group throughout a 100 km TT and subsequent to a maximal exercise test (McSwiney *et al.,* 2018). An investigation in ultra-marathon runners demonstrated that participants' experienced simultaneous increases in blood pH and lactate during a 100 km running trial (Jastrzębski *et al.,* 2015). Our investigation lacks the specificity of Carr *et al.'s* (2018), however one could postulate based on PRAL scores (Table 5.3) and the homogeneity of high-carbohydrate and LCKD participants lactate responses during exercise (McSwiney *et al.,* 2018), that non-elite endurance

trained individuals may possess the same 'blood buffering capacity' as elite athletes following a 12 week adaptation to a LCKD and that NEAP calculations may not be applicable to non-elite endurance athletes. Although a more thorough investigation which examines changes in blood bicarbonate and pH is required.

Limitations. This investigation has a number of limitations, namely; 1) small sample size, 2) findings may be limited to the population sample at hand (i.e., male endurance athletes), 3) findings may be limited to the geographical and cultural location (i.e., Ireland), 4) weighed food diaries are an imperfect method of collecting data (i.e., high possibility of human error and/or chance of under or over reporting of nutrients to conform with researchers expectations) and 5) dietary analysis software may lack the delicacy to identify nutrients lost or salvaged through various cooking methods (i.e., grilling versus boiling).

5.6 Conclusions

Noteworthy findings were the LCKD groups reported reduced iron intake and reduced MCV and MCHC, but maintenance of serum ferritin. This may re-highlight a limitation previously outlined (accuracy of food diaries) or perhaps, that iron bioavailability is increased on a LCKD. Irrespective of dietary approach, endurance athletes should be made aware of the dangers of low iron and encouraged to monitor blood work with a medical professional. In an attempt to increase their carbohydrate intake, high-carbohydrate participants restricted dietary fat to <20% of total energy and appeared to be lacking in some essential vitamins, namely vitamin A, D, E and vitamin B_{12}. Therefore, individuals following a high-carbohydrate diet should be advised to keep dietary fat to >20-30% of total calories and to prioritise polyunsaturated and monounsaturated fatty

acids to ensure a diverse range of fat soluble vitamins. Additionally, nutritional guidelines recommend macronutrient intakes (g·kg) to optimise performance (Jeukendrup, 2004; Burke *et al.,* 2011). Although not always the case (Thomas *et al.,* 2016), some editions of nutritional guidelines recommend 'nutrient-rich carbohydrate foods' but fail to stipulate forms (Jeukendrup, 2004; Burke *et al.,* 2011). These guidelines may be taken out of context, which may lead athletes to overly concentrate on energy and macronutrient intake (g·kg) and to prioritise carbohydrate dense foods such as pastas, breads and cereals, which lack the micronutrient density of less calorically and carbohydrate dense foods such as fruits and green leafy fibrous vegetables, as observed within the current investigation. Therefore, athletes adhering to a high-carbohydrate diet should be reminded to consume a diet with appropriate micro-nutrient density (European Food Safety Authority, 2017) as well as adhering to sports specific macronutrient guidelines (Jeukendrup, 2004; Burke *et al.,* 2011).

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Authors Contributions

FMS & LD carried out the investigation, analysis and wrote the paper.

Conflicts of Interest

The authors declare no conflicts of interest.

Chapter 6

Effects of Acute Ingestion of Ketone Ester on Exercise Metabolism and 10 km TT Running Performance in Well-Trained Endurance Athletes

6.1 Abstract

Background

Acute ketone ester supplementation produces nutritional ketosis, which alters metabolic responses' to exercise and may positively impact performance. Despite a number of investigations' taking place in cyclists', impacts' on running performance remains unexplored.

Methods

On two occasions, *n* = 5 well-trained endurance athletes' (*n* = 4 males; *n* = 1 female) performed 60 minutes' on a motorised treadmill at 65% VO_{2peak} (pre-load) followed by a simulated 10 km (time trial) TT in a double-blind, randomised crossover design. Following breakfast (1 g·kg carbohydrates), participants' consumed 500 ml of an 8% carbohydrate-electrolyte solution (CES) containing ketone ester (KET) (286 $mg \cdot kg^{-1}$ BM) or placebo (PLA). An additional three boluses were consumed during the submaximal run, each containing 200 ml of CES, with two containing KET (drink 2&4; ~143 $mg \cdot kg^{-1}$ BM) or PLA. Participants completed a blinded (time and speed) 10 km TT as fast as possible and consumed 200 ml of CES at 5 km. Heart rate, rating of perceived exertion (RPE) and expired air were recorded incrementally throughout pre-load. Plasma βeta-hydroxybutyrate (βHB), lactate and glucose were monitored at rest, during pre-load (20-

, 40- and 60 minutes) and post 10 km TT via serial venous blood sample and subsequently assayed.

Results

Plasma βHB increased to 0.8 ± 0.1 mM ($P < 0.001$) 30 minutes post KET ingestion and peaked at ~1.2 ± 0.2 mM during submaximal exercise. No time x condition interaction effect for plasma glucose or lactate and heart rate or RPE were observed (*all* $P > 0.05$). Time trial performance was improved by 2.9% following KET condition (-70.8 ± 40.9 seconds; $P = 0.018$, ES: 0.78).

Conclusions

Acute ketone ester ingestion produced nutritional ketosis and improved 10 km TT running performance, but did not alter perceptual or metabolic responses to submaximal exercise in well-trained endurance athletes'.

6.2 Introduction

Many nutritional strategies' exist within endurance sports to optimise and prolong energy provision. Guidelines recommend the consumption of a high-carbohydrate diet (Burke *et al.,* 2011) and optimising carbohydrate provision prior- to and during exercise (Burke, 2015b), but there's growing interest in alternative methods, including 'fat adaptation' (Maunder, Kilding and Plews, 2018), 'keto-adaptation' (Volek *et al.,* 2016; Webster *et al.,* 2016) and the use of exogenous ketone supplements (Cox *et al.,* 2016; Evans, Cogan and Egan, 2016). The ketone bodies β-hydroxybutyrate (βHB) and acetoacetate (AcAc) are endogenously produced in hepatic tissues during periods of carbohydrate restriction, fasting or starvation to provide an alternative fuel source for the brain in the absence of glucose. However, exogenous ketone supplementation in the form of ketone salts and esters provide an opportunity to acutely increase plasma βHB to ~2.0-3.0 mM 20 minutes post-ingestion in the absence of carbohydrate restriction/fasting (Cox *et al.,* 2016). With regards to an athletic population, this acute increase in plasma βHB represents a number of effects on exercise metabolism, which may impact performance (Evans, Cogan and Egan, 2016).

Ketone salts alter metabolic responses' to exercise and cause moderate increases in plasma βHB (<1.0 mM), however their impact on performance is negligible (Cox *et al.,* 2016; O'Malley *et al.,* 2017; Rodger *et al.,* 2017), while ingestion is commonly associated with gastrointestinal symptoms due to the high-salt load (Evans *et al.,* 2018). Therefore, ketone salts' are not considered ergogenic in their current form. In contrast, ketone ester supplementation causes substantially greater increases in plasma βHB (~2.0-3.0 mM) (Cox *et al.,* 2016). Despite this, ergogenic benefits of ketone ester supplementation

in an endurance population remain mixed, with one investigation noting a +2% improvement in distance covered during a 30 minute time trial (TT) with no incidences of gastrointestinal discomfort (Cox *et al.,* 2016), while an additional investigation observed -2% impairment of 31.2 km TT performance and a high incidence of gastrointestinal distress in cyclists (~100%) (Leckey *et al.,* 2017a). Subsequent work in field sport athletes noted no effect on intermittent running performance, however symptoms' of gastrointestinal discomfort were highly prevalent post-ingestion (~82%) (Evans and Egan, 2018).

Previously, ketone esters were found to be glycogen sparing, by contributing 16-18% of total energy provision during a 45 minute cycle ranging from 40% to 75% watt max (W_{MAX}) attributing to markedly reduced blood lactate (Cox *et al.,* 2016). Previous attempts at glycogen sparing using carbohydrate restriction caused decreases in moderate and high performance (Havemann *et al.,* 2006; Stellingwerff *et al.,* 2006). Therefore, if tolerable, ketone esters have the potential to be a highly desirable substrate among endurance athletes. However, it remains to be seen in practical settings if this mechanism is found to elicit performance enhancing qualities or impair performance.

Given recent commercialisation and interest in ketone esters, there is a need to better understand its impact on performance modalities beyond cycling. Running (i.e., marathon/ultra-marathon) and swimming are cornerstones of endurance sports and two key components of ironman and triathlons. Further, it has been proposed that different exercise modalities, such as treadmill running elicit higher oxygen uptake than cycling on a cycle ergometer (Kravitz *et al.,* 1997) and potentially greater energy

expenditure at the same relative intensity (Sedlock, 1992), which may stimulate different metabolic demands on glycolytic and oxidative metabolism (Millet, Vleck and Bentley, 2009). This could enhance glycogen sparing properties previously observed by Cox *et al.,* (2016) at a relative intensity in a runners. Therefore, the aim of the current investigation is to examine the effects acute ingestion of ketone ester has on metabolic responses' and running performance in well-trained endurance athletes'.

6.3 Methods

Six well-trained endurance athletes' (*n* = 5 male, *n* = 1 female; Table 1) gave written informed consent to participate after receiving a verbal explanation of the procedures. Ethical approval was obtained from the research ethics committee at Dublin City University, IE.

6.3.1 Experimental Design

Participants' visited the laboratory for exercise testing on four separate occasions. All tests were performed on the same treadmill (COSMED T150, Italy). Total body mass and height were measured using digital scales and a stadiometer (Holtain, UK). Body composition was assessed using a TANITA bioelectric impedance scales (TANITA DC-430U, Japan). On their first visit to the laboratory, participants' completed a submaximal incremental exercise test to establish their lactate threshold and subsequently completed an incremental exercise test to volitional exhaustion to establish peak oxygen uptake (VO_{2peak}). Visit-2 acted as a familiarisation for the 2 subsequent visits. Two experimental trials (visit 3&4), each comprised of a 60 minute pre-load at 65% VO_{2peak} and a blinded (speed & time) 10 km TT (Figure 6.1). Diet was prescribed 24 hours prior

to arrival using a meal plan designed using Nutritic's dietary analysis software (Nutritic's Professional v3.09, Nutritic's, Dublin, Ireland) supplying 6 g·kg of carbohydrates. Upon arrival, participants' consumed 1 g·kg of carbohydrate and a pint of water. Additionally, participants consumed 1.2 $gmin^{-1}$ of carbohydrate in the form of a carbohydrate-electrolyte solution (CES) (Lucozade Sport, Lucozade Ribena Suntory Ltd., UK) and Maltodextrin (BulkPowders, IE) 30 minutes prior to, during 60 minute pre-load (i.e., 20-, 40- and 60 minutes) and midway through the 10 km TT. Subsequent to the first experimental drink being ingested, participants provided a urine sample to assess hydration status (PalOSMO, VITECH Scientific, Japan). Visit-3 and 4 were identical with the exception of the additional bolus of fluid added to drink-1 (-30 minutes), -2 and -4 (Figure 6.1), namely the ketone ester (KET) supplying ~573 $mg{\cdot}kg^{-1}$ BM (HVMN®, San Francisco, CA) in 50:25:25 ratio or placebo (PLA). Experimental trials were performed in a double-blinded, cross-over design.

6.3.2 Incremental Exercise Tests

Lactate threshold and VO_{2peak} were performance in accordance with guidelines, previously described (Australian Institute of Sport, 2012). In brief, for determination of lactate threshold, participants' completed four 4 minute stages (3 minutes' running, 1 minute rest) typically performed between 15-21 km/h for males and 13-19 km/h for females. Starting speeds were 4 km/h below participants' reported 10 km TT personal best, each stage increased by 1 km/h until a blood lactate concentration (Lactate Pro 2, Japan) of 4 mM was exceeded. Treadmill elevation was set to 1%.

Subsequent to 15 minutes rest, participants' completed an incremental test to exhaustion. The initial stages were completed at a 1% gradient. Treadmill speed was set

4 km/h lower than the final stage completed during the submaximal test (lactate threshold), previously described as the 'critical speed' (Australian Institute of Sport, 2012). Thereafter, treadmill speed increased by 0.5 km/h until participants' reach their critical speed. Once critical speed was achieved, treadmill incline was elevated by 0.5% every 30-seconds until volitional exhaustion. Expired gas was collected (COSMED Quark b2, Italy) throughout the maximal test for determination of ventilation, namely VO_2 and VCO_2.

6.3.3 Pre-Trial Preparation

Participants' arrived for experimental trials between 07:00 and 11:00, but on an individual basis, participants' performed their second trial (visit-4) at the same time ± 1 hour as their first trial (visit-3). Proceeding visit-2, participants' received a meal plan designed using Nutritic's dietary analysis software (Nutritic's Professional v3.09, Nutritic's, Dublin, Ireland) containing common foods, providing 40 kcal per kilogram body weight and 6 g·kg of carbohydrates (% carbohydrate:fat:protein = 60:20:20) (Burke *et al.,* 2011). Each participant was asked to adhere to the meal plan for 24 hours' preceding visit 2-4 and requested to abstain from caffeine and strenuous exercise 24 hours prior to each trial. Male participants' two experimental trials were separated by 7-14 days. Whereas, due to the menstrual cycle (Oosthuyse and Bosch, 2010), the *n* = 1 female participants' experimental trials' were separated by 7-days and within the early to mid-luteal phase of their menstrual cycle.

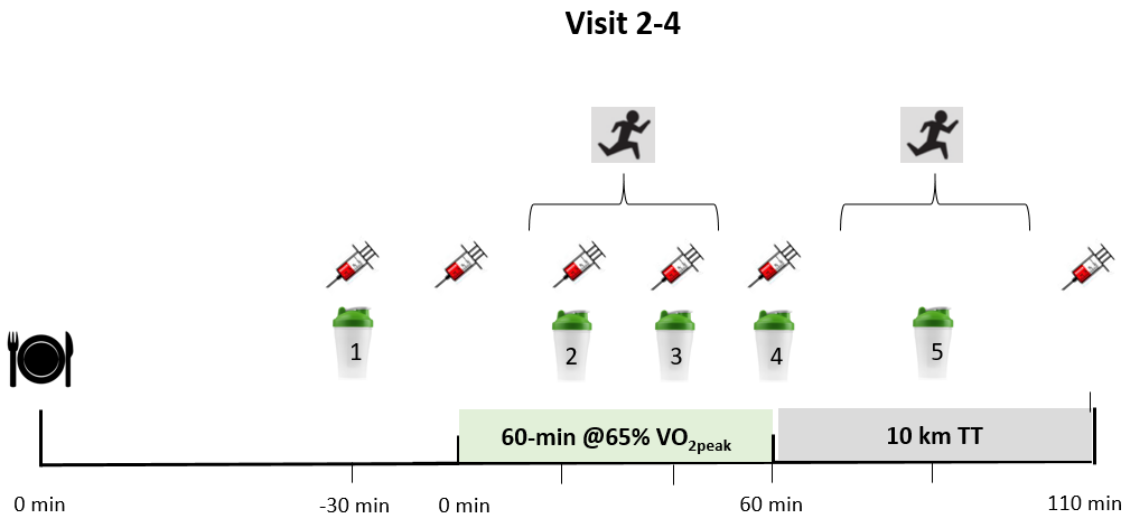

Figure 6.1. Experimental protocol implemented at visit 2-4

6.3.4 Experimental Trials

As previously outlined, experimental trials' were performed in a double-blinded, randomised cross-over design and were identical except for the drinks consumed. Sixty minute' prior to commencement of exercise, an indwelling catheter was introduced into an antecubital vein for serial blood sampling at: -30 minutes, 0 minutes, time points 20-, 40- and 60 minutes during 60 minute pre-load at 65% VO_{2peak} and immediately after 10 km TT (Figure 6.1).

During each trial, a bolus of a given drink was ingested 30 minute prior to exercise (drink-1), at 20 minute intervals during the 1 h pre load (drinks 2 to 4) and at the 5 km mark during each 10 km time trial (drink 5). During PLA, an 8% glucose carbohydrate-electrolyte solution (Lucozade Sport, Lucozade Ribena Suntory Ltd., UK) was provided at a rate of ~1.0 $g \cdot min^{-1}$ of exercise. During KET, an 8% carbohydrate solution was provided at a rate of ~1.0 $g \cdot min^{-1}$ combined with 573 $mg \cdot kg^{-1}$ BM of a D-βHB (R)1,3-butanediol ketone ester (HVMN, San Francisco, USA). The ketone ester was mixed directly with the carbohydrate-electrolyte solution for ingestion in three boluses (50:25:25) i.e., at 30 minute prior to exercise (drink 1) and at 20- (drink 2) and 60 minutes (drink 4) sub-maximal exercise (Figure 6.1), respectively. During PLA, drinks-1, 3 and 5, were flavoured with a bitter additive and in both trials, drinks 3 and 5 were provided as the unadulterated carbohydrate-electrolyte solution.

Following ingestion of their first experimental drink and prior to completing a 5 minute warmup at 8 km/h, participants' provided a urine sample for hydration testing (PalOSMO, VITECH Scientific, Japan). Participants' then began a ~2 hour endurance running protocol, which was previously found to have a within-subject coefficient

variation for 10 km TT of 1.00 ± 0.25% in 8-endurance runners (*n* = 4 males; *n* = 4 females) (Russell *et al.,* 2004). The initial phase of the exercise trial comprised of a 60 minute pre-load at speeds predicted to bring participants' to 65% VO_{2peak}, based off aforementioned submaximal and maximal tests. During the first 10 minutes of the pre-load, participants expired air was collected (COSMED Quark b2, Italy) for determination of ventilation, namely VO_2, VCO_2 and respiratory exchange ratio (RER). Heart rate (Polar, Finland) was recorded during the 9th minute and rating of perceived exertion (RPE) (Borg scale) were noted once the mask was removed. Thereafter, respiratory values were analysed to determine at what percentage of participants' VO_{2peak} participants' were exercising. Visit-2 acted as a familiarisation for participants' and an opportunity for researchers' to elicit the desired target %VO_{2peak}. During visit-2, participants begun exercise at a speed (km) that was calculated to bring participants' to 65% VO_{2peak}. Thereafter, if participants were exercising above or below this desired relative intensity (i.e., 65% VO_{2peak}), minor speed adjustments (±0.1-0.2 km/h) took place to elicit the desired relative intensity. Visit-2 was precisely the same as visit-3 and 4, apart from the absence of the ketone ester and/or placebo from experimental drinks. During visit 3, if speeds required further adjustments' to bring participants' to the desired range (i.e., 65% VO_{2peak}), the time at which speeds were adjusted was noted and replicated during visit-4.

At the 25th and 53rd minute, participants' were instructed to hold the handrails and to briefly hop to the sides of the treadmill to fit the face mask, in order to recommence gas collection. Exhaled gas was recorded for 5 minutes at these two time points. Heart rate was recorded in the 29th and 57th minute, RPE was noted once the mask was removed subsequent to the second and third respiratory collection. Similarly, at time point's 20-

and 40 minutes, participants' were instructed to hold the handrails and to momentarily hop to sides of the treadmill to receive a blood sample and to ingest drink-2 and drink-3, thereafter (Figure 6.1). Drink 2&3 contained 200 ml of an isotonic drink (63.6 kcal / 16 g carbohydrates), while merely drink-2 contained KET (~143.25 $mg \cdot kg^{-1}$ BM) or PLA. Once participants' had completed the 60 minute pre-load an additional blood sample was obtained and drink-4 was ingested. Drink-4 contained 200 ml of an isotonic drink and KET (~143.25 $mg \cdot kg^{-1}$ BM) or PLA. If required, participants' were allocated time to go the toilet prior to beginning the 10 km TT. Time taken between the steady state and 10 km TT was recorded during visit-3 and replicated during visit-4; routinely <5 minutes.

Prior to beginning the 10 km TT, heart rate monitor was removed and speed (km/h) and time (minute:second) displays were covered on the treadmill. Therefore, during the 10 km TT, participants' were only aware of distance covered (meters). Participants' only instruction/encouragement was to complete the 10 km TT as fast as possible. At the midway point (5 km), participants' were handed their final drink (drink-5) and instructed to keep running. Drink-5 contained 200 ml of an isotonic drink. Following visit-3 and -4, participants' filled out a questionnaire which was designed to examine if any gastrointestinal discomfort was experienced. Following successful completion of visit-4, participants' were asked to identify whether they could identify the trial in which they received the ketone ester, based on taste and/or performance. Subsequently, participants' were informed of their 10 km TT times' from visit-3 and visit-4. Both the researchers' and participants' were informed of the blinding once each participant had completed visit-4.

6.3.5 Blood Analysis

Blood samples (4 mL) were collected in plastic tubes containing sodium fluoride/potassium oxalate (Vacuette Glucose tubes, Greiner-Bio-One, Germany) for subsequent analysis. Samples were stored on ice before centrifugation at 3000 g for 10 minutes at 4°C, after which three aliquots of plasma were separated for storage at −80°C until later analysis of plasma βHB, lactate and glucose (RX Daytona, Randox Laboratories, UK; assay codes RB1007, LC2389 and GL364, respectively).

6.3.6 Statistical Analysis

IBM Statistics SPSS 24 (Illinois, Chicago, USA) was used for statistical analysis. Data are presented as mean ± SD. Each of the data points were tested for normality using the Shapiro-Wilk test. A two-way (time x condition) repeated measures analysis of variance (ANOVA) was used to determine differences between the two experimental trials. When a main effect of condition or an interaction effect between condition and time was indicated, post-hoc testing with Bonferroni adjusted *P* values was applied to compare KET to PLA at respective time points. Additionally, effect sizes were evaluated as: η^2_p = 0.01 (small effect), η^2_p = 0.09 (medium effect) and η^2_p = 0.25 (large effect) (Cohen, 1988). The alpha level for significance was set at *P* = 0.05 for each test.

6.4 Results

6.4.1 Participants

Five participants' (*n* = 4 males; *n* = 1 female) successfully completed each experimental trial and have been included for analysis (Table 6.1). One participant (*n* = 1 male)

dropped out of the investigation. Blood samples were obtained from four participants' (*n* = 3 males; *n* = 1 female).

6.4.2 Plasma βHB, Glucose and Lactate Responses.

No differences for fasting plasma concentrations of βHB (0.1 ± 0.0 mM KET, 0.1 ± 0.0 mM PLA), glucose (4.2 ± 0.6 mM KET, 3.8 ± 0.8 mM PLA) and lactate (1.2 ± 0.2 mM KET, 1.0 ± 0.1 mM PLA) were observed between experimental trials (*all P* > 0.05).

Beta-hydroxybutyrate

A main effect of time and condition (*both P* < 0.005) and time x condition interaction effect (*P* < 0.001) were observed for plasma βHB. Concentrations' increased by +0.8 ± 0.1 mM from resting values (*P* = 0.004 ES: 0.95) in the KET condition (Figure 6.2). Thereafter, βHB increased from time point 20- to 40 minutes (+0.4 ± 0.1 mM; *P* = 0.003 ES: 0.96) and decreased from time point 40- to 60 minutes (-0.59 ± 0.1 mM; *P* = 0.006 ES: 0.94) in the KET condition (Figure 6.2). Plasma βHB was greater following KET versus PLA condition at time point's 0 minutes (*P* = 0.004 ES: 0.95), 20 minutes (*P* = 0.004 ES: 0.95), 40 minutes (*P* = 0.004 ES: 0.95), 60 minutes (*P* = 0.001 ES: 0.99) and post-exercise (*P* = 0.007 ES: 0.93) (Figure 6.2).

Glucose and Lactate

No main effect or time x condition interaction effect was observed for glucose or lactate (*all P* > 0.05). However, a significant time effect for glucose and lactate was observed (*both P* < 0.005). Glucose increased from 5.4 ± 0.2 mM to 7.7 ± 1.0 mM (*P* = 0.011 ES: 0.91) from time point 60 minutes to post-exercise during the KET condition and increased from 3.2 ± 0.6 mM to 5.9 ± 0.6 mM (*P* = 0.015 ES: 0.89) at 20- to 40 minutes

(*P* = 0.015 ES: 0.89) during the PLA condition (Figure 6.3). Lactate decreased from 1.0 ± 0.3 mM to 0.7 ± 0.2 mM (-0.3 ± 0.1 mM; *P* = 0.027 ES: 0.84) from time points' 20- to 40 minutes and increased from 60 minutes to post-exercise (0.6 ± 0.2 mM to 9.0 ± 2.8 mM; *P* = 0.011 ES: 0.95) during the KET condition (Figure 6.4). Lactate increased from time point 60 minutes to post-exercise (0.8 ± 0.2 mM to 7.9 ± 1.3 mM; *P* = 0.001 ES: 0.97) during the PLA condition (Figure 6.4).

6.4.3 Heart Rate and Rate of Perceived Exertion

No main effect or time x condition interaction effect for heart rate or RPE was observed (*all P* > 0.05) (Table 6.2).

Respiratory Exchange Ratio

No main effect or time x condition interaction effect was observed for RER (*all P* > 0.05) (Table 6.2).

10 km TT performance

Time to complete 10 km TT was 70.8 ± 40.9 seconds less during the KET condition (*P* < 0.05). Individual TT times' are presented within Figure 5.

10 km TT performance – 2 km Splits

Time – Seconds

A significant time and condition effect for 2 km TT times' occurred (*both P* < 0.05), however no time x condition interaction effect was observed (*P* > 0.05). Two kilometre TT splits are presented within Table 3. Time to complete 2 km splits decreased from 0-2 km to 2-4km (*P* = 0.010; ES 0.842), 4-6 km to 6-8 km (*P* = 0.010 ES: 0.837) and from 6-8

km to 8-10 km (*P* = 0.001 ES: 0.956) during KET condition. Similarly, time to complete 2 km splits decreased from 0-2 km to 2-4 km (*P* = 0.008 ES: 0.856) 4-6 km to 6-8 km (*P* = 0.027 ES: 0.746) and from 6-8 km to 8-10 km (*P* = 0.048 ES: 0.664) during PLA condition.

6.4.4 Interviews

6.4.4.1 Ketone ester identification

Four participants answered 'don't know' when asked to differentiate experimental trials. One participant correctly highlighted the KET condition, based on 'performance'. Therefore, no participant identified KET/PLA condition based on taste, therefore, blinding was successful. One participant' noted, both the KET/PLA were "equally horrible".

6.4.4.2 Gastrointestinal discomfort

Upper Abdominal Symptoms

Two participants' reported reflux/heartburn (*n* = 1 KET; *n* = 1 PLA), four participants' reported belching (*n* = 1 KET; *n* = 3 PLA) and one participant' reported bloating (*n* = 1 KET), stomach pain/cramps (*n* = 1 KET) and nausea (*n* = 1 KET).

Lower Abdominal Symptoms

One participant' reported lower ab cramps (*n* = 1 KET), flatulence (*n* = 2 PLA), three participants' reported urges to defecate (*n* = 2 KET; *n* = 1 PLA). No participants' experienced cramp, diarrhea, vomiting or intestinal bleeding.

Table 6.1. Subject Characteristics			
	Mean ± SD	Range	Shapiro-Wilk *p* Value
Age, years	29.3 ± 6.0	25-37	0.016
Height, m	179.5 ± 7.8	168-188.5	0.994
Body Mass, kg	66.6 ± 9.9	55.5-79	0.229
Body Fat, %BF	7.8 ± 4.8	3-16.2	0.749
VO_{2peak}, L/min	4.2 ± 0.4	3.6-4.8	0.537
VO_{2peak}, ml·kg^{-1}min^{-1} BM	63.6 ± 6.0	58-74.9	0.125

Abbreviation: m = meter; kg = kilogram; %BF = percentage body fat; VO_{2peak} = maximal oxygen consumption per minute in litres; ml·kg = millilitres of oxygen used per minute per kilogram body mass.

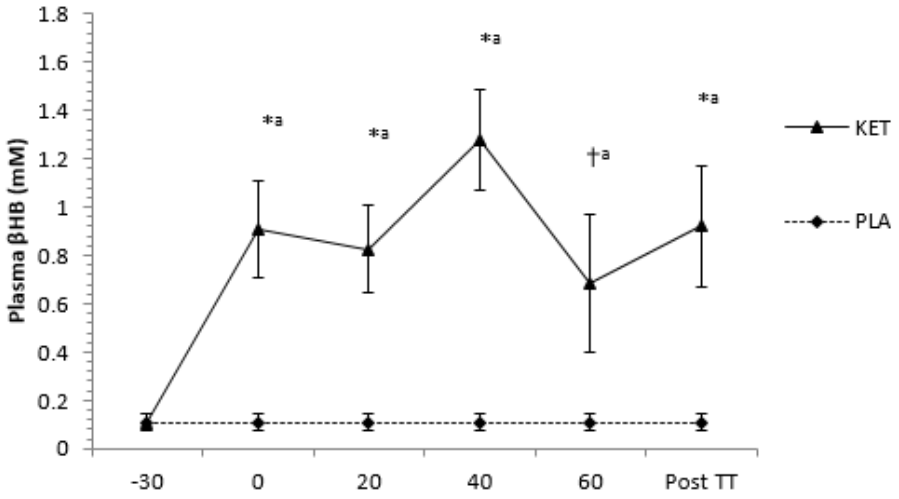

Figure 6.2. Serum βeta-hydroxybutyrate (βHB) responses at rest, during submaximal exercise (~65% VO_{2peak}) and immediately post 10 km TT
Abbreviations: KET = ketone ester; PLA = placebo.
* Significant ($P < 0.05$) within group (KET) increase in serum βHB from previous time point.
† Significant ($P < 0.05$) within group (KET) decrease in serum βHB from previous time point.
[a] Serum βHB was significantly ($P < 0.05$) greater in KET than PLA condition at this time point.

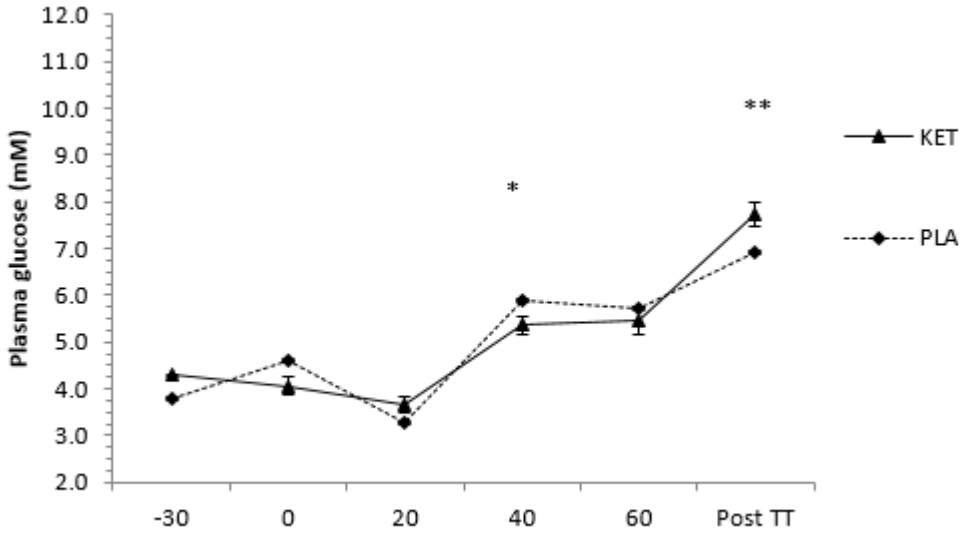

Figure 6.3. Serum glucose (mean ± SD) responses at rest, during submaximal exercise (~65% VO_{2peak}) and immediately post 10 km TT

Abbreviations: KET = ketone ester; PLA = placebo.
* Significant ($P < 0.05$) within group (PLA) increase in serum glucose from previous time point.
** Significant ($P < 0.05$) within group (KET) increase in serum glucose from previous time point.

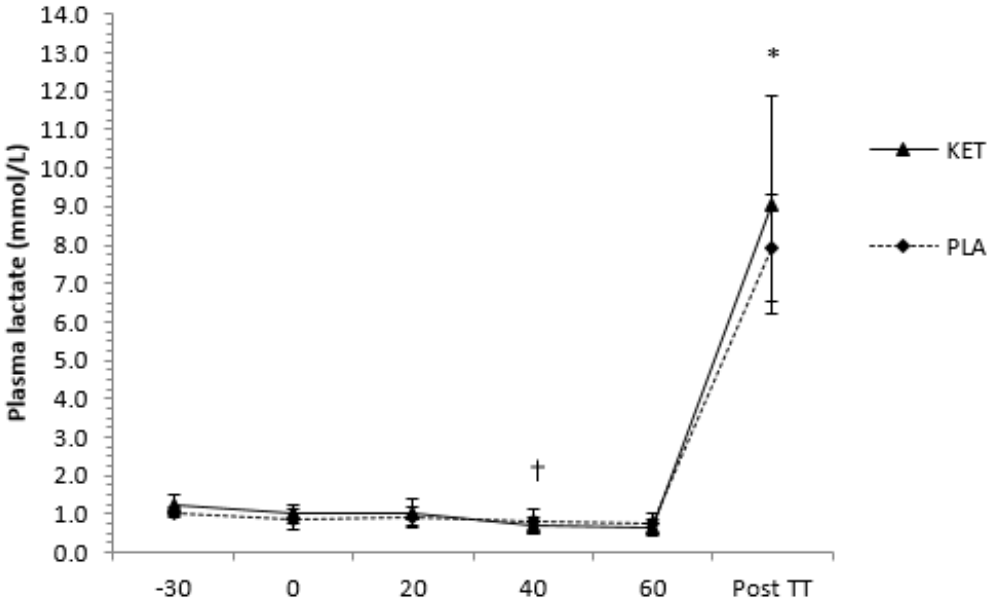

Figure 6.4. Serum lactate (mean ± SD) responses at rest, during submaximal exercise (~65% VO_{2peak}) and immediately post 10 km TT

Abbreviations: KET = ketone ester; PLA = placebo.
* Significant (P < 0.05) within group (KET and PLA) increase in serum lactate from previous time point.
† Significant (P < 0.05) within group (KET) decrease in serum lactate from previous time point.

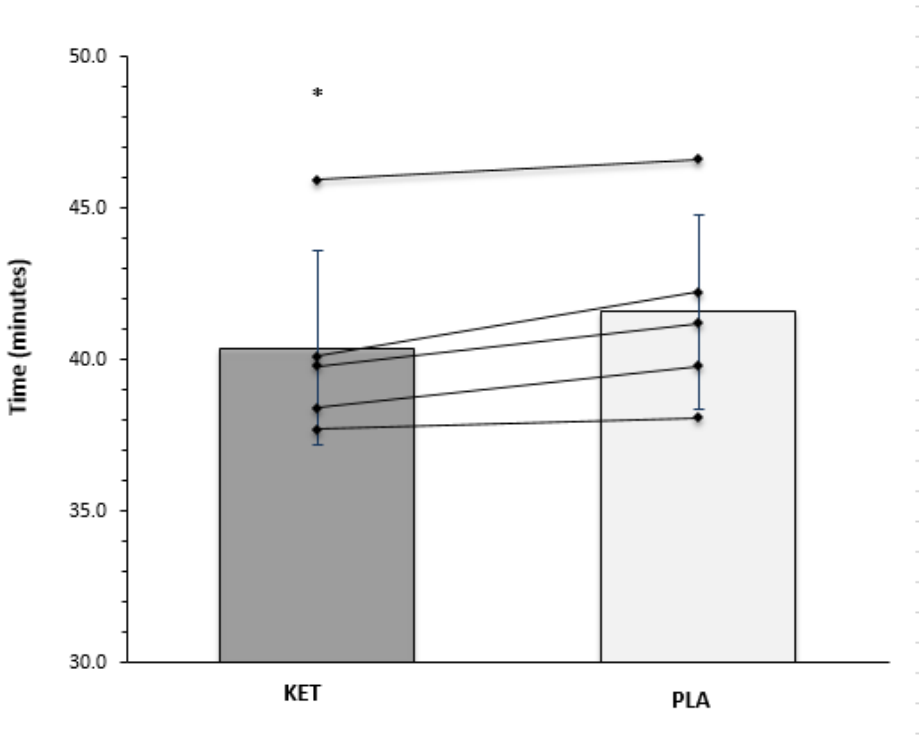

Figure 6.5. 10 km TT times' (mean ± SD) and participants' individual TT times.

Abbreviations: KET = ketone ester; PLA = placebo.
* Significant ($P < 0.05$) decrease in 10 km TT time between conditions.

Table 6.2. Respiratory, physiological and subjective responses' to exercise in ketone ester (KET) and placebo (PLA) trials

	KET (*n* = 5) Time points			*PLA (n* = 5) Time points		
	0 – 10 min	25 - 30 min	53 - 58 min	0 – 10 min	25 - 30 min	53 - 58 min
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
RER	0.950 ± 0.030	0.934 ± 0.043	0.932 ± 0.039	0.948 ± 0.019	0.926 ± 0.039	0.914 ± 0.035
Heart rate, bpm	145.6 ± 7.9	145.0 ± 9.6	149.4 ± 12.5	147.6 ± 12.3	145.0 ± 9.6	149.4 ± 12.5
RPE	9.6 ± 1.6	10.2 ± 1.4	11.2 ± 1.0	11.5 ± 1.7	10.2 ± 1.4	11.2 ± 1.0
$\%VO_{2peak}$	66.0 ± 7.1	67.3 ± 3.8	64.3 ± 0.6	67.1 ± 6.2	67.1 ± 3.1	66.4 ± 3.3

Abbreviations: KET = ketone ester; PLA = placebo; RER = respiratory exchange ratio; bpm = beats per minute; RPE = rating of perceived exertion; percentage of maximal oxygen consumption.

6.5 Discussion

The aim of the current investigation was to examine the effect, if any, acute ingestion of a ketone ester had on submaximal exercise and simulated 10 km TT running in well-trained male and female endurance athletes'. The investigations' main findings' were, 1) ketone ester supplementation produced acute nutritional ketosis (i.e., βHB >0.5 mM) 30 minutes post-ingestion and remained elevated thereafter, 2) heart rate and RPE remained unchanged, 3) plasma glucose and lactate responses remained consistent during submaximal exercise and 4) 10 km TT performance improved following ketone ester ingestion.

Ten kilometre TT performance improved by ~2.9% following 573 mg·kg^{-1} BM ketone ester ingestion during the current investigation. Figure 5 presents' individual TT times and highlights the homogenous improvement in performance. Performance improvements ranged from 21 to 126 seconds (0.9% to 5.2%), respectfully. Interest in exogenous ketones developed in response to a key publication by Cox *et al.,* (2016). Acute ketone ester ((R)-3-hydroxybutyl (R)-3-hydroxybutyrate ketone monoester) supplementation increased distance covered during a 30 minute blinded cycling TT by +2% (411 ± 162 m). However, subsequent work examining ketone esters impact on performance has been mixed, Leckey *et al.'s* (2017) investigation found that ketone ester supplementation (R,S-1,3-butanediol acetoacetate diester) (D'Agostino, Dean and Phillа, 2012) caused a -3.7% decreases in power output during a 31.2 km cycling TT in professional cyclists, attributing to increased time to completion (+58.2 seconds), while Evans and Egan (2018) investigation displayed no benefit to ketone ester supplementation ((R)1,3-butanediol ketone ester) (KE4 KetoneAid, USA) during an

intermittent running performance in team sport athletes. Important dissimilarities among aforementioned investigations' is the form of ketone ester and the concentrations of βHB achieved following supplementation. The βHB ketone monoester implemented by Cox *et al.,* (2016) and Evans and Egan (2018) increased plasma βHB to ~2.3 mM in well-trained cyclists and ~1.5-2.5 mM in field athletes when co-ingested with a carbohydrate solution, respectfully, while the AcAc ketone diester utilised by Leckey *et al.,* (2018) increased plasma βHB to 1.2 mM during exercise. It's thought that ergogenic benefits of ketone ester supplementation is unlikely unless blood ketone concentrations exceed ~2 mM. Further, consuming a βHB versus AcAc ketone ester is likely to cause greater increases in plasma βHB, potentially yielding greater performance benefits, as AcAc must be reduced to βHB prior to entering skeletal muscle (Evans, Cogan and Egan, 2016). Additionally, the use of D-βHB (also known as R-βHB) versus an L-βHB isomer is more suited to an athletic population (Stubbs *et al.,* 2018), as D-βHB is more readily available for oxidative metabolism, as the D-βHB is released by the liver, while the L- βHB is an intracellular metabolite (Webber and Edmond, 1977; Desrochers *et al.,* 1992). During the current investigation, plasma βHB increased to ~0.95 mM at the commencement of exercise following ingestion of ~286.5 mg·kg^{-1} BM of ketone ester and peaked at ~1.27 mM 40 minutes thereafter, which was 20 minutes subsequent to their second bolus containing an additional ~143.25 mg·kg^{-1} BM. The final ~143.25 mg·kg^{-1} BM was ingested once participants' completed the 60 minute pre-load at 65% VO_{2peak}, totalling their intake at ~573 mg·kg^{-1} BM. Previous investigations' have consumed 500 mg·kg^{-1} BM (Leckey *et al.,* 2017) and 750 mg·kg^{-1} BM (Cox *et al.,* 2016; Evans and Egan, 2018), respectfully. Cox *et al.*'s (2016) preliminary investigation achieved plasma βHB concentrations of ~2.0-3.0 mM within 20 minutes of ingestion and

throughout a 90 minute exercise bout. However, despite consuming the same dose as Cox *et al.*'s (2016) participants' (i.e., 750 mg·kg^{-1} BM), team sport athletes' contained within Evans and Egan's (2018) achieved ~2.0 mM 65 minutes post-ingestion. This delay and 33% decrement in plasma βHB elevation was attributed to differing feeding strategies among investigations', Cox *et al.,*'s (2016) participants' consumed the ketone ester in a fasted state, while team sport athletes' consumed the ketone ester in a postprandial state (3 g·kg carbohydrates) (Evans and Egan, 2018). Previously, consuming a ketone ester (~282 mg·kg^{-1} BM) in a postprandial state versus a fasted state was found to lower mean plasma βHB concentrations' from 3.3 mM to 2.2 mM (B. J. Stubbs *et al.,* 2017). Notably, well-trained cyclists within Leckey *et al.'s* (2018) investigation consumed 2 g·kg of carbohydrates' on the morning of their trial respective trial and 250 mg·kg^{-1} BM of ketone ester 30-miniutes prior to an incremental warm-up and an additional 250 mg·kg^{-1} BM immediately prior to the warm-up. However, judging from 'Figure 1' and 'Figure 5B' (contained within the manuscript), plasma βHB peaked 20 minutes post-exercise, at ~123 minutes post-ingestion. Therefore, one may have expected greater increases in plasma βHB during the current investigation, as participants consumed fewer carbohydrates prior to exercise (1 g·kg versus 2 g·kg) and a larger dose ketone ester (~573 mg·kg^{-1} BM versus 500 mg·kg^{-1} BM) than Leckey *et al.,* (2018). Importantly however, both Cox *et al.,* (2016), Evans and Egan (2018), Leckey *et al.,* (2018) and the current investigation incorporated varying ketone esters. Therefore, notwithstanding any potential concerns with quality/potency, dosing strategies may need to be individualised for each ketone ester to optimise increases' in plasma βHB.

Previously, ketone ester supplementation attenuated an increase in plasma lactate in well-trained cyclists during a 60 minute bout of exercise at 75% W_{MAX} (Cox *et al.,* 2016) and caused a ~10 to 30% reduction in plasma lactate during an incremental running protocol (Evans and Egan, 2018). This phenomenon was explained by Cox *et al.,* (2016) as a reduction in glycolytic flux and a sparing of muscle glycogen during submaximal exercise, attributing from increased energy contribution from ketone bodies and intramuscular triglycerides. In advance of the current body of literature, Evans, Cogan and Egan (2016) hypothesised that ketone bodies would most likely be of benefit to endurance athletes, since type I muscle fibres are highly expressed within this population and because ketone bodies are transported across the skeletal muscle membrane by monocarboxylate transporters (MCT_2). Consistent with the lack of effect on plasma lactate during each experimental trial (i.e., 60 minute at 65% VO_{2peak}), its evident participants' were exercising below their lactate threshold (i.e., ~4 mM) (Yoshida *et al.,* 1987) (Figure 6.4). Therefore, given similarities in respiratory quotient (RQ) values' for ketone bodies' (1.00 = AcAc; 0.89 = βHB) (Frayn, 1983) and carbohydrates' (1.00) being metabolised (Table 6.2), it's plausible that ketone bodies' (i.e., βHB) were being oxidised at a high-rate during the 60 minute pre-load at 65% VO_{2peak} in the absence of a lactate lower effect, perhaps attributing up to ~18% of total energy provision, as previously observed within cyclists at <75% W_{max} (Cox *et al.,* 2016). However, this 'glucose sparing' mechanism doesn't help explain improved 10 km TT performance observed within each of the *n* = 5 participants' (Figure 6.5), as participants' followed an optimal carbohydrate based feeding strategy 24 hours' proceeding and during each of the experimental trials, thus, glycogen depletion/fatigue should not have been a concern. Although an altogether different paradigm, low-carbohydrate high-fat

and/or low-carbohydrate ketogenic diets (LCKD) are discussed within this 'glycogen/glucose sparing' context. The premise for glucose sparing via exogenous ketones or carbohydrate restriction is the inhibition of glycolytic flux via inhibition of pyruvate dehydrogenase (PDH) and phosphofructokinase by increases in NADH:NAD+, acetyl-CoA:CoA or citrate (Evans, Cogan and Egan, 2016). However, impaired performance under submaximal and high-intensities has been observed following acute dietary induced increases in fat oxidation (Havemann *et al.,* 2006; Stellingwerff *et al.,* 2006). This was in spite participants' being in a state of glycogen availability (i.e., carbohydrate restoration), attributing to the proposed mechanism being labelled, 'glucose impairing' (Burke, 2015). Conversely, Evans and Egan observed no decrement in 15 x 15 m sprint performance spanning across a 75 minute intermittent running protocol, an activity highly dependent on the ATP-phosphocreatine (PCr) system and anaerobic glycolysis for energy provision. As previously outlined, ~10-30% lower plasma lactate concentrations were observed during this investigation, with a reduction being observed during 20 m variable intensity shuttles and 15 m sprints ('Part A'), suggesting a reduction in glycolytic flux, which ultimately did not impact upon 15 m running performance, lasting < 3 seconds. Additional work is needed to determine the mechanism by which performance was sustained.

Nine incidents of upper abdominal symptoms (*n* = 5 KET; *n* = 4 PLA) and six incidents of lower abdominal symptoms (*n* = 3 KET; *n* = 3 PLA) of gastrointestinal discomfort were reported. The homogenous response observed in each experimental trial and lack of effect on heart rate and RPE (Table 6.2) may demonstrate difficulties in tolerating ~1.3 litres of isotonic fluid in ~110 minutes in some individuals', rather than 573 $mg \cdot kg^{-1}$ BM

of ketone ester. Incidents of gastrointestinal discomfort were considerably less than previously observed (Leckey *et al.,* 2017; Evans and Egan, 2018). These investigations incorporated larger doses of ketone ester, which ultimately may have attributed to increased incidents and perhaps, decreased performance in one instance, as participants' nomination of their gastrointestinal symptoms' as a distraction or interference to performance (Leckey *et al.,* 2017).

6.4.1 Limitations and Conclusions

This investigation has a number of limitations, namely 1) small population sample, which ultimately impeded our ability to observe time x condition statistical significance, specifically in the context of 2 km times' (seconds) and 2 km speeds (km/h). With a greater *n* size, statistical significance may have been observed and uncovered pacing mechanisms for improvement (i.e., faster running speeds during the final 2-4 km), 2) due to the nature of treadmill running, blood samples' were unable to obtained safely during the 10 km TT, therefore, potential changes that may have occurred and attributed to enhanced performance remain unexplored, 3) in order to ensure participants' received equal carbohydrate feeding each experimental trial, the KET condition received additional energy contained within the ketone ester (~97-139.2 kcal), which may have attributed to improved performance and 4) results may be limited to the exercise protocol (i.e., 60 minutes pre-load at 65% VO_{2peak} + 10 km TT) and population sample at hand (i.e., well-trained male and female athletes').

6.4.2 Conclusion

In conclusion, 573 mg·kg^{-1} BM of ketone ester was well tolerated and produced acute nutritional ketosis in well-trained endurance athletes'. Increases in βHB were associated with improvements in simulated 10 km TT running performance, versus a group with equal carbohydrate feeding. Notably however, increases in βHB did not alter perceptual or metabolic responses to submaximal exercise in well-trained endurance athletes'. Therefore, additional work is needed to determine the mechanism which enhanced performance.

Chapter 7

Discussion

Following a notable gap within the scientific literature since the early 1980's (Phinney *et al.,* 1983) and a decade of failed attempts to harness fat adaptation as an ergogenic strategy towards endurance athletes (Burke, 2015), interest in low-carbohydrate ketogenic diets (LCKD) (re)emerged (Burke, 2017) to improve athletic performance and health (Volek, Noakes and Phinney, 2015). In addition, there is growing interest in recently developed exogenous ketones, in the form of ketone salts and esters which are capable of acutely inducing nutritional ketosis and thereby, altering fuel selection and potentially, enhancing performance (Evans, Cogan and Egan, 2016).

The aim of this research was to monitor changes in measures of performance and health relevant to endurance athletes following a 12 week adaptation to a LCKD versus a traditional high-carbohydrate (HC) diet. Additionally, this dissertation aimed to examine the impact acute ingestion of a (R)-3-hydroxybutyl (R)-3-hydroxybutyrate ketone monoester, had on metabolic responses to submaximal exercise and simulated 10 km TT running performance in well-trained endurance athletes.

7.1 Main Research Findings

Study 1: Subsequent to a 12 week adaptation period, 1) a LCKD was associated with maintenance of 100 km TT performance 2) and improvements in relative power outputs during a SS sprint and CPT; increases in relative sprint performance were at least partly due to 3) decreases in body mass (kg) within LCKD group.

Study 2: In accordance with decreases in body fatness, 1) a LCKD was associated with an increase in total cholesterol, 2) maintained other homeostatic markers of wellness, including glycaemic control, triglycerides and inflammation and oxidative stress. 3) In addition, there was a relationship between change in body mass and leptin, while increased βHB were related to increased blood glucose, reduced leptin and reduced markers of inflammation (IL-8 and M-CSF) and oxidative stress (protein carbonyl).

Study 3: The LCKD was reportedly deficient in fibre, vitamin C and copper and contained in excess of nutritional guidelines for saturated fat. In contrast, high-carbohydrate diet participants failed to meet sports specific guidelines for carbohydrate and consumed <20% of total energy from dietary fat, attributing to low intakes of selenium, iodine, vitamin A, D, E and B12 and riboflavin. Despite differing nutrient content, no effect on serum ferritin was observed.

Study 4 Despite not measurably altering perceptual or metabolic responses to submaximal exercise, acute ingestion of 573 $mg \cdot kg^{-1}$ BM (R)-3-hydroxybutyl (R)-3-hydroxybutyrate ketone monoester improved simulated 10 km TT running performance in well-trained endurance athletes.

Keto-adaptation

Prior to embarking on this journey in 2014, little experimental evidence was available to suggest whether an adaptation to a LCKD would yield ergogenic or ergolytic effects. In 2015, Volek, Noakes and Phinney provided rationale why to 'rethink fat as a fuel', while Burke (2015) was cautious of this dietary approach, as there was only one experimental investigation carried out previously which had successfully achieved increases in plasma βHB >0.5 mM and assessed endurance performance (Phinney *et al.,* 1983). Therefore, the current experiment was designed to address calls within the literature, for an investigation to assess performance following an adaptation period >3-4 weeks (Phinney, 2004) and ideally, following a number of months allocated to keto-adaptation (Volek and Phinney, 2012; Volek, Noakes and Phinney, 2015).

While the current investigation was underway, Volek *et al.'s* (2016) cross-sectional investigation illustrated the effects of long-term keto-adaption, achieving resting plasma βHB values of ~0.5 mM and ~1.0 mM during 3 hours of submaximal exercise and rates of fat oxidation 'two-fold higher than ever recorded within the scientific literature'. However, subsequent physiological responses observed within elite race walkers raised an important question (Burke *et al.,* 2017), a question the current investigation was constructed around, i.e., the length of time necessary to become keto-adapted. Participants contained within Burke *et al.'s* (2017) investigation achieved resting plasma βHB values of ~1.0 mM in 21-days, 50% greater than athletes who adhered to a LCKD for a number of months (Volek *et al.,* 2016) and greater rates of fat oxidation, therefore, it remains unclear what additional physiological adaptations, if any, are necessary to be categorised as keto-adapted, as increases in fat oxidation and plasma βHB can be

achieved within as little as 3 weeks in elite athletes. As discussed within Chapter 5, an additional measure of adaptation may be the maintenance of blood glucose, as Burke *et al.,* (2017) noted decreases in blood glucose at rest and during a 25 km walk, while both Volek *et al.,* (2016) Webster *et al.,* (2016) and participants contained within the current investigation maintained blood glucose at rest and during exercise (Volek *et al.,* 2016; Webster *et al.,* 2017) after consumption of a LCKD for >3 months. This suggests a greater efficiency at producing glucose from non-carbohydrate sources if adaptation length is beyond 21 days.

While the current investigation demonstrated 100 km TT performance can be maintained and relative power outputs improved following a 12 week adaptation to LCKD, maintenance of endurance performance was previously observed following a 4 week adaptation period. Therefore, it must be acknowledged that perhaps, a 12 week adaptation period is not necessary to regain endurance performance, 4-6 weeks may suffice, as previously observed (Phinney *et al.,* 1983). However, one of the key contributions to the literature the current investigations has made is how the experimental exercise trial assessed performance. One of the initial goals of this investigation was to assess performance in as practical a manner as possible, therefore, a 100 km TT was chosen versus a TTE at 65% VO_{2peak}, as previously observed (Phinney *et al.,* 1983). The maintenance of performance during a TTE at 65% VO_{2peak} provides practitioners with valuable information on fuel utilisation and exercise metabolism, however, it has poor transferability to real world athletes for a number of reasons, namely, 65% VO_{2peak} does not equate to the physiological demands placed on athletes during endurance events, such as multi-stage cycling (>70% VO_{2peak}) (Fernandez-Garcia

et al., 2002) and does not account for cognitive awareness and mental toughness to ensure appropriate pacing. In addition, a TTE trial normally compares performance in a fasted state, as was the case within Phinney *et al.'s* (1983) high-carbohydrate/control group. Generally speaking, high-carbohydrate athletes are not accustomed to performing endurance exercise >60-90 minutes without a pre-exercise meal and using exogenous carbohydrates during exercise. Therefore, the maintenance of 100 km TT performance versus a well-matched high-carbohydrate group in a fed state is all the more impressive and transferable to real world athletes.

Although the current investigation demonstrates this feat can be achieved in 12 weeks. There is a need for an investigation to monitor changes in performance in response to a LCKD. Ideally, this investigation would be a RCT, obtain a larger sample size and leaner (8-10% BF) population sample than the current investigation to ensure a more homogenous group and to remove differences and changes in body mass as confounding variables, whilst monitoring a number of performance variables, ranging from <30 seconds in length, to sprint and endurance events ranging from 4-60 minutes to >2-3 hours in length on a weekly or biweekly basis over an extended period of time (>8-12 weeks), as well as other measures of adaptation such as, plasma βHB, fat oxidation and blood glucose. Such an elaborate study design would provide a framework capable of demonstrating if additional time accolated to keto-adaptation helps regain performance or demonstrate exercise intensities where performance remains impaired and if so, by how much, versus a well-matched group consuming a high-carbohydrate diet. Although such an investigation would be a large undertaking, an elaborate

experiment is needed to resolve the contentious discussion surrounding keto-adaptation and performance (Burke, 2015; Volek, Noakes and Phinney, 2015).

Keto-adaptation and Performance

The current experimental investigation is the first to demonstrate endurance performance can be maintained following a 12 week adaptation to a LCKD in endurance trained males. This efficiency is in line with previous experimental investigations (Phinney *et al.,* 1983) and cross sectional studies (Volek *et al.,* 2016; Webster *et al.,* 2016) in well-trained athletes, while Zinn *et al.,* (2017) noted decreases in TTE in recreationally active individuals following a similar adaptation period.

However, one of the most thought provoking findings from the current investigation, was improvements in relative power outputs during the SS sprint and CPT, which is not a renowned feature of keto-adaptation (Zajac *et al.,* 2014; Burke *et al.,* 2017). Optimal athletic performance in many endurance sports, such as cycling requires an athlete to have a 4^{th} and 5^{th} gear (Jeukendrup, Craig and Hawley, 2000). Thus, having an ability to achieve and sustain high power outputs is a necessity and therefore, a desirable finding. Spriet (2014) highlighted that fat as a primary fuel source becomes limited as exercise intensity increases >75% VO_{2peak}, as the body's ability to deliver environmental oxygen to the muscle mitochondria nears maximum capacity. During the current investigation, although not a measured component, it's unlikely the longer adaptation period miraculously allowed keto-adapted athletes to convert fat to adenosine triphosphate (ATP) in the absence of oxygen.

Prior to discussing potential mechanisms for improvement in power outputs, it's important to re-highlight that decreases in body mass meant participants were working against less resistance, however, absolute power outputs were maintained ($P > 0.05$) (see appendix K). Three additional factors which likely contributed to maintenance of absolute power were, the population sample, performance test chosen and when the performance test was performed. The CPT was performed subsequent to a 100 km TT, when participants were possibly in a glycogen depleted and/or fatigued state. Previously, it was noted that completing a 30-second sprint followed by a 2 minute rest period prior to completing a CPT impaired CPT performance (average power/W) in 7 habitually trained males (~49 $ml \cdot kg^{-1}min^{-1}$), with the authors attributing decreases in performance to muscle phosphocreatine depletion (Vanhatalo and Jones, 2009). Jeukendrup, Craig and Hawley (2000) demonstrated that moderately trained males completing a 1000 m TT cycle (1:00 minute) relied on 10% lactic, 40% anaerobic and 50% aerobic metabolism to fuel performance and the authors postulated that well-trained or elite athletes would have greater energy contribution from anaerobic metabolism. Therefore, given a CPT is 3x times the length of the aforementioned investigation (Jeukendrup, Craig and Hawley 2000), was carried out in a fatigued state in non-elite athletes, decreased carbohydrate consumption (<50 g/d) within the LCKD group was unlikely to substantially impact on CPT performance following the 100 km TT, as although the CPT was a 'maximal test', it likely a large proportion of the test was fuelled by aerobic metabolism versus had a more conventional Wingate test been performed and/or completed in a non-fatigued state and using highly-trained individuals.

Previous work using an acute LCHF diet has shown contradictory results, with impairment of 1 km (Havemann *et al.,* 2006) and maintenance of 5 km sprint performance (Rowlands and Hopkins, 2002) during endurance and ultra-endurance exercise trials. The current investigation demonstrates that 3 minute sprint performance can be maintained subsequent to a 100 km TT and 12 weeks of LCKD. Had the CPT been performed prior to the 100 km TT, the results would likely have favoured the high-carbohydrate group, however specific research in keto-adapted athletes is absent. Although the pre-exercise SS sprint was sufficient to examine peak power, the duration of the test was not appropriate to challenge any energy system beyond the ATP-phosphocreatine system (Egan and Zierath, 2013). Therefore, future work examining performance responses to a LCKD should examine anaerobic performance using tests such as a Wingate and CPT in a non-fatigued and fatigued state.

The maintenance of 3 minute sprint performance following a 12 week adaptation to a LCKD versus high-carbohydrate group in fed state is an intriguing finding and has considerable relevance within the right context. Endurance athletes who compete in endurance events ranging from 2-3 hours in length would likely not be too concerned if sprint performance in a non-fatigued state was impaired due to decreases in glycolyic flux (Egan and Zierath, 2013), granted endurance and sprint performance in a fatigued state was sustained or relatively improved, as is the case during the current investigation (McSwiney *et al.,* 2018). Although not always the case, break-aways, hill climbs and sprint finishes are likely to occur mid-way through an event or during the final stages of an endurance event, when fatigue is likely a contributing factor to optimal athletic performance. Although not a measured component, Havemann *et al.,* (2006) previously

examined sprint performance in a fatigued state following an acute LCHF diet (Table 6.3), by incorporating 4x 1 km sprints during a 100 km TT; to find that sprint performance was impaired versus high-carbohydrate group. Following promising findings from the current investigation, an additional elabourate investigation similar to Havemann *et al.,*'s (2006) would be desirable to examine if repeated sprint performance in a fatigued state or during exercise was sustained with sufficient time to adapt, which based on current evidence would be 12 weeks (McSwiney *et al.,* 2018) or more. Such a study design would help practicionerrs and athletes alike better understand physiological responces to endurance exercise varying in intensity and therefore, further highlight sports specific curcumstances where keto-adaptation may be beneficial or at least, not completely detrimental to performance. Finally, although carbohydrate restoration and/or carbohydrate feeding during exercise on a LCKD was not component of the current investigation, a recent study by Webster and colleagues (2017) documented the performance of an elite-level (77 $ml \cdot kg^{-1}min^{-1}$) 'low-carbohydrate/fat-adapted' endurance athlete. The participant involved in this 7 week crossover investigation had consumed a low-carbohydrate diet (~80g/d) for 2 years. The study concluded that a train low (low-carbohydrate diet), race high (carbohydrate supplementation during exercise) likely benefited high-intensity endurance-type exercise (4-30 minutes), but did not benefit short sprint or prolonged endurance performance versus a train low, race low approach. Though this investigation is limited by a small population sample (n = 1) and lack of a control group (HC diet), additional experimental work in this area could help to optimise dietary prescription for habituated low-carbohydrate/LCKD athletes.

Energy Contribution of Ketone Bodies once Keto-adapted

Respiratory exchange ratio values for LCKD participants contained within the current investigation demonstrated that fat was the predominant fuel source up until the final stages of the 100 km TT (Figure 3.2, Chapter 3). Although it's difficult to differentiate between fat, carbohydrate and ketone bodies being metabolised due to similarities in respiratory quotient of carbohydrates (1.00) and the ketone bodies, βHB (0.89) and AcAc (1.00) (Frayn, 1983), its unlikely βHB contributed significant energy to skeletal muscle. This assumption is based on two investigations, namely Volek *et al.,* (2016) and Cox *et al.,* (2016). As previously outlined, Volek *et al.'s* (2016) participants had resting βHB values of ~0.5 mM, which coincides with resting βHB values of the population sample in question (McSwiney *et al.,* 2018). Unlike the current investigation, changes in plasma βHB were monitored throughout and post-exercise; increasing to ~1.0 mM during 3 hours of submaximal exercise. Although the aforementioned investigation was carried out on a treadmill and the current investigation was carried out on a cycle ergometer (WattBike), which likely elicited different metabolic responses to exercise (Millet, Vleck and Bentley, 2009), assuming participants on the cycle ergometer experienced similar increases in plasma βHB, energy contribution from βHB to contracting muscles would have been most likely negligible. Previously, Cox *et al.,* (2016) configured a way of estimating energy contribution of ketone bodies (albeit from exogenous ketones) despite similarities in RER/RQ values. As discussed within Chapter 2 and 6, βHB was estimated ~16-18% of total energy during submaximal exercise (<75% W_{MAX}). Therefore, based on previous knowledge (Cox *et al.,* 2016; Volek *et al.,* 2016), energy contribution

from ketone bodies during the current investigation was perhaps, in the region of ~8-9%.

Keto-adaptation, Inflammation, Oxidative Stress and Recovery

From an athletic standpoint, one of the main drivers for endurance athletes adopting a LCKD is anecdotal reports of improved recovery contained within the literature (Volek, Noakes and Phinney, 2015) and lay press (Volek and Phinney, 2012). However, specific experimental research, particularly in a well-trained endurance athlete population is absent to support such claims. The current investigation failed to examine these claims, as blood samples were taken subsequent to a 2 day break from training. This is not a criticism of the current investigation, rather to highlight that it examined a LCKDs impact on various markers of health rather than a LCKDs impact on inflammation or oxidative stress in response to prolonged or intense exercise. Current claims within the literature are that ultra-endurance athletes can return to training within days, not weeks following a 100-mile ultra-marathon. Although speculative, Volek *et al.'s* (2016) cross-sectional study highlighted that ultra-endurance burned greater amounts of fat than high-carbohydrate athletes and as previously outlined, obtained plasma βHB of ~1.0 mM during a 3 hour exercise protocol. Perhaps, increases in oxidative metabolism during exercise, in addition to βHB provide protection against inflammation and oxidative stress during a ~100 mile race, as previously observed (Shimazu *et al.,* 2013) and hypothesised (Miller, Villamena and Volek, 2018). Although Cox *et al.'s* (2016) investigation highlighted that increases in plasma βHB could coexist with normal glycogen stores and insulin/glucose following ingestion of an exogenous ketone ester, it's unlikely βHB derived from hepatic tissues would co-exist in such circumstances

(Evans, Cogan and Egan, 2016), although specific research in this area is absent. Notably however, as previously outlined within investigations contained within Table 2, increases in fat oxidation following an adaptation to a LCHF diet are sustained following a period of carbohydrate restoration/loading (24-48 hours). Therefore, within the context of this claim within the literature (i.e., 100 mile ultra-endurance athletes), its plausible improved recovery stems from increases in fat oxidation/oxidative metabolism and not necessarily, 'ketones'. Given interest and lack of experimental knowledge in this highly desirable reported side effect of keto-adaptation, more work is needed to better understand the nuances of why keto-adapted persons reported enhanced recovery.

Keto-adaptation, Health and Well-Being

Body mass index is negatively correlated with longevity and disease free life (Stenholm *et al.,* 2017), therefore decreases in BMI in the LCKD group were a welcome consequence of keto-adaptation, as the lowest mortality rate is associated with persons with a BMI of <25 $kg \cdot m^2$ (Fontana and Hu, 2014). As outlined within Chapter 2 and Chapter 5, keto-adaptation is associated with many improvements in bodily health in an overweight population. However, large improvements in HOMA-IR previously observed (Volek *et al.,* 2009) were unnecessary during the current investigation, as it involved healthy individuals.

In addition, 'low-carbohydrate diets' were once noted for causing increases in inflammation and oxidative stress (Djuric *et al.,* 2001; Erhardt *et al.,* 1997). However, these diets contained 40-60% dietary fat and 20-40% of energy from carbohydrates. During the current investigation, dietary fat accounted for 77.2 %/kcal, while

carbohydrates were restricted to <50 g/d (41.1 ± 13.3 g/d) and no increases in inflammation or markers of oxidative stress were observed.

In fact, better safeguarding against increases in IL-6 and positive moderate effects in ICAM-1, MCSF and TNF-α occurred, while a relationship between changes in βHB and reduced IL-8 and reduced IL-8, M-CSF and protein carbonyl was observed. Maintenance of inflammatory biomarkers may have been partially affected by decreases in adipose tissue, as levels of body fatness and pro-inflammatory cytokine production are considered to be intertwined (Conde *et al.,* 2014). However, beyond the relationship between changes in body mass and leptin, body mass was not correlated with any changes in inflammatory or oxidative stress marker. Currently, it must be assumed that positive associations were in fact due to increases in βHB and/or potentially, other unmeasured variables. In conclusion, with a LCKD being primarily comprised of dietary fat, some segments of modern society are preconditioned to be alarmed, however, the current investigation demonstrates a LCKD can have beneficial impacts on body composition whilst maintaining or causing moderate improvements in key markers of bodily health, including glycaemic control and inflammation. With that being said, additional work examining the lipoprotein fractions of athletes following a LCKD long term and their inflammatory and oxidative stress responses to exercise would be desirable.

Despite resting serum βHB only reaching 0.5 ± 0.4 mM within LCKD participants who completed the training intervention (McSwiney *et al.,* 2018), plasma βHB concentrations of ~0.8 mM in mice consuming a LCKD (% carbohydrate:fat:protein = 1:89:10) was associated with an 8.8% increase in median lifespan and a reduced incidence of tumors

at the time of death (Roberts *et al.,* 2017) versus a low-carbohydrate high-fat diet (% 10:70:20) and control diet (% = 65:17:18). This increase in lifespan was associated with increases in beta-oxidation and a shift away from glycolysis, a characteristic evident within the current population during exercise (RER) (Figure 3.2, Chapter 3) (McSwiney *et al.,* 2018). Despite these promising findings in mice, longitudinal studies in humans are currently absent and an area of research which warrants further exploration.

Prior to this investigation, to the author's knowledge, no investigation had examined the nutrient density of a LCKD in an endurance athlete population. This was a concern, given the restrictive nature of a LCKD and that poor implementation of a 'well-formulated LCKD' may lead to nutrient or vitamin deficiency. Thereafter, iron deficiencies became a concern within the LCKD group, as reported intakes of iron, vitamin C and copper were reduced. However, following further investigation, no decreases in concentrations of serum ferritin were observed. This may have been due to inaccuracy of food diaries or perhaps, due to increased bioavailability of iron due to a reduced concentrations of phytates in the diet. Whatever the mechanism, the current investigation demonstrates a LCKD appears proficient at sustaining normal iron stores despite reported dietary deficiencies, following a 12 week adaptation in male endurance athletes. Although this is a positive finding, endurance athletes, irrespective of dietary approach (HC or LCKD) should continue to be screened regularly by a medical professional (Thomas *et al.,* 2016).

Adherence to a LCKD remains a concern based on modest increases in βHB in some individuals within the current investigation, as outlined within Figure 1 in Chapter 4. In addition, carbohydrate consumption was 41.1 ± 13.3 g/d in LCKD participants, meaning

some participants were consuming in excess of the ~50 g/d threshold. Previously, Saslow *et al.,* (2017) and Tay *et al.,* (2015) carried out 1-year LCKD studies in participants with type-2 diabetes mellitus. At the midway point, carbohydrate consumption was 54 ± 07 g/d (Tay *et al.,* 2015) and 44.1 g/d (Saslow *et al.,* 2017), respectfully, however by the 1-year mark carbohydrate intake had crept up to 74.0 ± 18.1 g/d (Tay *et al.,* 2015) and 73.7 g/d (Saslow *et al.,* 2017), respectfully, indicating long-term adherence to a LCKD (<50 g/d CHO) may be an issue in some individuals. As outlined within Table 2 in Chapter 5, fibre intake was beneath RDA's for European males within the LCKD group. The authors postulated that a low-fibre intake on a LCKD may not be as problematic as a low-fibre intake on a non-ketogenic diet, as ketogenesis is associated with hepatic production of butyrate, an important short-chain fatty acid typically produced via bacterial fermentation in the gut following ingestion of fibre butyrate (Kanauchi *et al.,* 1999). If poor dietary adherence to a LCKD is present a person following a 'ketogenic diet' could conceivably not stimulate ketogenesis all the while, excluding foods high in fibre such as, wholegrains and starchy vegetables and therefore, put themselves at an increased risk of various cancers (Kritchevsky, 1986). Given these reasonable concerns, it remains to seen in future longitudinal investigations and on population level the sustainability of a LCKD.

Exogenous Ketones and Performance

Prior to the current investigation, the examination of exogenous ketones as an ergogenic tool had only been assessed in well-trained cyclists (Cox *et al.,* 2016; Leckey *et al.,* 2017) and field sport athletes (Evans and Egan, 2018). Therefore, the current investigation set out to examine if their implementation in a well-trained runners would

be of benefit. During the current investigation, acute ingestion of 573 mg·kg^{-1} BM of (R)-3-hydroxybutyl (R)-3-hydroxybutyrate ketone monoester (HVMN®, San Francisco, CA) increased plasma βHB to 0.8 ± 0.1 mM 30 minutes post-ingestion and peaked 70 minutes post-ingestion during submaximal exercise at 1.2 ± 0.2 mM. These increases in plasma βHB coincided with 1 g·kg of carbohydrate feeding prior to exercise and 1.2 g·min^{-1} during exercise. Plasma βHB values were twofold those achieved by participants within the LCKD performance investigation who followed a LCKD for 12 weeks (McSwiney *et al.,* 2018), illustrating why the topic of exogenous ketones was described as an exciting area of research for sports science practitioners (Evans, Cogan and Egan, 2016).

This investigations findings that acute ingestion of 573 mg·kg^{-1} BM ketone monoester enhances simulated 10 km TT running performance in well-trained athletes coincide with findings by Cox *et al.,* (2016) that 573 mg·kg^{-1} BM of ketone monoester enhances 30 minute TT performance in well-trained cyclists. Increases in plasma βHB were less during the current investigation, than previously observed by Cox *et al.,* (2016). However, as previously noted, participants during the current investigation incorporated a pre-exercise meal and co-ingested the ketone ester with carbohydrate electrolyte solution, whereas, Cox *et al.,* (2016) participants co-ingested βHB with carbohydrate-electrolyte solution. Based on previous learnings, the presence of the pre-exercise meal likely subsided increases in plasma βHB (Stubbs *et al.,* 2017).

Given moderate increases in plasma βHB and submaximal exercise was performed below participants lactate threshold (<4 mM) (Yoshida *et al.,* 1987), the current investigation did not measurably observe metabolic responses to exercise such as decreased lactate, which are a consequence of ketone monoester ingestion (Cox *et al.,*

2016; Evans and Egan, 2018). Therefore, the mechanism by which each of the *n* = 5 participants improved simulated 10 km TT running performance remained unknown. However, given similarities in RER values during the current investigation (0.926 ± 0.039) and RER/RQ values for βHB (0.89) (Frayn, 1983), it's plausible ingested βHB was oxidised at a high-rate in the absence of a lactate lowering effect, perhaps attributing up to 16-18% of total energy, as previously observed by Cox *et al.,* (2016). Exogenous ketones, specifically ketone monoester ingestion presents an opportunity to alter and enhance endurance performance in well-trained runners and cyclists (Cox *et al.,* 2016). Despite observing a homogenous improvement in simulated 10 km TT running performance during the current investigation, an additional more nuanced experiment is required to determine the ergogenic mechanism

Chapter 8

Conclusion

In conclusion, despite a welcome resurgence in experimental and cross sectional studies examining LCKDs and performance in endurance athletes, experimental knowledge remains in its infancy. Therefore, a comprehensive endorsement of this dietary paradigm at this time is difficult to make. However, an endorsement of any 'diet' in all circumstances is unwise. This is why current guidelines recommend a personalised, periodised approach (Burke *et al.,* 2011; Jeukendrup, 2017). Based on current knowledge, it would appear unwise to adopt a LCKD <4 weeks prior to an endurance event ranging from 2-3 hours in length. Thereafter, endurance performance at sub-maximal intensities appears to be maintained in well-trained and trained individuals, while the performance of highly-glycolytic modes of exercise in non-fatigued states, such as sprinting, likely remains impaired, however, long-term experimental work in this area is absent. From a health and well-being standpoint, a LCKD appears effective at sustaining homeostatic measures of health. Specifically, its implementation in *ad libitum* settings caused substantial decrease in body mass, preservation of lean body mass and better protection against increases in inflammation versus a high-carbohydrate group completing the same training intervention. The area of exogenous ketone esters is fast emerging and appears promising. Their implementation is most likely to be of benefit in well-trained individuals who have a greater capacity to oxidise ketone bodies, however, additional sport specific experimental work is needed to better understand dosing strategies to hopefully optimise performance and minimise gastrointestinal issues frequently reported.

8.2 Study Implications

8.2.1 Performance

An endurance athlete who adopts a LCKD in *ad libitum* non-highly controlled settings can expect to observe decreases in body fatness and maintenance of lean body mass. These observations are common within the literature and may be particularly attractive to athletes who struggle with maintaining competitive race weight using a carbohydrate based diet and a caloric deficit.

Improvements in body composition may contribute to improvements in athlete's power to weight ratio and relative power outputs, as observed during the current investigation. However, although not a measured component of the current investigation, athlete's ability to perform at 90-100% VO_{2peak} will likely remain impaired, via inhibition of pyruvate dehydrogenase and phosphofructokinase.

The current investigation demonstrated 100 km TT performance could be maintained following a 12 week adaptation to a LCKD. As participants' maximal oxygen consumption was not assessed in a non-fatigued state, the percentage of their peak oxygen consumption they were exercising at during the 100 km TT cannot be accurately quantified. However, from examining and comparing lactate responses and RER values to Webster *et al.,* (2016) cross sectional investigation carried out in keto-adapted endurance athletes on a cycle ergometer, its estimated participants were exercising at 70-72% VO_{2peak} during the 100 km TT within the current investigation. Therefore, the current investigation demonstrated 100 km TT performance at 70-72% VO_{2peak} was maintained. This relative intensity is sufficient for recreationally active and trained

individuals, however, elite athletes compete a large proportion of 2-3 hour endurance events at >70% VO_{2peak} (Fernandez-Garcia *et al.,* 2002), necessitating the need for a greater carbohydrate intake.

8.2.2 Health

Body Mass Index and Well Being

As previously discussed, being a healthy body weight and in a hypocaloric state are two of the most profound things which can be done to optimise health, i.e., glycemic control, mitochondrial function and inflammation. As some participants within the LCKD group were overweight at pre-intervention testing (i.e., BMI >25 kg/m^2), adopting a LCKD across the lifespan could have considerable beneficial impacts on health if adherence was sustained. That being said, the current investigation only monitored a number of markers of wellness across a 12 week period. Therefore, other unmeasured variables and longitudinal changes remain unknown, but warrant further exploration given the growing interest in LCKD.

Saturated Fat

Although experimental evidence has shown higher intake of saturated fat can be safe once energy is being oxidized, the worry would be an athlete would adopt a LCKD, restrict carbohydrates and lose 'fear' associated with foods high in saturated fat, but longitudinally start to reintroduce more carbohydrates into their diet. As previously discussed, this has been observed within two investigations in overweight patients following a LCKD for 12 months (Tay *et al.,* 2015; Saslow *et al.,* 2017). This increase in carbohydrate was associated with weight re-gain. Therefore, this hypothetical

athlete/person would be in a hypocaloric state and consuming a larger proportion of energy from saturated fats than is recommended, which we know from epidemiological studies to be problematic.

Fibre

Debate exists to whether the reduced fiber content of a LCKD is problematic, due to endogenous production of fatty acids (i.e., butyric acid) when in a keto-adapted state (i.e., ketogenesis). As there is currently no experimental evidence within humans to suggest this is not an area of concern, athletes should be encouraged to consume adequate fibre until compelling evidence becomes available; illustrating that a lower fibre intake on a LCKD is not problematic.

8.2.3 Exogenous Ketones

Performance

The current investigation has shown beneficial effects of ketone ester ingestion for simulated 10 km TT running performance subsequent to one hour of submaximal exercise. However, the investigation failed to determine the mechanism for enhanced performance. Therefore, it's difficult to postulate where and how their use may be of benefit to real world athletes. Until more research emerges to demonstrate mechanisms by which they can positively impact on endurance running performance, they should not be recommended for use in non-highly controlled settings.

8.3 Recommendations for Future Research

8.3.1 Keto-adaptation and Performance

Duration of Adaptation

To quantifiably measure changes in performance responses to a period keto-adaptation, assess baseline performance under various stimulus, i.e., sprint (10 seconds), sprint endurance (<1 hour), endurance (1-3 hours), ultra-endurance (>3-5 hours) and strength when participants are in a state of carbohydrate availability. Thereafter, using an RCT replicated crossover design, monitor changes in performance each week for a number of months (i.e., >3-6 months). Such an elaborate investigation would provide evidence if additional time accolated to keto-adaptation helps regain performance or demonstrate exercise intensities where performance remains impaired and if so, by how much, versus a well-matched group consuming optimal carbohydrate feeding.

Intensity

Despite the best intentions of current investigation to assess performance in as practical a manner as possible for endurance athletes and to reflect the metabolic demands placed on real world athletes, it would appear participants completed the 100 km TT at sub-maximal intensities (~70% VO_{2peak}). Therefore, future work aiming to assess endurance performance should incorporate an exercise trial similar Havemann *et al.*'s (2008) trial, which included a 100 km TT and a number of 4 km and 1 km sprints throughout, to ensure participants are exercising under aerobic and anaerobic conditions.

8.3.2 Keto-adaption and Health

Longitudinal Effects and Response to Exercise

Study two demonstrated a LCKD for 12 weeks is proficient at maintaining homeostatic measures of wellness. Future work should examine its longitudinal effects (i.e., >12 weeks) and inflammatory and oxidative stress responses to prolonged and intense exercise.

Gut Microbiota

One of the key concerns which arose from the current investigation was that the LCKD was reportedly deficient in fibre. Some low-carbohydrate proponents suggest this is not problematic, due to endogenous production of ketone bodies and short chain fatty acids, which are traditionally derived through bacterial fermentation within the gut. However, if a state of ketogenesis provides an optimal state for the gut remains to be explored within experimental settings. Key questions are, 1) what increase in βHB is necessary to produce adequate production of butyrate for the gut, 2) what volume of foods high in butyrate are necessary to supplement endogenous production and finally, 3) are short chain fatty acids produced within the gut (i.e., bacterial fermentation of fibre) more optimal and/or equal to short chain fatty acids produced within hepatic tissues (i.e., ketogenesis).

Iron

Serum ferritin remained unchanged during the current investigation, however, haemoglobin non-significantly decreased to marginally above undesirable levels. This decrease was associated with reduced iron intake and a decrease in MCH and MCHC.

Therefore, future work is needed to determine the mechanism which enabled the maintenance of iron stores, but decreased LCKD participant's ability to utilise and transport it.

8.3.3 Exogenous Ketones and Performance

Dosing

Including the current investigation, four investigations have been carried out in well-trained individuals and exogenous ketone esters. The current investigation and Cox *et al.*'s (2016) dose of ketone ester were identical and well tolerated. However, other investigations incorporated larger doses (Evans and Egan, 2018) and two large doses with fizzy cola (Leckey *et al.,* 2017) and were not well tolerated (i.e., increased incidence of GI symptoms). Therefore, future work should follow dosing stagey implemented by Cox *et al.'s* (2016) research group, who were involved in the manufacturing and development of the 'Oxford ketone ester', therefore, it's likely a number of preliminary unpublished investigations were undertaken to perfect the tolerability of the dose.

Metabolic Responses to Exercise

The current investigation failed to measurably observe metabolic changes to exercise performance post-ingestion. To avoid this in the future, it would be beneficial to have participants exercising at or near their lactate threshold, therefore, if there is a glucose sparing effect, it would be measureable using basic laboratory equipment, such as a COSMED, lactate meter or an ELISA.

Glucose Sparing or Impairing

There is a considerable evidence to suggest adaptation to a LCHF or LCKD impairs glycolytic flux by inhibiting pyruvate dehydrogenase and phosphofructokinase. However, it remains to be seen if increases in βHB via the use of exogenous ketone esters are an allosteric inhibitor to glucose metabolism. Preliminary work by Evans and Egan (2018) demonstrated no impairment of intermittent running or 15 m sprint performance, while the current investigation noted similar increases in plasma glucose and a greater increase in plasma lactate immediately after the 10 km TT. However, more work is needed to determine if exogenous ketone esters impair high intensity exercise performance (i.e., >90-95% $VO2_{peak}$) and/or spare muscle glycogen.

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Additional Conference Proceedings and Publications

Poster Presentation. Russ Kline Symposium Columbus Ohio (2016)
Vincent J. Miller, Richard A. LaFountain, Emily C. Barnhart, Teryn N. Sapper, Parker N. Hyde, Ryan Dickerson, Jay Short, Madison Bowling, **Fionn T. McSwiney**, William J. Kraemer and Jeff S. Volek. "Effects of Keto-Adaptation and Exercise Training on Mitochondria Function in Human Skeletal Muscle"

Poster Presentation. Annual Meeting, World Congress on Exercise is Medicine (2017) Parker N. Hyde, Nathan Lamba, Christopher Crabtree, Debbie Scandling, Jay A. Short, Richard A. LaFountain, Teryn N. Sapper, Madison L. Bowling, Vincent J. Miller, **Fionn T. McSwiney**, Ryan M. Dickerson orlando P. Simonetti and Jeff S. Volek. "Analysis of Visceral Fat Tissue via Dual-energy X-ray Absorptiometry and Magnetic Resonance Imaging".

Poster Presentation. Russ Kline Symposium Columbus, Ohio (2017)
Vincent J. Miller, Richard A. LaFountain, Emily C. Barnhart, Teryn N. Sapper, Parker N. Hyde, Ryan Dickerson, Jay Short, Madison Bowling, **Fionn T. McSwiney**, William J. Kraemer and Jeff S. Volek. "Effects of Keto-Adaptation and Exercise Training on Mitochondria Function in Human Skeletal Muscle" (see appendix I).

Poster Presentation. Russ Kline Symposium Columbus, OH 2018 – Poster Presentation (see appendices I).
Ryan Dickerson, Parker N. Hyde, Teryn N. Sapper, Vin J. Miller, Rich A. LaFountain, Emily Barnhart, **Fionn T. McSwiney**, Jay Short, Madison Bowling and Jeff S. Volek.
"Carbohydrate Restriction Results in Favorable Outcomes in Plasma Lipid Composition Despite High Saturated Fat Consumption"

Poster Presentation. Russ Kline Symposium in Columbus OH, 2018 – Poster Presentation (see appendices J).
Vincent J. Miller, Richard A. LaFountain, Emily C. Barnhart, Teryn N. Sapper, Parker N. Hyde, Ryan Dickerson, Jay Short, Madison Bowling, **Fionn T. McSwiney**, Carl M. Maresh, William J. Kraemer and Jeff S. Volek.
"Nutritional Ketosis Uniquely Enhances Mitochondrial Function in Human Skeletal Muscle During Adaptation to Exercise"

Submitted for Publication

Journal Article. Narrative Review: Submitted to 'European Journal of Sports Science' in February 2018.
Fionn T. McSwiney, Caryn Zinn, Daniel J. Plews and Lorna Doyle
"Keto-adaption, an ergogenic aid for endurance athletes, what evidence? A narrative review" (submitted – see appendices N)

To be submitted for Publication.

Journal Article. Invited Review which has been written and will be submitted shortly to 'The Journal of the American College of Nutrition'.
Parker N Hyde, Richard A Lafountain, Teryn Sapper, **Fionn T. McSwiney**, Jay Short, Emily Barnhart, Madison Bowling, Jeff S Volek
"Construction and Composition of a Well-Formulated Ketogenic Diet for Scientific Research"

Journal Article. To be submitted to Lipids in July 2018.
Fionn T. McSwiney, Ryan Dickerson, Parker N. Hyde, Vincent J. Miller, Richard A. LaFountain, Teryn Sapper, Bruce Wardrop, Jay Short, Emily Barnhart, Madison Bowling, Jeff S. Volek, Lorna Doyle
'12 Week Ketogenic Diet increases Total Cholesterol with increased Beta-hydroxybutyrate (βHB) levels associated with reduced resting measures of Inflammation and Oxidative Stress in Endurance Athletes'

Journal Article. To be submitted to Nutrients in July/August 2018.
Fionn T. McSwiney, Lorna Doyle
"Comparison of Nutrient Intake of a Low-Carbohydrate Ketogenic Diet versus, a High-Carbohydrate Diet in *ad libitum* non-highly controlled settings in Endurance Athletes"

Journal Article. To be submitted to Metabolism Clinical and Experimental.
Fionn T. McSwiney, Lorna Doyle, Brendan Egan
"Increased rates of fat oxidation during graded exercise after a short-term ketogenic diet in endurance-trained males"

Journal Article. Following additional data collection, manuscript will be prepared for publication.
Mark. Evans, **Fionn T. McSwiney**, Brendan Egan
Running title: "Effects of Acute Ingestion of Ketone Ester on Exercise Metabolism and 10 km TT Running Performance in Well-Trained Endurance Athletes"

Appendices

Appendix A. Participant Information Sheet

PARTICIPANT INFORMATION SHEET

Project Title: To determine if a high fat ketogenic diet or a high-carbohydrate diet is optimal for endurance performance.

Lead Investigators:

Fionn Mc Swiney (Postgraduate Researcher): fionnmc@hotmail.com

Dr. Lorna Doyle (Principal Investigator): lmdoyle@wit.ie

INFORMATION TO POTENTIAL PARTICIPANTS

1. What is the purpose of the project?

A significant amount of research has been carried out over the last two decades on high fat and high-carbohydrate diets and their impacts on performance in endurance athletes. Previous research has focused on the short term effects of consuming a high fat or a high-carbohydrate diet, with adaptation periods ranging from 3 days to 6 weeks. This majority research recommends that consuming a high-carbohydrate is optimal for endurance performance. Despite these findings many endurance athletes have begun to lose faith with this method due to the limited supply of glycogen and risk of early fatigue during an event and issues related to consuming a high-carbohydrate diet such as gastrointestinal stress and weight gain. The long term consequences of consuming a high fat diet are unknown, the principle objective of this research is to determine the performance effects of consuming a high fat ketogenic diet (carbohydrate is restricted to <50g/d) or a high-carbohydrate diet (diet contains 71% carbohydrate) following a 12 week dietary and training intervention.

2. Why have I been selected to take part?

We are looking to access endurance athletes aged 18-40years with a minimum of 2 years training experience (7 hours a week training for the past 12 months), a category to which your preliminary details place you.

3. What will I have to do?

You will be asked to attend the human performance laboratory at Waterford Institute of Technology on two separate occasions separated by a 12 week dietary and training intervention. You will receive a DXA scan and complete two exercise protocols (100km time trial & a critical power test) (lasting 3-4 hours). Prior to the exercise protocol we will take a resting venous blood sample (withdrawn by a qualified phlebotomist). During 100km time trial participant's heart rate (HR), blood lactate, respiratory exchange ratio (RER) will be measured at the beginning and at 20km intervals and at 10 minutes after the critical power test. The

following 12 weeks will comprise of participants consuming a high fat ketogenic diet (150g/d for 2 weeks, 100g/d for 2 weeks and 50g/d of carbohydrate for the remaining 8 weeks) or a high-carbohydrate diet (71% carbohydrate, 20% fat & 9% protein for 12 weeks) and taking part in a training intervention; daily bouts of high intensity exercise (10X1 minute sprints at 60% maximal power output), endurance exercise (56-68% VO2max for minimum 7 hours a week) and strength training (6X10reps at 70-80% one repetition maximum 3 days a week). At the end of this 12 week intervention you will be asked to return to the human performance laboratory at WIT and repeat the pre intervention testing.

4. What are the exclusion criterion (i.e., are there any reasons why I should not take part)?

People under the age of 18 or over the age of 40yrs. Has a minimum of 2 years endurance training experience (minimum 7 hours a week for the previous 12 months).

5. Will my participation involve any physical discomfort?

Maximal and submaximal exercise is challenging even to the fittest individuals and therefore carries exertional discomfort during participation. DOMS is commonly experienced in the days preceding high intensity exercise. This soreness is not serious and normally subsides after 24-72h varying from individual to individual.

Furthermore, for the purpose of measuring blood biomarkers we will require a qualified phlebotomist to take venous blood samples before the exercise protocol. This procedure is considered extremely safe and carries minimal risk when performed by appropriately trained and certified individuals.

6. Will my participation involve any psychological discomfort or embarrassment?

No.

7. Will I have to provide any bodily samples (i.e., blood, saliva)?

We will require a small venous blood sample for the purpose of measuring inflammatory markers. The blood sample will be taken prior to exercise protocol. During exercise participants blood lactate will be measured prior to the exercise protocol and at 20km intervals during the exercise protocol and 10 minutes after the final exercise protocol. This measurement requires a blood sample from a finger or ear lobe and is relatively non-invasive as the blood sample is minimal.

8. How will confidentially be assured?

The research team will put into place a number of procedures to protect the confidentially of participants. These include:

You will be allocated a participant code that will always be used to identify any data that you provide. Your name or other personal details will not be associated with your data, for example the consent form you sign will be kept separate from your data.

All paper records will be stored in a locked filing cabinet, accessible only to the research team and all electronic information will be stored on a password protected computer. In general all of the information you provide will be treated in accordance with the Data Protection Act 1998 & 2003.

You will not be identifiable from any academic publications that may arise from this study.

9. Who will have access to the information that I provide?

Any information and data gathered during this research study will only be available to the research team identified in the information sheet. Should the research be presented or published in any form, then that information will be generalised (i.e., your personal information or data will not be identifiable).

10. How will my information be stored / used in the future?

All information and data gathered during this research will be stored in line with the Data Protection Act 1998 & 2003 and will be destroyed 5 years following the conclusion of the study. During this time the data may be used by members of the research team only for purposes appropriate to the research question, but at no point will your personal information or data be revealed.

11. Has this investigation received appropriate ethical clearance?

Yes, the study and its protocol have received full ethical approval from the Waterford Institute of Technology Research Ethics Committee. If you require confirmation of this please contact the Secretary to the Ethics Committee Suzanne Kiely skiely@wit.ie stating the title of the research project and the name of the principal investigator.

12. Will I receive any financial reward / travel expenses for taking part?

No. Your participation in this study is entirely voluntary. However, we may offer feedback from your tests, which can be used to modify and enhance training and nutritional programmes.

13. Are there any risks associated with low-carbohydrate diets?

At the beginning of an athlete's adaptation period to a high fat ketogenic diet, an athlete may feel fatigued, nausea, cravings or experience migraines. These symptoms occur at the start of an adaptation period, when the body is not efficient at burning fat as a fuel. These symptoms

can last one to two weeks in extreme circumstances; if the side effects persist participants are asked to contact the research team immediately. If this occurs the individual should also increase sodium and potassium content of their diet by eating plenty of green leafy vegetables and slightly increase carbohydrate intake. A slow reduction of carbohydrate intake is recommended to minimize any potential adverse effects.

14. How can I withdraw from the project?

Participants reserve the right to withdraw from the investigation at any time. Please notify the research team on your decision to withdraw from the investigation as soon as possible (contact details attached).

After you have completed the research you can still withdraw your personal information / data by contacting one of the research team (contact details attached) referencing your participant number or if you have lost this give them your name.

If for any reason, you wish to withdraw your data please contact the investigator within a month of your participation. After this date, it may not be possible to withdraw your individual data as the results may already have been published. However, as all data are anonymised, your individual data will not be identifiable in any way.

15. If I require further information who should I contact and how?

Fionn Mc Swiney

School of Health Science,

Dept. Health, Sport & Exercise Science,

Waterford Institute of Technology,

Waterford,

Ireland.

Mob: 0862032735

Email: fionnmc@hotmail.com

Waterford Institute *of* Technology
INSTITIÚID TEICNEOLAÍOCHTA PHORT LÁIRGE

Appendix B. Informed Consent Form

Waterford Institute *of* Technology
INSTITIÚID TEICNEOLAÍOCHTA PHORT LÁIRGE

Department of Health, Sport and Exercise Science

INFORMED CONSENT FORM

TITLE OF PROJECT: To Determine if a High Fat Ketogenic Diet or a High-carbohydrate Diet is Optimal for Endurance Performance

PARTICIPANT ID NUMBER:

Please read and complete this form carefully.

Please tick if applicable

I have read and understand the Participant Information Sheet.
I have had an opportunity to ask questions and discuss this study and I have received satisfactory answers.
I understand I am free to withdraw from the study at any time, without having to give a reason and without prejudice.
I agree to take part in this study.
I confirm that I have disclosed relevant medical information before this study.
I know that the results may be published, but they will not be linked to me.

I am aware of any possible risks and discomfort.

I agree to inform the researcher immediately if I am in pain or if I feel uncomfortable.

I would like to receive feedback on the overall results of the study at the email address given below.

Email address……

Signature of participant…… Date……

(NAME IN BLOCK LETTERS)……

Signature of researcher…… Date……

(NAME IN BLOCK LETTERS)……

This consent is specific to the particular test described in the information sheet attached and shall not be taken to imply my consent to participate in any subsequent experiment or deviation from that detailed here.

Appendix C. Ethical Approval from Waterford Institute of Technology, 2015

Institiúid Teicneolaíochta Phort Láirge Waterford Institute *of* Technology

Port Láirge, Éire.
T: +353-51-302000
info@wit.ie

Waterford, Ireland.
T: +353-51-302000
www.wit.ie

Ref: 15/HSES/03

4th March, 2015.

Mr. Fionn McSwiney,
Brambly,
27, The Anchorage,
Williamstown Road,
Waterford.

Dear Fionn,

Thank you for submitting your amended documentation in relation to your project *'To determine if a high fat ketogenic diet or a high carbohydrate diet is optimal for endurance performance'* to the WIT Research Ethics Committee.

I am pleased to inform you that we now fully approve WIT's participation in this project and we will convey this to Academic Council.

We wish you well in the work ahead.

Yours sincerely,

Prof. John S. Wells,
Chairperson,
Research Ethics Committee

cc: Dr. Lorna Doyle

Appendix D. High-Carbohydrate Information Handout

Waterford Institute *of* Technology
INSTITIÚID TEICNEOLAÍOCHTA PHORT LÁIRGE

High-carbohydrate Group Handout

Enter participants code
Enter todays date

Fionn Mc Swiney

School of Health Sciences,

Dept. of Health, Sport & Exercise Science,

Waterford Institute of Technology,

Waterford.

(Enter participant's name)

This information hand out is designed to make your dietary intervention as easy and maintainable as possible.

The hand out includes information on:

- High-carbohydrate Diet, what it consists of
    - Carbohydrate
    - Fat
    - Protein
    - Vitamins & Minerals

- Frequently Asked Questions
    - Why is Carbohydrate important?
    - How much Carbohydrate do I need?
    - Which foods are good sources of Carbohydrate?
        - Categories of Carbohydrate
        - Acute fuelling strategies
    - Glycaemic Index explained
    - When is Carbohydrate important?

- Food Portions
    - Supplying 50g of Carbohydrate

- Example of a High-carbohydrate Diet
    - Supplying 3,236kcal
    - With 75% of energy coming from Carbohydrate

What does a High-carbohydrate Diet consist of?

- **Carbohydrate (70 - 75% energy intake)**
    - High (75% of a 3,000kcal+ diet)
- **Fat (20% energy intake)**
    - Moderate
- **Protein (9% energy intake)**
    - Low

Carbohydrates

Athletes benefit the most from the amount of carbohydrates stored in the body. In the early stages of moderate exercise, carbohydrates provide 40 to 50% of the energy requirement. Carbohydrates yield more energy per unit of oxygen consumed than fats. Because oxygen often is the limiting factor in long duration events, it is beneficial for the athlete to use the energy source requiring the least amount of oxygen per kilocalorie produced. As work intensity increases, carbohydrate utilization increases.

Complex carbohydrates come from foods such as spaghetti, potatoes, lasagne, cereals and other grain products. Simple carbohydrates are found in fruits, milk, honey and sugar. During digestion, the body breaks down carbohydrates to glucose and stores it in the muscles as glycogen.

During exercise, the glycogen is converted back to glucose and is used for energy. The ability to sustain prolonged vigorous exercise is directly related to initial levels of muscle glycogen. The body stores a limited amount of carbohydrate in the muscles and liver. If the event lasts for less than 90 minutes, the glycogen stored in the muscle is enough to supply the needed energy. Extra carbohydrates will not help any more than adding gas to a half-full tank will make the car go faster.

For events that require heavy work for more than 90 minutes, a high-carbohydrate diet eaten for two to three days before the event allows glycogen storage spaces to be filled. Long distance runners, cyclists, cross-country skiers, canoe racers, swimmers and soccer players report benefits from a pre-competition diet where 70 – 75% of the calories comes from carbohydrates.

For continuous activities of three to four hours, make sure that glycogen stores in the muscles and liver are at a maximum. Consider taking carbohydrates during the event in the form of carbohydrate solutions. The current recommendation is a 6 to 8% glucose solution.

- You can make an excellent home-brewed 7.6 percent sports drink with reasonable sodium amounts. Add 6 tablespoons sugar and 1/3 teaspoon salt to each quart of water. Dissolve sugar and cool. The salt translates into a sodium concentration of 650 mg/litre. This small amount is good for marathon runners.

Electrolyte beverages can be used if the athlete tolerates them, but other electrolytes are not essential until after the event. Experiment during training to find the best beverage for you.

Eating sugar or honey just before an event will not provide any extra energy for the event. It takes about 30 minutes for the sugar to enter the blood stream. This practice may also lead to dehydration. Water is needed to absorb the sugar into the cells. Furthermore, sugar eaten before an event may hinder performance because it triggers a surge of insulin. The insulin causes a sharp drop in blood sugar level in about 30 minutes. Competing when the blood sugar level is low leads to fatigue, nausea and dehydration.

A diet where 70 – 75% of calories come from carbohydrates for three days prior to the event is sometimes helpful for endurance athletes. Water retention often is associated with carbohydrate loading. This may cause stiffness in the muscles and sluggishness early in the event. A three-day regimen minimizes this effect. The previously suggested seven days of deprivation/repletion is not recommended due to increased risks of coronary heart disease. In addition, electrocardiograph abnormalities may occur and training during the deprivation phase may be difficult.

Fats

Fat also provides body fuel. For low - moderate exercise, about half of the total energy expenditure is derived from free fatty acid metabolism. If the event lasts more than an hour, the body may use mostly fats for energy. Using fat as fuel depends on the event's duration and the athlete's condition. Trained athletes use fat for energy more quickly than untrained athletes. Consumption of fat should not fall below 15% of total energy intake because it may limit performance. Athletes who are under pressures to achieve or maintain a low body weight are susceptible to using fat restriction and should be told that this will hinder their performance.

Fat may contribute as much as 75% of the energy demand during prolonged aerobic work in the endurance-trained athlete. There is evidence that the rate of fat metabolism may be accelerated by ingesting caffeine prior to and during endurance performance. However, insomnia, restlessness and ringing of the ears can occur with caffeine consumption. Furthermore, caffeine acts as a diuretic and athletes want to avoid the need to urinate during competition.

Protein

After carbohydrates and fats, protein provides energy for the body. Exercise may increase an athlete's need for protein, depending on the type and frequency of exercise. Extra protein consumed is stored as fat. In the fully grown athlete, it is training that builds muscle, not protein per se. The ADA reports that a protein intake of 10 to 12% of total calories is sufficient. Most authorities recommend that endurance athletes eat between 1.2-1.4 grams protein per kg of body weight per day; resistance and strength-trained athletes may need as much as 1.6-1.7 grams protein per kg of body weight (1kg equals 2.2 pounds).

Japanese researchers demonstrated that "sports anaemia" may appear in the early stages of training with intakes of less than 1 gram/kg of body weight per day of high quality protein. To calculate your protein needs, divide your ideal weight by 2.2 pounds to obtain your weight in kilograms. Then multiply kilograms by the grams of protein recommended.

A varied diet will provide more than enough protein as caloric intake increases. Furthermore, Americans tend to eat more than the recommended amounts of protein. Excess protein can deprive the athlete of more efficient fuel and can lead to dehydration. High-protein diets increase the water requirement necessary to eliminate the nitrogen through the urine. Also, an increase in metabolic rate can occur and

therefore, increased oxygen consumption. Protein supplements are unnecessary and not recommended.

Vitamins and Minerals

Increased caloric intake through a varied diet ensures a sufficient amount of vitamins and minerals for the athlete. There is no evidence that taking more vitamins than is obtained by eating a variety of foods will improve performance. Thiamine, riboflavin and niacin (B vitamins) are needed to produce energy from the fuel sources in the diet. However, plenty of these vitamins will be obtained from eating a variety of foods. Carbohydrate and protein foods are excellent sources of these vitamins. Furthermore, the B vitamins are water soluble and are not stored in the body, so toxicity if not an issue. Some female athletes may lack riboflavin, so ensuring adequate consumption of riboflavin-rich food is important, like milk. Milk products not only increase the riboflavin level but also provide protein and calcium. The body stores excess fat-soluble vitamins A, D, E and K. Excessive amounts of fat-soluble vitamins may have toxic effects.

Minerals play an important role in performance. Heavy exercise affects the body's supply of sodium, potassium, iron and calcium. Sweating during exercise increases the concentration of salt in the body. Consuming salt tablets after competition and workouts is not advised as this will remove water from your cells, causing weak muscles. Good sodium guidelines are to: 1) avoid excessive amounts of sodium in the diet and 2) beverages containing sodium after endurance events may be helpful.

Consuming potassium-rich foods such as; oranges, bananas and potatoes throughout training and after competition, supplies necessary potassium.

Iron carries oxygen via blood to all cells in the body and is another important mineral for athletes. Female athletes and athletes between 13 and 19 years old may have inadequate supplies of iron due to menstruation and strenuous exercise. Female athletes who train heavily have a high incidence of amenorrhea, the absence of regular, monthly periods and thus conserve iron stores. Iron supplements may be prescribed by a physician if laboratory tests indicate an iron deficiency. Excess iron can cause constipation. To avoid this problem, eat fruits, vegetables, whole grain breads and cereals.

Calcium is an important nutrient for everyone as it is important in bone health and muscle function. Female athletes should have an adequate supply of calcium to avoid calcium loss from bones. Calcium loss may lead to osteoporosis later in life. Choosing low-fat dairy products provide the best source of calcium.

Frequently Asked Questions

Why is carbohydrate important?

Carbohydrate is a key fuel source for exercise, especially during prolonged continuous or high-intensity exercise. The body stores carbohydrate as glycogen in the muscles and liver, however its storage capacity is limited. When these carbohydrate stores are inadequate to meet the fuel needs of an athlete's training program, the results include fatigue, reduced ability to train hard, impaired competition performance and a reduction in immune system function. For these reasons, athletes are encouraged to plan carbohydrate intake around key training sessions and over the whole day according to their carbohydrate requirements as an exercise fuel.

How much carbohydrate do I need?

Carbohydrate requirements are dependent on the fuel needs of the athlete's training and competition program. Exactly how much is required is dependent on the frequency, duration and intensity of the activity. Since activity levels change from day to day, carbohydrate intake should fluctuate to reflect this. On high activity days, carbohydrate intake should be increased to match the increase in activity. This will help to maximise the outcomes from the training sessions and promote recovery between sessions. Alternatively, on low or no training days, carbohydrate intake should be reduced to reflect the decreased training load. A clever way to adjust carbohydrate intake from day to day is to schedule carbohydrate-rich food choices at meals or snacks around the important training sessions. As the sessions increase in their carbohydrate demands, so should the athlete increase their carbohydrate intake before, during or after exercise?

Not only does this strategy help the athlete to keep track of their total carbohydrate needs, but it ensures that the timing of the carbohydrate is best suited to fuel the session.

The table on this page provides some general targets for daily carbohydrate intake goals across a range of activity levels. Each athlete should fine-tune their carbohydrate intake with individual consideration of total energy (kilojoule) needs, specific training demands and feedback from training performance. Additional guidelines outline the specific ways in which carbohydrate intake can be timed to enhance carbohydrate availability for key sessions.

Which foods are good sources of carbohydrate?

Many everyday foods and fluids contain carbohydrate, but have different features. For this reason, carbohydrate-containing foods and fluids are often divided into categories for comparison. Previously, carbohydrates were classified as either simple or complex and more recently, the terms low and high glycaemic index (GI) are being used (more on GI below). From a sports nutrition point of view, it is more helpful to classify carbohydrates as nutrient-dense, nutrient-poor or high-fat.

Categories of Carbohydrates

Category	Description	Examples	Use for athletes
Nutrient-dense carbohydrate	Foods and fluids that are rich sources of other nutrients including protein, vitamins, minerals, fibre and antioxidants in addition to carbohydrate	Breads and cereals, grains (e.g. pasta, rice), fruit, starchy vegetables (e.g. potato, corn), legumes and sweetened low-fat dairy products	Everyday food that should form the base of an athlete's diet. Helps to meet other nutrient targets
Nutrient-poor carbohydrate	Foods and fluids that contain carbohydrate but minimal or no other nutrients	Soft drink, energy drinks, lollies, carbohydrate gels, sports drink and cordial	Shouldn't be a major part of the everyday diet but may provide a compact carbohydrate source around training
High-fat carbohydrate	Foods that contain carbohydrate but are high in fat	Pastries, cakes, chips (hot and crisps) and chocolate	'Sometimes' foods best not consumed around training sessions

Daily Needs for Fuel & Recovery

Situation	Carbohydrate Targets	
Light	Low-intensity or skill-based activities	3–5g per kg BM
Moderate	Moderate exercise programme (~1 hr / day)	5-7g per kg BM
High	Endurance programme (i.e. moderate-to-high intensity exercise of 1-3 hr / day)	6-10g per kg BM
Very High	Extreme commitment (i.e. moderate-to-high intensity exercise of >4-5 hr / day)	8-12g per kg BM

Acute Fuelling Strategies

Situation	Carbohydrate Targets	
General fuelling up	Preparation for events < 90 min exercise	7-12g/kg per 24 hr as for daily fuel needs
Carbohydrate loading	Preparation for events >90 min of sustained/intermittent exercise	36-48 hours of 10-12g/kg BM per 24 hour
Pre-event fuelling	Before exercise > 60 min	1-4g/kg BM (consumed 1-4 hr pre-competition)
During brief exercise During sustained high-intensity exercise During endurance exercise including "stop and start" sports During ultra-endurance exercise	<45 min 45-75 min 1-2.5 hours 2.5-3 hours	Not required Small amounts including mouth rinse 30-60 g/hr Up to 90 g/hr using multiple transportable carbohydrates (glucose:fructose mix)
Speedy refuelling	<8 hr recovery between two fuel demanding sessions	1-1.2 g/kg BM every hour for first 4 hr then resume daily fuel needs

What about glycaemic index?

Glycaemic Index (GI) is a ranking of how quickly carbohydrate foods raise blood glucose levels (BGLs) in the body following ingestion. High GI foods are rapidly digested and absorbed by the body and raise BGLs quickly. Low GI foods, on the other hand, are much slower to be digested and absorbed and result in more gradual rise in blood glucose levels.

In sport, it is important to consider immediate requirements and what a whole food or snack can provide (such as protein, vitamins and minerals) rather than looking at only one component of any food. For example, higher GI foods can be useful immediately after exercise to promote a faster recovery of muscle glycogen stores. Daily requirements, based on physique and performance goals should also be considered when making such food choices

When is carbohydrate important?

An individual's carbohydrate requirements before, during and after training or competition depend on a number of factors including:

- Type, intensity, duration of exercise
- Frequency of exercise or time available for recovery between sessions
- Body composition goals
- Environmental conditions

- Training background
- Performance goals for the session.

While the recommendations provided above consider the overall carbohydrate needs over the day, it is also important to consider the timing of carbohydrate around training and competition.

Carbohydrate ingestion before exercise should assist in topping up blood glucose levels as well as glycogen stores in the muscle and liver. This is especially important if the competition or training is undertaken first thing in the morning or if the event is high intensity or will continue beyond 90 minutes in duration.

The replacement of carbohydrate during prolonged exercise can benefit sports performance, both through effects on the muscle (reducing / delaying the decline in exercise intensity with time) and the brain/central nervous system (reducing/delaying the decline in concentration and mental skills, as well as reducing/delaying the decline in pacing strategies with time). Using specific training sessions to practice consuming specific carbohydrate foods is also important if it is intended to be consumed during a competition.

Carbohydrate intake after exercise is essential for optimum recovery of glycogen stores. Often athletic performance is dependent upon the ability to recover from one session and do it all again in the next session. Incomplete or slow restoration of muscle glycogen stores between training sessions can lead to a reduced ability to train well and a general feeling of fatigue. In competition, it may also reduce subsequent performances where efforts are repeated within or across days (such as in a tournament, a swim or athletics meet or a rowing regatta).

Food Portions

(Providing 50 g of Carbohydrate)

CEREAL	
Wheat biscuit cereal (e.g. Weetabix)	60g (5 biscuits)
'Light' breakfast cereal (e.g. Cornflakes)	60 g (2 cups)
'Muesli' flake breakfast cereal	65 g (1-1.5 cups)
Toasted muesli	90 g (1 cup)
Porridge - made with milk	350 g (1.3 cups)
Porridge - made with water	550 g (2.5 cups)
Rolled oats	90 g (1 cup)
Bread	100 g (4 slices white or 3 thick wholegrain)
Bread rolls	110 g (1 large or 2 medium)
Pita and Lebanese bread	100 g (2 pita)
Chapatti	150 g (2.5)
English muffin	120 g (2 full muffins)
Crumpet	2.5
Muesli bar	2.5
Rice cakes	6 thick or 10 thin
Crispbreads and dry biscuits	6 large or 15 small
Fruit filled biscuits	5
Plain sweet biscuits	8-10
Cream filled/chocolate biscuits	6
Cake style muffin	115 g (1 large or 2 medium)
Pancakes	150 g (2 medium)
Scones	125 g (3 medium)
Iced fruit bun	105 g (1.5)
Croissant	149 g (1.5 large or 2 medium)
Rice, boiled	180g (1 cup)
Pasta or noodles, boiled	200 g (1.3 cups)
Canned spaghetti	440 g (large can)
FRUIT	
Fruit crumble	1 cup
Fruit packed in heavy syrup	280 g (1.3 cups)
Fruit stewed/canned in light syrup	520 g (2 cups)
Fresh fruit salad	500 g (2.5 cups)
Bananas	2 medium-large
Large fruit (mango, pear, grapefruit etc.)	2-3
Medium fruit (orange, apple etc.)	3-4
Small fruit (nectarine, apricot etc.)	12

AIS Sports Nutrition (2014)

CEREAL	
Grapes	350 g (2 cups)
Melon	1,000 g (6 cups)
Strawberries	1,800 g (12 cups)
Sultanas and raisins	70 g (4 Tbsp)
Dried apricots	115 g (22 halves)
VEGETABLES	
Potatoes	350 g (1 very large or 3 medium)
Sweet potato	350 g (2.5 cups)
Corn	300 g (1.2 cups creamed corn or 2 cobs)
Green Beans	1,800 g (14 cups)
Baked beans	440 g (1 large can)
Lentils	400 g (2 cups)
Soy beans and kidney beans	400 g (2 cups)
Tomato puree	1 litre (4 cups)
Pumpkin and peas	700 g (5 cups)
DAIRY PRODUCTS	
Milk	1 litre
Flavoured milk	560 ml
Custard	300 g (1.3 cup or half 600 g carton)
'Diet' yoghurt and natural yoghurt	800 g (4 individual tubs)
Flavoured non-fat yoghurt	350 g (2 individual tubs)
Ice cream	250 g (10 Tbsp)
Fromagefrais	400 g (2 tubs)
Rice pudding/creamed rice	300 g (1.5 cups)
SUGARS and CONFECTIONERY	
Sugar	50 g
Jam	3 Tbsp
Syrups	4 Tbsp
Honey	3 Tbsp
Chocolate	80 g
Mars Bar and other 50-60 g bars	1.5 bars
Jubes and jelly babies	60 g
MIXED DISHES	
Pizza	200 g (medium -1/4 thick or 1/3 thin)
Lasagne	400 g serve
Fried rice	200 g (1.3 cups)
DRINKS	
Fruit juice - unsweetened	600 ml
Fruit juice - sweetened	500 ml

CEREAL	
Cordial	800 ml
Soft drinks and flavoured mineral water	500 ml
Fruit smoothie	250-300 ml
SPORTS FOODS	
Sports drink	700 ml
Carbohydrate loader supplement	250 ml
Liquid meal supplement	250-300 ml
Sports bar	1-1.5 bars
Sports gels	2 sachets
Glucose polymer powder	60 g

Example of a High-carbohydrate Diet

Food item	Calories	Grams carbohydrate
Breakfast		
8 ounces orange juice	120	28
1 cup oatmeal	132	23
1 medium banana	101	26
8 ounces low-fat milk	102	12
1 slice whole wheat toast	60	12
1 tablespoon jelly	57	15
Lunch		
2-ounce slice ham	104	0
1 ounce Swiss cheese	105	1
2 slices whole wheat bread	120	25
1 leaf lettuce	1	0
1 slice tomato	3	1
8 ounces apple juice	116	30
8 ounces skim milk	85	12
2 cookies	96	14
Dinner		
3 cups spaghetti	466	97
1 cup tomato sauce with mushrooms	89 5	19 1
2 tablespoons Parmesan cheese	45	0
4 slices French bread	406	78
1 slice angel food cake	161	36
1/4 cup sliced strawberries	13	3
1/2 cup ice cream	133	16
Snack		
16 ounces grape juice	330	83
6 fig cookies	386	81
TOTAL	**3236**	613
	(75% of total calories)	

Appendix E. Ketogenic Group Information Handout

Waterford Institute *of* Technology
INSTITIÚID TEICNEOLAÍOCHTA PHORT LÁIRGE

KETOGENIC DIET HANDOUT

Enter participant code

Enter today's date

Fionn Mc Swiney

School of Health Sciences,

Dept. of Health, Sport & Exercise Science,

Waterford Institute of Technology,

Waterford.

(Enter participant's name),

This hand out is designed to make your dietary intervention as easy and maintainable as possible.

It hand out includes information on:

- Dietary breakdown, what does a high fat ketogenic diet consist of?
    - Fat
    - Protein
    - Carbohydrate

- Some frequently asked questions.
    - When should I consume certain foods and why?
    - How will I feel once I start the intervention?
    - What should I do if I am feeling fatigued? Is this because of the low-carbohydrate diet? Should I increase my carbohydrate consumption?
    - I have a real sweet tooth, what can I eat without interrupting my adaptation?

- Brief introduction to a high fat ketogenic diet; what foods you should eat & what foods you should avoid.

- A shopping list of the foods you will need. This list contains all the foods you will need to optimise your performance.

- Nutritional information of foods; so that you can count how much fat, protein and carbohydrate is in each serving.

- Example of a high fat ketogenic diet plan.

What does a High Fat Ketogenic Diet consist of?

Fat (70-75% energy from fat)

- High 2,500kcal
    - Monounsaturated fatty acids 55%
    - Saturated fatty acids 27%
    - Polyunsaturated fatty acids 18%
    - Figures based on fat biopsy, fatty acid composition.

Protein (15-20%)

- Moderate 1.76 – 2.2 g·kg lean mass.
    - Excess protein is anti-ketogenic, so intake must be kept moderate.

Carbohydrate (5-10%)

- Low (<50g/d)
    - 5 – 10g protein based food
    - 10 – 15g vegetables
    - 5 – 10g nuts seeds
    - 5 – 10g fruits
    - 5 – 10g miscellaneous

Frequently Asked Questions

When should I consume certain foods and why?

Consuming the correct foods at the correct time of day is vitally important if you want to optimise the results you get from your diet and your training. When you wake up in the morning you are in a state of fasting (we have not eaten for the previous 6 – 7 hours) so our bodies are burning fat. As soon as you consume food high in carbohydrate or sugar our bodies stop burning fat and switches to breaking down the carbohydrate / sugar rich food for energy. Once this food has been broken down you feel hungry / or if it's during exercise you fatigue. Once you have consumed a meal high in carbohydrate it can take hours (following morning realistically), until you are back using fat as a fuel.

It is important that when you wake up in the morning you avoid carbohydrate rich foods and instead reach for foods high in fat e.g eggs, avocados, olives, butter or cream. Your body will immediately begin to break down the fat and protein that you have consumed; once it is gone your body will continue to break down fat from the adipose tissue. An athlete with 8 – 10% body fat has on average 20,000 kcal in store in the form of fat. Over the 12 week intervention your body will become more efficient at utilizing this fat as a fuel.

Carbohydrate should be consumed following exercise; carbohydrate will not be utilized as a fuel. You will only consume enough carbohydrate to replace muscle glycogen and rebuild muscle.

How will I feel once I start this intervention?

Low energy and dizziness – This can occur in the first few days of the diet, as your body has not realised it should be burning fat for fuel. Even though you will be consuming a diet in excess of 2,000 kcal a day, your body acts like its being starved causing a drop in energy.

- Adding a little lemon juice to sparkling water or black tea can give you a tiny blood sugar jolt that changes things without spoiling your adaptation to the diet.
- My advice, hang tight the feeling will pass!

Grumpiness – headaches and irritability. This is a side effect of sugar withdrawal at the beginning of your intervention.

- Resist the urge to give in these feeling will only last 1 – 2 days. Just make sure you are keeping well hydrated as dehydration can make the symptoms worse.

Bowel changes – if you are used to eating a high fibre, high-carbohydrate diet much of the food you eat comes out in waste. As you cut down on carbohydrates and certain fruits and vegetables, the waste will reduce. Toiled visits should be as frequent.

Bad breath – It doesn't happen to everyone, but some people do experience bad breathe as they are going into ketosis.

- If you're worried about it, try some mint tea or one to two drops of Japanese peppermint to a glass of water and tip of your tongue.

What should I do if I am feeling fatigued? Is this because of the low-carbohydrate diet? Should I increase my carbohydrate consumption?

I have experienced this feeling myself; it feels as if your body is crying out for some carbohydrate. But if you have been consuming your 150 / 100 or 50g/d you are taking enough carbohydrate on board to replenish muscle glycogen stores. So what is causing me to feel this way?

One of the mechanisms behind a Ketogenic Diet is a reduction in insulin levels; insulin has many roles in the body; such as telling fat cells to store fat. But another thing the insulin does is tell the kidneys to hold on to sodium.On a High Fat Ketogenic Diet your insulin levels go down and your body starts to secrete excess levels of water and sodium. However sodium is a crucial electrolyte in the body, it can become problematic when the body secretes to much of it.

- This is the main reasons people get negative side effects on a High Fat Ketogenic Diet, such as; light-headedness, fatigue, headaches and even constipation.

What's the best way of replacing lost salt?

- Add more salt to your foods.
- Add a sodium supplement to your water bottle during exercise.
- Add a bouillon cube to a cup of hot water; drink it like a cup of soup. This method actually tastes good and contains 2g of sodium.

If symptoms persist….

If you are sure that you are properly hydrated and have supplemented sufficient levels of sodium and symptoms persist consume some low glycaemic index foods (white rice, sweet potato, couscous, Weetabix). These foods should only be consumed in the evening following your training session, consuming a large quantity of carbohydrate prior to training will inhibit your ability to metabolise fat.

I have a real sweet tooth, what can I eat without interrupting my adaptation?

Artificial sweeteners –and other sugar substitutes are found in a variety of food and beverages marketed as "sugar-free" or "diet," including soft drinks, chewing gum, jellies, baked goods, candy, fruit juice and ice cream and yogurt. If you really feel like you need some “sweets” in your life, add an artificial sweetener such as Splenda to a cup of tea or coffee.

My advice:

Avoid temptation; try your culinary skills at making some Mousses (no culinary skills required), your performance will thank you for it!

Casein Mousse

- 10 – 15g Casein Protein Powder
- 65ml double cream

1. Spoon the protein powder into a bowl and add one table spoon of water. Mix into a thick paste.
2. Now add the cream and mix so it forms a thick mousse.
3. Place in the fridge for 10 minutes to set further.

Or

Coconut Mousse

- 30g CO YO coconut yogurt or mascarpone
- 10 – 15g casein protein powder
- 35ml double cream

1. Spoon the coconut yogurt into a bowl and start to add the protein powder, about half a teaspoon at a time.
2. As it gets thick and difficult to mix add a tiny dash of water and a splash of cream and keep mixing. If you have any cream left at the end mix it in well. If you want to make it thinner, add a little more water.
3. Place in the fridge for 10 minutes to set further.

Dietary Information

Fats and Oils

Since the majority of calories on a ketogenic diet will come from dietary fats, choices should be made with digestive tolerance in mind. Most people cannot tolerate eating a large amount of vegetable oil, mayonnaise or even olive oil over time. And this is a good thing, since vegetable oils are high in polyunsaturated Omega-6 fatty acids.

The Omega-6 fatty acids (found in nut oils, margarines, soybean oil, sunflower oil, safflower oil, corn oil and canola oil) should be limited due to the inflammatory effect they trigger within the body. Most nuts (with the exceptions of macadamias and walnuts) are high in Omega 6 fatty acids as well, so go easy on them). Your intake of polyunsaturated fats should be balanced between Omega 6 and Omega 3 types. Eating wild salmon, tuna and shellfish will provide balancing Omega 3 fatty acids and are important part of a low carb food list. If you don't like seafood, then consider taking small amounts of a fish or krill oil supplement.

Saturated and monounsaturated fats such as butter, macadamia nuts, coconut oil, avocado and egg yolks are tolerated more easily by most people and since they are chemically stable, they are less inflammatory. Fats and oils can be combined in sauces, dressings and other additions to basic meals. Over time, it will become a habit to add a source of fat to each meal.

Avoid hydrogenated fats such as margarine to minimize trans fats intake. If you use vegetable oils (olive, canola, sunflower, safflower, soybean, flaxseed and sesame oils) choose "cold pressed." Keep cold pressed oils like almond and flaxseed refrigerated to avoid rancidity. Avoid heating vegetable oils. Use clean non-hydrogenated lard, beef tallow, coconut oil, ghee and olive oil for frying, since they have high smoke points.

What foods should I eat?

- **Whole meats, poultry and seafood** in any form, preferably grass fed or wild caught (for instance, imitation crabmeat is not wild caught and has sugar in it.) You can choose from beef, pork, chicken, turkey, shellfish, fish and the higher the fat content of the meat, the better.
- **Eggs, in any form.** Devilled eggs make great snacks and eggs in many forms are typically are a breakfast foundation food. Quiche, scrambled eggs, omelettes, poached eggs, hard boiled eggs are all good choices.
- **Natural Fats:** I believe one should only eat real organic fats like butter, cream and coconut oil, but if you want to include commercial mayonnaise, vegetable oils and olive oil, you can. However, I do advise leaning toward butter, olive oil and coconut oil and limiting your intake of refined vegetable oils (soybean, canola, safflower, sunflower or corn oil) as they are high in Omega 6 fatty acids and cause a long list of health issues associated with inflammation.

- **Green leafy vegetables** such as lettuce, spinach, kale, collards, cucumbers and high fibre cruciferous vegetables such as broccoli, cauliflower and cabbage. You'll need to track how much of the sweeter vegetables you eat (tomatoes, peppers and summer squash) because these are higher in carbohydrates. A whole plate of tomatoes will quickly put you over your carb limit.

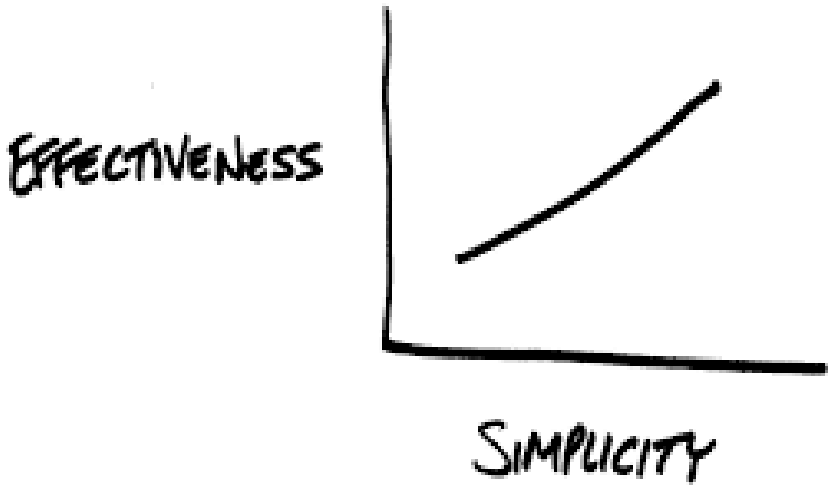
What foods should I avoid?

- **Sugars** and sweetened foods: read labels and avoid any foods which contain brown sugar, powdered sugar, cane sugar, corn syrup, sorghum, honey, maple syrup, sucrose, maltose, fructose, glucose, lactose and the sugar alcohols such as sorbitol, xylitol, mannitol and maltitol. If it tastes sweet, you should avoid it. This of course, rules out candy.
- **All Grain and Grain Products** (wheat, barley, rye, sorghum, tricale, teff, spelt, rice, etc..) and products made from grain flours: bread, waffles, pancakes, pasta, muffins, cold cereals, hot cereals, bread crumbs, tortillas, crackers, cookies, cakes, pies, pretzels, etc..
- **Corn** products, including cornbread, tamale wrappers, corn chips, grits, polenta, popcorn and cornmeal. Corn is in everything as high fructose corn syrup or thickeners or preservatives, so read the labels.
- **Potatoes** and other starchy tubers such as sweet potatoes and potato products such as hash browns, potato chips, tater tots, etc..
- **Starchy vegetables**, such as corn, sweet potatoes, lima beans, peas, okra and artichokes.
- Canned soups and stews, as most canned products contain hidden starchy thickeners.
- **Boxed processed foods,** because most are high in wheat and sugar and are the worst high carb foods to eat because of the added preservatives and fillers.
- **Fruit** of any kind (dried, fresh and frozen): Fruit is high in carbs and fructose. Fructose, even from natural fruit, puts a serious metabolic load on your liver if eaten in large amounts. Berries are the lowest in carbohydrate, so if you have to have something sweet, you could eat 1 or 2 strawberries on a ketogenic diet, but the fructose might halt ketosis.
- **Beans** and lentils, which are high in starch.
- **Beers**, as they are made from grain (there are low carb beers, but since you only have so many carbs per day, you have to decide if you want to spend them on beer.)
- **Dessert wines** such as Icewine, Beerenauslese, Trockenbeerenauslese, Ruster Ausbruch, Moscato, Riesling. These are high in sugar.
- Non-diet **sodas**: Sweetened soda pop or soft drinks are probably the most damaging food product around. They contain large amounts of high fructose corn syrup (HFCS), which is extremely damaging to your liver, plus all kinds of other chemicals not so good for you. And it's easy to consume many of them in one day.
- **Juices made from fruit and vegetables.** These are just the concentrated sugar of the original product.
- **Milk**, especially skim and 1%. Milk is full of lactose, a type of sugar. Fermented milk products like cheese and yogurt have less lactose because the bacteria used to ferment the milk eats up all the lactose during the fermentation process.

Shopping List

Meats	Fish	Dairy	Vegetables	Fruit	Nuts	Other
Optimal	**Optimal**	**Optimal**	**Optimal**	**Optimal**	**Optimal**	**Optimal**
Rib Mince	Mackerel	Butter	Broccoli	Avocados	Macadamia	CO YO Yogurt
Mince Beef	Herring	Cream	Cucumbers	Olives	Cashews	Eggs
Tallow	Tuna	Cream Cheese	Cabbage		Almonds	Coconut Oil
Steak	Salmon	Hard Cheeses	Cauliflower	**OK**	Pecan	Olive Oil
Bacon	Fish	Greek Yogurt	Lettuce	Berries	Walnuts	
			Spinach			
OK		**OK**	Kale		**Avoid**	
Ham		Full Fat Milk	Onions		Legumes	
Chicken			Bean Sprouts			
Turkey		**Avoid**	Zucchini			
		Soft Cheeses	Garlic			
Avoid		Low Fat Products				
Processed Meats		Greek Style Yogurts	**Avoid**			
			Root Vegetables			

Your Diet



Day 1 Example

Breakfast

- Americano – blitz with a generous teaspoon of butter and coconut oil. (FAT)
    - Good meal replacement if you're rushing out the door or to add to a meal at breakfast.

Break

- Americano – cream. (FAT)

Scrambled Eggs.

- Chia Seeds (FIBRE)
- Eggs – cooked in butter X 1 - 2 (PROTEIN / FAT)
- Avocado (FAT)

Lunch

Salad – Mix all together.

- Salmon (PROTEIN)
- Cream Cheese (FAT)
- Avocado (FAT)
- Coconut Oil (FAT)
- Spinach – baby spinach Lidl (CARBOHYDRATE – Minimal)
- Salted cashew & almond nuts – (PROTEIN & FAT)

Dinner

- Steak (FAT & PROTEIN)
- Roasted Green vegetables (CARBOHYDRATES)

Snack

- Greek Yoghurt (PORTEIN)
    - Pouring Cream – 1 tbsp (FAT)
    - Raspberries / Blueberries (FIBRE)
    - Dark Chocolate
    - Chia seeds (PROTEIN & FAT)

Day 2 Example

Breakfast

- Greek Yoghurt (FAT / PROTEIN / CARBOHYDRATES)
    - Added full fat pouring cream (FAT)
    - Blueberries
    - Raspberries
    - Americano – cream (FAT)

Break

- Americano – cream. (FAT)
- Chia Seeds (FIBRE)
- Chicken 50- 80g – Fried in butter (PROTEIN)
- Avocado (FAT)

Lunch

All ingredients except an avocado can be got at a service station if you haven't prepped lunch. If I'm rushing out the door, I grab some cream (for my coffee) and an avocado to mix to lunch like so.

Salad

- Eggs (PROTEIN)
- Bacon (PROTEIN / FAT)
- Cheddar Cheese (FAT)
    - Avocado (FAT)
- Lettuce / Spinach (CARBOHYDRATE – Minimal)

Dinner

- Turkey Burger (PROTEIN / FAT)
- Mashed cauliflower and butter (FAT)
    - Alternative to mash potatoes.

Snack

- Mixture of Nuts (FAT / PROTEIN)
- Raspberries / Blueberries (FIBRE)
- Dark Chocolate
- Chia seeds (PROTEIN & FAT)

Day 3 Example

Breakfast

- Americano (CREAM)
- Bacon (FAT & PROTEIN)
- Fried Egg (FAT /PROTEIN)
- Avocado (FAT)
- Banting Bread
    - Recipe attached.

Break

Salad – mix all together.

- Salmon / Chicken / Ham etc.
- Avocado (FAT)
- Almonds & Cashew - Salted (FAT / PROTEIN)
- Coconut Oil (FAT)
- Cheese (FAT)
- Spinach (CARBOHYDRATES)
- Greek Yoghurt – 1 - 2 tablespoons (PROTEIN / FAT) – Mix it in, delicious!

- Coffee (cream, butter, coconut oil) (FAT)

Dinner

Salad – Mix together.

- Almonds & Brazil Nuts (FAT)
- Greek Yoghurt 1 - 2 Tablespoons(FAT / PROTEIN / CARBOHYDRATE)
- Spinach (LOW GI CARBOHYDRATE)
- Coconut Oil (FAT)
- Cheese (PROETIN & FAT)

Snack

- Greek Yoghurt (FAT / PROTEIN / CARBOHYDRATES)
    - Added full fat pouring cream (FAT)
    - Raspberries / Blueberries (FIBRE)
    - Dark Chocolate.

Day 4 Example

Breakfast

- Eggs – Fried in Coconut Oil or Butter (FAT)
- Chorizo – fried at medium heat (FAT / PROTEIN)
- Kale – fried (Fibre)

Break

- Coffee (cream, butter, coconut oil) (FAT)

Lunch

Salad – Mix together.

- Spiralized Courgette fried in coconut oil (FAT / CARBOHYDRATES)
- Salmon (PROTEIN / FAT)
- Cucumber (LOW GI CARBOHYDRATE)
- Avocado (FAT)
- Greek Yoghurt 1 - 2 Tablespoons(FAT / PROTEIN / CARBOHYDRATE)
- Cheese (PROETIN & FAT)

Dinner

- Oats (CARBOHYDRATE)
- Mixed with Whey Protein (25g) (PROTEIN / FAT)

Snack

- Greek Yoghurt (FAT / PROTEIN / CARBOHYDRATES)
  - Added full fat pouring cream (FAT)
  - Raspberries / Blueberries (FIBRE)
  - Dark Chocolate.

Day 5 Example – Weekend

Breakfast

- Bacon (PROTEIN & FAT)
- Mushrooms (cooked in cream) (CARBOHYDRATE – VERY LITTLE)
- Grilled Avocado (FAT)
- Melted cheese (FAT)

Lunch

- Eating out? Go for the healthiest option on the menu. Avoid chips, potatoes and sauces.
    - This may be a chicken salad, steak or a burger (with no bun) and salad. Ask for olive oil to garnish the dish.

Dinner

- Steak with garlic butter (homemade) (PROTEIN)
- Streaky spinach & broccoli (LOW GI – CARBOHYDRATES)

Snack

- COYO Yoghurt (FAT & PROETIN) – it's expensive, but great as a treat!
    - Raspberries / Blueberries (FIBRE)

Nutritional Information

Fatty Fruits							
Food	**Energy Kcal**	**CHO**	**Protein**	**Fat**	**Monounsaturated**	**Saturated**	**Polyunsaturated**
Olives 100g	81	5.6	1.0	6.3	14%	77%	9%
Avocados (100g)	160	8.5	2.0	14.7	15%	71%	13%

Oils							
Food	**Energy Kcal 1 T/S**	**CHO**	**Protein**	**Fat**	**Monounsaturated**	**Saturated**	**Polyunsaturated**
Coconut oil	39	0.0	0.0	4.5	0.3	3.9	0.1
Palm oil	120	0.0	0.0	13.6	14%	75%	11%

Nuts							
Food	**Energy Kcal (28g)**	**CHO**	**Protein**	**Fat**	**Monounsaturated (g)**	**Saturated (g)**	**Polyunsaturated (g)**
Macadamia	2.3	1.6	1.2	25.6	16%	82%	2%
Cashews	163	9.3	4.3	13.3	21%	62%	18%
Almonds	163	6.3	6.0	14.0	8%	66%	26%
Pecan	2.1	3.1	2.7	25.1	9%	62%	29%
Walnuts	185	3.9	4.3	18.5	10%	14%	76%

Dairy							
Food	**Energy Kcal (100g)**	**CHO**	**Protein**	**Fat**	**Monounsaturated (g)**	**Saturated (g)**	**Polyunsaturated (g)**
Egg 1L	155	1.1	13	11	4.1	3.3	1.4
Cheese (cheddar)	402	1.3	25	33	9.0	21	0.9
Cheese (Swiss)	380	5	27	28	7.0	18	1.0
Cream Cheese	342	4.1	6.0	34	9.0	19	1.4

Butter	717	0.1	0.8	81	21	51	3
Greek Yoghurt	59	3.6	3.2	0.4	0.1	0.1	0.0
Cream (heavy) (1 T/S)	52	0.4	0.3	5.6	66%	30%	4%
Meat & Fish							
Food	**Energy Kcal (100g)**	**CHO**	**Protein**	**Fat**	**Monounsaturated (g)**	**Saturated (g)**	**Polyunsaturated (g)**
Steak	251	0.0	27.4	14.48	6.2	5.8	0.5
Beef Steak	252	0.0	27.29	15.01	6.2	5.8	0.5
Ground Beef (cooked)	276	0.0	25.36	18.58	8.5	7.1	0.5
Ground Beef (70% lean)	332	0.0	14.38	30.0	13.1	11.2	0.6
Ground Beef (85% lean)	215	0.0	18.59	15.0	6.5	5.8	0.4
Ground Beef (95% lean)	137	0.0	21.94	5.0	2.1	2.25	0.2
Food	**Energy Kcal (100g)**	**CHO**	**Protein**	**Fat**	**Monounsaturated (g)**	**Saturated (g)**	**Polyunsaturated (g)**
Beef Tallow	902	0.0	0.0	100	42	50	4
Fresh Ham	272	0	26.72	17.54	7.8	6.4	1.6
Ham Sliced	163	3.83	16.6	8.6	4.3	2.9	0.7
Deli Sliced Ham	162	2.28	18.26	8.39	3.9	2.7	0.9
Chicken Breast	195	0.0	29.55	7.72	3.0	2.1	1.6
Roasted or Broiled Breast	164	0.0	30.76	3.54	1.23	1.0	0.7
Salami (28g)	336	2.4	22	26	11	9.0	2.5
Pepperoni (28g)	494	0.0	23	44	17	15	3.4
Bacon (3 slices)	133	0.3	8.7	10.5	37%	50%	13%
Pork	145	1.5	21	6.0	2.6	1.8	0.5
Turkey	161	0.3	30	4.0	1.1	1.0	0.0
Shrimp	144	1.24	27.59	2.35	0.3	0.4	0.9

Tuna (in water)	116	0	25.51	0.82	0.15	0.23	0.33
Tuna (in oil)	198	0.0	29.13	8.21	2.94	1.53	2.88
Tuna Baked or Broiled	153	0.41	27.3	3.96	1.52	0.81	1.16
White Tuna Fish	128	0.0	23.62	2.97	0.78	0.79	1.1
Salmon	146	0.0	21.62	5.93	2.13	1.26	1.99
Baked or Broiled Salmon	126	0.33	21.94	3.44	1.28	0.68	1.14
Atlantic Salmon (farmed)	183	0.0	19.9	10.85	3.8	2.1	3.9
Pink Salmon	116	0.0	19.94	3.45	0.9	0.5	1.3
Wild Atlantic Salmon	142	0.0	19.84	6.34	2.1	0.9	2.5
Canned Salmon	144	0.0	20.57	6.28	1.8	1.5	2.0
Scallops	217	10.49	18.14	10.96	4.415	2.216	3.36
Fish	84	0.0	17.76	0.92	0.16	0.195	0.364

Vegetables

Food	Energy Kcal (100g)	CHO	Protein	Fat	Monounsaturated (g)	Saturated (g)	Polyunsaturated (g)
Bell Peppers	100	4.6	0.9	0.2	0.0	0.1	0.1
Broccoli	100	7.0	2.8	0.4	0.0	0.0	0.0
Cucumbers	16	3.6	0.6	0.1	0.0	0.0	0.0
Cabbage	25	6.0	1.3	0.1	0.0	0.0	0.0
Cauliflower	25	5.0	1.9	0.3	0.0	0.1	0.0
Lettuce	15	2.9	1.4	0.2	0.0	0.0	0.1
Spinach	23	3.6	2.9	0.4	0.0	`0.1	0.2
Kale	49	9.0	4.3	0.9	0.1	0.1	0.3
Onions	40	9.0	1.1	0.1	0.0	0.0	0.0`
Bean Sprouts	23	2.1	4.0	0.7	0.1	0.1	0.4

Zucchini	17	3.1	1.2	0.3	0.0	0.0	0.1
Garlic	149	33	6.0	0.5	0.0	0.1	0.2

Appendix F. Training Logs

Training Logs

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Endurance Training

Participants are required to spend 7+ hours a week doing moderate intensity (56 – 68% VO_{2peak}) exercise.

In the table below please enter the amount of hours you have completed each day.

Week	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							
11							
12							

Strength Training

Participants are required to incorporate strength training into their training regime at least 2 days per week. Complete a leg press (machine) or free squat at 70-80% of their one repetition maximum (1RM), for 6 sets of 8 - 10 reps.

In the table below please enter 'Weight Lifted' during each strength training session.

Week	Day 1	Day 2
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		
11		
12		

High Intensity Interval Training

Participants are required to do high intensity interval training on a bike at least two times a week. The HIIT consists of 10 sets of 1 minute bouts at 70% peak power with 1 minute recovery.

In the table below; please tick (✔) to show completion of a High Intensity Interval Training session.

Week	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							
11							
12							

Appendix G. Data Collection Sheet

Participant ID: _____ **Time WK1:** _____

_____

D.O.B: _____

Email: _____

Mobile: _____

Date: _____ **Time WK12:** _____

_____

Baseline Measurements:

Height:
Weight:
DXA (Lean Body Mass):
DXA (Muscle Mass):
Blood Pressure and Heart Rate:
Blood Sample:

100km Time Trial

	RER	Lactate	VO_2 / VCO_2
0km			
20km			
40km			
60km			
80km			
100km			
CPT			
After			

Critical Power Test

0secs
30sec
60secs
90secs
120secs
150secs
180secs

Post Exercise Tests

Weight:

Mid Intervention Testing (Week 6)

Weight:
DXA (Lean Body Mass):
DXA (Muscle Mass)

Measurements to Record after the 12 week intervention:

Height:
Weight:
DXA (Lean Body Mass):
DXA (Muscle Mass):
Blood Pressure and Heart Rate:
Blood Sample:

100km Time Trial			
	RER	Lactate	VO_2 / VCO_2
0km			
20km			
40km			
60km			
80km			
100km			
CPT			
After			

Critical Power Test

0secs	
30sec	
60secs	
90secs	
120secs	
150secs	
180secs	

Post Exercise Tests

Weight:	

Appendix H. Abstract for Nutrition Society Meeting 2017

Proceedings of the Nutrition Society (2017), **76 (OCE3)**, E72 doi:10.1017/S0029665117001458

Irish Section Meeting, 21–23 June 2017, What governs what we eat?

Effect of a 12 week low carbohydrate ketogenic diet versus a high carbohydrate diet on blood count indicators of iron status in male endurance athletes

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Low carbohydrate ketogenic diets (LCKD) where carbohydrate intake is <50 g/d have become popular is many populations, including endurance athletes(1). The complete effect of this diet on nutrient absorption and its potential to influence health has not been fully uncovered(2). A 14 day ketogenic diet in females demonstrated no effect on RBC, Hb or Hct levels in healthy females(3), but longer duration research in an athletic population is lacking. The aim of the current research is to examine the effect of a LCKD versus a high carbohydrate diet, on blood count indicators of iron status in endurance athletes.

Following ethical approval 19 participants selected into a high carbohydrate (HC) (65 % kcal CHO, 20 % kcal fat, 14 % kcal protein) or LCKD (>75 % kcal fat, 10–15 % kcal protein and <50 g/d CHO) group for 12 weeks. Participants also completed an endurance training protocol during the trial. Whole blood samples were analysed using the haematology analyser before and after selection into each dietary group. RM ANOVA was used to assess changes in indicators of iron status in participants over time. Paired t-tests assessed changed within group over time.

	HC diet ($n = 10$)				LCKD diet ($n = 9$)				RM ANOVA		
	Pre		Post		Pre		Post		*P* Value		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Time	Group	Time*Group
RBC (10^{-6}µl)	4·59	0·33	4·30	0·63	4·67	0·42	4·44	0·61	0·038*	0·620	0·783
Hb (g/dl)	14·15	1·25	13·36	1·87	14·29	0·99	12·95	1·62[a]	0·013*	0·816	0·487
Hct (%)	0·41	0·03	0·38	0·05[a]	0·41	0·03	0·39	0·05	0·015*	0·846	0·744
MCV (fL)	90·47	4·52	89·35	4·78	89·19	3·14	88·31	2·40	0·082	0·430	0·825
MCH (pg/cell)	30·81	1·78	31·14	2·00	30·88	1·75	29·27	1·63[a]	0·113	0·239	0·021*
MCHC (g/dl)	33·66	1·95	34·85	1·52	34·59	0·87	33·14	1·33[a]	0·801	0·417	0·017*
RDW (%)	12·65	0·70	12·52	0·69	12·89	0·44	13·83	2·34	0·253	0·116	0·137

RBC – red blood cell; Hb – haemoglobin; Hct – haematocrit; MCV – mean corpuscular volume; MCH – mean corpuscular haemoglobin; MCHC – mean corpuscular haemoglobin concentration; red cell distribution width.
* Significant difference at $P < 0·05$; [a] Significant difference within group over time at $P < 0·05$.

RBC, Hb, and Hct significantly decreased over time. Within group Hct decreased between weeks 1 and 12 in the HC participants and Hb, MCH and MCHC decreased within the LCKD participants.

Endurance training has been associated with increased plasma volume with exercise induced inflammation also implicated as a possible cause for iron deficiency in athletes(4). It is possible the endurance training added during this trial had a similar effect on iron status within these participants. However it does not explain the difference in Hb, MCH and MCHC between HC and LCKD participants. Due to the effect iron status has on oxygen transport to muscle, further research is warranted to investigate the effect of a ketogenic diet on iron status in endurance athletes.

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2. Paoli A, Rubini A, Volek JS *et al.* (2013) *Eur J Clin Nutr* **67**, 789–796.
3. Nazarewicz RR, Ziolkowski W, Vaccaro PS *et al.* (2007) *Rejuvenation Res* **10**, 435–439.
4. Hinton PS (2014) *Appl Physiol Nutr Metab* **39**, 1012–1018.

Appendix I. Translational Research on Mitochondria, Metabolism, Aging and Disease Symposium (TRiMAD). October, 2017. Pittsburgh, PA

Effects of a Ketogenic Diet with Exercise Training on Mitochondrial Substrate Oxidation in Human Skeletal Muscle

Vincent J. Miller, Richard A. LaFountain, Emily C. Barnhart, Teryn N. Sapper, Parker N. Hyde, Ryan Dickerson, Jay Short, Madison Bowling, Fionn Mc Swiney, Carl M. Maresh, William J. Kraemer, Jeff S. Volek

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Background: Impairment of mitochondrial function, particularly in regard to reactive oxygen species (ROS), contributes to many pathologies. In lower-level organisms, inhibition of carbohydrate metabolism increases fat oxidation with a concomitant but transient increase mitochondrial ROS production that upregulates endogenous antioxidant defense, ultimately decreasing oxidative stress. This response, referred to as mitohormesis, is a potential target for the treatment and prevention of metabolic disease. Ketogenic diets and exercise increase reliance on fat oxidation and may therefore induce mitohormesis, particularly when implemented in combination. Therefore, an overarching goal of this prospective research is to search for evidence of mitochondrial adaptation and mitohormesis in human skeletal muscle in response to nutritional ketosis combined with exercise.

Methods: This study is ongoing with intent of enrolling 15 subjects into a 12-week feeding and exercise intervention designed to improve physical fitness and maintain β-hydroxybutyrate (BHB) levels above 1 mM. Matched controls follow the same exercise protocol, but consume a high-carbohydrate diet, and are excluded from this analysis due to limited sample size. Capillary BHB and glucose were measured daily with a hand-held meter. Muscle biopsies were obtained from the *Vastus lateralis* pre- and post-intervention. Following isolation of muscle mitochondria, O_2 consumption and membrane potential ($\Delta\Psi$) were simultaneously measured with a Clark-type electrode fitted with a tetraphenylphosphonium (TPP) electrode. H_2O_2 and ATP production were measured using fluorescence (Amplex Ultra Red) and luminescence (luciferase) assays, respectively. Each test was repeated with pyruvate (Pyr), palmitoyl-L-carnitine (PC), and BHB+acetoacetate (Ket), representing carbohydrate-, fat-, and ketone-derived substrates, respectively. Respiratory control ratio (RCR) was calculated as the ratio of maximal O_2 consumption rates after (state 3) and before (state 4) introduction of ADP. $\Delta\Psi$ was calculated using the Nernst equation with TPP measurements.

Results: These results are preliminary and limited to pre- vs. post-intervention comparisons for the 11 participants (26.2±6.7 yrs, 85.4±7.4 kg, 25.7±5.5% body fat) who completed the diet plus exercise protocol. Daily mean BHB concentration was 1.2±0.4 mM, indicating high compliance with the diet protocol. For PC, RCR increased 25% (4.48±0.1 to 5.60±0.2, n=10, p=0.016), with trends for increased maximal rate of state 3 O_2 consumption (84.18±5.7 to 107.22±4.3 $nmol \cdot mg^{-1} \cdot min^{-1}$, n=10, p=0.073) and ATP production (15.44±1.9 to 20.51±2.2 $nmol \cdot mg^{-1} \cdot min^{-1}$, n=9, p=0.110). For Pyr, there was a trend for increased RCR (6.29±0.1 to 7.20±0.2, n=10, p=0.093) and a significant increase in the ADP to oxygen (ADP/O) ratio (3.14±0.1 to 4.12±0.1, n=10, p=0.002). For Ket, oxidation was negligible at both pre- and post-intervention. Maximal rates of state 3 O_2 consumption with Ket were less than 20% of those for PC and Pyr, and RCRs with Ket were less than 2. $\Delta\Psi$ increased only with PC (169.15±1.4 to 176.42±1.3 mV, n=9, p=0.039). Dissipation of $\Delta\Psi$ during state 3 respiration decreased with PC (25.17±0.8 to 22.24±0.6 mV, n=8, p=0.039), but with Pyr, there was a trend for an increase (18.47±0.5 to 21.71±0.6 mV, n=8, p=0.078). No significant changes in H_2O_2 production were observed. However, for PC, post-intervention H_2O_2 production per unit of O_2 consumption was negatively correlated with the percentage of days BHB was above 1.0 mM (ρ=-0.66, p=0.044).

Conclusions: Consistent with the accelerated rate of whole body fat oxidation observed in the keto-adapted state, these results show that 12 weeks of a ketogenic diet plus exercise training increased skeletal muscle mitochondrial oxidative capacity, particularly with PC, suggesting upregulation of β-oxidation. In contrast, the increased dissipation of $\Delta\Psi$ with Pyr, combined with the lack of change in ATP production, suggests increased mitochondrial uncoupling during carbohydrate oxidation. This, along with the inverse correlation between H_2O_2 production and nutritional ketosis, is suggestive of upregulated antioxidant defense and supports the occurrence of mitohormesis. Ongoing analysis will provide further insight on this preliminary conclusion. The low O_2 consumption and RCR with Ket suggests that ketones are not a primary source of ATP production in human skeletal muscle, even in the keto-adapted state.

Appendix J. Journal Article Published in Metabolism Clinical and Experimental, 2018

METABOLISM CLINICAL AND EXPERIMENTAL 81 (2018) 25–34

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Metabolism

www.metabolismjournal.com

Keto-adaptation enhances exercise performance and body composition responses to training in endurance athletes

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ABSTRACT

Background. Low-carbohydrate diets have recently grown in popularity among endurance athletes, yet little is known about the long-term (>4 wk) performance implications of consuming a low-carbohydrate high fat ketogenic diet (LCKD) in well-trained athletes.

Methods. Twenty male endurance-trained athletes (age 33 ± 11 y, body mass 80 ± 11 kg; BMI 24.7 ± 3.1 kg/m^2) who habitually consumed a carbohydrate-based diet, self-selected into a high-carbohydrate (HC) group (n = 11, %carbohydrate:protein:fat = 65:14:20), or a LCKD group (n = 9, 6:17:77). Both groups performed the same training intervention (endurance, strength and high intensity interval training (HIIT)). Prior to and following successful completion of 12-weeks of diet and training, participants had their body composition assessed, and completed a 100 km time trial (TT), six second (SS) sprint, and a critical power test (CPT). During post-intervention testing the HC group consumed 30–60 g/h carbohydrate, whereas the LCKD group consumed water, and electrolytes.

Results. The LCKD group experienced a significantly greater decrease in body mass (HC −0.8 kg, LCKD −5.9 kg; P = 0.006, effect size (ES): 0.338) and percentage body fat percentage (HC −0.7%, LCKD −5.2%; P = 0.008, ES: 0.346). Fasting serum beta-hydroxybutyrate (βHB) significantly increased from 0.1 at baseline to 0.5 mmol/L in the LCKD group (P = 0.011, ES: 0.403) in week 12. There was no significant change in performance of the 100 km TT between groups (HC −1.13 min·s, LCKD −4.07 min·s, P = 0.057, ES: 0.196). SS sprint peak power increased by 0.8 watts per kilogram bodyweight (w/kg) in the LCKD group, versus a −0.1 w/kg reduction in the HC group (P = 0.025, ES: 0.263). CPT peak power decreased by −0.7 w/kg in the HC group, and increased by 1.4 w/kg in the LCKD group (P = 0.047, ES: 0.212). Fat oxidation in the LCKD group was significantly greater throughout the 100 km TT.

Conclusions. Compared to a HC comparison group, a 12-week period of keto-adaptation and exercise training, enhanced body composition, fat oxidation during exercise, and specific measures of performance relevant to competitive endurance athletes.

1. Introduction

Traditional sports nutrition guidelines recommend consumption of high-carbohydrate diets for endurance performance [1,2], yet a growing number of athletes have adopted a LCKD

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approach [3,4]. Endurance performance is limited when endogenous carbohydrates are the dominant fuel [5,6], necessitating provision of exogenous carbohydrate during exercise [7]. A LCKD increases oxidation of endogenous fat stores [8] partially relieving an athlete's dependency on glucose [4]. There is no universally agreed definition for a LCKD. The level of carbohydrate and protein restriction required to induce nutritional ketosis varies, however, some guidelines recommend consuming >75% of energy from fat, moderate protein (1.76–2.2 g per kg lean mass), and <50 g/d carbohydrate [5].

There is a scarcity of investigations examining the effects of a LCKD on performance [9–12] with a greater number of investigations examining low-carbohydrate high fat (LCHF) diets and performance [13–17]. A recent review [3] defined a LCHF diet to contain >60% energy from fat, with moderate levels of carbohydrate restriction (<25% energy). This definition of a LCHF diet is similar to a LCKD diet, both are higher in dietary fat than a traditional diet, and restrict carbohydrates. However, a LCHF diet may not optimise metabolic adaptations associated with accelerated fat oxidation and ketone-related metabolic and signaling effects [4,8]. LCHF diet investigations have focused on short (7–14 days) [13–15], to medium term adaptation periods (14–35 days) [16–17] in athletes. These investigations have reported consistent alterations in fuel utilization, and exercise metabolism in fasted, and carbohydrate depleted states, but fail to test the hypothesis surrounding long-term keto-adaptation and exercise performance [8]. When well-formulated ketogenic diets are implemented for a minimum of four weeks, enhanced fat oxidation rates are observed, with no decrement in aerobic capacity [9]. What happens to exercise performance beyond 4 weeks of keto-adaptation remains unclear, but empirically several endurance athletes using this approach remain highly competitive [8].

Changes in performance due to consumption of LCHF diets are mixed [13–17]. A recent cross sectional study examined the metabolic characteristics of keto-adapted ultra-endurance athletes who consumed a LCKD for 9–36 months [8]. Peak and sub-maximal fat oxidation rates during exercise in keto-adapted participants were more than two-fold higher compared to HC counterparts, and 50% higher than peak rates previously reported [18]. Two of the most notable differences between the LCKD investigation [4], and the current body of LCHF research are the level of carbohydrate restriction, and the length of the adaptation period.

LCKD research on performance has focused on short to medium term adaptation periods (21–30 days) [9–12], possibly due to challenges of long term dietary interventions. Two of these investigations should not be categorised as "ketogenic", since protein [11], and carbohydrate [10], were not sufficiently restricted. Nonetheless, strength, and time to exhaustion were not negatively affected [9–12], however two trials reported a decreased ability to perform at higher intensities [11], and decreased exercise economy [12]. Despite a lack of experimental scientific literature advocating clear performance benefits of adapting to a LCKD diet, interest in this dietary paradigm has continued to gather traction [3,4,19]. Keto-adaptation is believed to unlock a much larger fuel tank versus a carbohydrate-based diet [4,5]; hence reducing an athlete's need for carbohydrate supplementation during exercise. Thus, unlike previous long term cross-sectional LCKD investigations where keto-adaptation had already taken place [8,20] we designed an experimental study to investigate the long-term (12-week) performance implications of consuming a LCKD diet on performance relevant to competitive endurance athletes, and tested the hypothesis that a keto-adapted athlete can maintain/improve performance on a LCKD. This research also involved incorporation of training programme to enhance mitochondrial biogenesis and hence fuel utilization, an aspect not incorporated within previous research.

2. Methods

2.1. *Experimental Approach*

This was a non-randomised control trial comparing long term performance implications of consuming a HC and LCKD, in male endurance trained athletes. A non-randomised approach was chosen due to the length of the adaptation period, and to promote dietary adherence. Participants were informed of the purpose, and any risks associated with taking part, prior to written consent being obtained. The investigation was approved by the research ethics committee at Waterford Institute of Technology, IE. At baseline participants completed a DXA scan, SS sprint, 100 km TT and CPT. Following baseline testing both groups began a 12-week dietary and training intervention (endurance, strength, and HIIT). Participants returned at the end of week 12 and repeated the testing protocol.

2.2. *Participants*

Forty-seven male endurance trained athletes (18–40 years) were enrolled. Twenty participants completed all requirements associated with the current study (Table 1). For this

Table 1 – Subject characteristics.

	HC diet (n = 11)		LCKD diet (n = 9)		t-Test
	Mean ± SD	Range	Mean ± SD	Range	P Value
Age, years	32.1 ± 6.4	20.0–38.0	33.8 ± 6.9	19.0–40.0	0.566
Height, cm	181.2 ± 4.9	177.0–192.1	183.1 ± 5.5	175.5–191.6	0.408
BMI, kg/m^2	23.9 ± 2.9	20.0–30.5	25.6 ± 3.0	22.2–31.2	0.090

investigation, an endurance athlete was an athlete who competed in endurance events, completed >7 h/week training with >2 years training experience. The reasons for dropout were: an injury or illness not related to the intervention (HC $n = 7$; LCKD $n = 9$), intervention too time consuming (HC $n = 1$; LCKD $n = 1$), dietary intervention too difficult to adhere to (LCKD $n = 5$), participants unable to complete post-intervention testing (LCKD $n = 2$), strength and HIIT training too difficult to incorporate into training week (HC $n = 1$), and technical difficulty at post-intervention testing (LCKD $n = 1$). Participants were recruited by contacting clubs, and via social media; Cycling Ireland, Triathlon Ireland, and Irish Triathlon. The following sports were represented: triathlon ($n = 6$), cycling ($n = 5$), Ironman ($n = 4$), marathon runners ($n = 3$), ultra-marathon runner ($n = 1$), and adventure racer ($n = 1$).

2.3. *Pre–Participation Screening*

Screening took place to ensure all participants were; male endurance athletes for >2 years, 18–40 years, and currently consumed a carbohydrate based diet (>50% kcal). Exclusion criteria included; diseases or conditions known to affect performance, use of pharmaceuticals that may affect any measurements of performance, and Illness or injury prior to start date.

2.4. *Pre-Intervention Testing*

Participants avoided racing or training 48 h prior to testing and maintained habitual carbohydrate based diet. Participants reported to the Human Performance Laboratory at 09:00 following 12 h fast (Fig. 1). Upon arrival, weight was recorded to the nearest 0.1 kg (SECA 711, Hamburg, Germany) and height was recorded to the nearest 0.1 cm (SECA 213, Hamburg, Germany). Body composition was measured by DXA (Norland XR-46) via whole-body scan set to a resolution of 4.5 × 9.0 mm and a scan speed 260 mm/s. Fasting blood samples were collected from an antecubital vein using a 21G BD Vacutainer blood collection set (BD Diagnostics). Blood samples were centrifuged and the resultant serum stored at −80 °C for later analysis.

This initial phase of testing was completed by 09:30 and participants were allowed 2 h to "fuel up" prior to the exercise trial. "Fueling up" included consumption of the individual's habitual breakfast, or pre-exercise nutrition (%carbohydrate:protein:fat HC = 52:20:28; LCKD = 64:16:20). Each group was allowed to self-select their pre exercise carbohydrate based meal, to ensure habitual dietary practices and performance measurements were obtained. Participants returned to the Human Performance Laboratory at 11:30, set up their bike position (Wattbike Ltd., Nottingham, UK), and began a 10 min warm up. The Wattbike is reported to sufficiently track performance changes in trained and untrained athletes with a reliability coefficient of 2.2% in a trained population and 6.7% in an untrained population [21]. After warm up participants completed a SS sprint on the Wattbike to determine peak and average power output. During the SS sprint a relative load of 0.5 of air resistance was applied for every 5kgs of body weight. Participants were then connected to the MOXUS Metabolic System (AEI Technologies, Chicago, IL) via mouthpiece (2700 series (large) 2 way T-shape non-rebreathing valve with saliva collector Hans Rudolph, Shawnee, KS) for the determination of energy expenditure, and began their 100 km TT. Participants were instructed to complete the 100 km TT as fast as possible. During the 100km TT, air resistance on the Wattbike was self selected. VO_2 and VCO_2 were averaged every 15 s for the first 5 min of every 20 km, and used to calculate oxygen uptake, minute ventilation, and carbohydrate and fat oxidation; which were presented as a respiratory exchange ratio (RER) value

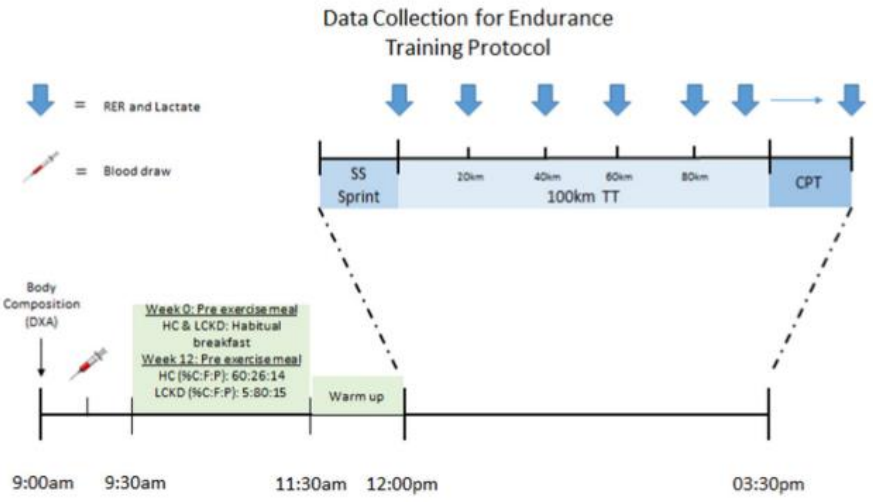

Fig. 1 – Experimental protocol implemented at pre and post-intervention testing.

(VCO_2/VO_2). During the final 30 s of these 5 min, a capillary blood sample was obtained and blood lactate concentrations analysed using a lactate analyser (Lactate Pro, Arkay, Shiga, Japan). Measurements were repeated at 20 km intervals up until 80 km (20 km, 40 km, 60 km & 80 km), the final measurement was the concluding 2500 m of the 100 km TT. Immediately after the 100 km TT, participants completed a CPT on a Wattbike, where peak VO_2, peak power (watts), and average power (watts) were recorded. Participants were instructed to maintain as high a power output as possible while remaining seated for 3-min, during which, the same resistance applied during the SS sprint, was applied. Absolute power measurements were converted to relative power (RP) by dividing watts by body mass in kg. Following completion of the CPT, the mouthpiece was removed and the final lactate measurement was recorded. Participants then completed a gradual self-selected cool down.

2.5. Intervention

2.5.1. Diet

Food diaries were obtained at baseline using a 3 day weighed food diary (2 week days and 1 weekend day). These were analysed using Nutritic's dietary analysis software (Nutritic's Professional v3.09, Nutritic's, Dublin, Ireland). The dietary and training intervention began the day after pre-intervention testing. The macronutrient goals were: HC 65% CHO, 20% fat and 14% protein, or LCKD >75% fat, 10–15% protein and <50 g/d CHO. HC participants were instructed to consume carbohydrates based on their daily energy requirements [7], whereas LCKD participants were instructed to adhere to carbohydrate and protein guidelines, and consume dietary fat ad libitum. 3 day food diaries were also obtained at week 12 and analysed.

2.5.2. Nutritional Counselling

Subsequent to pre-intervention testing each participant received a detailed handout, and nutritional counselling from the researcher. The researcher contacted each participant weekly to ensure dietary adherence, and a weighed food diary was submitted each week. The HC group's nutritional handout included guidelines on how to formulate a HC diet according to their daily energy requirements [7]. The LCKD group's handout included information on how to formulate a LCKD diet, a shopping list and example meal plans. To preclude orthostatic symptoms, LCKD participants were recommended to supplement salt to taste at meal times, consume electrolytes and water when exercising, and supplement 1–2 g/d of sodium from bouillon cubes, or homemade broth [5,9].

2.5.3. Training

Each group received the same training intervention, with endurance training (cycling and running), strength training and high intensity interval training (HIIT) to encourage mitochondrial biogenesis [22–23]. Each participant completed 7+ h a week endurance training (moderate intensity 56–68% VO_2max), 2 strength sessions; 6 sets of 8–10 reps on a leg press, or free squat (70–80% of participants 1RM), and 2 HIIT sessions/week (10 sets of 1 min bouts at 70% peak power with 1 min recovery). During endurance training the LCKD group minimised carbohydrate intake prior to training and limited food consumption during exercise.

2.6. Post-Intervention Testing

Post-intervention testing was similar to pre-intervention testing with the exception of fuelling prior to, and during the exercise trial. Following an overnight fast and preliminary tests, HC participants consumed a HC breakfast (%carbohydrate:fat:protein, 60:26:14) to meet dietary guidelines [2], and LCKD participants consumed a high fat breakfast (5:80:15) to maximize fat oxidation [24].

Participants were again allocated 2 h rest. Once exercise had commenced, participants in the HC group consumed 30–60+ g/h of carbohydrate (glucose, maltodextrin, sucrose and fructose), according to carbohydrate recommendations [2], whereas LCKD participants consumed water, and zero calorie electrolytes.

2.7. Blood Analysis

Fasting Beta-hydroxybutyrate (βHB) concentrations were determined at baseline and post-testing via colorimetric enzymatic assay (Sigma-Aldrich; St. Louis, MO). Intra-assay coefficient of variation was <10%. All samples were thawed one time prior to analysis.

2.8. Statistical Analyses

IBM Statistics SPSS 24 (Illinois, Chicago, USA) was used for statistical analysis. Data was tested for normality, with parametric tests used for normally distributed data or non-parametric, for data not normally distributed. Independent sample t-tests or Mann Whitney *U* test (if data was not normally distributed) were used to determine differences between HC and LCKD groups at baseline, with the alpha level for significance set at $P < 0.05$. Effects for each group were analysed using ANCOVA, with pre-intervention measures acting as a covariate. ANCOVA with baseline body fat (kg) as an additional covariate was carried out, due to a significant difference in body fat between HC and LCKD groups at baseline. As a measure of effect size, partial eta-squared (η_p^2) was used. Effect sizes were evaluated as: $\eta_p^2 = 0.01$ (small effect), $\eta_p^2 = 0.09$ (medium effect), and $\eta_p^2 = 0.25$ (large effect) [25]. Paired samples t-tests or Wilcoxon signed ranks test (if data was not normally distributed) examined changes over time within each group, if ANCOVA P value was <0.05.

3. Results

3.1. Baseline Subject Characteristics, Diet and Performance Measurements

Body fat (kg) ($P = 0.046$), and carbohydrate intake (g) ($P = 0.028$) were significantly different between the HC and LCKD group at baseline. All other physical characteristics (Table 1), dietary (Table 2), performance and anthropometric measurements (Table 3) were not statistically significant between groups ($P > 0.05$).

Table 2 – Daily energy intake, macronutrient distribution, and circulating ketones pre and post-intervention.

Dependent variables	HC diet (n = 11) [a]			LCKD diet (n = 9) [a]			ANCOVA		
	Pre	Post	Change	Pre	Post	Change			
	Mean ± SD	Mean ± SD	Mean	Mean ± SD	Mean ± SD	Mean	F – value	P value	ES [b]: η_p^2
Energy, kcal/d	2440.2 ± 773.8	2643.6 ± 358.0	+203.4	2843.8 ± 558.4	3022.3 ± 911.1	+178.5	(1,17) = 0.646	0.433	0.037
CHO, g/d	315.6 ± 107.5	400.3 ± 102.7 [d]	+84.7	454.8 ± 152.0 [c]	41.1 ± 13.3 [d]	−413.7	(1,17) = 77.71	0.000 *	0.821
CHO, g/kg BM	4.2 ± 1.6	5.3 ± 1.4	+1.1	5.2 ± 1.4	0.5 ± 0.2 [d]	−4.7	(1,17) = 95.93	0.000 *	0.849
Fat, g/d	77.7 ± 33.5	55.2 ± 10.7 [d]	−22.5	64.7 ± 39.1	259.3 ± 83.4 [d]	+194.6	(1,17) = 62.41	0.000 *	0.786
Fat, g/kg BM	1.0 ± 0.5	0.8 ± 0.5	−0.2	0.7 ± 0.1	3.2 ± 0.9 [d]	+2.5	(1,17) = 86.67	0.000 *	0.836
Protein, g/d	118.9 ± 31.8	90.9 ± 23.6	−28.0	110.3 ± 25.5	130.7 ± 35.8	+20.4	(1,17) = 8.270	0.010 *	0.327
Protein, g/kg BM	1.6 ± 0.5	1.2 ± 0.3	−0.4	1.2 ± 0.3	1.6 ± 0.4	+0.4	(1,17) = 5.306	0.034 *	0.238
βHB (mmol/L)	0.2 ± 0.3	0.1 ± 0.0	−0.1	0.1 ± 0.1	0.5 ± 0.4 [d]	+0.4	(1,17) = 8.780	0.011 *	0.403

Abbreviation: CHO, Carbohydrate.
[a] Original means and standard deviations, i.e. without adjustment for covariate (i.e. pre-treatment data).
[b] ES = effect size. η_p^2 = 0.01 (small effect), η_p^2 = 0.09 (medium effect), η_p^2 = 0.25 (large effect) [25].
[c] Significant difference between groups at baseline.
[d] Significant difference within group between pre and post-intervention.
* ANCOVA significant difference at $P < 0.05$.

3.2. *Diet and Exercise Adherence*

Mean duration of the intervention was 84 ± 2.8 days for the HC group, and 81.2 ± 4.9 days for the LCKD group. Energy intake remained unchanged in each group (Table 2). Mean carbohydrate intake significantly increased in the HC group (+85 g/d) and significantly decreased (−414 g/d) in the LCKD group (Table 2). Fat intake significantly decreased (−23 g/d) in the HC group, and significantly increased in the LCKD group (+195 g/d) (Table 2). Protein intake was significantly greater post intervention in LCKD group compared to the HC group (P = 0.010). There was no significant difference in HC and LCKD group's number of HITT (HC: 18.2 ± 2.0, versus LCKD: 19.7 ± 2.3), strength sessions (HC: 17.8 ± 2.1, versus LCKD: 18.3 ± 3.9) or hours endurance training (HC: 11.1 ± 1.7, versus LCKD: 13.0 ± 2.8) completed per week. βHB non-significantly decreased in the HC group (from 0.2 down to 0.1 mmol/L) and significantly increased in the LCKD group (from 0.1 up to 0.5 mmol/L) (P = 0.021).

3.3. *Body Composition*

Body mass significantly decreased in the LCKD group, with a loss of 5.9 kg compared to 0.8 kg in the HC group (Table 3). The significant change in body mass resulted from LCKD participants losing more body fat compared to the HC group (LCKD = −4.6 kg vs HC = −0.5 kg, P = 0.002). Despite significant loss in body mass, both groups maintained lean body mass (HC = +0.1 kg, LCKD = +0.3 kg). When baseline body fat was added as a covariate to body composition changes, similar levels of difference between HC and LCKD groups were obtained (body mass P = 0.009; lean mass P = 0.281).

Table 3 – Body composition and performance variables for pre and post-intervention.

Dependent variables	HC diet (n = 11) [a]			LCKD diet (n = 9) [a]			ANCOVA		
	Pre	Post	Change	Pre	Post	Change	P value		
	Mean ± SD	Mean ± SD	Mean	Mean ± SD	Mean ± SD	Mean	F – value	P value	ES [b]: η_p^2
Body mass, kg	76.5 ± 9.9	75.7 ± 8.7	−0.8	86.3 ± 14.3	80.4 ± 13.4 [d]	−5.9	(1,17) = 8.682	0.006 *	0.338
Lean mass, kg	63.6 ± 5.4	63.7 ± 5.0	+0.1	67.6 ± 9.0	67.9 ± 9.4	+0.3	(1,17) = 1.239	0.167	0.068
Body fat, kg	10.6 ± 6	10.1 ± 5.6	−0.5	15.8 ± 7.2 [c]	11.2 ± 5.0 [d]	−4.6	(1,17) = 13.05	0.002 *	0.434
Body fat, %	12.8 ± 5.1	12.1 ± 4.7	−0.7	17.5 ± 5.5	12.3 ± 4.7 [d]	−5.2	(1,17) = 8.998	0.008 *	0.346
Bone density (g/cm^2)	1.13 ± 0.12	1.12 ± 0.11	−0.01	1.16 ± 0.11	1.14 ± 0.11	−0.02	(1,17) = 0.001	0.978	0.000
VO_2max, ml/kg/min	52.6 ± 6.4	57.2 ± 6.1	+4.6	53.6 ± 6.8	57.3 ± 6.7	+3.7	(1,17) = 0.002	0.968	0.000
TT, min·s	169.57 ± 9.36	168.44 ± 9.14	−1.13	166.00 ± 12.38	161.53 ± 8.44	−4.07	(1,17) = 4.152	0.057	0.196
SS peak RP (w/kg)	13.9 ± 2.7	13.8 ± 2.2	−0.1	13.7 ± 1.4	14.5 ± 1.1 [d]	+0.8	(1,17) = 6.064	0.025 *	0.263
SS Av RP (w/kg)	12.2 ± 1.5	12.5 ± 1.7	+0.3	12.3 ± 1.3	12.8 ± 1.1	+0.5	(1,17) = 0.982	0.336	0.055
CPT peak RP (w/kg)	9.1 ± 2.6	8.4 ± 2.2	−0.7	8.3 ± 2.2	9.7 ± 2.3 [d]	+1.4	(1,17) = 4.574	0.047 *	0.212
CPT Av RP (w/kg)	4.5 ± 2.2	4.6 ± 2.2	+0.1	3.9 ± 0.7	4.0 ± 0.6	+0.1	(1,17) = 0.362	0.555	0.021

[a] Original means and standard deviations, i.e. without adjustment for covariate (i.e. pre-treatment data).
[b] ES = effect size. η_p^2 = 0.01 (small effect), η_p^2 = 0.09 (medium effect), η_p^2 = 0.25 (large effect) [25].
[c] Significant difference between groups at baseline.
[d] Significant difference within group between pre and post-intervention.
* ANCOVA significant difference at $P < 0.05$.

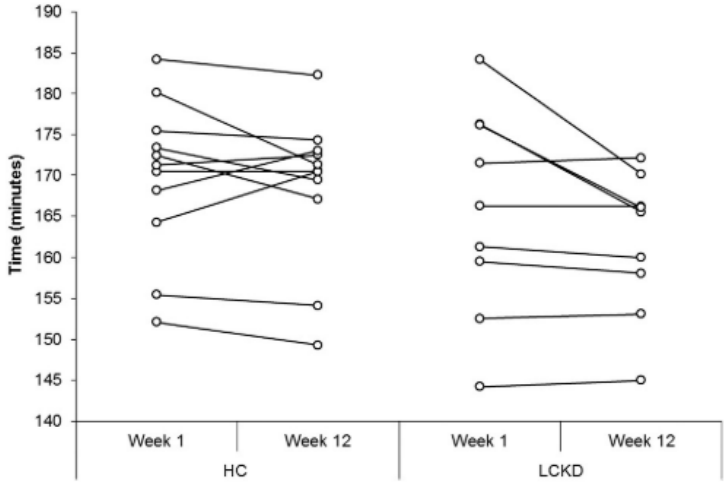

Fig. 2 – Individual 100 km TT times for HC, and LCKD groups at pre and post-intervention testing.

3.4. *Performance and Fuel Utilization*

VO_2max changed similarly in HC (+8.7%) and LCKD (+6.9%) groups ($P = 0.968$). Time required to complete the 100 km TT was not significantly different between groups ($P = 0.057$, ES: 0.196) (Table 3), but change was numerically greater within the LCKD group (−4.07 min·s) compared to the HC group (−1.13 min·s) (Table 3). Participants individual 100 km TT times are shown in Fig. 2. Improvements in time were observed in 6 out of 9 LCKD participants, and 7 out of 11 HC participants. When baseline body fat was added as a covariate to time trial performance, performance differences between group remained insignificant ($P = 0.137$).

There was a significant difference in SS peak power between groups ($P = 0.025$) with a significant increase in the LCKD group (Table 3), but no changes to participants average power observed ($P = 0.336$). Similar to the SS Sprint, peak power in the CPT was significantly different between groups; decreasing in the HC group (−0.7 w/kg), and increasing in the LCKD group (1.4 w/kg) ($P = 0.047$) (Table 3), while average power during the CPT remained unchanged. Significant changes found in SS peak power ($P = 0.024$) and CPT peak power ($P = 0.045$) remained when baseline body fat was considered. Significant differences in RER were observed at 20 km ($P = 0.000$), 40 km ($P = 0.000$), 60 km ($P = 0.000$), 80 km ($P = 0.000$) and at 100 km ($P = 0.040$) (Fig. 3). These differences were present due to significant changes within the LCKD group. No changes were found in blood lactate responses to exercise for the HC and LCKD groups (Fig. 4). RER and blood lactate results remained similar when baseline fat was considered as a covariate.

4. Discussion

This study examined the effects of a consuming a 12 week LCKD diet versus a HC diet, while incorporating a training intervention, on exercise performance and body composition. We show compared to athletes consuming a HC diet, 12-weeks of keto-adaptation is associated with greater improvements in body composition, fat oxidation and peak power, with endurance performance being maintained in both groups. A 12-week period of keto-adaptation reduced total body mass and fat mass, while maintaining lean body mass and increased peak and average relative power during the SS sprint and CPT compared to HC participants. Performance of 100 km TT improved over the course of the intervention, but was not statistically different between groups.

Carbohydrate intake was greater within LCKD compared to HC participants at baseline (4.2 vs 5.2 g/kg). Increased fuel availability may have accounted for slightly better performance of LCKD participants at baseline, but this performance difference was not statistically significant. Due to high carbohydrate intake within LCKD participants at baseline, effect of carbohydrate reduction on fuel utilization was extreme, resultantly; LCKD participants reported fatigue and tiredness during initial adaptation period. The HC groups increase in carbohydrate intake to meet current carbohydrate recommendations [2] was moderate but enabled HC participant's equal LCKD baseline intake. Even though the HC participants consumed 5.3 g/kg of carbohydrate for 12 weeks, they did not equal the LCKD participant's mean time trial performance post intervention.

One of aims of this investigation was to determine if a HC athlete could improve/maintain endurance performance when keto-adapted. The findings here suggest endurance performance can be maintained, and in some cases improved when compared to a HC diet. This is evident in 3 LCKD participants, as 100 km TT performance improved, with maintenance observed in 6 LCKD participants. LCKD participants with the greatest improvements possessed the slowest baseline times. HC participants with similar baseline times did not experience similar improvements in performance, despite similar VO_2max gains. Strength training in cyclists alters muscle fibre type recruitment patterns, and improves exercise economy by increasing maximal strength of

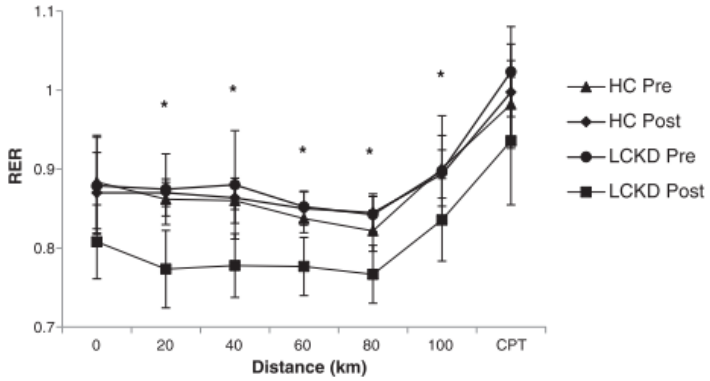

Fig. 3 – RER for HC and LCKD groups at 0 km, 20 km, 40 km, 60 km, 80 km, 100 km and CPT data points, at pre and post-intervention testing. *Indicates significant ($P < 0.05$) difference from ANCOVA, with changes within the LCKD group.

type 1 muscle fibres, and postponing activation of less economical type 2 muscle fibres [26]. Ability to maintain/increase performance may be due to favourable mitochondrial and oxidative enzyme adaptations occurring within muscle [27], due to diet and/or training effect within some LCKD participants. This challenges decades of conventional wisdom advocating a high-carbohydrate diet to optimise performance [3,28].

Two important factors in this study likely to contribute to the positive responses to training are diet composition and prolonged adaptation period.

4.1. *Diet Composition*

Nutritional counselling in this investigation focused on the fundamentals of a well-formulated LCKD diet with carbohydrate restricted to <50 g/day, protein consumed in moderation, and remaining energy derived from natural fat sources [5]. Dietary fat accounted for 81% of energy, protein 1.9 g/kg LBM, and carbohydrate 41 g/day. Macronutrient profile is similar to previous research [9,12], but inconsistent with others [10–11]. Studies where fat accounts for 55–70% of total energy [10,11], with higher protein intakes will fail to adequately induce nutritional ketosis, and may hinder adaptation [10,11]. Evidence of this derives from participant's haematology results [11]; although βHB significantly increased to 0.15 mmol/L, it was below the threshold of nutritional ketosis (>0.5–3.0 mmol) [5], failure of participants to reach nutritional ketosis may explain negative effects on performance, observed within the study by Zajac and colleagues. In contrast, βHB significantly increased from 0.1 at baseline to 0.5 mmol/L at week 12 in LCKD participants. Although nutritional ketosis was achieved [5], concentrations of βHB were less than previously observed in experimental trials (>1.0 mmol/L) [9,12], however, these trials were feeding trials, with βHB concentrations monitored throughout intervention periods. Greater control and accuracy of dietary prescription may contribute to greater βHB concentrations. A LCHF diet that does not achieve nutritional ketosis provides endurance athletes with a dilemma. When carbohydrates are restricted and protein consumed to a point that induces nutritional ketosis, ketones supply the brain with energy [5,29]. Consuming a high fat non-ketogenic diet may increase fat oxidation, but the brain is unable to use long-chain fatty acids for fuel. An interruption or inadequate supply of glucose

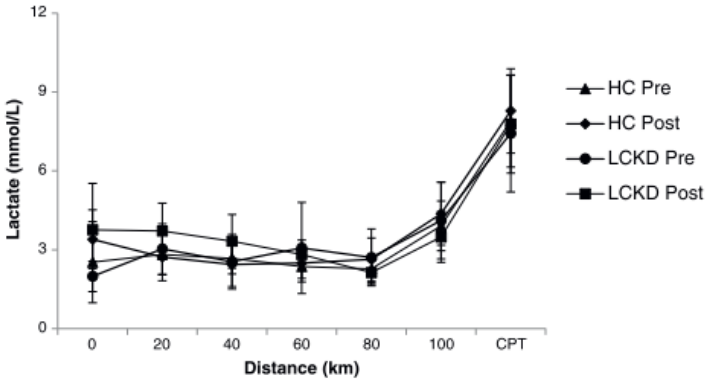

Fig. 4 – HC and LCKD groups lactate responses at 0km, 20km, 40km, 60km, 80km, 100km and CPT data points, at pre and post-intervention testing. P 0.05 at all time-points from ANCOVA.

to the brain in the absence of nutritional ketosis is the metabolic basis for reduced performance and bonking/hitting the wall. Phinney and colleagues [9] identified time to exhaustion was maintained following 28 days of LCKD diet with βHB concentrations >1.0 mmol/L, however, Burke et al. [12] recent investigation found performance adaptations were negated with βHB concentrations of >1.0 mmol/L following 21 days of a LCKD diet. Burke et al. [12] investigation did not assess endurance performance, as the 10 km race implemented was not sufficient to deplete muscle glycogen stores [2], with exercise times <46 min. It is important to highlight that the LCKD diet may not be suitable for everyone, 5 participants found the LCKD diet too difficult to adhere to, and two participants were unable to complete post-intervention testing. Participants unable to complete post-intervention testing were subsequently found to have βHB levels <0.2 mmol/L.

4.2. *Length of Keto-Adaptation*

Previous research incorporated 21–30 day adaptation periods [9–12], with one investigation in 'recreational endurance athletes' adopting a 10-week dietary protocol [30]. Previous LCKD investigations with 21–30 day adaptations noted decreased performance [31], decreases in exercise efficiency, and increases in rates of perceived exertion [12]. Decreased performance was also reported in a 10-week LCKD protocol [30]. However Zinn and colleagues [30] did not incorporate additional training into their study protocol, which the current trial possessed. A significant decrement in peak power was also reported [30] but study participants lost −4 ± 3.1 kg. The effect body mass loss had on power was not considered, decreased power reported by Zinn and colleagues [30] cannot be compared to current results, which are presented as relative power due to body mass loss. During this investigation, LCKD participants noted a drop in energy levels, and performance during the first 7–10, and a "lag" in performance for the first 4–6 weeks. The collective lag in performance, and that 5 LCKD participants dropped out because the dietary intervention was too difficult indicates a LCKD diet may not be for every athlete, should not be undertaken 4–6 weeks prior to an event, or without consideration of individual's dietary preferences.

Phinney et al. [31] first coined the term keto-adaptation to describe the physiological adaptations an individual goes through following consumption of a LCKD diet. They showed performance decreased 20% after one week of consuming a LCKD diet despite significant increases in fat oxidation, but time to exhaustion increased 155% at week 6. The temporal pattern and full scope of the keto-adapted phenotype have not been rigorously studied. The available evidence indicates at least 4–6 weeks is necessary to return to performance and additional time may be necessary to observe consistent increases in performance. Thus, we examined performance after 12-weeks of keto-adaption.

4.3. *Body Composition*

LCKD participants had greater body fat (kg) pre-intervention which may have distorted fat loss findings. When differences in baseline data and body fat are considered, significance values remain similar. Despite LCKD participants losing 4.4 kg body fat, lean body mass increased 0.3 kg. Calorie intake in each group slightly increased during the trial, which contradicts how weight loss occurred. Weight loss is attributed to increased energy output, due to added training. Added weight loss within LCKD participants could be due to slightly greater volume of training undertaken each week. Loss of 4.4 kg body fat within LCKD participants and 0.7 kg body fat within HC participants resulted in both groups having very similar body fat post-intervention (HC 12.1%; LCKD 12.3%). Theses figure are similar to Ackland and colleagues finding that collegiate endurance athletes carry 5–11% body fat [32] and resulted in current study participants possessing the ideal body fat range for endurance athletes [33]. A LCKD diet is a useful tool for achieving weight loss in an untrained population [34], recreational athletes [30] and resistance trained males [35]. These findings suggest a LCKD diet could be a valuable aid to endurance athletes who struggle to maintain race weight, or athletes required to make competitive weight. Furthermore a recent cross-sectional study [36] positively correlated fat mass with increases in inflammatory biomarkers in male endurance trained athletes (17.1 ± 5.1% body fat), therefore reduced body fat is desirable.

4.4. *Performance*

The 100 km TT is short enough to encourage participants to work at high work rates, yet long enough to challenge fuel availability. During exercise there was a significant shift in fuel utilization in the LCKD group, with rates of fat oxidation significantly increasing throughout exercise. This pronounced shift in fuel utilization is a hallmark of keto-adaptation [3,8,9,37]. In attempts to simulate actual race conditions HC group athletes were allowed to fuel following standard dietary recommendations (30–60 g CHO/h). The observed shift in fuel utilization allowed LCKD athletes utilize a greater amount of endogenous lipid stores [4]. 100 km TT improved by 01:13 min·s (0.7%) in the HC group, and 04:07 min·s (2.5%) in the LCKD group (ES: 0.196). A 01.13 and 04:07 min·s increase in performance in 12-weeks in well-trained endurance athletes is practically significant, considering the difference between winning and losing the Tour de France may be seconds [38]. The training programme was designed to enhance mitochondrial biogenesis [22–23]. Although mitochondrial adaptations were not measured during this trial, it is possible that improvements in participant's mitochondrial density enhanced participant's abilities to utilize oxygen, attributing to increases in VO_2max, and performance. Prolonged keto-adaptation may result in an increased transcription and translation of lipid metabolism machinery. This may allow for an enhanced rate of lipolysis and subsequent energy production via oxidative pathways, and potentially explain the performance enhancement observed.

The SS sprint was performed prior to 100 km TT to determine participants' exercise capacity at higher intensities, in a non-fatigued state. Relative peak power increased in LCKD participants as did relative average power, with no changes observed in the HC group. SS sprint, due to the very

short duration, is primarily reliant on the phosphocreatine (PCr) energy system. Previous research indicates a carbohydrate restricted diet does not impair strength performance [10]. Our findings show 12-weeks keto-adaptation is associated with improvement in all-out short-duration exercise capacity, implying no detrimental effects on the phosphagen energy system.

The CPT was performed directly following the 100 km TT to mimic a sprint finish at the end of an endurance event. Previous research examining the effect of a non-ketogenic low carbohydrate diet for 2 weeks, demonstrated no effect on power [16], with other research indicating decreased performance at higher intensities [11]. This investigation incorporated a strength training programme and resulted in a significant increase in relative peak power, with no change in the HC group. Incorporating endurance training with strength training has resulted in increased maximal power output [26]. Improvements in power output observed in the LCKD group may be due to LCKD participants improved power to weight ratio. It is unlikely improvements in power output resulted from strength training, since HC participants had similar levels.

Increases in VO_2max were partially due to decreases in body weight (l/kg/min), and potentially due to improvements in participant's aerobic capacity from the new training stimuli and/or volume of aerobic training. There is little evidence to suggest strength training is an effective mode of improving an athlete's VO_2max [26], however investigations have positively correlated endurance training, and HIIT with improvements in aerobic parameters [39–41]. In a previous trial involving well trained keto-adapted athletes, muscle glycogen stores were not different to well-trained HC athletes after 180 min sub-maximal exercise [8]. Both LC and HC athletes demonstrated similar glycolytic and glycogen synthesis rates during and after exercise [8]. Although muscle glycogen was not assessed in this study, similar lactate responses between groups suggest keto-adaptation did not impair glycolysis during high-intensity exercise.

In summary, a LCKD diet may benefit some athletes, particularity those who struggle with maintaining competitive race weight. Adaptation to a LCKD diet for 12 weeks did not negate measures of performance relevant to endurance athletes, and caused more favourable adaptations to take place in some individuals. Thus, implementation or avoidance of this dietary protocol should be based on an individual's own dietary preference. Despite the concept of keto-adaption being over 30 years old [31], we are still in the early stages of understanding this dietary paradigm. The finding that 12-weeks of keto-adaptation improved aerobic and anaerobic exercise capacity, as well as body composition, in endurance athletes most certainly implies there is potential for using LCKD to improve performance and metabolism. In striving for a more individualistic approach to dietary prescription, keto-adaptation is one approach worth considering.

Contributions of Authors

The study was designed by Fionn McSwiney, Lorna Doyle, and Bruce Wardrop; data was collected by Fionn McSwiney, Lorna Doyle, and Bruce Wardrop; data interpretation and manuscript preparation were undertaken by Fionn McSwiney, Lorna Doyle, Bruce Wardrop, Parker Hyde, Richard LaFountain and Jeff Volek. All aforementioned authors approved the final version of the article.

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Conflicts of Interest

Dr. Volek receives royalties from books on nutrition and exercise.

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Appendix K. Letter to the Editor, Published in Metabolism Clinical and Experimental, 2018

METABOLISM CLINICAL AND EXPERIMENTAL 83 (2018) e1–e2

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Letter to the editor

Dear Sir,

I read with interest the recent paper by McSwiney et al. [1] examining the effects of a long-term ketogenic diet on both body composition and exercise performance in a group of endurance athletes. Such a study is timely given the recent research and debate in this field, both scientific [2,3] and contemporary [4].

Briefly, the authors put 47 trained endurance athletes through a 12-week dietary intervention, whereby the subjects self-selected a high-carbohydrate (HC) diet, or a low-carbohydrate high fat ketogenic diet (LCKD). Such a long-term dietary intervention is no doubt difficult, and the authors should be congratulated for overcoming this logistical challenge. 27 subjects dropped out, leaving twenty for pre- and post-intervention analysis.

However, I have some concerns regarding the reporting of the results. In the abstract, the authors make the headline claim that "the LCKD group experienced a significantly greater decrease in body mass… and percentage body fat percentage." Whilst this is true, there were significant differences between the groups in terms of both body mass and body fat percentage at baseline, such that the LKCD group were an average of 9.8 kg heavier and had 4.7 percentage points greater body fat values. Once the post-intervention fat loss was adjusted for baseline differences, there were no differences between the groups; a finding buried within the text.

Secondly, the authors report greater improvements in power in the LKCD vs HC group, but report these changes relative to body weight. Given that the LKCD group lost significantly more weight during the intervention (because they were much heavier at baseline), these performance improvements appear to be a function of weight loss as opposed to anything else. Indeed, once this data is corrected to the absolute values, we see decreases in many of these values within the LCKD group.

As intervention type was self-selected, did the authors determine why particular subjects chose the LKCD diet over the HC diet? This could have an important impact on the results.

Finally, in a three-day diet assessment at the end of the intervention, the LKCD group consumed an average of 379 kcal/d more than the HC group. If we take this as indicative of energy intake throughout the intervention (which wasn't assessed), the LKCD group consumed far more energy than the HC group, but lost far more body fat; does this indicate differences in training intensity and/or volume? Was this measured by the authors?

In summary, this is an interesting study, but the main, headlines findings are perhaps somewhat misleading. Further research with matched energy intake, monitored training loads, and baseline body mass and body fat (a big ask!) are required to fully elucidate the benefit, if any, of a LCKD on endurance performance.

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Keto-adaptation enhances exercise performance and body composition responses to training in endurance athletes

Dear Editor,

We appreciate the opportunity to address concerns raised by Mr. Pickering.

His first comment was that the differences in body composition responses between groups were no longer statistically significant after accounting for baseline

differences in body fat, "a finding buried in text" as he suggested. Perhaps we did bury it, because we scoured every word of our paper and could not find it. We acknowledged in the paper there were significant differences in body composition between groups at baseline. We clearly state in the statistical section that body fat was used as a covariate. We clearly state in the results section that the greater loss of body fat remained significant after using this covariate. Moreover, the accusation is misplaced because loss of body fat and improved body composition in the LCKD group was an expected positive outcome, one based on a robust body of literature [1,2].

His second point that performance decreased in the LCKD group when accounting for weight loss is also off base. Measures of absolute power were not different between groups (see Supplemental Table 1). The power outputs were presented in the manuscript relative to body mass because relative power is highly correlated with cycling performance [3–5]. In the real World, competitive cyclists are not penalized if they lose body fat while maintaining lean muscle, which is what happened in the LCKD group. Perhaps Mr. Pickering thinks Tour de France champion Chris Froome should be handicapped with a 20-pound sack of potatoes because he lost that much weight on a low-carbohydrate diet [6]. The ability of a low-carbohydrate diet to promote an improved power to weight ratio is an asset, not a liability.

We allowed participants to self-select diet group, but did not ascertain specific reasons for their choice. Our rationale for not performing a randomized study was that in the real-World, athletes have a choice what foods they want to eat. In a randomized trial, potential subjects need to be informed about both diet options. If we accurately presented the two eating patterns and potential benefits, this introduces a significant enrolment bias. Further, since the athletes were often interacting and training together, any perceived benefits for one diet would 'pollute' adherence to the other diet.

Regarding the question of energy intake and training records, diet logs indicated no significant differences in caloric intake or number of training sessions between groups, intensity of training volume over the intervention was not quantified. It is well known that diet records are notoriously inaccurate [7], so we caution reading too much into the numerical non-significant difference reported between groups.

The concept that athletes could perform well on a ketogenic diet is not new [8], but it has taken >3 decades for that spark to be ignited [9–11]. While we disagree that our paper was misleading, we do appreciate that Mr. Pickering has given us the opportunity to shed additional light on this topic that has lived in the shadows for too long.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.metabol.2017.11.016.

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Appendix L – Abstract from Russel Kline Research Symposium in 2018

Carbohydrate Restriction Results in Favorable Outcomes in Plasma Lipid Composition Despite High Saturated Fat Consumption

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Background: Metabolic Syndrome (MetS), a cluster of symptoms including obesity, insulin resistance and dyslipidemia, is an ever-expanding global health crisis. Recent evidence has demonstrated the superiority of low carbohydrate diets for improving the metabolic syndrome phenotype. Fatty acid composition (FAcomp) is widely considered a proxy measure of cellular membrane health that can be significantly altered by diet and predictive of future disease risk. MetS FAcomp is associated with elevated lipogenic fatty acids (FA) and reduced polyunsaturated fatty acids (PUFA).
Objective/Hypothesis: To determine the effects of a high, moderate and low-carbohydrate diet on both triglyceride (TG) and phospholipid (PL) FAcomp.
Methods: Twelve patients (male n=8; women n=4) with MetS completed three 4-wk controlled feeding periods in a randomized and balanced manner. A 2-week washout period separated each feeding period. The diets were all isocaloric and isonitrogenous, but consisted of low (LC), moderate (MC), and high (HC) carbohydrate. Venipuncture was conducted after each feeding phase. Both TG and PL fractions were extracted from plasma, separated using a silica gel, and FA species were quantified using a targeted gas chromatography approach.
Results: FAcomp in TG fractions indicated LC resulted in lower composition of C14:0, C14:1, C16:0 and C16:1n7 compared to diets higher in carbohydrates. HC trended toward higher total SFA in TG but did not reach significance. Total PUFA were highest in LC and exhibited a dose-dependent reduction with increasing levels of carbohydrate intake. In the PL fraction, LC resulted in increased proportion of C20:4n6 (arachidonic acid), C22:6n3 (DHA), and a decrease in precursors such as C20:3n6 compared to MC and HC diets. Total PUFA in PL also decreased significantly from LC to HC groups. There were no significant changes in PL SFA content between diets.

Conclusions: LC intake was associated with decreased lipogenic fatty acids and increased highly unsaturated omega 3 and omega 6 fatty acids. A decrease in several SFA despite high saturated fat intake in the context of LC intake is indicative of both higher oxidative metabolism and decreased synthesis of saturated fat. The increased proportion of arachidonic acid and DHA after a LC diet, but lower amounts of their biosynthetic precursors, may be due to decreased reactive oxygen species-mediated destruction of highly unsaturated FA and therefore better preservation in membranes.
Acknowledgments: Funding was provided by Dairy Management, Inc. FA analysis was completed at Lipid Technologies, Inc. (Austin, MN).

Appendix M. Abstract from Russel Kline Research Symposium in 2018

Nutritional Ketosis Uniquely Enhances Mitochondrial Function in Human Skeletal Muscle During Adaptation to Exercise

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Background: Exercise-induced changes in mitochondria can be augmented by nutritional ketosis. However, the underlying biological adaptations are largely unknown, particularly in humans. Therefore, the objective of this research is to characterize changes in skeletal muscle mitochondrial function induced by nutritional ketosis during adaptation to chronic exercise.

Methods: Twenty-nine participants completed a 10-12-week exercise program while following a ketogenic diet (LC, n=15, males=14) or their habitual high-carbohydrate diet (HC, n=14, males=13). Muscle biopsies were collected from the *Vastus lateralis* pre- and post-intervention. Oxygen consumption, membrane potential, and H_2O_2 and ATP production were measured in isolated mitochondria. Each test was repeated with a carbohydrate-, fat-, and ketone-based substrate.

Results: Participants were matched by age, gender, and body fat (LC vs HC: 27.4±6.8 vs 24.6±9.0 yrs, 25.6±5.0% vs 22.0±8.6%). Mean daily blood β-hydroxybutyrate concentration for LC was 1.2±0.2 mM. An effect of time was observed for increases in mitochondrial protein (5.56±0.2 to 6.02±0.2 µg/µL, $p<0.00001$) and respiratory control ratio (RCR, 4.22±0.3 to 4.61±0.3, $p<0.01$). Time x diet interactions were observed indicating a lesser increase in H_2O_2 ($p=0.013$) and a relative increase in ATP ($p<0.01$) for LC, as well as a relative increase in uncoupling for HC (based on ATP/O_2, $p<0.001$). With the fat-based substrate, mean RCR and median ATP production increased for LC (4.68±0.3 to 5.62±0.2, $p=0.013$; 15.20 to 30.97 nmol/mg/min, $p=0.027$), but not HC. For all conditions, ATP production with the ketone-based substrate was more than 4-fold lower than with other substrates.

Conclusions: While the effects of time indicate exercise-induced enhancement of mitochondrial function, the time x diet interactions indicate augmentation of this enhancement by nutritional ketosis, particularly for fat metabolism. When normalized to O_2 consumption, the changes in H_2O_2 and ATP production are in opposite directions for LC, but not HC, indicating a shift in metabolic efficiency from carbohydrate to fat.

Appendix N. Original Article. Submitted to European Journal of Sports Science in February 2018

Keto-adaption, an ergogenic aid for endurance athletes, what evidence? A narrative review

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Abstract

The low-carbohydrate ketogenic diet (LCKD) is both a highly debated, and misunderstood topic within sports nutrition. Despite a lack of consensus in the scientific literature around its impact on performance, LCKDs continue to gather traction amongst athletes wishing to attain an 'edge' on their opponent. This is likely due to benefits reported anecdotally by athletes themselves, and by sports science practitioners who have applied it in a practical setting. Keto-adaptation is attractive to endurance athletes largely because of its ability to enhance fat oxidation, and reduce the need for aggressive high-carbohydrate fuelling strategies during exercise. In a keto-adapted state; the body's skeletal muscle relies almost exclusively on the oxidation of endogenous fatty acids, whilst the brain relies on endogenous ketones produced primarily by the liver. Keto-adaptation is believed to maintain/enhance endurance performance, and improve body composition but limit performance as exercise intensity increases. A recent flurry of scientific literature examining LCKDs and performance has emerged; which has

created a need to re-examine this dietary paradigm. Though a LCKD may not be preferable to everyone, a better universal understanding of its potential positive attributes, and frailties to endurance athletes is required. The aim of this narrative review is to provide clarity on the current body of scientific literature surrounding LCKDs and measures of performance relevant to an endurance athlete. Despite the scarcity of experimental investigations, this review scrutinises the available literature, shares what is currently understood, and highlights scope for future research.

Keywords*:* *Ketogenic diet, low-carbohydrate, high-fat diet, endurance, athlete, performance*

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Introduction

Since the 1930s it has been widely accepted in mainstream nutritional science that having high levels of pre-exercise muscle glycogen is a precursor for optimal athletic performance (Christensen & Hansen, 1939). However, like most areas of science, many athletes and scientists have opposing theories (Phinney, 2004; Brukner, 2015; Olsen, 2014; Volek, Noakes & Phinney, 2015; Noakes, Volek & Phinney, 2014; Noakes & Windt, 2016). The LCKD may be one of the more polarising concepts in sports nutrition, with opinions on this dietary paradigm appearing to divide scientists into believers, and non-believers, with little or no room for a neutral standpoint. Debate and deliberation is not only good, it's necessary to progress the science and practice of sports nutrition for optimal performance, and athlete health.

The science around the LCKD and sports performance lacks consensus, but despite this, popularity in LCKD has continued to gather traction, particularly among, but not exclusive to endurance, and ultra-endurance athletes. The appeal of a LCKD is it causes a shift in substrate utilisation, i.e., increasing energy contribution from endogenous fatty acids, and ketones; thus, reducing the body's reliance on exogenous carbohydrates. With keto-adaptation, the ultimate goal for athletes is to be metabolically flexible i.e., be able to easily switch between using fat and carbohydrate for fuel as required. However, specific research in this area is lacking. Undoubtedly, athletes adopt this approach because they feel they're attaining an 'edge' on their opponents. Keto-

adaptation is associated with ease of weight management, and a host of other anecdotal, and physiologically documented benefits; including decreased inflammation (Zinn *et al.,* 2017), and accelerated recovery (Volek, Noakes & Phinney, 2015), however, sufficient scientific literature examining its impact on performance has been lacking to date.

Due to the contentious nature of this area of nutrition amongst opposing scientists, and practitioners (Noakes & Windt, 2016) there is an increased risk of experimental bias, misinterpretation, miss-presentation, or over-reaching conclusions when conducting and interpreting the science, in order to reinstate the individual's original beliefs (Robinson, 1921). The broad aim of this review is to profile the current state of knowledge surrounding LCKDs and endurance performance. We also aim to enhance understanding of the benefits and shortcomings of adopting LCKDs for endurance athletes so that researchers, practitioners and athletes can be guided appropriately in their endeavours if using this dietary approach.

Definitions

One of the key limitations in this area of research is there is no agreed definition of what constitutes a 'low-carbohydrate diet'. As a result many different protocols have been applied in research studies (Paoli *et al.,* 2012; Zajac *et al.,* 2014; Rhyu & Cho, 2014; Burke *et al.,* 2017). Though many definitions exist, the following is a commonly used three-tiered system describing various levels of carbohydrate restriction (Feinman *et al.,* 2015; Noakes & Windt, 2016).

Tier 1: Moderate carbohydrate diet (26-45% of daily calories)

Tier 2: Low-carbohydrate high-fat (LCHF) (<130g of carbohydrate (CHO)/day)

Tier 3: Low-carbohydrate, ketogenic (LCKD) (>75% of total energy from fat, <50 g/d of CHO)

Reduced carbohydrate diets restrict carbohydrates to below the lowest threshold of the range stipulated in the Dietary Guidelines for Americans (i.e., 45-56% of total energy intake) (Noakes & Windt, 2016). When examining low-carbohydrate literature, it's imperative to appreciate that although LCHF and LCKDs are similar in the sense that both restrict carbohydrates, and are higher in dietary fat than a traditional diet, they are metabolically different. Carbohydrate restriction to any degree will result in increased energy contribution from fatty acids, however only a LCKD will cause a metabolic shift referred to as 'nutritional ketosis' (Volek & Phinney, 2012; Volek *et al.,* 2015). In this

glucose/carbohydrate restricted metabolic state, ketone bodies; namely acetone, acetoacetate, and beta-hydroxybutyrate (βHB) replace glucose as the primary fuel for peripheral tissues, such as the brain, heart, and skeletal muscle (Evans, Cogan and Egan, 2016).

Phinney *et al.,* (1980) first discovered the concept of keto-adaptation and its initial wavering impact on performance during an investigation in a metabolic ward in the 1980s. The investigation involved 6 clinically obese participants who consumed a high-carbohydrate diet for 1 week (%carbohydrate:fat:protein = 54:40:15), followed by 6 weeks of a eucaloric LCKD *(>80% dietary fat, 1.2g/kg of protein, <10g/d CHO*). Participants completed a time to exhaustion trial on a treadmill at 60% VO_2max at baseline, the end of week 2, and week 6. Time to exhaustion decreased to 80% following 1 week of carbohydrate restriction; despite participants achieving significant increases in fat oxidation. To the investigators' surprise, time to exhaustion increased to 155% in week 6. This rebound effect an individual goes through following extreme carbohydrate restriction was later coined '*keto-adaptation'* (Phinney *et al., 1983)*. For the purpose of this review, we will be focusing on literature involving LCKDs, and endurance athletes.

Methods

Data Sources

This review involved keyword searches on electronic databases including PubMed, Science Direct, and Google Scholar. Search terms included 'endurance performance', AND 'athlete', AND 'ketogenic', AND 'keto', AND 'keto-adapted', AND 'carbohydrate', AND 'fat', AND 'low-carbohydrate', AND 'high-fat', AND 'low-fat', AND 'high-carbohydrate'. Search terms were entered in a number of combinations. Manual searches were also conducted using the reference lists of other narrative, and meta-analytic reviews on LCHF/LCKD performance literature.

Eligibility Criteria for Selecting Studies

We assessed control trials, and case studies for eligibility and inclusion. To be included, control trials, and case studies must have examined 1) a LCKD, or studies whereby authors state a LCKD is applied, and 2) measures of endurance performance relevant to an endurance athlete, 3) in endurance trained athletes (>2 years' experience), and 4) have been published in English in a peer-reviewed journal. Scientific databases were searched in October 2017, and again in February 2018.

Ketogenic diets and performance within endurance athletes: A historical perspective

The subsequent review is focused on six studies which met our inclusion criteria (Table 1); as well as brief references to other original articles, and control trials which have helped shape research interest in this area, and provide the reader with a historical overview of the literature.

Table I. Summary of studies involving low-carbohydrate ketogenic diets and measures of performance in trained individuals.

Author	Population Sample & Study Design	Adaptation Period, LCKD & HC Diets, & Performance Nutrition	Performance Test	Ketosis Achieved? (Ketones >0.5mmol/L)	Results
Phinney *et al.,* (1983)	*n* = 5 well-trained male cyclists (*>65 VO_2max)* Crossover design	28 days, HC (57% CHO, 29% fat, 14% protein) LCKD (<20gCHO, 85% fat, 15% protein), overnight fast, no CHO during	TTE at 60% VO_2max	Yes Serum βHB 1.16-2.44mmol/L	TTE: 151 min LCKD vs 147 min HC (*P* > 0.05)
Zajac *et al.,* (2014)	*n* = 8 moderately trained male off-road cyclists (*>55 VO_2max)* Crossover design	28 days, HC (50% CHO, 30% fat, 20% protein) LCKD (15% CHO, 70% fat, 15% protein), during not stated	VO_2max (incremental test)	No Serum βHB 0.15mmol/L	Max workload: LCKD 350W vs HC 362W (*P* = 0.037) Body fat: -1.8kg LCKD group (*P* < 0.001)
Burke *et al.,* (2017)	*n* = 9 HC, *n* = 8 PCHO, *n* = 10 LCKD, elite male race walkers (*>61 VO_2max)* Parallel group design	21days, HC (65% CHO, 20% fat, 15% protein) PCHO (65% CHO, 20% fat, 15% protein) LCKD (<50g CHO, 78% fat, 15% protein)HC & PCHO – 2g/kg CHO prior, 60g/h CHO during. LCKD energy equivalent LCHF snack	VO_2max and economy testing (treadmill - incremental test), 10km race, 25km walk	Yes Serum βHB >1.0mmol/L	10km TT: HC (6.6% *P* < 0.01), and PCHO (5.3% *P* < 0.01) performance improved, LCKD (-1.6% decrease) LCKD, HR & RPE interaction (*P* < 0.001); decreased exercise economy, increased RPE LCKD, RER decreased 25km walk (*P* < 0.05)

Table I. Continued.

Author	Population Sample & Study Design	Adaptation Period, LCKD & HC Diets, & Performance Nutrition	Performance Test	Ketosis Achieved? (Ketones >0.5mmol/L)	Results
Zinn *et al.,* (2017)	*n* = 5 recreationally active endurance athletes (4 female, 1 male) (*>44 VO_2max)* Case study	70 days (10 weeks) LCKD (<50g CHO, 75% fat, 15% protein) Fasted	VO_2max (incremental test), TTE at 60% VO_2max, peak power	Yes Blood βHB >0.5-1.9mmol/L (finger stick)	TTE -2mins (*P* = 0.004 ES: 0.53) Body weight -4kgs (*P* = 0.046, ES: 0.62) Enhanced well-being, improved recovery, improved skin condition, and inflammation
McSwiney *et al.,* (2017)	*n* = 11 HC, *n* = 9 LCKD Male endurance athletes (*>52 VO_2max)* Non-randomised control trial	84 days (12 weeks) HC (65% CHO, 14% protein, 20% fat) LCKD (6% CHO, 17% protein, 77% fat) 30-60 g/h (HC), water + electrolytes (LCKD)	Six-second sprint, 100km TT, CPT (VO_2max retrieved during CPT)	Yes Serum βHB ~0.5mmol/L	100km TT: -1.13 min HC, -4.07 min LCKD (*P* = 0.057. ES: 0.196) Body fat: -0.7% HC, -5.2% LCKD (*P* = 0.009, ES: 0.338) CPT peak power: -0.7 w/kg HC, +1.4 w/kg LCKD (*P* = 0.047, ES: 0.212)
Heatherly *et al.,* (2017)	*n* = 8 middle-age male runners(*>49 VO_2max)* Case study	21 days (3 weeks) LCKD (7% CHO, 64% fat, 29% protein) Fasted	VO_2max (treadmill - incremental test), 5km TT (running)	Yes Blood βHB ~0.7mmol/L (finger stick)	Body mass (-2.5 kg *P* < 0.001) 5km TT (-0.47 min *P* = 0.25)

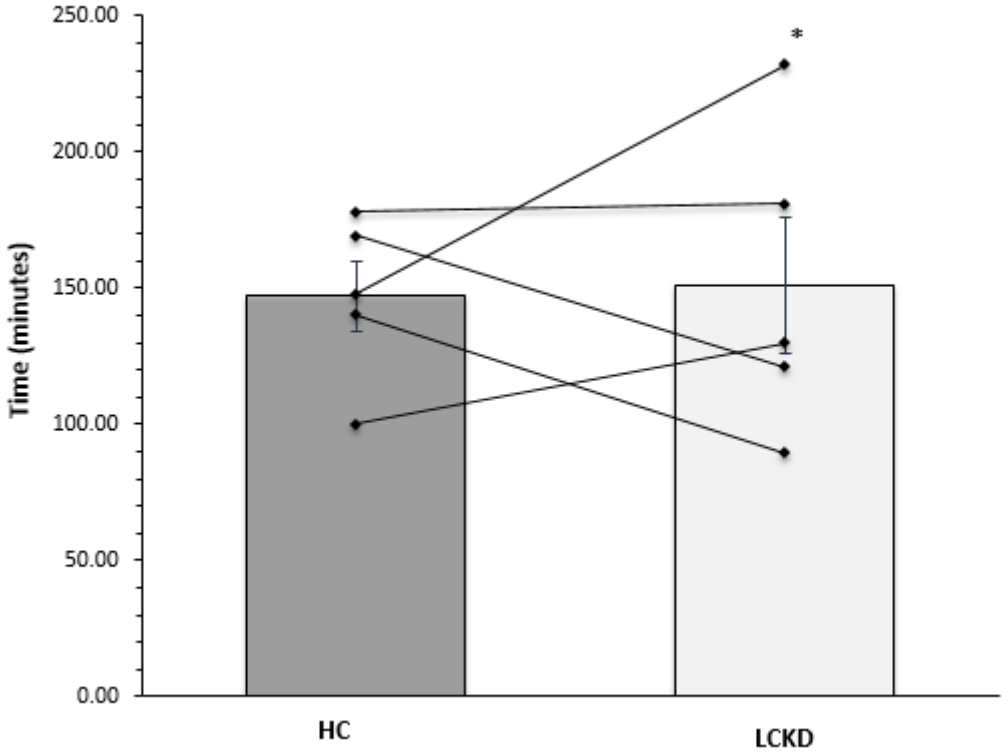

Figure 1. Data presents mean ± SD changes in time to exhaustion at 62-62% VO_{2peak}. Redrawn figure from Phinney *et al.,* (1983).

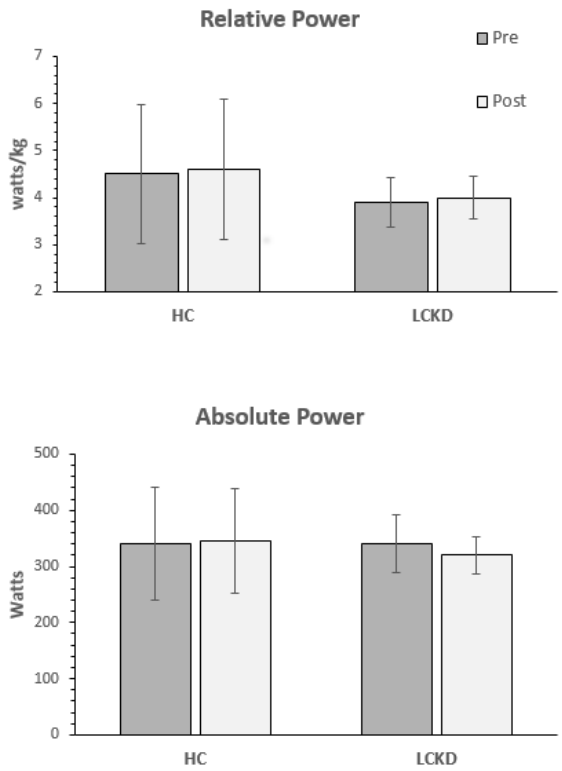

Figure 2. Data presents changes in absolute power (watts) and relative power (watts/kg) in high-carbohydrate (HC) and low-carbohydrate ketogenic (LCKD) participants contained within McSwiney *et al.,* (2018).

Phinney *et al.,* (1983) conducted the first trial to examine keto-adaptations impact on endurance performance in 5 well-trained cyclists (Table 1). During the LCKD trial participants consumed a carbohydrate rich diet (*57% carbohydrate*) for 1 week, followed by 4 weeks of a LCKD (*85% fat, 1.2g/kg of protein, <20g/d carbohydrates*). Resting blood ketones i.e., beta-hydroxybutyrate (βHB) rose from 0.04mmol/L at baseline to 1.28mmol/L at the end of week 5; indicating participants were successful in achieving *nutritional ketosis* (fasting βHB >0.5mmol/L) (Volek & Phinney, 2012; Volek *et al.,* 2015). Endurance performance was measured following an overnight fast at baseline and at the end of week 2 and week 5. Despite lay claims that this investigation noted a performance enhancement in the LCKD group, the LCKD caused maintenance of endurance performance on a cycle ergometer (time to exhaustion (TTE) at 62-64% VO_2max), at best (151 minutes in the HC group and 147 minutes in the LCKD). These mean values suggest that the HC and LCKD groups were well matched; however there was one outlier which skewed the mean values; participant JP's time to exhaustion improved by 63.7% (*84 minutes*) on the LCKD trial (Figure 1). JP's results have been interpreted in two ways: 1) JP's performance improvement was not by chance, and that he and other endurance athletes could benefit from experimenting, and individualising their carbohydrate consumption, 2) JP was a significant outlier, and unimportant. A magnitude based inferences approach carried out by Burke (2015) revealed an unclear outcome; with the chances of substantially positive, trivial, and substantially negative outcome being 32, 32, and 36%. Irrespective of how findings are interpreted, the investigation illustrated that athletes react in different manners to different dietary protocols; particularly extreme carbohydrate restriction.

Despite these thought provoking findings, the following investigation that set out to assess LCKDs and endurance performance in athletes didn't take place until three decades later. Zajac *et al.,* (2014) examined exercise metabolism, and performance responses in well-trained off-road cyclists (Table 1). Participants' βHB levels significantly increased from 0.04 mmol/l to 0.15 mmol/L following this LCKD trial (28 days), indicating participants were unsuccessful in achieving *nutritional ketosis;* as blood ketones were <0.5 mmol/L (Volek & Phinney, 2012; Volek *et al.,* 2015). Grams of carbohydrate per day were not specified, instead it stated that 15% of total calories (3865 kcal) came from carbohydrate, which is approximately ~145 g/d of carbohydrate. This is significantly higher than *<50 g/d* guideline for achieving nutritional ketosis (Volek & Phinney, 2012;

Volek *et al.,* 2015). This point highlights the importance of correct dietary prescription, and good dietary adherence. Nevertheless, the 'non-ketogenic LCKD groups' max work load (W) and work load during a lactate threshold test both decreased in the LCKD group; indicating LCKD participants struggled to maintain performance as exercise intensity increased, despite improvements in VO_2max, and significant increases in fat oxidation (RER).

Burke (2015) identified that LCKDs were a growing trend within endurance sports and was dismissive of the current body of scientific literature, identifying that there was just one performance trial to date where nutritional ketosis was achieved in athletes (Phinney *et al.,* 1983). Burke *et al.,* (2017) designed an investigation to examine the effect of a LCKD on exercise performance and efficiency following an intensive 21-day training camp involving elite race walkers (Table 1). Three different diets were implemented; a high-carbohydrate availability diet (*n* = 9, %carbohydrate:fat:protein = 65:20:15), periodised CHO availability diet (*n* = 8, %carbohydrate:fat:protein = 65:20:15), and a LCKD (*n* = 10, %carbohydrate:fat:protein = 7:78:15). The macronutrient profile of the two high-carbohydrate diets were the same; however the timing of carbohydrates differed; high-carbohydrate availability consumed carbohydrates prior to exercise, whereas the periodised carbohydrate diet incorporated periods of fasting and practised carbohydrate back loading (majority of carbohydrates consumed post-exercise). LCKD participants achieved significant increases in whole body fat oxidation; rates were greater than peak figures previously observed by Webster *et al.,* (2016) (*1.2* g·min^{-1}) and Volek *et al.,* (2016) (*1.57* g·min $^{-1}$). Both Webster and Volek's investigations were cross sectional studies on self-reported keto-adapted athletes; who had consumed a LCKD for >6 months (*6–36 months*). Peak rates of fat oxidation were observed at 72% VO_2max (Webster *et al.,* 2016), and 70.3% VO_2max (Volek *et al.,* 2016), during a 2-hour steady state cycle, and during a 3-hour run on a treadmill, respectfully. Whereas, peak rates of fat oxidation observed by Burke *et al.,* (2017) were achieved during the later stages of 2 hours of exercise at 80% VO_2max (*50km race pace*). This 21 day metabolic shift in substrate metabolism came at a cost to LCKD participants; exercise economy decreased from baseline, as there was a significant diet versus test interaction effect on heart rate and rates of perceived exertion (RPE); both of which significantly increased following the LCKD trial. Similarly, the O_2 cost of exercise significantly increased during the later stages of the 10km and 25km walking trials. Whereas, the O_2 cost of exercise, heart rate, and

RPE remained consistent in both groups consuming carbohydrate based diets. These markers indicate that LCKD participants were exercising at a higher metabolic cost while experiencing increased exertion following a 21 day adaptation period. Prior to the 10km, and 25km races; βHB ranged from 0.3-0.78 mmol/L, indicating participants achieved nutritional ketosis. Decreases in exercise economy, and increased perceived exertion did not significantly negate 10km and 25km performance, however each of the high-carbohydrate groups improved times following 21 days of intensive training. Burke et al.'s (2017) investigation illustrates that elite race walkers can achieve high rates of fat oxidation following just 21 days of keto-adaptation, however such an acute adaptation can negate training adaptations.

Shortly after Burke et al.'s (2017) investigation, Zinn *et al.,* (2017) shared a unique investigation (Table 1). It was the first investigation to monitor changes in performance >28 days, with a 10 week period of keto-adaptation. Although this investigation was a case study, and in recreationally-active individuals, it appears to be a good blue print for longitudinal changes that may occur, if performed in well-trained athletes. Blood ketones were monitored daily using 'FreeStyle' ketone meters and strips, and ranged from 0.5-1.9mmol/L. Interestingly, nutritional ketosis (βHB >0.5 mmol/L) was achieved following just ~14 days (2 weeks) of consuming a LCKD. Despite an elevation in blood ketones, during the first 1-5 weeks of the intervention period, participants specifically noted a reduction in power while exercising, claiming "*it was embarrassing to the point where I just got to the point where you just don't have any energy*", and "*I got too tired and I got to the point where I might have had some big runs, 4 or 5 h runs and wake in the night before hand, worried about it. I'd think, how am I going to do that tomorrow…it's going to be hard*". This indicates that although nutritional ketosis was objectively achieved, participants sustained a reduced ability to maintain/increase power, which may indicate reduced metabolic efficiency in fuel utilization. This suggests that being 'keto-adapted' is not defined exclusively by an elevation in blood ketones (βHB); mitochondrial adaptations within the muscle may also be necessary in order for an athlete to feel 'adapted'. This process appears to take ~5 weeks in recreationally-active males, and females; which may, or may not be an accurate reflection of how elite athletes adapt to a LCKD. Despite lay calls for longer adaptation periods in the literature, the 10 week adaptation period caused a significant decrease in time to exhaustion on a cycle ergometer (-2 mins). In accordance with Zajac *et al.,* (2014), and Burke *et al.,* (2017)

investigations, the LCKD group in the current investigation appeared to struggle as exercise intensity increased; max work load non-significantly decreased (-18 watts). Performance decrements at higher-intensities on low-carbohydrate (LCHF/LCKD) diets are attributed to alterations in metabolic pathways that impair oxidative metabolism (glycogen) (Burke and Hawley, 2002; Stellingwerff *et al.,* 2006; Yeo *et al.,* 2011). Specifically, adaptation to a low-carbohydrate (LCHF/LCKD) diet causes down-regulation of pyruvate dehydrogenase; a carbohydrate oxidative enzyme that helps link the glycolytic pathway with the Krebs cycle, via conversion of pyruvate to acetyl-coenzyme A (Peters and Leblanc, 2004). The down regulation of PDH occurs in response to reduced circulating insulin, and increased levels of circulating free fatty acids (Peters and Leblanc, 2004).

Two authors of this narrative review later published a 12 week dietary, and training intervention involving male endurance athletes (McSwiney *et al.,* 2018) (Table 1). One of the novel aspects of this investigation was the feeding strategy implemented during exercise; at baseline participants in each group consumed their habitual high-carbohydrate pre-exercise meal, and carbohydrates throughout (~30-60 g/h). In contrast, following a pre-exercise meal (HC or LCKD), participants in the HC group consumed 30–60 g/h of carbohydrate throughout post-intervention testing, whereas the LCKD group consumed only water, and electrolytes (to prevent orthostatic symptoms). This feeding strategy was implemented to improve the translational quality of the work, and to put claims of; 'no need for exogenous carbohydrates during exercise when on a LCKD' to the fore. Fasting serum βHB significantly increased from 0.1 up to 0.5mmol/L in the LCKD group, indicating nutritional ketosis was achieved. 100km TT performance improved in each group, however the improvement was numerically greater in the LCKD group (HC 0.71%, LCKD 2.48%). Interestingly, two LCKD participants were unable to complete post-intervention due to fatigue/hitting the wall. Participants were later found to have βHB <0.2 mmol/l; indicating the importance of having elevated βHB to fuel the brain if an athlete chooses to avoid carbohydrate supplementation prior to and during endurance exercise. A critical power test (CPT) (a 3-minute all out sprint against a set resistance) was performed immediately following a 100km TT. Imaginably, each group was fatigued, and in a carbohydrate depleted state. CPT peak power decreased by -0.7w/kg in the HC group, and increased by 1.4w/kg in the LCKD group (P = 0.040), while average power increased to a similar extent in each group (0.1 w/kg).

This would indicate an improvement in high-intensity exercise performance on a LCKD, a first for the LCKD literature. However, given the LCKD group's significant decrease in body mass (HC -0.8 kg, LCKD -5.9 kg), participants' power-to-weight ratio significantly improved, which resulted in improved relative performance (Figure 2).

In this landmark year for LCKD research; Heatherly and colleagues (2017) published a fourth investigation examining endurance performance where nutritional ketosis was achieved in 2017 (Table 1). This recent flurry of investigations is representative of the interest in 'ketogenic diets' and endurance sports in recent years (Burke *et al.,* 2015; Volek *et al.,* 2015). Heatherly et al.'s (2017) investigation examined the effects *ad libitum* LCKD had on measures of performance in recreationally active male runners following a 21 day adaptation period. The *ad libitum* LCKD (*n* = 8, %carbohydrate:fat:protein = 7:64:29) caused significant weight loss (~2.5 kg), and a significant increase in fat oxidation during exercise (RER). Mean 5 km TT performance was not significantly affected (HC = 23.92 ± 2.57 min, versus LCKD = 23.45 ± 2.25 min), however, the authors conceded that because this investigation was not a parallel arm design; there was a potential for an ordering effect, which perhaps, may explain the mean performance improvement. The *ad libitum* nature of the diet and the subsequent weight loss indeed suggests an ergogenic aid (improved power-to-weight ratio) to LCKD, however, weight lost may have impacted on the investigations findings in the research by Heatherly *et al.,* 2017. Unlike cycling on a cycle ergometer; running is very much a weight-bearing exercise. Thus, weight lost during the intervention period means that participants were completing less work following the intervention period. Previously, Phinney *et al.,* (1980) got participants to wear a back pack containing weight lost (kg) during the intervention period to control for this confounding variables impact on performance. However, in field settings an endurance athlete is not penalised for possessing an advantageous power-to-weight ratio (kg). The exercise protocol consisted of a 50 minute run, a 20 minute rest, and a 5 km TT. This long rest period does not reflect real world practices, which limits its transferability to real world athletes.

Applications

Body composition. Ad libitum LCKD appears an effective method of improving an athlete's body composition (Zajac *et al.,* 2014; Zinn *et al.,* 2017; McSwiney *et al.,* 2018; Heatherly *et al.,* 2017). Thus, a LCKD may be of benefit to endurance athletes who

struggle with maintaining competitive race weight. Athletes should be advised that improvements in body composition can be achieved on any 'diet'; with increased protein, and appropriate calorie restriction (Aragon *et al.,* 2017). However, some athletes appear particularly drawn to the '*ad libitum*' nature of LCKD, and may be more suited to a periodised approach to their nutrition (Jeukendrup, 2017).

Endurance/ultra-endurance. Current knowledge suggests that endurance performance (>2 hours) can be sustained on a cycle ergometer in endurance athletes following >28 days – 12 weeks of keto-adaptation (Phinney *et al.,* 1983; McSwiney *et al.,* 2018), however, acute (<21 days) ingestion of a LCKD causes poor training adaptations, decreases in exercise economy, and increased RPE in elite race walkers completing <1 hour of work (Burke *et al.,* 2017). Although it's unwise to compare elite and non-elite athletes; Burke *et al.,* (2017) may have further accounted for LCKD participants' initial decreases in performance; as subsequent investigations noted performance lagged until week 5-6 (Zinn *et al.,* 2017; McSwiney *et al.,* 2018). This additional time to 'adapt' would account for initial decreases in training intensity/volume, and may allow for better optimisation of low-carbohydrate metabolic pathways, perhaps leading to greater exercise efficiency, as observed previously at <72% VO_2max (Webster *et al.,* 2016; Volek *et al.,* 2016). Based on current knowledge, it would be unwise to adopt a LCKD <6 weeks prior to an endurance event.

Higher-intensity exercise. Optimal athletic performance in many endurance sports requires an athlete to have a 4^{th} and 5^{th} gear. Thus, having an ability to achieve and sustain high power outputs is a necessity. Zajac *et al.,* (2014) and Burke *et al.,* (2017) noted decreases in LCKD participants' ability to perform as exercise intensity increased. Spriet (2014) highlighted that fat as a primary fuel source becomes limited as exercise intensity increases >75% VO_2max; as the body's ability to deliver environmental oxygen to the muscle mitochondria nears maximum capacity. Interesting however, McSwiney et al.'s (2017) investigation did not note significant decreases in absolute power (Figure 2). As authors of McSwiney *et al.,* (2017), we do not believe that the longer adaptation period miraculously allowed keto-adapted athletes to convert fat to adosine triphosphate (ATP) in the absence of oxygen. If the CPT were conducted in a non-fatigued state, the results would likely have been different (most likely in favour of the HC group). However, when designing this investigation we aimed to assess measures of endurance performance in as practical a manner as possible. Thus, we placed a 3-minute

sprint finish (CPT) at the end of an endurance event (100 km TT). This preliminary work suggests that a keto-adapted athlete's 'inability to perform high-intensity exercise' may not be as 'significant' as one might think; at least in a fatigued state.

Limitations

A number of limitations exist within the current literature.

1. Positive performance responses observed may be limited to the population at hand (i.e., male endurance athletes), and may be limited to the performance tests at hand (i.e., CPT, time to exhaustion trial at 60% VO_2max, and 100km TTs).
2. Although recent investigations have opted for a 10-12 week adaptation periods, what happens beyond 12 weeks remains unknown. However, cross sectional studies suggest that greater exercise efficiency may be achieved (Volek *et al.,* 2016; Webster *et al.,* 2016).
3. Protein intake is often higher in LCKD groups (g/kg), versus high-carbohydrate/control groups (McSwiney *et al.,* 2018; Heatherly *et al.,* 2017). Due to the satiating effects of protein (Argon *et al.,* 2017); this may explain *ad libitum* weight loss in LCKD participants, and oftentimes lack thereof in high-carbohydrate/control groups, as dietary protein is lower. Conversely, a LCKD is said to have greater satiating effects than a 'non-ketogenic diet' with matched protein (g/kg) (Johnstone *et al.,* 2008); perhaps due to leptin, and its impact on appetite regulation. Previously, Miller *et al.,* (2017) indicated that leptin is an important predictor of increased fat oxidation; ultra-endurance athletes who consumed a LCKD for an average of 24 months had significantly lower concentrations of leptin (62.5 pg/ml LCKD), versus, a well-matched group of ultra-endurance athletes consuming a HC diet (112 pg/ml HC), however, specific research in this area is lacking.

Scope for future work

1) Noakes (2004) stressed the importance of having an exercise protocol with sufficient duration to induce near total glycogen depletion. The 10-25km races implemented by Burke *et al.,* (2017) would not have been long enough to do so (<50 minutes). Future work should examine circumstances where a ketogenic diet is most likely to be of benefit to athletes i.e., endurance/ultra-endurance exercise trials; where near glycogen depletion is a possibility.

2) ‘Real world LCKD athletes’ report reintroducing moderate amounts of carbohydrates during exercise to maintain ‘top level performance’ (>75% VO_2max). A recent study by Webster and colleagues (2017) documented the performance of an elite-level low-carbohydrate/fat-adapted endurance athlete. The participant involved in this 7 week crossover investigation had consumed a low-carbohydrate diet (~80g/d) for 2 years. The study concluded that a train low (low-carbohydrate diet), race high (carbohydrate supplementation during exercise) likely benefited high-intensity endurance-type exercise (4-30 min), but did not benefit short sprint, or prolonged endurance performance, versus a train low, race low approach. Though this investigation is limited by the small population sample (*n* = 1), and lack of a control group (HC diet); additional work in this area is needed.
3) In order to improve the translational quality of future work to real world endurance athletes, each group should be given equal opportunity to perform i.e., equal dietary protein (g/kg), appropriate carbohydrate feeding for high-carbohydrate/control group during exercise (60-90 g/h). Fasted time to exhaustion trials provide practitioners with valuable information regarding fuel utilisation, and exercise metabolism, however limits transferability to real world practice.

Conclusion

Despite a welcome resurgence in investigations examining LCKDs and endurance performance, the body of knowledge representing LCKDs remains in its infancy. Thus, a comprehensive endorsement of this dietary paradigm at this time is difficult to make. However, an endorsement of any ‘diet’ in all circumstances is unwise. This is why current guidelines recommend a personalised, periodised approach (Burke *et al.,* 2011; Jeukendrup, 2017). Nevertheless, the LCKD literature is beginning to show promise, and athletes continue to report anecdotal success on this diet. Thus, the implementation, or avoidance of this dietary paradigm should come down to an athlete’s own dietary preference. Despite a high-carbohydrate diet remaining the ‘evidence based choice for elite athletes’ (Helge, 2017); the concept of keto-adaptation does not appear to be dead and buried as previously outlined (Burke, 2015).

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Appendix O. Informed Consent Form and PAR-Q Form

Dublin City University

School of Human Health and Performance

Lonsdale Building

Glasnevin Campus

Dublin 9

Effect of ketone ester supplementation on simulated 10km treadmill time trial performance

Investigation into the potential performance enhancing effect of adding ketones to optimal carbohydrate based sports nutrition guidelines

INFORMED CONSENT FORM

Participant Name: _____

1. I confirm that I have read and understood the information leaflet dated 27/02/2018 for the above research study and received an explanation of the nature, purpose, duration and foreseeable effects and risks of the study and what my involvement will be.
2. In consideration of the researchers facilitating this study I hereby voluntarily release, relinquish and waive all or any causes of action for personal injury, loss and damage whether the same shall arise by the negligence of the researchers or otherwise, occurring to me because of my participation in the study and my use of any equipment relating thereto. I accept that I am solely responsible for the safe use of the equipment and acknowledge and accept that the researchers will have no legal liability whatsoever for any injury, loss or damage sustained by me while participating in the study or using any equipment.
3. I understand that data collected during the study will be stored in electronic format on a computer. I understand my identity will remain confidential and will not appear alongside any of this data. I understand that my data can be withdrawn only before the master data sheet is destroyed.
4. I had time to consider whether to take part in this research study. My questions have been answered satisfactorily and I have received a copy of the Participant Information Leaflet.
5. I understand that my participation is voluntary (my choice) and that I am free to withdraw at any time without my legal rights being affected.
6. I have to the best of my knowledge informed the investigator of my previous or present illnesses/medication and of any consultation that I have had with a doctor for the last four months. I have not participated in any other clinical trial in the past four months.
7. I will inform the research investigator immediately if I suffer any unexpected or unusual symptoms during the study.
8. I agree to take part in the above research study.
9. Please indicate whether you agree with your data being de-identified and stored indefinitely as per the study description. *Circle one* **YES NO**

…… ……..		
Name of participant (in block letters)	Date	Signature
……. ……..		
Name of Person taking consent (if different from researcher)	Date	Signature
……. ……..		
Researcher	Date	Signature

Physical Activity Readiness
Questionnaire - PAR-Q
(revised 2002)

PAR-Q & YOU

(A Questionnaire for People Aged 15 to 69)

Regular physical activity is fun and healthy, and increasingly more people are starting to become more active every day. Being more active is very safe for most people. However, some people should check with their doctor before they start becoming much more physically active.

If you are planning to become much more physically active than you are now, start by answering the seven questions in the box below. If you are between the ages of 15 and 69, the PAR-Q will tell you if you should check with your doctor before you start. If you are over 69 years of age, and you are not used to being very active, check with your doctor.

Common sense is your best guide when you answer these questions. Please read the questions carefully and answer each one honestly: check YES or NO.

YES	NO		
☐	☐	1.	**Has your doctor ever said that you have a heart condition <u>and</u> that you should only do physical activity recommended by a doctor?**
☐	☐	2.	**Do you feel pain in your chest when you do physical activity?**
☐	☐	3.	**In the past month, have you had chest pain when you were not doing physical activity?**
☐	☐	4.	**Do you lose your balance because of dizziness or do you ever lose consciousness?**
☐	☐	5.	**Do you have a bone or joint problem (for example, back, knee or hip) that could be made worse by a change in your physical activity?**
☐	☐	6.	**Is your doctor currently prescribing drugs (for example, water pills) for your blood pressure or heart condition?**
☐	☐	7.	**Do you know of <u>any other reason</u> why you should not do physical activity?**

If you answered YES to one or more questions

Talk with your doctor by phone or in person BEFORE you start becoming much more physically active or BEFORE you have a fitness appraisal. Tell your doctor about the PAR-Q and which questions you answered YES.

- You may be able to do any activity you want — as long as you start slowly and build up gradually. Or, you may need to restrict your activities to those which are safe for you. Talk with your doctor about the kinds of activities you wish to participate in and follow his/her advice.
- Find out which community programs are safe and helpful for you.

NO to all questions

If you answered NO honestly to <u>all</u> PAR-Q questions, you can be reasonably sure that you can:

- start becoming much more physically active – begin slowly and build up gradually. This is the safest and easiest way to go.
- take part in a fitness appraisal – this is an excellent way to determine your basic fitness so that you can plan the best way for you to live actively. It is also highly recommended that you have your blood pressure evaluated. If your reading is over 144/94, talk with your doctor before you start becoming much more physically active.

DELAY BECOMING MUCH MORE ACTIVE:

- if you are not feeling well because of a temporary illness such as a cold or a fever – wait until you feel better; or
- if you are or may be pregnant – talk to your doctor before you start becoming more active.

PLEASE NOTE: If your health changes so that you then answer YES to any of the above questions, tell your fitness or health professional. Ask whether you should change your physical activity plan.

<u>Informed Use of the PAR-Q</u>: The Canadian Society for Exercise Physiology, Health Canada, and their agents assume no liability for persons who undertake physical activity, and if in doubt after completing this questionnaire, consult your doctor prior to physical activity.

No changes permitted. You are encouraged to photocopy the PAR-Q but only if you use the entire form.

NOTE: If the PAR-Q is being given to a person before he or she participates in a physical activity program or a fitness appraisal, this section may be used for legal or administrative purposes.

"I have read, understood and completed this questionnaire. Any questions I had were answered to my full satisfaction."

NAME _____

SIGNATURE _____ DATE _____

SIGNATURE OF PARENT or GUARDIAN (for participants under the age of majority) _____ WITNESS _____

Note: This physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if your condition changes so that you would answer YES to any of the seven questions.

Supported by: Health Canada Santé Canada

continued on other side...

Appendix P. Advertisement for Study 4

Ketone Ester Supplementation and Time Trial Performance

A **Ketone Ester** is a new sport supplement being used in endurance sports to improve performance. We are investigating whether they have any benefit for distance runners.

Who we are looking for: Male distance runners aged 18-35 that can visit the Human Performance Lab in DCU 4 times, each visit lasts 3-4 hours.

Benefits: Free VO2max/lactate threshold testing and feedback based on your results.

What do the visits involve?

1. Participants will complete a battery of iPad based reaction tests to assess whether supplementation affects brain function.

2. Ingestion of a ketone ester, 1 hour of treadmill running followed by a 10-km treadmill based time trial.

For more info please contact: **fionnmcswiney@gmail.com**

IRISH RESEARCH COUNCIL
An Chomhairle um Thaighde in Éirinn

Appendix Q. Data Collection Sheet from Visit 1 from Study 4

Lactate and VO2max Protocol Data Collection Sheet

Name: _____

Date of Birth: _____/_____/_____

Height: _____ cm

Weight: _____kg

Date of Test: _____/_____/_____ **Time of Test:** _____

Lactate Threshold Test (Stage 5 at 10km race pace, stage 4 at -1km h^{-1} etc.)

*Only if lactate 4mM not established, possible will only reach max 2mM

Stage	Time (min)	Speed (km h^{-1})	Gradient (%)	HR (bpm)	Blood Lactate (mM)	RPE
Warm Up	5 min		1.0			
1	0-4		1.0			
2	5-9		1.0			
3	10-14		1.0			
4	15-19		1.0			
5	20-24		1.0			

VO_{2max} Test (Starts at 2km h^{-1} lower than last stage of LT if threshold reached)
(Starts at last speed of LT if threshold not reached)

Stage	Time (min)	Speed (km h^{-1})	Gradient (%)	HR (bpm)	RPE
1	0-2		1.0		
2	2-4		1.0		
3	4-6		1.0		
4	6-7	-	2.0		
5	7-8	-	3.0		
6	8-9	-	4.0		

7	9-10	-	5.0		
8	10-11	-	6.0		
9	11-12	-	7.0		
10	12-13	-	8.0		
11	13-14	-	9.0		
12	14-15	-	10.0		

Confirmation Trial

Stage	**Time (min)**	**Speed (km h^{-1})**	**Gradient (%)**	**Predicted VO2 (ml)**	**Actual VO2 (ml)**	**Predicted HR**	**Actual HR**	**Adjustments**
60%	0-5		1.0					
65%	5-10		1.0					
70%	10-15		1.0					

Lactate Threshold (km h^{-1}): _____

VO_{2max}: _____

Speed @65 VO_{2max}: _____

Appendix R. Data Collection Sheet from Visit 2-4 from Study 4

Data Collection Sheet Visit ___

Name: _____
Date of Birth: _____/_____/_____
Height: _____ cm
Weight: _____kg **Body Fat:** ____%
Date of Test: _____/_____/_____
Time of Test: _____

Collection periods	Min	Speed	Gradient (%)	VO2 (predicted)	VO2 (actual)	HR (bpm)	RPE	Speed adjustments
1	0-10		1.0					
2	25-30		1.0					
3	55-60		1.0					

Pre Load (1 h @ 65% VO2max)

10km Simulated Time Trial

Km	Time	Av. Speed	Gradient	HR (bpm)
0-2			1.0	
2-4			1.0	
4-6			1.0	
6-8			1.0	
8-10			1.0	

TTF: _____

Gastrointestinal Symptoms (Please tick box)

1. Lower Abdominal Symptoms	2. Lower Abdominal Symptoms
☐ Reflux/Heartburn	☐ Lower abdominal cramps
☐ Belching	☐ Side ache/Stitch
☐ Bloating	☐ Flatulence
☐ Stomach pain/cramps	☐ Urge to defecate (urgency)
☐ Vomiting	☐ Diarrhea
☐ Nausea	

Notes

Appendix S. Checklist for Visit 2-4 from Study 4

Visit 2-4 Checklist	
Morning	
Make breakfast (1g·kg CHO)	
Set up bloods station	
Vacutainers	
Eppendorfs	
Ice box	
COSMED in lab	

Pre-exercise	
Breakfast w/pint of water	
Body fat	
Calibrate COSMED	
Make mask	
Drinks ready	
60 min – Brendan insert canula	
Hydration testing	
90 min – first drink and blood sample 1	
110 min – start warm up (8km/h for 5 min)	
120 min – blood sample 2	
Instructions – tie shoes, bathroom etc.	

Pre-load	
0-10 min = Gas, HR, RPE	
20 min = Drink 2, blood sample 3	
25-30 min = Gas, HR, RPE	
40 min = Drink 3, blood sample 4	
53-58 min = Gas, HR, RPE	
60 min = Drink 4, blood sample 5	

Time Trial	
Pre = Instructions, bathroom	
2k splits	
5k = Drink 5	
End = Blood sample 6	

Post Exercise	
Spin bloods and store in freezer	

Appendix T. Internship at The Ohio State University (OSU)

My supervisor and I corresponded with Prof. Jeff Volek during 2015/2016 so that he agreed to supervise me for a 5 month internship at Ohio State University from Aug 2016-Dec 2016. I attended the Kinesiology Department at The Ohio State University. The goal going over was to get research experience on ongoing studies and carry out analysis on blood samples obtained, from the performance investigation I ran in Ireland (Chapter 3). Additionally Prof. Volek very generously agreed to provide the finance to analyse the blood samples from the performance trial.

When I arrived at OSU, the CHEESE study had already started. This is still an ongoing feeding study looking at varying levels of fat in the diet (low, medium and high) versus a high-carbohydrate diet on markers of health in an overweight population with the metabolic syndrome. Unlike a number of investigations outlined within Chapter 2, weight loss was not an objective of this investigation. Therefore, meals that were prepared on-site (by myself and other PhD/MSc students 3 days a week) were provided to participants at maintenance calories. Non-exercise activity thermogenesis, resting metabolic rate and body composition was measured frequently to ensure participants weren't losing weight. Unfortunately, due the intensive nature of the study (food preparation etc.,), there were only eight participants being put through the investigation at the time. Therefore, analysis of blood samples or preparation of the manuscript did not take place when I was there. However, through data collection I became very proficient at working with participants within a clinical setting, which was very new to me, as well as receiving training in phlebotomy. In addition, I also got to experience some pretty cool techniques, such as determining visceral fat using an MRI machine and a computer program developed by an undergrad student at tOSU.

The second study I helped out on at OSU was TANK or 'tactical athletes in nutritional ketosis'. This investigation started 2 weeks after I arrived, so it was a great to be involved in the investigation from start to finish. The investigation was funded by Quest Nutrition, so it was another feeding study but this time involving United States Marines. The study design and participants was something I was very familiar and comfortable with, so it was something I could contribute to from the outset. The investigation examined body

composition, strength, agility, high-intensity interval performance and hormonal and mitochondrial responses to a LCKD versus HC diet subsequent to an intensive training intervention. Unlike my own investigation, training took place on campus. So there frequent contact with participants throughout. Despite this, dropout was still quite high, which is testament to the difficulties in carrying out long term dietary intervention studies. With this study, I was involved in all aspect from recruitment, training, check-ins, blood processing, 1RM back squat and bench testing, agility testing and helping out with muscle biopsies. In addition, I got to experience one of the Midwest's most cherished traditions, i.e., American football and tail gating.

Upon completion of the internship Prof. Volek agreed to be my co-supervisor for PhD.

Appendix U. Internship at Dublin City University (DCU)

Following my transfer to PhD register in September 2017, Dr. Brendan Egan who was my external transfer examiner and an associate professor at DCU, asked would I be interested in helping out on an upcoming investigation he wanted to carry out, on a recently commercialised ketone ester. Dr. Egan advised completion of this investigation would enable achievement of my PhD.

I arrived in DCU in February 2018, and began planning the investigation outlined within Chapter 6. This was a very enjoyable research study. Prior to this, I had only been involved in a cross sectional study for my undergraduate thesis and experimental studies involving dietary and/or training interventions. Therefore, it was good experience to be involved in a double blind crossover investigation involving a supplement.

I got to analyse blood samples at Dublin City University to determine concentrations of βHB, glucose and lactate, respectfully, using a Daytona. In addition, the study came with its own challenges, i.e., tying to minimise confounding variables such as, order effect by implementing visit 2 to ensure participants were familiar with aspects of the investigation prior to experimental visits (3 and 4), successfully blinding the 'jet fuel' taste off the ketone ester and minimising the potential impact of menstrual cycle in our female participant.

Finally, prior to this investigation I had only used a MOXUS for respiratory assessment. However, during my time at DCU I became very proficient at using a COSMED for maximal tests and steady state assessment, as outlined within methods of Chapter 6.