Changes in energy metabolism and intermittent sprint performance in healthy active individuals following a 6-week low carbohydrate eating plan

by
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Declaration

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Abstract

Most field- and court sports are characterized by intermittent sprint movement patterns. With intermittent sprints, it is well-established that the anaerobic glycolytic pathway is downregulated during later sprints, to diminish metabolic by-product induced muscle fatigue. This study is, to my knowledge, the first to investigate the merits of a low-carbohydrate (LC) diet as a nutritional approach for intermittent sprints. It was hypothesised that carbohydrate (CHO) restriction will stimulate upregulation in the two main energy systems; ATP-PCr and aerobic metabolism. Measures of the metabolic energy systems and power output during intermittent sprints (6 x 10 s cycle sprints; 2 min recovery) was performed in 15 recreationally active participants (7 men, 8 women), on their baseline habitual diet (HD: 35% CHO, 45% fat, 20% protein) and 2-weekly throughout a 6-week LC intervention (7% CHO, 66% fat, 28% protein). Pre- and post-intervention, maximal aerobic capacity tests were performed and weekly blood [ketone] and wellness scores obtained. A linear increase in absolute ATP-PCr energy contribution occurred every 2 weeks to achieve a statistically significant change at LC week-6 (+22.0 ± 43.15 Joule; $p = 0.019$; ES = 0.47). Expressed as a percentage of total energy output, a *large* (2.1 ± 2.61%) increase in ATP-PCr contribution from baseline to LC week 2 was evident ($p = 0.072$: ES = 0.81), with *very large* significant changes at LC week 4 (2.5 ± 2.29%; $p = 0.011$; ES = 1.10) and 6 (3.5 ± 2.36%; $p = 0.002$; ES = 1.50). A significant *moderate* decrease in absolute anaerobic glycolytic contribution occurred at LC week 2 (-14.4 ± 28.16 Joules; $p = 0.031$; ES = -0.10) and remained low throughout the LC intervention. This change reflected a *very large* significant (-3.0 ± 2.91%) decline in percentage contribution by LC week 6 ($p = 0.028$: ES = -1.04). No significant change was, however, evident in absolute aerobic energy contribution ($p = 0.85$). These energy system adaptations resulted in *moderately* lower onset of fatigue by LC week-6 after a supercompensation-curved adaptation (7.4 ± 3.92% vs. 5.7 ± 2.64; $p = 0.332$; ES = -0.50). Peak power output during a graded exercise test was unchanged over the LC intervention (271.6 ± 60.19 W vs. 272.7 ± 54.48 W; $p = 0.772$; ES = 0.02), accompanied by a significant (5.3 ± 5.66%) increase in relative VO_2max ($p = 0.005$; ES = 0.32), a *very large* (-19.1 ± 18.34%) significant reduction in peak lactate ($p = 0.005$; ES = -1.00) and a significant (12.1 ± 12.77%) improvement in power at aerobic threshold ($p = 0.002$; ES = 0.43). Mean blood

ketone levels of of 0.8 ± 0.47 mmol.L^{-1} for the LC 6-weeks, were significantly higher than at baseline (0.3 ± 0.09 mmol.L^{-1}; $p = 0.002$), while a *moderate* improvement in wellness scores was evident during the LC phase ($p = 0.062$; ES = 0.54). These outcomes suggest that the LC intervention stimulated significant and favourable adaptations in anaerobic energy metabolism, resulting in supercompensation-curved changes in intermittent sprint performance.

Opsomming

Die meeste veld- en baansportsoorte word gekenmerk deur onderbroke naelloop bewegingspatrone. Dit is goed gevestig in onderbroke naelloop energie-metabolisme fisiologie dat die anaërobiese glikolitiese energiesisteem afgereguleer word gedurende latere naellope, om sodoende metaboliese byproduk-geïnduseerde spieruitputting te verminder. Hierdie studie is egter, na my beste wete, die eerste om die meriete van 'n lae-koolhidraat (LC) dieet as 'n voedingsbenadering vir onderbroke naellope te ondersoek. Dit was gehipotetiseer dat koolhidraat (CHO)-beperking opregulering sal stimuleer in die ander twee hoof energiesisteme: ATP-PCr en aërobiese metabolisme. Metings van die metaboliese energiesisteme en krag-uitset gedurende onderbroke naellope (6 x 10 s fietsergometer naellope; 2 min hersteltyd) is deur 15 aktiewe deelnemers (7 mans, 8 vrouens) uitgevoer, tydens hulle gewone basislyn-dieet (HD: 35% CHO, 45% vet, 20% proteïen) en 2-weekliks deur die loop van 'n 6-weke LC intervensie (7% CHO, 66% vet, 28% proteïen). Pre- en post-intervensie maksimale aërobiese kapasiteitstoetse was uitgevoer en weeklikse bloed [ketone] en welstand metings was verkry. 'n Liniêre toename in absolute ATP-PCr energie bydrae het elke 2 weke plaasgevind om 'n statisties-beduidende verandering teen LC week 6 teweeg te bring (+22.0 ± 43.15 Joules; p = 0.019; ES = 0.47). Uitgedruk as persentasie van totale energie-uitset, was 'n *groot* (2.1 ± 2.61%) toename in ATP-PCr-bydrae van basislyn tot LC week 2 duidelik (p = 0.072: ES = 0.81), met *baie groot* beduidende verandering by LC week 4 (2.5 ± 2.29%; p = 0.011; ES = 1.10) en 6 (3.5 ± 2.36%; p = 0.002; ES = 1.50). 'n Beduidende *matige* afname in absolute anaërobies glikolitiese bydrae het plaasgevind by LC week-2 (-14.4 ± 28.16 Joule; p = 0.031; ES = -0.10), en het laag gebly regdeur die hele LC intervensie. Hierdie anaërobies glikolitiese sisteemverandering het 'n *baie groot* (-3.0 ± 2.91%) beduidende afname in persentasie-bydrae in LC week 6 (p = 0.028: ES = -1.04) getoon. Geen beduidende verandering was egter duidelik in absolute aërobiese energie-bydrae nie (p = 0.85). Hierdie energiesisteem-aanpassings het gelei tot *matige* laer uitputtingsvlakke in LC week-6 na afloop van 'n superkompensasie-kurwe aanpassing (7.4 ± 3.92% vs. 5.7 ± 2.64; p = 0.332; ES = -0.50). Maksimale aërobiese kapasiteit piek krag-uitset is gehandhaaf oor die LC intervensie (271.6 ± 60.19 W vs. 272.7 ± 54.48 W; p = 0.772; ES = 0.02), vergesel deur 'n beduidende

(5.3 ± 5.66%) toename in relatiewe VO_2maks (p = 0.005; ES = 0.32), 'n *baie groot* (-19.1 ± 18.34%) beduidende afname in pieklaktaat (p = 0.005; ES = -1.00) en 'n beduidende (12.1 ± 12.77%) verbetering in aërobiese draaipuntkrag (p = 0.002; ES = 0.43). Gemiddelde bloed [ketoon] van 0.8 ± 0.47 mmol.L^{-1} vir die LC 6-weke was beduidend hoër as by basislyn (0.3 ± 0.09 mmol.L^{-1}; p = 0.002), terwyl 'n *matige* verbetering in welstandtellings gevind was vir die LC-fase (p = 0.062; ES = 0.54). Hierdie uitkomste maak dit duidelik dat die LC intervensie beduidend en gunstige aanpassings in anaërobiese energiemetabolisme gestimuleer het, wat gelei het tot superkompensasie-kurwe verandering in uitputting tydens onderbroke naellope.

Abbreviations

Acetyl-CoA:	Acetyl co-enzyme A
ADP:	Adenosine diphosphate
AT:	Aerobic threshold
ATP:	Adenosine triphosphate
[BLa]:	Blood lactate concentration
CHO:	Carbohydrate
CI:	Confidence interval
CK:	Creatine kinase enzyme
CK_{mito}:	Mitochondrial isoform of creatine kinase enzyme
CPT:	Carnitine palmityl transferase
ES:	Effect size
GOF:	Goodness of fit
H^+:	Hydrogen ions
HD:	Habitual diet
FAD:	Flavin adenine dinucleotide
FFA:	Free fatty acids
[ketone]:	Ketone concentration
k-LCHF:	Ketogenic low-carbohydrate, high-fat diet
LC:	Low carbohydrate
LCHF:	Low-Carbohydrate, High-fat
LPL:	Lipoprotein lipase
MPO:	Mean power output
mTOR:	Mammalian Target of Rapamycin
NAD:	Nicotinamide adenine dinucleotide
nk-LCHF:	non-ketogenic low-carbohydrate, high-fat diet

O_2:	Oxygen
P^+:	Phosphate ions
PCr:	Phosphocreatine
PDH:	Pyruvate dehydrogenase
P_i:	Inorganic phosphate
PPO:	Peak power output
RC:	Respiratory compensation point
rpm:	Revolutions per minute
PQ:	Respiratory quotient
s:	Seconds
SD:	Standard deviation
SEM:	Standard error of the mean
TTE:	Time to exhaustion
W:	Watts
WAnT:	Wingate Anaerobic Test
1RM:	One repetition maximum

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Chapter 1
Problem statement

1.1 Introduction

Although some of the world`s most popular field and court sports consist of short-intermittent sprint movement patterns (Spencer *et al.*, 2005), the effects of carbohydrate restriction has not, to my knowledge, been investigated in this exercise modality. Interestingly, at metabolic level during intermittent sprints, energy metabolism is already mainly dependent on phosphocreatine (ATP-PCr) and aerobic metabolism, while anaerobic carbohydrate (CHO) metabolism is down-regulated (Bogdanis *et al.*, 1996; Gaitanos, 1993; Parolin *et al.*, 1999; Trump *et al.*, 1996) to diminish metabolic by-product induced fatigue from anaerobic glycolysis (Parolin *et al.*, 1999). The present study therefore set out to investigate the effects of a 6-week low-carbohydrate (LC) diet on intermittent sprint energy metabolism and performance.

1.1.1 Fuel metabolism and exercise intensity

Fuel metabolism is exercise intensity specific. It is therefore advisable that sport nutrition guidelines be in line with exercise intensity specific physiology frameworks.

Physiology textbooks often refer to "high intensity exercise" as the later stages of incremental maximal aerobic capacity exercise protocols, where fuel metabolism shifts from predominantly fat to predominantly CHO (Powers & Howley, 2012). Hence, it is generally accepted that only CHO can provide energy at sufficiently fast rates during high intensity exercise, and that CHO supplementation is necessary for optimal athletic performance. It is further believed that the body is dependent on CHO during all types of high intensity exercise, mainly because fat metabolism produces energy at a rate that is too slow to maintain high workloads. Importantly, however, exercise intensities during the end stages of an incremental exercise test is still much lower than the intensities of most high intensity sports (MacLaren & Morton, 2012).

During low-intensity endurance exercise, associated with the beginning of graded maximal aerobic capacity laboratory tests, the rate at which ATP is needed is low

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enough so that aerobic metabolism can provide most of the required ATP. Fat is the fuel source that yields the most ATP/energy per unit and produces minimal metabolic by-products, that could disrupt homeostasis during exercise. The rate at which ATP is produced from fat, however, is theoretically slower, due to longer biochemical reactions (i.e. β-oxidation, Krebs cycle and electron transport chain) in the cell's mitochondria. During high intensity exercise, ATP is required at elevated rates and are usually met by a greater contribution from anaerobic metabolism, which involves shorter biochemical reactions. Since CHO can produce fast energy and in the absence of oxygen, it is believed that high intensity exercise is dependent on CHO as the main fuel source. However, the potential contribution of another major anaerobic fuel source, namely ATP-PCr, has, to my knowledge, not previously been considered in the debate on macronutrient requirements during high intensity sport performance.

Figure 1.1 illustrates the fuel metabolism pattern and relative fuel source contributions during short sprints of "maximal intensity" exercise. It is evident that the ATP-PCr energy system is the main contributor to energy outputs during short, maximal intensity bouts (< 10 s), while the aerobic system makes the least contribution.
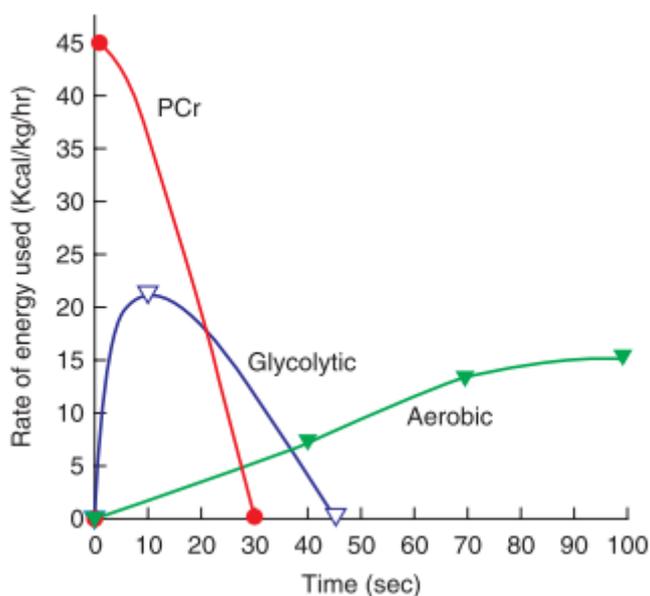
Figure 1.1. Energy continuum (from MacLaren & Morton, 2012, p.4)

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According to traditional sport nutrition guidelines, the consumption of high-carbohydrate diets for all types of sport are recommended (i.e. 6 - 10 g CHO / kg body weight / day and < 20% of total energy intake from fat (Jeukendrup, 2017; Rodriguez *et al.*, 2009). These guidelines, however, do not take into account the diverse fuel metabolism patterns at different exercise intensities. Thus, athletes who engage in continuous or intermittent high intensity activities may need alternative tailored guidelines.

Volek *et al.* (2016) reported on endurance athletes who successfully employed a low-carbohydrate-high-fat (LCHF) nutritional approach during training and competition. Their observations were in line with intensity-specific fuel metabolism as viewed from a graded exercise test perspective. During graded exercise test protocols, exercise at the lower intensities is associated with typical endurance events when fat is the major energy source. Additionally, if athletes are adapted to a LCHF diet, ketone bodies, produced by the liver during nutritional ketosis, become another 'fast' source of aerobic ATP production. A shift in fuel utilization towards predominantly ketone and fat metabolism, and less dependence on CHO, is thus associated with LCHF diet strategies. Volek *et al.* (2016) and Webster *et al.* (2016) hence proposed a ketogenic LCHF diet as an alternative exercise intensity-specific dietary strategy for endurance sport.

1.1.2 Intermittent sprint exercise

Some of the world's most popular team sports, such as soccer, rugby, basketball, netball, hockey and court sports, are characterised by repeated bouts of short, intermittent sprints (Spencer *et al.*, 2005). Performance in field-based team sports is determined by players` ability to repeatedly produce near-maximal power outputs or sprint speeds. A review of the literature on the movement patterns of field-based team sports found that mean sprint distances of 10 – 20 m (2 – 3 s) and 6 - 7 repetitions best represent these sports (Spencer *et al.*, 2005). Pre-eminently, these movement patterns involve intensities that is far greater than the "high-intensities" observed at the end of graded maximal aerobic exercise tests. Nutritional guidelines based on fuel metabolism at "high-intensities" of graded laboratory tests, may

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accordingly result in erroneous nutritional guidelines for performance in intermittent sprint sports.

Surprisingly, even though these sports attract worldwide attention, research on the effects of nutrition on the specific metabolic demands of intermittent sprint exercise is limited. In the textbook, *Biochemistry for Sport and Exercise Metabolism* (2012) it was stated: *"In contrast to endurance exercise, there are relatively few studies which have examined the influence of nutritional status on the regulation of substrate utilization during HIE* (high-intensity, intermittent exercise)…." *"To our knowledge, there are no studies to date which have examined the effects of fat-loading or high-fat pre-exercise meals on metabolism and performance during HIE."* (MacLaren & Morton, 2012: 206 - 207).

Upon examination of the physiological mechanisms and unique fuel metabolism patterns during intermittent sprints, it is even more surprising that LC interventions have not previously been employed as a sport-specific nutritional strategy in this exercise modality. In this regard, several authors described the down-regulation of anaerobic glycolysis and a subsequent greater reliance on ATP-PCr and aerobic metabolism during intermittent sprints (Bogdanis *et al.*, 1996; Gaitanos, 1993; McCartney *et al.*, 1986; Parolin *et al.*, 1999; Trump *et al.*, 1996). It was suggested that the reduced contribution of anaerobic glycolysis assists in the maintenance of homeostasis, thereby minimizing fatigue during the later sprints of intermittent sprint protocols (Parolin *et al.*, 1999). Hence, lowering CHO intake through a LC intervention could theoretically decrease disruptions in homeostasis during intermittent exercise, with a resultant reduction in the onset of muscle fatigue.

Although a "30 - 15 Intermittent Fitness Test" has recently been implemented in LC interventions (Cipryan *et al.*, 2018; Dostal *et al.*, 2019), the 30 s shuttle runs in these studies are still far removed from the observed short sprint movement patterns of field-based sports (Spencer *et al.*, 2005). Some LC investigations also utilized single bout, maximal intensity exercise or muscle strength tests (Fleming *et al.*, 2003; Greene *et al.*, 2018; Lambert *et al.*, 1994; McSwiney *et al.*, 2017; Paoli *et al.*, 2012). Findings from these investigations may thus shed light on the effect of LC strategies on performance in activities where the ATP-PCr energy system is the main contributor to energy metabolism.

Indeed, promising findings during short (< 10 s), maximal-intensity exercise emerged from previous studies. For instance, strength performance was maintained in eight elite artistic gymnasts (men) after a 4-week LC diet (54.8% fat, 40.7% protein, 4.5% CHO) (Paoli *et al.*, 2012). Olympic- and power lifting performance was also maintained in competitive, intermediate and elite level lifting athletes following a 12-week LCHF diet (< 50 g or < 10% CHO) (Greene *et al.*, 2018). Furthermore, McSwiney *et al.* (2017) reported a significant improvement in 6 s cycle sprint power output in nine endurance trained men after a 12-week k-LCHF diet intervention (77% fat, 6% CHO).

During the first few seconds (≤ 10 s) of maximal intensity exercise, as observed in the short sprint and strength tests employed in the studies of Greene et al. (2018), McSwiney *et al*. (2017) and Paoli *et al*. (2012), the ATP-PCr energy system produced the majority of ATP from stored PCr in the muscle. In light of this fact and in conjunction with their finding of improved 6 s sprint performance, McSwiney *et al.* (2017) speculated that the k-LCHF intervention had no detrimental effects on the capacity of the ATP-PCr energy system. These findings provide encouraging outcomes and suggest that the effects of a LC intervention on the adaptations of the ATP-PCr energy system should be quantitatively measured and further investigated.

Quantitative measurements related to the adaptations in the aerobic- and glycolytic energy pathways to LC interventions are fairly common in the literature (Cipryan *et al.*, 2018; Dostal *et al.*, 2019; Fisher *et al.*, 1983; Phinney *et al.*, 1983; Vogt *et al.*, 2003; Volek *et al.*, 2016; Waldman *et al.*, 2018; Zajac *et al.*, 2014). Yet, to my knowledge no previous investigations have quantified, or monitored, the adaptation in the ATP-PCr energy system in response to a LC intervention.

A few studies also utilized 30 s Wingate cycle sprint tests (WAnT) to study the effects of a LC nutritional strategy on anaerobic exercise performance. According to the energy continuum model, peak power output (PPO) (reached within the first 5 s of a 30 s WAnT), has been reported to reflect the utilization of the ATP-PCr energy system to ATP production, while mean power output (MPO) over the 30 s reflects the utilization of the glycolytic energy system (Smith & Hill, 1991). Thus, from this standardized exercise test, it is possible to indirectly estimate the contributions of these energy systems to ATP production.

To my knowledge, only three LC studies implemented WAnT tests (Fleming *et al.*, 2003; Lambert *et al.*, 1994; Langfort *et al.*, 1997). A consistent finding in these studies, namely a drop in MPO, seemed to discourage further investigations into high intensity exercise and LC interventions. However, MPO during a WAnT test is associated with the anaerobic glycolytic energy system contribution (Smith & Hill, 1991). Since dietary CHO intake, the fuel for glycolysis, is restricted on a LC diet, it is thus not surprising that a LC intervention impaired anaerobic glycolytic metabolism and MPO. However, Lambert *et al.* (1994) and Langfort *et al.* (1997) both observed either the maintenance of, or a small improvement in PPO following their LC interventions. Since PPO during the WAnT reflects the utilization of the ATP-PCr energy system, it supports the suggestion of McSwiney *et al.* (2017) that a LC diet should not have a detrimental effect on the capacity of this fast energy system.

Fleming *et al.* (2003) included a 2 x 30 s WAnT with 2 min rest in their test battery. A finding not emphasized by the authors, was that PPO (W/kg) during bout two was maintained after the 6-week LC intervention (Fleming *et al.*, 2003). Accordingly, research on exercise metabolism during repeated sprints and high-intensity intermittent exercise have shown reductions in the contribution of glycolysis during later bouts, with ATP-PCr and aerobic metabolism contributing more to ATP production (Bogdanis *et al.*, 1996; Gaitanos, 1993; Parolin *et al.*, 1999). In light of this knowledge, it seems plausible that CHO restriction in this study (Fleming *et al.*, 2003) explained the unchanged PPO during bout two of the repeated WAnT.

1.1.3 Time-course of adaptation to carbohydrate restriction

In sport science training theory, the time-course of adaptation in response to a physiological "challenge"/stimulus (periodization) follows a "supercompensation" curve (Cole, 1998; Furnas, 2000). In this model, performance initially declines after application of the "challenge"/stimulus. Thereafter, the body adapts physiologically by over-compensating to the specific "challenge" and consequently performance in that capacity is improved. The phases in this process are illustrated in Figure 1.2.
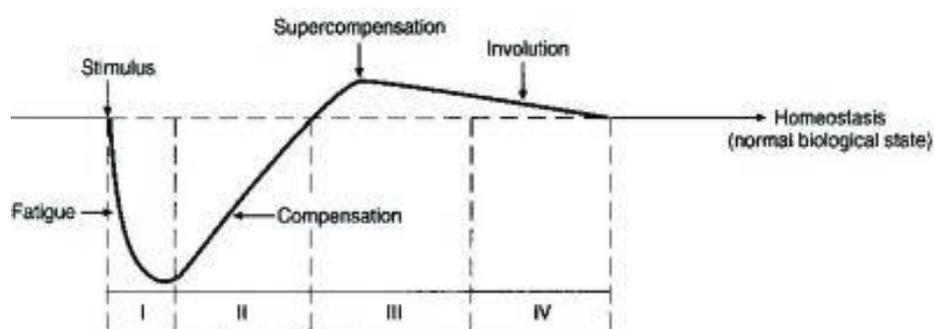
Figure 1.2: Supercompensation curve depicting adaptation to a stimulus (from Cole, 1998)

Traditionally, only exercise training was regarded as stimulus for this model (Cole, 1998). However, nutrition is now also acknowledged as a stimulus for physiological adaptation and regarded as part of the integrated, multifactorial approach to performance improvements (Mujika *et al.*, 2018). The time course of supercompensation is also different for diverse stimuli (Mujika *et al.*, 2018), and investigation of the diverse metabolic adaptations in response to the stimulus of prolonged nutritional ketosis is still a novel field of research. The term, keto-adaptation, emerged from this literature with reference to metabolic adaptation in response to chronic nutritional ketosis. On this issue, Sherrier & Li (2019) conducted a narrative review which integrated outcomes from various studies to cumulatively propose time frames of several metabolic adaptations involved in this process of keto-adaptation.

The process of keto-adaptation in athletes is still poorly understood. Most LC intervention studies in sport did not monitor the adaptation process over time and only included pre- and post- intervention measurements (Carr *et al.*, 2018; Fleming *et al.*, 2003; Greene *et al.*, 2018; Lambert *et al.*, 1994; McSwiney *et al.*, 2017; Paoli *et al.*, 2012; Zajac *et al.*, 2014). However, investigations on the time course of keto-adaptation through repeated measures are less common (Dostal *et al.*, 2019; Phinney *et al.*, 1983; Prins *et al.*, 2019; Waldman *et al.*, 2018).

Nonetheless, the time course of changes in performance in response to a LC intervention has already been observed in one of the earliest studies in this field, although not in athletes (Phinney *et al.*, 1980). In their study, six moderately obese, untrained participants (5 women, 1 man) completed a 6-week protein-supplemented fast (< 10 g CHO; 1.2 g of protein/ kg ideal body weight). Time to exhaustion (TTE)

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during treadmill uphill walking with weighted backpacks (to compensate for body mass reductions) were conducted at week one and week six on the protein-supplemented fast. TTE decreased to 80% of baseline walking performance in week one, reflecting phase one ("fatigue") on the supercompensation curve (Figure 1.2). There after TTE performance increased to 155% of baseline performance at the 6-week time point (Phinney *et al.*, 1980), reflecting supercompensation in performance.

It is clear from the literature that there is scope for studies on training and performance adaptations during carbohydrate restriction, as well as investigations into other modes of exercise, than endurance training.

1.2 Purpose of the study

To my knowledge, no previous study investigated the effect of CHO restriction on energy metabolism or performance during intermittent sprints. Similarly, no previous study has quantified or monitored adaptations in the ATP-PCr energy system in response to a LC intervention. Lastly, most LC intervention studies in sport are limited to pre- and post- intervention measurements (Carr *et al.*, 2018; Dostal *et al.*, 2019; Fleming *et al.*, 2003; Greene *et al.*, 2018; Lambert *et al.*, 1994; McSwiney *et al.*, 2017; Paoli *et al.*, 2012; Zajac *et al.*, 2014), while monitoring the time course of adaptation has rarely been documented (Dostal *et al.*, 2019; Phinney *et al.*, 1980; Prins *et al.*, 2019; Waldman *et al.*, 2018).

The purpose of this study was to investigate the effect of a 6-week self-selected LC dietary eating plan on the metabolic adaptations and physical performance of healthy, active men and women during all-out effort intermittent sprint exercise.

1.2.1 Research aims and hypothesis

Primary aims:

1. To quantify the changes in the contributions of the metabolic energy systems in healthy active individuals during intermittent sprint cycle exercise throughout a 6-week self-selected CHO restricted eating plan (< 50 g/day).

H₁: It is hypothesised that adaptation in the phosphocreatine energy system will occur, so that energy production from this pathway will increase throughout the LC diet intervention during maximal intensity intermittent cycle exercise.

H₂: It is hypothesised that restriction of fuel for anaerobic glycolysis on a LC diet will down-regulate this energy system during maximal intensity intermittent cycle exercise.

H₃: It is hypothesised that adaptation in aerobic fat metabolism will occur, so that energy production from this pathway will increase throughout the LC diet intervention during maximal intensity intermittent cycle exercise.

2. To determine whether 6-week CHO restriction affects the intermittent sprint cycle performance (fatigue and MPO) of healthy active individuals.

H₄: It is hypothesised that participants` fatigue and total session MPO performance will follow a supercompensation curve, as previously described by Cole (1998).

3. To determine if changes occur in maximal aerobic capacity and aerobic exercise metabolism in response to a 6-week LC diet.

H₅: It is hypothesised that relative substrate utilization will notably shift towards greater fat oxidation, with a consequent reduced reliance on anaerobic CHO metabolism, and endurance performance (PPO) will be maintained.

Secondary aims:

1. To quantify the nutritional intake of healthy, active individuals during the adoption of a self-selected LC dietary plan.
2. To monitor the wellness of the participants during their adoption of a self-selected LC dietary plan.
3. To monitor blood [ketone] levels of the participants during their adoption of a self-selected LC dietary plan.

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Objectives:

1. To calculate the contributions of the ATP-PCr, glycolytic and aerobic energy systems during the intermittent cycle sprints and compare baseline measurements on their habitual diet to LC diet measurements at 2-weekly intervals over six weeks.

2. To measure the participants' work capacity (power output in Watts) during intermittent cycle sprints and compare baseline measurements on their habitual diet to LC diet measurements at 2-weekly intervals over six weeks.

3. To measure changes in maximal aerobic capacity and cardio-metabolic parameters at baseline and at the end of the 6-week intervention, while participants are still on the LC diet.

4. To measure blood [ketone] once a week during the six weeks on the LC diet.

5. To monitor the dietary intake and analyse the macronutrient intake of the participants in the last week on their habitual diet, as well as during week one, five and six of the LC intervention.

1.2.2 Rationale for the study

It is proposed that the phosphocreatine and aerobic energy systems are the main contributors to energy metabolism during repeated all-out effort intermittent sprints, with a down-regulation of glycolysis during later sprints in order to maintain homeostasis and performance (Bogdanis *et al.*, 1996; Gaitanos, 1993; Parolin *et al.*, 1999). Thus, theoretically, the adoption of a LC eating plan, where the fuel for glycolysis is restricted, should not have a negative effect on intermittent sprint exercise performance. Restriction of CHO may also possibly stimulate adaptations in the other two energy systems that are dominant during this exercise modality.

The focus of most previous research on the effect of a LC nutritional intervention on energy metabolism and sport performance is generally limited to endurance type sport. Nonetheless, many popular team sports, as well as racquet sports, consist of intermittent sprints, but this exercise modality has not been previously investigated in conjunction with a LC nutritional intervention. Due to the many health benefits of a LC lifestyle and its adoption by an increasing number of the health-conscious general population and sport participants, it is conceivable that individuals

participating in intermittent sprint sport may be interested to know if a LC eating plan will have a negative effect on their performances.

1.3 Overview of chapters

Chapter 2 will provide an overview of current knowledge on intermittent sprint metabolism in order to gain an understanding of the underlying physiology behind the primary hypothesis of this thesis. I will provide a review of the literature on the biochemical pathways for ATP production, known as the energy systems. The theory behind energy system physiology during intermittent sprint exercise will be discussed to gain an understanding of this exercise modality`s unique fuel utilization pattern. Thereafter, an overview of current literature on the influence of CHO restriction on exercise metabolism and -performance will be provided.

Chapter 3 will describe the methods employed in the study. Details regarding research design, study participants, research procedures and instruments, statistical analysis and ethical considerations will be provided.

Chapter 4 will present the results of the study. This will include participant characteristics, nutritional characteristics of the habitual- and LC diet phases, intermittent sprint energy metabolism and performance, maximal aerobic capacity parameters, blood ketone responses and wellness scores.

In Chapter 5 the results of the study will be discussed in relation to current literature.

Chapter 6 will conclude with the main outcomes of the study and the contribution of the present study to current knowledge. The study's limitations, practical implications of the study findings and recommendations for future research are also presented in this chapter.

Chapter 2
Literature review

2.1 Introduction

Competitive athletes often demonstrate immense discipline and dedication to rigorous training regimens with the goal to progressively stimulate adaptations in the most prominent metabolic pathways utilized in their specific sport. Typical training adaptations resulting in enhanced sport performance include increased mitochondrial density and enzyme activity, higher fat oxidation rates and higher lactate thresholds. Recent research has shown that adaptation to a low-carbohydrate, high-fat (LCHF) diet also results in a combination of metabolic changes in energy metabolism in response to the stimulus of prolonged nutritional ketosis (McSwiney *et al.*, 2017; Sherrier & Li, 2019; Volek *et al.*, 2016). These metabolic adaptations are similar to and could be complimentary to those induced through exercise training (Sherrier & Li, 2019). Over and above the widely reported health advantages of carbohydrate (CHO) restriction and ketone metabolism (Gano *et al.,* 2014; Miller *et al.*, 2018; Newman & Verdin, 2014; Rojas-Morales *et al.*, 2016; Stafford *et al.*, 2010; Veech 2004; Veech *et al.*, 2017; Vidali *et al.*, 2015), these additional metabolic adaptations to a LCHF eating plan might well become an incentive for competitive athletes to implement this dietary strategy.

Many of the desired adaptations that result in enhanced sport performance occur in the various metabolic pathways. Creatine phosphate, muscle glycogen, and free fatty acids (FFA) are the three main fuel sources of ATP production during exercise. Different sports utilize different combinations of these fuel sources based on the characteristics of the specific activity.

Intermittent sprint exercise has a unique energy metabolism pattern. Glycolysis is down regulated, while the other fuel sources, phosphocreatine and aerobic oxidation of FFA, are the preferred and more efficient fuel sources (Bogdanis *et al.*, 1996; Gaitanos, 1993; Parolin *et al.*, 1999). Down-regulation of glycolysis during intermittent sprint exercise happens even when an athlete follows a traditional (CHO-rich) diet. Thus, the question is: What would be the effect of a change in

macronutrient intake, such as restricting carbohydrate, on metabolic adaptations and sport performance during intermittent sprint exercise?

2.2 The biochemistry of energy

2.2.1 ATP production rate and exercise intensity

The substrates that provide fuel for exercise are metabolised along various metabolic pathways and have different biochemical reactions that produce and replenish ATP at varying rates. Anaerobic metabolism (PCr degradation and glycolysis) have shorter biochemical pathways than the aerobic reactions involving β-oxidation, the Krebs cycle and electron transport chain, thus producing ATP at a faster rate.

The rate of ATP utilization is, among other factors, dependent on exercise intensity and duration. The higher the exercise intensity, the faster the rate of ATP utilization. Refer to Chapter one (Figure 1.1, p.2) for an illustration of the energy continuum, which shows the interaction and relative contributions of the three energy systems (MacLaren & Morton, 2012). At any intensity or time point on the continuum, a combination of the different energy systems contributes to the total ATP turnover. However, the percentage contribution of each biochemical pathway is governed by the rate of total ATP turnover needed for the specific energy demands at a given exercise intensity.

The energy continuum can be used to explain the relationship between exercise intensity and fuel source contribution to ATP synthesis. During the first phase (up to 10 s) of a maximal intensity sprint, the rate of ATP turnover is maximal and therefore highly dependent on the contribution of the fastest possible ATP producing source, namely ATP-PCr. However, the intramuscular storage capacity of PCr is small, leading to quick depletion during all-out efforts. Sprint intensity will thus decline when PCr stores drop as this necessitates greater contributions by the slower ATP production processes, namely glycolysis and oxidative phosphorylation.

One of the purposes of exercise training is to improve the capacity and efficiency of the dominant energy system(s) involved in a particular sport. An energy system's capacity is improved with enhanced intramuscular storage of its applicable energy

substrate (PCr, glycogen or triacyl-glycerol), while adaptations that enhance an energy system's efficiency is related to enhanced enzyme activity, lower substrate utilization to produce the same exercise intensity, faster ATP resynthesis and lower O_2, NAD, FAD cost of the reaction. Physiological adaptations occur in the different biochemical pathways to improve the capacities and / or the efficiency of each system. Although exercise training is the more commonly employed stimulus for adaptation (Gibala *et al.*, 2006; Ross & Leveritt, 2001; Tunstall *et al.*, 2002), nutrition has also been explored as an adjunct stimulus (Cameron-Smith *et al.*, 2003; Hawley *et al.*, 2011).

2.2.2 Maximal intensity intermittent sprint metabolism

Field based sports such as rugby, netball, hockey, soccer, basketball, and racquet sports are characterized by maximal or near maximal bouts of exercise (i.e. all-out efforts), with brief recovery intervals of either complete rest, or low intensity activity, during games that vary from one to four hours in duration (Spencer *et al.*, 2005). Exercise performance in these sport activities rely heavily on the availability and replenishment of quick, anaerobic energy. However, the duration of typical matches (longer than one hour) also necessitate a well-developed endurance capacity.

Intermittent sprints have a unique pattern of energy metabolism during which ATP-PCr and aerobic energy metabolism seems to be the preferred fuel sources, while anaerobic glycolysis is down-regulated during later sprints (Bogdanis *et al.*, 1996; Gaitanos, 1993; McCartney *et al.*, 1986; Parolin *et al.*, 1999; Trump *et al.*, 1996). ATP-PCr provides ATP at fast rates during the sprint, while aerobic metabolism is utilized during recovery to rephosphorylate Cr to PCr for the next sprint. During later sprints, aerobic metabolism also makes a larger contribution to ATP production.

The importance of the ATP-PCr energy system during intermittent sprints has been widely observed (Bogdanis *et al.*, 1996; Casey *et al.*, 1996; Gaitanos, 1993; Trump *et al.,* 1996). For instance, Bogdanis *et al.* (1996) quantified the robust utilization and fast recovery of PCr during two all-out bouts of exercise in eight men, using muscle biopsies. After the first of two 30 s cycle sprints, PCr was depleted to approximately 17% of resting levels, indicating a high rate of utilization. Then, the four min recovery between bouts allowed for rapid PCr replenishment to

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approximately 80% of resting values, thus enabling this energy system to once again contribute significantly to ATP production during the following sprint (Bogdanis *et al.*, 1996). Various studies also demonstrated that higher PCr resynthesis rates during recovery is associated with higher total power output across intermittent sprint protocols (Aaserud *et al.*, 1998; Bogdanis *et al.*, 1995; Casey *et al.*, 1996; Crisafulli *et al.*, 2018). Furthermore, intermittent sprint performance is typically enhanced after as little as five days of oral creatine supplementation (Aaserud *et al.*, 1998; Greenhaff *et al.*, 1993).

The contribution of anaerobic glycolysis during intermittent sprints has also been well documented. A consistent finding is the down-regulation of anaerobic glycolysis during later sprints within a session or match (Bogdanis *et al.*, 1996; Gaitanos, 1993; McCartney *et al.*, 1986; Parolin *et al.*, 1999; Trump *et al.*, 1996). For example, Bogdanis *et al.* (1996) reported a 45% decrease in anaerobic glycolysis contribution in the second of two consecutive 30 s sprints.

The drop in the contribution of glycolysis during repeated sprints has been attributed to the allosteric effects of H^+ ions that are released from lactic acid (Parolin *et al.*, 1999). H^+ ions inhibit the rate-limiting enzyme of glycolysis, phosphofructokinase, thereby reducing the supply of ATP through the glycolytic pathway, as well as the further production of lactic acid. H^+ ions also allosterically upregulate pyruvate dehydrogenase (PDH), which converts pyruvate to acetyl-CoA for uptake into the Krebs-cycle (Parolin *et al.*, 1999). High levels of H^+ ions released from lactic acid, in conjunction with other metabolic by-products, lower muscle pH. Low skeletal muscle pH disrupts muscle contraction by interfering in various steps of the cross-bridge cycle, as well as down-regulating various enzymes involved in energy metabolism. Cumulatively, these effects contribute to the onset of muscle fatigue and are thus detrimental to exercise performance.

With regards to the aerobic energy system contribution during intermittent sprints, it was observed that its contribution to ATP production increased during later sprints, commonly reflected by a greater oxygen uptake as the number of sprints increase (Bogdanis *et al.*, 1994; Bogdanis *et al.*, 1996; Gaitanos, 1993). Also, Bogdanis *et al.* (1996), who mainly focussed on quantifying anaerobic system contributions, additionally observed that, while total anaerobic ATP production drops due to down-regulation of glycolysis, total work does not decrease proportionately. This supports

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the view that aerobic metabolism makes a significant contribution to the provision of energy with the onset of fatigue during later sprints.

Most of the mentioned studies, however, utilized 30 s all-out efforts, whereas intermittent sprint patterns commonly observed in sport settings are much shorter (Spencer *et al.*, 2005). Hence, the study by Gaitanos (1993), using 10 x 6 s sprints with 30 s rest intervals, better reflects sport-specific intermittent sprint exercise. Gaitanos (1993) quantified the contributions of the anaerobic energy systems (ATP-PCr and anaerobic glycolysis) in eight healthy male physical education students (age: 26.7 ± 8.4 years) through muscle biopsies. A progressive decline in power output was observed from the first to the last sprint, mainly attributed to increasingly less ATP contribution from the glycolytic pathway. It was calculated that approximately 50% of anaerobic ATP production during the first sprint was provided by the ATP-PCr system and 45% from glycolysis. In the last sprint the contribution of glycolysis decreased to 16% and the relative contribution of ATP-PCr to anaerobic ATP production increased to 80%. Thus, while repeated short (6 s) sprints are more sport-specific, the pattern of energy system contribution is consistent with findings from the more commonly employed 30 s sprint studies (Bogdanis *et al.*, 1996; McCartney *et al.*, 1986; Parolin *et al.*, 1999; Trump *et al.*, 1996),

2.2.3 ATP-PCr-mitochondrial coupling

It is clear from the literature that intermittent sprint exercise requires both high rate, anaerobic ATP production, as well as aerobic ATP production. Even with maximal rates of ATP utilisation during an all-out intensity sprint, ATP levels in skeletal muscle cells are kept relatively constant through the combined contributions of all the energy systems. The ATP-PCr energy system is a high-rate anaerobic system and of particular importance during intermittent sprint exercise (Bogdanis *et al.*, 1996). Its biochemical reaction is very short and involves PCr directly donating a P_i that binds to ADP to produce ATP. Once PCr levels drop, this fast reaction cannot provide enough P_i to maintain ATP levels and the slower fuel sources cannot sustain the high rate of ATP turnover. When this happens, muscle fatigue sets in. Reportedly, the maintenance of power output during later sprints in an intermittent

sprint protocol is correlated to the percentage of PCr resynthesis (r = 0.74; p < 0.05) during recovery periods (Bogdanis *et al.*, 1995).

Creatine, however, cannot be rephosphorylated during a bout of exercise since the available ATP is used to maintain the highest possible intensity of muscle contractions. For this reason, the ATP-PCr energy system is only available for the first few seconds (about 10 s) of all-out effort exercise (MacLaren & Morton, 2012). Small intramuscular PCr stores are also rapidly depleted during short bouts of maximal intensity exercise as observed during intermittent sprints. Thus, since PCr is the fastest source of ATP, replenishment of these stores are metabolically prioritised during the recovery phases of intermittent sprints in order to maximize energy availability in the following maximal effort (Dawson *et al.*, 1997).

There are two chemical reactions involved in the ATP-PCr metabolic system. The first reaction occurs during muscle contraction. Stored PCr is broken down and a phosphate is donated for ADP rephosphorylation. The second reaction occurs during the recovery phase after muscle contraction. During this reaction Cr is rephosphorylated through donation of a phosphate from ATP to form ADP and PCr. Both these reactions are enzymatically catalysed by various creatine kinase (CK) isoforms. The CK isoform found in the cytosol of skeletal muscles regulates the reaction during muscle contraction. Rephosphorylation of PCr from Cr during recovery after contraction is catalysed by another isoform, CK_{mito}, in the mitochondrial intermembrane space. PCr is thus rephosporylated through aerobically produced ATP. To illustrate this proposed reliance of PCr resynthesis on oxidative ATP production in the mitochondria, investigators who applied blood flow occlusion to the muscle during recovery after a fatiguing isometric contraction (Sahlin *et al.*, 1979) and aerobic exercise (Yoshida & Watari, 1997), reported significantly impaired PCr restoration.

During intermittent sprint exercise, higher rates of PCr restoration during the recovery phase commonly resulted in higher power outputs in later sprints (Gaitanos, 1993; Aaserud *et al*, 1998; Bogdanis *et al.*, 1995; Casey *et al.*, 1996; Crisafulli *et al.*, 2018). The role of Cr rephosphorylation for intermittent sprint performance therefore highlights the importance of the action of CK_{mito} and aerobic ATP production in energy metabolism and performance during this type of exercise.

Although the effect of CHO restriction on the functioning of the ATP-PCr energy system has not previously been investigated, researchers reported that mitochondrial biogenesis and mitochondrial enzyme activity are more enhanced following LCHF plus exercise training interventions, compared to exercise training alone (Cheng *et al.*, 1997; Fisher *et al.*, 1983; Hyatt *et al.*, 2016; Morton *et al.*, 2009). Thus, the direct connection between Cr rephosphorylation and mitochondrial metabolism (at the level of CK_{mito}), could suggest that a CHO restricted diet might induce advantageous adaptations in the ATP-PCr energy system. The mitochondria in skeletal muscle cells may consequently emerge as an important site of adaptation in response to a LCHF diet.

2.3 Methodological considerations in the quantification of energy metabolism during exercise

Various experimental protocols have been used to quantify energy metabolism during intermittent sprint exercise. Most often cycling is used as mode of exercise as the measurements of power output and metabolic variables can be reliably collected in a controlled environment and with the least amount of extraneous noise in the data. Energy metabolism is further affected by the duration of sprint- and recovery intervals, mode of recovery (active or passive), number of sprint bouts, training status of participants, as well as the mathematical models utilized to calculate the energy system contributions.

2.3.1 Sprint duration

In their review of the literature on repeated sprint ability, Spencer *et al.* (2005) concluded that the running sprint duration most representative of court- and field sports is 2 – 3 s. However, on electronically braked cycle ergometers that are often used in laboratory settings, flywheel inertia impairs initial acceleration. Hence, 6 s (Balsom *et al.*, 1993; Dawson *et al.*, 1997; Gaitanos, 1993) and 10 s cycle sprints (Pearcey *et al.*, 2015; Stathis *et al.*, 1999) are the shortest sprint durations used in laboratory settings to investigate the physiological and metabolic responses during repeated sprint activities.

Even though repeated, longer duration sprints are not per definition classified as repeated or intermittent sprints in team sport (Collins *et al.*, 2018; Olivier *et al.*, 2011), a few classic metabolism studies have employed repeated 30 s tests (Bogdanis *et al.*, 1996; McCartney *et al.*, 1986; Parolin *et al.*, 1999). Considering that PCr contribution to ATP production is diminished within the first 10 s of a 30 s sprint, these longer duration tests will generally produce larger amounts of lactic acid during the first sprint, due to a greater glycolytic contribution to the energy requirements. Nonetheless, the unique sequence of metabolic events when sprints are repeated was still observed and even magnified in these protocols.

2.3.2 Recovery intervals

During repeated sprints, the duration of the recovery phase between sprints greatly affects the degree of peak power recovery across repeated bouts. Remarkably, power output recovery after a sprint follows a similar time-course as PCr resynthesis (Aaserud *et al.*, 1998; Bogdanis *et al.*, 1995; Casey *et al.*, 1996; Crisafulli *et al.*, 2018). Gaitanos (1993) observed incomplete PCr resynthesis with 30 s rest periods between 6 s sprints. Parolin *et al.* (1999), on the other hand, used 4 min of rest between 30 s maximal sprints and observed that it was sufficient to almost complete PCr resynthesis. Bogdanis *et al.* (1995) compared power output and muscle metabolites between 90 s, 3 min and 6 min recovery, respectively, between two 30 s maximal cycle ergometer sprints. After the second 30 s sprint, PCr levels were depleted to roughly 20% of resting levels and raised to 65% following 90 s recovery and 85% following 6 min recovery. They also determined that the average half-time of the biochemical reaction for PCr resynthesis is approximately 57 s.

In a 15 x 40 m running-based repeated sprint protocol, Balsom *et al.* (1992) compared sprint times with 30, 60 and 120 s rest intervals, respectively. The seven moderately to well-trained men were able to maintain their initial acceleration with the 60 s and 120 s rest intervals, but not with the 30 s rest interval. It could mean that 30 s rest periods are inadequate to replenish sufficient PCr to produce the initial fast burst of ATP production. The 60 s and 120 s rest protocols resulted in similar post-exercise blood lactate concentrations, whereas the 30 s rest protocol resulted in higher blood lactate levels. Overall, the participants' sprint performances declined

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by about 10% from the first to the last sprint during the 30 s rest protocol, but only by 3% and 2% during 60 s and 120 s rest protocols, respectively.

On a metabolic level, these results may indicate that longer recovery durations allow more time for the replenishment of the PCr stores and removal of lactate through aerobic metabolism. Power output during the following sprint is thus compromised if the recovery duration is insufficient (i.e. 30 s).

2.3.3 Number of sprints

In previous research the number of sprint repetitions in test protocols ranged from two to three 30 s WAnT cycle sprint protocols (Bogdanis *et al.*, 1996; Fleming *et al.*, 2003; Parolin *et al.*, 1999) and up to ten or more shorter (< 10 s) running or cycle sprints (Balsom *et al.*, 1993; Balsom *et al.*, 1992; Gaitanos, 1993). With regards to the practical application in sport settings, a time-motion analysis study in elite field-hockey players found that 4 to 7 consecutive running sprint repetitions were the maximum amount of repetitions performed in a game, before longer recovery periods occurred naturally (Spencer *et al.*, 2004).

Balsom *et al.* (1992) investigated the effects of changes in the duration and number of sprint bouts on repeated running sprint performance in seven moderately to well-trained men. Three test protocols were performed on separate days by all participants. Total distance (600 m) and recovery periods (30 s) were kept constant for all three protocols, while sprint distances (15, 30 and 40 m) and number of bouts (40, 20 and 15) varied. The participants were able to maintain their 40 x 15 m sprint time from the first to the last sprint [last vs. first sprint time (s): 2.63 (SEM = 0.04) vs. 2.62 (SEM = 0.02)], whereas significant increases in 40 m sprint times were observed after only three repetitions (p < 0.05).

2.3.4 Type of recovery

The type of recovery between bouts (active vs. passive) is another factor that influences performance during intermittent sprints. Signorile *et al.* (1993) compared the effects of 30 s passive- and active recovery (60 rpm @ 1 kg resistance) during

8 x 6 s cycle ergometer sprints in six power athletes (men aged 18 - 40 years). The participants achieved higher total and peak power outputs ($p < 0.0001$) with the active recovery test protocol. Even though no metabolites were measured during this study, the authors suggested that the better performance in the active recovery trials might have been caused by greater removal of metabolic by-products and utilization of lactate in type I muscle fibres.

However, even though performance has been shown to be better with active recovery protocols (Signorile *et al.*, 1993), studies that investigated exercise metabolism during intermittent sprints, generally employ passive recovery protocols to eliminate any additional metabolic activity during recovery periods (Balsom *et al.*, 1992; Bogdanis *et al.*, 1994, 1996; Parolin *et al.*, 1999).

2.4 Summary of intermittent sprints metabolism literature

It was highlighted so far that the dominant fuel source(s) and biochemical reactions to produce ATP are governed by the exercise intensity requirements of the particular physical activity. A unique substrate metabolism pattern during intermittent sprints emerged from the available literature. This pattern involved down regulation of anaerobic glycolysis during later sprints in order to reduce metabolic by-product production, to sustain homeostasis and decrease the onset of muscle fatigue. Consequently, intermittent sprint metabolism relies greatly on the ATP-PCr and aerobic energy systems for ATP production.

2.5 Metabolic characteristics of a low-carbohydrate, high-fat diet

Investigations into the effects of CHO-restriction on endurance exercise metabolism and performance is a rapidly growing field of research. It has been recognised that CHO-restriction up-regulates fat oxidation rates, and since low-intensity endurance activities are mainly fuelled by fat, this topic developed into an exciting field of research.

The nature of the nutritional stimulus is determined by the macronutrient composition of the diet, as well as the duration of the imposed stimulus (diet). The physiological adaptations that have been reported so far are therefore governed by the level of CHO-restriction, the permitted amount of protein intake and the duration of the dietary intervention.

In light of the merits of LCHF diets in endurance sport, this topic has recently become a rapidly growing field in sport nutrition research. Hence, various definitions emerged for these nuanced endurance specific diet strategies, and non-uniform definitions have consequently caused confusion among researchers. In order to address this issue, several researchers contributed to a published paper, "*Toward a Common Understanding of Diet–Exercise Strategies to Manipulate Fuel Availability for Training and Competition Preparation in Endurance Sport,*" where uniform definitions for various nutritional interventions were formulated (Burke *et al.*, 2018).

According to the proposed definitions, a non-ketogenic low-CHO, high-fat (nk-LCHF) diet allows for 15 – 20% energy from CHO (< 2.5 g/kg/day), 15 – 20% protein and 60 – 65% fat. A ketogenic low-carbohydrate, high-fat (k-LCHF) diet of > 75% fat, 15 - 20% protein and < 50 g/day CHO, however, was a greater stimulus for keto-adaptation (Burke *et al.*, 2018). Some investigators, however, utilized low carbohydrate nutritional interventions with protein contents of > 20% of daily energy intake, which does not fit the definition of LCHF diets. Due to the variety in macronutrient compositions employed in various studies, this thesis will in most cases refer to low-carbohydrate (LC) interventions in general.

Ketone bodies are derivatives of fat metabolism and can be oxidized as a fuel source in all body tissues. During fasting, prolonged exercise, or with a k-LCHF diet,

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production of ketone bodies in the liver is stimulated. This metabolic state is often referred to as nutritional ketosis. However, unlike glucose that can produce ATP through anaerobic or aerobic metabolism, ketone bodies can only be oxidized aerobically. Hence, mitochondria play an important role in energy production when the body is in nutritional ketosis. Besides acting as an alternative fuel source, ketone bodies additionally act as a cell signalling metabolite that induce metabolic adaptations throughout the body (Bough *et al.*, 2006; Newman & Verdin, 2014; Veech, 2004; Veech *et al.*, 2017; Vidali *et al.*, 2015).

In a critical review, Veech *et al.* (2017) proposed that ketone body metabolism in the mitochondria also upregulates the body`s own anti-oxidant system, which protects cells from reactive oxygen species damage. These cell-protecting properties assist in the recovery from high-intensity exercise bouts and prolonged training, which may have positive effects on athletes' performance. Another 8-week, k-LCHF diet study in mice found enhanced maximal exercise capacity and fatigue recovery. These findings could be mechanistically explained by the adaptations in post-exercise biomarkers and anti-oxidation capacity (Huang *et al.*, 2018). It might therefore be inferred that enhanced endogenous anti-oxidant functioning through a LC diet may benefit   sports where competition occurs in a tournament format, or a multi-day stage-race.

2.5.1 Long-term adaptation to a LCHF diet

Traditional sport nutrition guidelines typically recommend a high-carbohydrate approach to exercise and training to ensure high muscle glycogen levels at the onset of exercise. However, studies on longer-term physiological adaptations, as opposed to acute physiological responses, revealed that aerobic adaptations in muscles might be blunted by high-CHO intake (Coffey & Hawley, 2007; Hawley *et al.*, 2006). In a review of the literature, Hawley *et al.* (2006) summarised the influence of nutrition in the modulation of training adaptations. They concluded that supplementation with CHO during exercise allows training at higher intensities and for longer (Hawley *et al.*, 2000), thus possibly providing a greater stimulus for training adaptation. However, they also concluded from the literature on molecular signalling pathways that training with low muscle glycogen is more conducive to

longer term metabolic adaptations (Keller *et al.*, 2001; Lane *et al.*, 2015; Pilegaard *et al.*, 2002).

One of the pioneering studies in the field of LC diets in exercise performance, investigated the time-course of adaptation in metabolism and exercise performance to six weeks of nutritional ketosis (Phinney *et al.*, 1980). Six moderately obese, untrained participants (5 women, 1 man) followed a 6-week protein-supplemented fast (<10 g CHO; 1.2 g of protein / kg ideal body weight) to induce nutritional ketosis. Time to exhaustion (TTE) during treadmill uphill walking with weighted backpacks (to compensate for body mass reductions) were conducted at week one and week six on the protein-supplemented fast. TTE decreased to 80% of baseline walking performance in week one on the nutritional intervention. However, after this initial decline in exercise performance, TTE improved to 155% of baseline levels at the end of the 6-week intervention (Phinney *et al.*, 1980).

This time-course of adaptation in physical performance resembles a typical supercompensation curve, similar to the reported response of performance following the stimulus to periodized training. The initial decrease in performance after the first week (with reference to Phinney *et al.,* 1980), indicated that nutritional ketosis induced a metabolic stimulus and was still challenging the body to adapt. The 55% increase in moderate exercise capacity after six weeks of nutritional ketosis indicated a robust "supercompensated" walking performance. It should be noted that the untrained status of participants in this investigation probably explains the large magnitude of change in endurance capacity. Even so, without a training intervention this study demonstrated the potential of nutritional ketosis to stimulate endurance performance improvements (Phinney *et al.*, 1980). The supercompensation-curved adaptation observed in this study (Phinney *et al.*, 1980), also emphasized that prolonged periods of ketosis are needed to stimulate the physiological adaptations in fuel metabolism.

More recent research on the molecular level reported an up-regulation in gene transcription involved in metabolic adaptation in response to low muscle glycogen and/or nutritional ketosis (Febbraio *et al.*, 2002; Keller *et al.*, 2001; Lane *et al.*, 2015; Newman & Verdin, 2014; Pilegaard *et al.*, 2002; Veech *et al.*, 2017). These authors also commented that the time-course of molecular level adaptations, through altered gene transcription and cell signalling, requires a longer period to alter phenotypic

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expression. Performance effects will therefore only be evident after sufficient adaptation time was employed. Consequently, the recommendations of acute pre-, during- and post-workout fuelling studies should not be generalised and recommended for athletes with the expectation that it will stimulate long-term training adaptations.

2.6 Adaptations in energy systems with carbohydrate restriction

One of the factors leading to improved sport performance is improvements in the efficiency of the energy systems that predominantly fuels a particular activity. Many of the molecular adaptations in response to a LCHF diet may involve changes in specific metabolic pathways.

2.6.1 Adaptations in the aerobic energy system

Since aerobic metabolism takes place in the mitochondria of the cell, mitochondrial biogenesis is an important exercise training adaptation that accounts for improved endurance performance. Ketone bodies, as a cell signalling metabolite, are also associated with mitochondrial biogenesis and enhanced mitochondrial enzyme activity (Cheng *et al.*, 1997; Hyatt *et al.*, 2016). Due to the invasive nature of mitochondrial analysis, many of these studies were performed in animals. For instance, Cheng *et al.* (1997) compared the effects of a 6-week high-fat diet (76% of daily energy intake from fat) combined with voluntary exercise to an exercise only intervention on rat skeletal muscle mitochondrial enzyme changes. They observed larger changes in mitochondrial density, β-oxidation capacity, and mitochondrial enzyme activity in the 6-week high-fat group, suggesting that exercise alone is not an optimal stimulus to maximize training effects.

In one of the first human studies where the effects of a k-LCHF diet was investigated (< 20 g CHO / day, 1.7 g/kg body weight per day protein and the remainder of energy intake from fat), five well-trained male cyclists underwent muscle biopsies before and after a 4-week intervention (Fisher *et al.*, 1983). Following the k-LCHF

adaptation period, the activity of Carnitine palmityl transferase (CPT) (a rate limiting mitochondrial fatty acid transporter) increased significantly by 35%, while no significant change was observed in the activity of malate dehydrogenase (Fisher *et al.*, 1983).

In a randomized cross-over study involving eleven male duathletes, however, the authors reported no difference in muscle mitochondrial density after the 5-week CHO-restricted (53% fat; 32% CHO; 15% protein) and high-CHO, low-fat period (17% fat; 68% CHO; 15% protein) (Vogt *et al.*, 2003). However, although fat intake was slightly higher during the high-fat phase of this intervention, 53% is still low, and the 32% CHO intake was too high to qualify as a LCHF diet (Burke *et al.*, 2018). Thus, it seems that changes in mitochondrial metabolism requires a certain degree of CHO-restriction, as well as a sufficiently high fat intake. Another methodological consideration is that no washout period was included in this study design. Since mitochondrial adaptations are induced by altered gene expression (Febbraio *et al.*, 2002; Keller *et al.*, 2001; Lane *et al.*, 2015; Newman & Verdin, 2014; Pilegaard *et al.*, 2002; Veech *et al.*, 2017), these adaptations take longer periods to reverse.

It is therefore evident that some molecular level evidence exists as mechanistic explanation for aerobic adaptations on LCHF diets.

2.6.2 Adaptations in the anaerobic glycolytic energy system

Anaerobic glycolysis is the energy system where glucose is catabolised for ATP production during high-intensity activities. Consequently, this energy system would be directly influenced by dietary restriction of CHO. Various methods to quantify glycolysis during exercise has been previously employed in LC studies. These include indirect measurement of CHO oxidation and respiratory quotient (RQ), as well as direct measurement of muscle glycogen utilization or glycolytic enzyme activity through muscle biopsies.

During intermittent sprint metabolism, where reduced glycolysis improves efficiency and sustainability of sprint power output, irrespective of nutritional status (Bogdanis *et al.*, 1996; Gaitanos, 1993; McCartney *et al.*, 1986; Parolin *et al.*, 1999; Trump *et al.*, 1996), glycogen sparing and sustained low levels of muscle glycogen may

hypothetically hold exercise performance benefits. However, present findings on the effects of LCHF adaptation on muscle glycogen levels and -utilization are still inconclusive.

Most researchers report significant reductions in muscle glycogen at the onset of a LCHF intervention (Fisher *et al.*, 1983; Lambert *et al.*, 1994; Phinney *et al.*, 1983; Phinney *et al.*, 1980; Vogt *et al.*, 2003). However, in some cases, muscle glycogen gradually recovered following the initial drop (Phinney *et al.*, 1980; Volek *et al.*, 2016). For instance, Volek *et al.*(2016) observed no differences in resting muscle glycogen levels between ultra-endurance athletes on habitually high-CHO and those on low-CHO diets for an average of 20 months. The restoration of "normal" muscle glycogen levels may thus be a long-term adaptation to a LCHF diet.

Opposed to these restored muscle glycogen levels, however, Webster *et al.* (2016) found that the rates of gluconeogenesis were not adapted to compensate for long-term restricted CHO intake. They compared seven well-trained cyclists on a long-term (8 – 24 months) LCHF habitual diet (72% fat, 7% CHO, 21% protein) to seven cyclists on a habitual mixed diet (33% fat, 51% CHO, 16% protein). It is therefore unclear whether long-term LCHF adaptations result in sufficient up-regulation of gluconeogenesis to compensate for low CHO intake.

In an investigation mentioned earlier, Phinney *et al.* (1980) observed a phenomenon called glycogen-sparing, where muscle glycogen remained unchanged from pre- to post-exercise due to reduced glycogenolysis and glycolysis in week six of their nutritional intervention in untrained participants. Similar glycogen-sparing responses to a LCHF diet became a regular observation in trained populations (Burke *et al.*, 2002; Fisher *et al.*, 1983; Phinney *et al.*, 1983; Stellingwerff, 2005; Yeo *et al.*, 2011). Fisher *et al.* (1983) reported a 46% reduction in hexokinase glycolytic enzyme activity and proposed this as a possible mechanism for this phenomenon. However, glycogen-sparing is not a consistent finding in the literature. Some studies also observed similar rates of glycogen breakdown and utilization following short- (2-week) (Lambert *et al.*, 1994) and long-term (~ 20 months) LCHF diets (Volek *et al.*, 2016). Therefore, the glycogenolysis and glycolysis responses to long term keto-adaptation is still inconclusive.

The respiratory quotient (RQ) is a popular, indirect measurement of fuel utilization that indicates the relative contributions of CHO and fat metabolism based on the ratio of CO_2 produced and O_2 uptake. Lower RQ values reflect greater contributions of fat metabolism and higher values reflect greater reliance on CHO metabolism. Since fat is the main macronutrient in LC diets, lower RQ values is a consistent finding in the literature (Cipryan *et al.*, 2018; Dostal *et al.*, 2019; Fisher *et al.*, 1983; Mcswiney *et al.*, 2017; Phinney *et al.*, 1983; Rubini *et al.*, 2015; Vogt *et al.*, 2003; Waldman *et al.*, 2018; Zajac *et al.*, 2014). Reported values ranged from 0.76 at baseline to 0.66 in five obese untrained participants after six weeks (Phinney *et al.*, 1980), to larger drops in RQ (-1.5 ± 0.1) in a moderately trained, LCHF group during high-intensity 30 s shuttle runs after four weeks (Cipryan *et al.*, 2018).

2.6.3 Anaerobic glycolysis and the lactate response with carbohydrate restriction

On a non-CHO restricted standard diet and during intermittent sprint exercise, anaerobic glycolysis is downregulated, resulting in lowered lactate production to maintain optimal efficiency of ATP production (Bogdanis *et al.*, 1996; Gaitanos, 1993; McCartney *et al.*, 1986; Parolin *et al.*, 1999). An understanding of the lactate response to a LCHF approach during exercise would thus be important. Although it makes intuitive sense that lower muscle glycogen stores, commonly observed during CHO restriction, would reduce anaerobic glycolysis and lactate production, evidence is still inconclusive on the lactate responses following LC interventions.

A nutrition protocol that involved a 5-day fat adaptation period followed by 1-day CHO restoration before an endurance event was followed by endurance trained male cyclists (Burke *et al.*, 2002; Stellingwerff, 2005; Yeo *et al.*, 2011). During 20 min cycling at 70% VO_2max, researchers found that decreased pyruvate dehydrogenase (PDH) activity was sustained, even after muscle glycogen stores were refilled during the one-day CHO consumption (Stellingwerff, 2005). PDH converts pyruvate to acetyl-CoA for aerobic oxidation, thus inhibiting conversion to lactate. They also did not find excessive lactate accumulation during exercise, since lower rates of glycogenolysis was also evident. However, this sustained glycogen-

sparing with CHO restoration contradicts the findings of other studies (Carr *et al.*, 2018).

Carr *et al.* (2018) compared the effects of a 21-day k-LCHF diet (< 50 g CHO per day, 75 – 80% fat, 15 – 20% protein) and a high CHO control diet (~8 g CHO per kg body mass for all training sessions) to a periodized CHO diet (same energy and macronutrient composition as the high-CHO diet, but alternated to provide high-CHO during some training sessions and low-CHO during other), in 24 non-randomized elite-level race walkers (17 men and 7 women). The authors reported significantly higher lactate levels in the periodized CHO group compared to the LCHF group. These significantly higher lactate levels could possibly be attributed to sustained down regulation of PDH (Stellingwerff, 2005), while during higher intensity exercise in the presence of restored muscle glycogen stores, glycogenolysis and anaerobic glycolysis might again be up-regulated.

An early review of the literature on the short-term (≤ 5 days) effects of glycogen depleting protocols without CHO restoration, on high-intensity exercise, concluded that these interventions consistently resulted in lower blood lactate levels (Maughan *et al.*, 1997). For instance, significantly lower lactate levels were observed after a 30 s WAnT sprint test following a 3-day LC intervention (5% CHO, 50% fat, 45% protein), compared to a mixed diet condition (50% CHO, 30% fat, 20% protein) in a randomized cross-over design in eight healthy men (Langfort *et al.*, 1997).

Findings on the lactate responses during endurance exercise after LCHF adaptation periods longer than two weeks are still inconclusive. Some studies observed lower during and / or post-exercise lactate values in highly trained populations (Lambert *et al.*, 1994; Vogt *et al.*, 2003; Zajac *et al.*, 2014), while other researchers reported no change in lactate levels in trained (Burke *et al.*, 2017; McSwiney *et al.*, 2017) and untrained, obese participants (Phinney *et al.*, 1980).

Notably, the effect of diet on the responses to interval exercise or repeated sprints, and the complexity of lactate kinetics during these exercise modalities have not been thoroughly studied.

2.7 The effect of low-carbohydrate interventions on sport performance

Studies on the metabolic effects of, and adaptations to a LC diet suggest it may be a useful nutritional strategy to stimulate peak performance. Nonetheless, investigations into the diet`s effects on sport performance has only recently gained real interest in the research community.

2.7.1 Endurance performance studies

Of the investigations on the effects of LC and k-LCHF on endurance performance that will be discussed here, ten studies were performed in highly trained endurance populations (Burke *et al.*, 2017; Carr *et al.*, 2018; Fisher *et al.*, 1983; Lambert *et al.*, 1994; McSwiney *et al.*, 2017; Phinney *et al.*, 1983; Vogt *et al.*, 2003; Volek *et al.*, 2016; Webster *et al.*, 2016; Zajac *et al.*, 2014), while five studies included recreationally active and moderately trained populations (Dostal *et al.*, 2019; Fleming *et al.*, 2003; Heatherly *et al.*, 2018; Prins *et al.*, 2019; Urbain *et al.*, 2017). It can be assumed that aerobic metabolic adaptations in response to training already occurred in well-trained individuals, thus it is expected that the effects of LC interventions would be different between trained and untrained populations. Minimal studies were performed in resistance trained athletes (Waldman *et al.*, 2018), or untrained, obese populations (Phinney *et al.*, 1980).

In most cases, recreationally active (Fleming *et al.*, 2003) and trained cyclists (Fisher *et al.*, 1983; Lambert *et al.*, 1994; McSwiney *et al.*, 2017; Phinney *et al.*, 1983; Vogt *et al.*, 2003; Webster *et al.*, 2016; Zajac *et al.*, 2014) are tested on cycle ergometers during laboratory assessments. Some researchers have also utilised the treadmill (Cipryan *et al.*, 2018; Dostal *et al.*, 2019; Prins *et al.*, 2019; Volek *et al.*, 2016; Waldman *et al.*, 2018) and outdoor running test protocols (Heatherly *et al.*, 2018; Vogt *et al.*, 2003). An uphill walking test protocol on a treadmill was utilized in a study involving untrained, obese participants (Phinney *et al.*, 1980), while a 10 km race walk (Burke *et al.*, 2017) and graded treadmill walking tests (Burke *et al.*, 2017; Carr *et al.*, 2018) were performed in elite race-walkers.

In response to LCHF interventions, metabolic adaptations occur at molecular level and through altered gene expression (Febbraio *et al.*, 2002; Keller *et al.*, 2001; Lane *et al.*, 2015; Newman & Verdin, 2014; Pilegaard *et al.*, 2002; Veech *et al.*, 2017). Changes in phenotypic expression, as reflected in shifts in performance, would therefore only be evident after the stimulus was applied for a sufficient period. It has also been observed that switching to a LC diet initially causes a decline in endurance capacity, but that supercompensation takes place once the metabolic adaptations have been made (Phinney *et al.*, 1980).

In this regard, many previous studies may not have been of sufficient duration to allow adequate fat adaptation to take place. For example, five studies employed interventions as short as 2 – 3 weeks (Burke *et al.*, 2017; Carr *et al.*, 2018; Heatherly *et al.*, 2018; Lambert *et al.*, 1994; Waldman *et al.*, 2018), while 4 - 5 week interventions were employed in five studies (Cipryan *et al.*, 2018; Fisher *et al.*, 1983; Phinney *et al.*, 1983; Vogt *et al.*, 2003; Zajac *et al.*, 2014). Four studies involved longer, 6-week interventions (Fleming *et al.*, 2003; Phinney *et al.*, 1980; Prins *et al.*, 2019; Urbain *et al.*, 2017), while only two studies lasted 12 weeks (Dostal *et al.*, 2019; McSwiney *et al.*, 2017). Furthermore, two cross-sectional investigations compared well-trained endurance athletes following long term, habitual k-LCHF diets to habitual high-CHO athletes for durations of 8 – 24 months (Webster *et al.*, 2016) and 9 - 36 months (Volek *et al.*, 2016). Due to the descriptive nature of these cross-sectional studies, the researchers focused mostly on metabolic characteristics rather than athletic performance.

Another consideration in the methodology of cross-over studies is whether an appropriate wash-out period was included. Regarding this factor, it is questionable whether randomized cross-over designs are suitable for these investigations. One study included a wash-out period of 2 weeks (Prins *et al.*, 2019), while two studies had no washout period (Vogt *et al.*, 2003; Zajac *et al.*, 2014). To my knowledge, it is not known how long the reversal of gene expression in response to a LC diet will take and whether two weeks is a sufficient wash-out duration. In light of the reported performance outcomes from these studies, one should be cognizant if this methodological issue.

Burke *et al.* (2018) defined a ketogenic low-carbohydrate, high-fat (k-LCHF) diet as > 75% fat, 10 - 15% protein and < 50 g/day or < 5% of daily energy from CHO,

while a non-ketogenic low-CHO, high-fat (nk-LCHF) diet allows for 15 – 20% energy from CHO (< 2.5 g/kg/day), 15 – 20% protein and 60 – 65% fat. Only six of the studies on endurance performance met the k-LCHF criteria (Burke *et al.*, 2017; Carr *et al.*, 2018; Fisher *et al.*, 1983; McSwiney *et al.*, 2017; Phinney *et al.*, 1980, 1983). Although it is desirable to consume sufficient amounts of fat and maintain protein intake below 20% to stimulate optimal metabolic adaptations, it is evident that many researchers only emphasised CHO restriction. Most interventions therefore employed sufficient CHO restriction and higher protein intake, yet < 60% of daily energy intake was still derived from fat (Cipryan *et al.*, 2018; Dostal *et al.*, 2019; Fleming *et al.*, 2003; Heatherly *et al.*, 2018; Lambert *et al.*, 1994; Prins *et al.*, 2019; Urbain *et al.*, 2017; Webster *et al.*, 2016). Some researchers, however, utilized interventions where the recommended CHO intake was exceeded, or were at the higher range of the nk-LCHF guidelines. Examples are 32% (Vogt *et al.*, 2003), 15% (Zajac *et al.*, 2014) and 14% (94 g per day) (Waldman *et al.*, 2018).

A likely, but over-simplistic rationale for studying the effect of CHO restriction on endurance performance is that fat is already the main fuel for exercise at low intensities. Nonetheless, at moderate to high exercise intensities, such as during competition, athletes rely more on CHO. However, muscle glycogen stores are limited, making CHO supplementation during high intensity exercise and competition necessary. Although one of the hallmarks of endurance training adaptations is a shift towards greater reliance on fat as fuel, evidence also exist that athletes become fat-adapted via training adaptations and a LC diet, their maximal fat oxidation rates may increase to such an extent that even higher exercise intensities are fuel by fat. This adaptation might therefore minimize the need to supplement with CHO during exercise (Volek *et al.*, 2016). However, evidence that this adaptation translates to improved performance in the field, is equivocal. Certainly, there are anecdotal evidence of world-class marathon, ultra-marathon and Ironman athletes who compete at international level and who follow a LCHF diet and only consume water during competition.

Even though metabolic changes and favourable shifts in fuel metabolism was observed in previous studies, findings on the effects on performance are equivocal. This can likely be attributed to the various methodological issues raised above. Moreover, although relative shifts in fuel metabolism towards greater reliance on fat

oxidation might be evident after relatively short LC interventions, absolute up-regulation in the ATP production rate from aerobic fat metabolism (β-oxidation and electron transport chain) will need prolonged stimulation from nutritional ketosis. Hence, performance improvements might only be evident after sufficient adaptation periods of the nutritional ketosis stimulus was applied.

In this respect, only two studies reported significant improvements in endurance performance on LC interventions (Lambert *et al.*, 1994; Phinney *et al.*, 1980). Lambert *et al.* (1994) found a significant improvement in a time to exhaustion (TTE) cycle test at 60% VO_2max (LC: 79.7 ± 7.6 min; High-CHO: 42.5 ± 6.8 min; p < 0.01) in five male cyclists after a very short (two-week) LC intervention. A two-week LC intervention is generally regarded as insufficient time for complete fat adaptation. However, a unique characteristic of this study was the packed test battery, namely:

1. Participants started the session with a force-velocity test; 5 x 5 s maximal cycling cadence bouts @ varying resistances, with 1 min recovery between bouts.

2. After 10 min recovery, a 30 s WAnT cycle sprint was performed.

3. 30 min after the WAnT sprint test, participants completed a high-intensity time to exhaustion ride at 90% VO_2max.

4. The moderate intensity endurance ride @ 60% of VO_2max, where the significant performance improvement was reported, was only performed 20 min following the high-intensity ride.

No significant performance changes occurred in the tests, other than the moderate intensity TTE test. Yet, a possible overlooked reason for the marked improvement in the moderate intensity, endurance ride, performed shortly after these strenuous exercise tests, could be the cyclists' enhanced ability to recover from the preceding high-intensity exercise tests when they were following the LC diet. Enhanced functioning of the endogenous mitochondrial anti-oxidant capacity has been proposed following LCHF diets (Huang *et al.*, 2018; Veech *et al.*, 2017). Therefore, this physiological adaptation might provide a plausible mechanistic explanation for the likely enhanced recovery rates after the strenuous exercise tests in this investigation by Lambert *et al.* (1994).

The study by Phinney *et al.* (1980), where a 55% increase in treadmill uphill-walking was observed, was unique in the fact that the study population consisted of untrained, obese participants. In untrained participants, the margin for physiological adaptations is greater than in trained populations, which probably explain the large improvements in endurance capacity of the participants. Furthermore, the intervention in this study was six weeks long and participants consumed < 10 g CHO per day. These factors may also have contributed to the significant improvement in walking performance.

Smaller improvements in endurance capacity were observed in the studies of Dostal *et al. (*2019) and McSwiney *et al.* (2017). Both studies included a 12-week adaptation period and good levels of CHO restriction (< 50 g CHO/day and 6% CHO intake, respectively). Dostal *et al.* (2019) reported a *large* improvement in time to exhaustion during a graded exercise test in the LCHF group (ES ± 95% CI: 1.03 ± 0.70, p = 0.005) compared to a *moderate* improvement in the control group (ES ± 95 CI: 0.86 ± 0.70, p = 0.018). McSwiney *et al.* (2017) reported a mean of 2.54 min greater improvement in a 100 km time trial performance in a k-LCHF-training intervention group compared to their training-only group. Although the difference between the groups was not statistically significant (p = 0.057; ES = 0.196), most competitive cyclists would regard a ~ 3 min change in performance as practically meaningful. Furthermore, the study involved well-trained cyclists where smaller improvements in performance in response to any kind of intervention can be expected.

Most investigations reported that participants maintained their performance following an LCHF intervention (Cipryan *et al.,* 2018; Fisher *et al.*, 1983; Heatherly *et al.*, 2018; Phinney *et al.*, 1983; Prins *et al.*, 2019; Vogt *et al.*, 2003; Waldman *et al.*, 2018). Although unchanged performance is not a negative research outcome, some methodological issues may have affected the outcomes of these studies. For instance, two studies employed short (2 – 3 weeks) interventions that may not have allowed enough time for keto-adaptation (Heatherly *et al.*, 2018; Waldman *et al.*, 2018). Two studies implemented randomized cross-over designs where either no (Vogt *et al.*, 2003), or a short (2 weeks) wash-out period was incorporated (Prins *et al.*, 2019). These limited wash-out periods could have been insufficient to reverse the adaptations in participants who were first assigned to the LC phase. Lastly, two

of these investigations implemented nutritional interventions where CHO restriction was not sufficient (32% of daily energy (Vogt *et al.*, 2003) and 14% (94 g per day) (Waldman *et al.*, 2018).

Lastly, three studies reported declines in endurance performance following CHO restriction (Burke *et al.*, 2017; Fleming *et al.*, 2003; Zajac *et al.*, 2014). Once again, the influence of some methodological considerations should not be overlooked. Since initial performance declines upon initiation of a LCHF diet, associated with "fatigue" on the supercompensation curve (Phinney *et al.*, 1980), performance results after a short (3 weeks) k-LCHF intervention (Burke *et al.,* 2017) may well reflect this initial phase. This investigation also involved elite-level race walkers, where lower margins of performance improvements is expected.

The investigation by Zajac *et al.* (2014) included no wash-out period. Thus, it is possible that there was a "carry-over" effect in participants who were first assigned to the LC phase, which could distort the final outcomes. Also, their nutritional intervention consisted of 15% of daily energy derived from CHO. The low blood ketone levels (0.15 mmol.L^{-1}) may further indicate that this nutritional intervention posed an insufficient stimulus for endurance performance adaptations.

Fleming *et al.* (2003) reported that 45 min work output was significantly decreased (~ 18%) in their LC group after six weeks on CHO restriction. However, the participants' protein intake increased significantly during the LC intervention (17 ± 4% to 30 ± 5%), which may have blunted their fat adaptations. Low blood ketone levels of 0.29 ± 0.09 mmol.L^{-1} also indicate that this nutritional intervention was not a sufficient stimulus for metabolic adaptations (Fleming *et al.*, 2003).

It is clear that the effect of LCHF diets on endurance performance is still equivocal. Differences in adaptation periods, macronutrient composition, training status of participants and the presence or absence of an exercise intervention also makes it difficult to draw comparisons between studies.

2.7.2 High intensity performance studies

Although most longer-term LC studies investigating sport performance focused on low-intensity, endurance type exercise, three studies included high-intensity

performance tests as a secondary aim (Fleming *et al.*, 2003; Lambert *et al.*, 1994; McSwiney *et al.*, 2017). A further five studies investigated strength or high-intensity performance as a main objective (Cipryan *et al.*, 2018; Dostal *et al.*, 2019; Greene *et al.*, 2018; Kephart *et al.*, 2018; Paoli *et al.*, 2012). Study populations included recreationally active individuals (Dostal *et al.*, 2019; Fleming *et al.*, 2003; Langfort *et al.*, 1997), recreational CrossFit trainees (Kephart *et al.*, 2018), endurance trained male cyclists (Lambert *et al.*, 1994; McSwiney *et al.*, 2017), elite male gymnasts (Paoli *et al.*, 2012) and intermediate to elite level Olympic lifting- and power-lifting men and women (Greene *et al.*, 2018).

High-intensity tests varied from an intermittent 30 s shuttle run test (Dostal *et al.*, 2019) and repeated 30 s WAnT sprints (Fleming *et al.*, 2003) to a continuous 400 m sprint (Kephart *et al.*, 2018). A single 30 s WAnT cycle ergometer sprint (Lambert *et al.*, 1994; Langfort *et al.*, 1997) and a short 6 s cycle sprint (McSwiney *et al.*, 2017) were employed in other studies. Two studies included sport-specific performance tests, e.g. gymnastics strength tests for gymnasts (Paoli *et al.*, 2012), competition lifts (jerk, clean and snatch) in Olympic-lifting athletes and bench press, squat, and deadlift in power-lifting athletes (Greene *et al.*, 2018).

The nutritional interventions varied in duration. Although chronic adaptations would not occur on a 3-day LC intervention (Langfort *et al.*, 1997), the acute responses of this short intervention will be discussed in this section. Other studies employed 2-week (Lambert *et al.*, 1994), 4-week (Paoli *et al.*, 2012) and 6-week (Fleming *et al.*, 2003) interventions, while 12-week interventions were implemented in four studies (Dostal *et al.*, 2019; Greene *et al.*, 2018; Kephart *et al.*, 2018; McSwiney *et al.*, 2017).

It was evident that the researchers focussed mainly on the restriction of CHO in their interventions. All the interventions restricted CHO to < 50 g per day, or < 10% of daily energy intake. In some cases, protein intake was not limited, and instructions merely stated "ad libitum protein and fat" consumption. While the desired protein intake for a k-LCHF intervention consists of 15 - 20% of daily energy intake (Burke *et al.*, 2018), some of these studies reported protein intakes up to 40.7% (Paoli *et al.*, 2012) and 45% (Langfort *et al.*, 1997) of daily energy intake. Paoli *et al.* (2012) also prescribed the intake of herbal extracts together with the LC intervention and their findings can therefore not be attributed to the CHO restriction alone. The 47%

protein intake on the LC intervention also does not typically qualify as a LCHF intervention.

Kephart *et al.* (2018) reported that only four of their seven participants completed the required food logs and therefore the reported CHO restriction of 15 ± 3 g per day may not be completely accurate. The protein to fat ratios of the intervention in other studies were closer to the LCHF guidelines, namely 31% protein, 61% fat (Fleming *et al.*, 2003) and 33% protein, 67% fat (Lambert *et al.*, 1994). However, only the nutritional intervention of McSwiney *et al.* (2017) (77% fat, 17% protein, 6% CHO) can be classified as a true k-LCHF intervention (Burke *et al.*, 2018).

Power-to-weight ratio is a performance indicator in most weight-bearing sports. Many power and short-duration high intensity sports compete in weight categories, where body mass reductions with uncompromised performance might pose a competitive advantage. Although the focus of this study was   not on weight loss, the maintenance of strength and power, in combination with significant body mass reductions (Greene *et al.*, 2018; Kephart *et al.*, 2018; McSwiney *et al.*, 2017; Paoli *et al.*, 2012) may also contribute to performance in intermittent sprint exercise.

All three studies that examined the effects of LC interventions on strength performance found that strength was maintained (Greene *et al.*, 2018; Kephart *et al.*, 2018; Paoli *et al.*, 2012), after intervention periods from 4 to 12 weeks. Similar to strength performance, power performance was also maintained after 12-week LC interventions (Greene *et al.*, 2018; Kephart *et al.*, 2018).

McSwiney *et al.* (2017) reported a significantly greater improvement ($p = 0.025$) in 6 s cycle sprint power output in a k-LCHF-training intervention group (0,8 Watts/kg) compared with a training-only intervention group (-0.1 Watts/kg). This performance improvement followed a 12-week k-LCHF diet intervention (77% fat, 6% CHO, 17% protein). This finding confirms the importance of a sufficiently long fat adaptation period, as well as the importance of implementing a ketogenic (i.e. very low CHO) intervention.

During the first few seconds (≤ 10 s) of maximal intensity exercise, as observed with short sprint and strength tests, the ATP-PCr energy system produces the majority of ATP from stored PCr in the muscle. The existing study findings are encouraging and suggest that the effects of k-LCHF interventions on the ATP-PCr energy system

should be further investigated and quantitatively measured. In this regard, based on their observed performance improvements in short-sprint exercise, McSwiney *et al.* (2017) suggested that ketogenic diets have no detrimental effect on the ATP-PCr energy system.

Conversely, the continuous 30 s duration of a WAnT test has been reported to require a greater contribution of the anaerobic glycolytic pathway for ATP production (Beneke *et al.*, 2002; Julio *et al.*, 2019). In this regard, a few studies used the 30 s Wingate test (WAnT) to study the effects of a LC diet on anaerobic exercise performance. In accordance with the energy continuum model, peak power output (PPO), reached within the first 5 s of a 30 s WAnT, reflects the contribution of the ATP-PCr energy system to ATP production, while mean power output (MPO) over the 30 s reflects the utilization of the glycolytic energy system (Smith & Hill, 1991). Thus, from this standardized exercise test, it is possible to indirectly estimate the contributions of these energy systems to ATP production.

In an early study by Langfort *et al.* (1997) the acute effects of a 3-day LC diet (5% CHO, 50% fat, 45% protein) on 30 s WAnT performance was compared to a mixed diet (50% CHO, 30% fat, 20% protein) in eight healthy men. In this investigation, MPO was significantly lower after the 3-day LC compared to the mixed diet (533 W vs. 581 W; $p < 0.05$). However, PPO was unchanged despite the lower mean power output (Langfort *et al.*, 1997). These results suggest that the capacity of the ATP-PCr system was uncompromised (maintained PPO), while the MPO reductions were consequently the result of a decline in ATP production from the anaerobic glycolytic metabolic system. In a similar study Lambert *et al.* (1994) reported a higher PPO after a 2-week LCHF intervention, compared to a high-CHO diet (mean [SEM]; 862 [94] W vs. 804 [65] W) in five well- trained male cyclists. These findings are in line with the suggestion by McSwiney *et al.* (2017) that CHO restriction does not impair the ATP-PCr energy pathway.

Fleming *et al.* (2003) employed a 2 x 30 s WAnT with 2 min rest in recreationally active men. In contrast to previous findings (Lambert *et al.*, 1994; Langfort *et al.*, 1997), PPO decreased in the first WAnT bout from 11.2 ± 0.5 W/kg to 10.2 ± 0.5 W/kg after six weeks in the LC diet group (61% fat; 8% CHO, 31% protein). However, a finding not emphasized by the authors was that PPO during bout two

was the same, or slightly improved (9.4 ± 0.5 W/kg to 9.5 ± 0.4 W/kg) (Fleming *et al.*, 2003).

Findings from previous studies where no nutritional intervention was employed, using repeated 30 s WAnT tests to quantify energy metabolism during these activities, consistently reported reductions in glycolytic contributions during later sprints (Bogdanis *et al.*, 1996; McCartney *et al.*, 1986; Parolin *et al.*, 1999). The possible inhibition of glycolysis through a LCHF dietary intervention might therefore lead to the   maintenance of power output during later sprints, as observed by Fleming *et al.*, (2003).

Outcomes of other longer duration high intensity exercise studies, e.g. continuous 400 m sprint (Kephart *et al.*, 2018) and an intermittent 30 s shuttle run test (Dostal *et al.*, 2019), following long, 12-week LC interventions, also revealed some encouraging results. Time to exhaustion in the 30 s shuttle run test improved slightly more in the LCHF-training intervention group compared to the training only high-CHO group (ES ± 95% CI: 1.93 ± 0.97 vs 1.50 ± 0.84). Although a continuous 400m track sprint would theoretically rely greatly on anaerobic glycolysis for ATP production, performance in this test was unchanged in CrossFit trainees after the 12-week LCHF intervention (Kephart *et al.*, 2018). These findings suggest that exercise durations that would generally be highly dependent on anaerobic glycolysis, may undergo positive adaptations if sufficient adaptation time is allowed.

2.8 Summary of the literature

a) Metabolic responses during intermittent sprints exercise:

- ATP-PCr and aerobic metabolism are the main sources of ATP production during intermittent sprint exercise, while anaerobic glycolysis is down-regulated.

- During recovery periods between sprints, rephosporylation of creatine occurs through the CK_{mito} enzyme action in the mitochondria.

- Anaerobic glycolysis is allosterically inhibited by the by-product of this energy pathway (i.e. lactate) during later sprints of repeated bouts through negative feedback.

- Anaerobic glycolysis inhibition during later sprints results in less lactate production, which may counter the onset of muscle fatigue.

b) Metabolic adaptations in response to a LCHF diet:

- A shift in fuel metabolism, namely less reliance on glycolytic CHO metabolism and greater aerobic fat oxidation rates, is evident from lower RQ and blood lactate levels during exercise with CHO restriction.

- Adaptations in aerobic metabolism are partly induced by altered gene transcription for mitochondrial biogenesis and -enzyme adaptations.

- Mitochondrial adaptations, at the level of gene transcription, may need long (> 6 weeks) LCHF adaptation periods.

c) Effect of CHO restriction on sport performance:

- The equivocal effects of CHO restriction on endurance performance can be attributed to short intervention periods, lack of wash-out periods, nutritional interventions with insufficient fat intakes or CHO restriction. Furthermore, most studies were performed in well-trained populations where margins for improvements are less, and where longer interventions are possibly necessary.

- Short (up to 6 s) maximal intensity exercise performance (where the ATP-PCr energy system would be the main energy source) was unchanged or improved following LC interventions.

- Peak power output achieved in the first 10 s (indicative of the ATP-PCr energy system) during 30 s WAnT sprint tests was unchanged or improved, while mean power output (indicative of anaerobic glycolysis contribution) was lower following short (3-day and 2-week) LC interventions.

- Longer high-intensity exercise performance, where anaerobic glycolysis typically contributes largely to ATP production (shuttle run and 400 m sprint), were slightly improved or maintained when long LCHF adaptation periods (12-weeks) were employed.

From the literature, it is evident that intermittent sprints exercise has a unique fuel utilization pattern, where anaerobic energy production from CHO as fuel is down regulated to sustain homeostasis and reduce fatigue during later sprints. On a LC nutritional intervention, fuel utilization also shifts towards greater fat oxidation with reduced reliance on CHO as fuel. It therefore seems like the metabolic state on a LC intervention might theoretically be complementary to intermittent sprint metabolism. However, to my knowledge, no previous investigation examined the effects of a LC diet on intermittent sprints performance or metabolism.

Quantification and measurement of the aerobic- and anaerobic glycolytic energy systems during exercise on LC interventions have also been commonly performed. However, to my knowledge no previous study has quantified changes in the ATP-PCr energy system on this nutritional intervention.

Chapter 3
Methodology

3.1 Study design

The study protocol was approved by the Health Research Ethics Committee (HREC) at Stellenbosch University (S19/05/100). All testing and laboratory procedures were performed in accordance to the Declaration of Helsinki (World Medical Association, 2013).

A time-series, single group, quasi-experimental design was employed, where each participant acted as his/her own control. It was assumed that the changes in intermittent sprint exercise performance and metabolism will reflect the degree to which a "real life" dietary intervention affects an individual's responses. Time-series or other within-participant analysis study designs are commonly employed in LC studies investigating sport performance (Fisher *et al.*, 1983; Heatherly *et al.*, 2018; Phinney *et al.*, 1983, 1980; Urbain *et al.*, 2017; Waldman *et al.*, 2018).

Participants completed two baseline / control intermittent sprints testing sessions two weeks apart, while on their habitual carbohydrate (CHO) diet (HD1 and HD2). The mean of these results was considered the baseline values. After the second HD test, participants implemented a 6-week low-carbohydrate (LC) intervention and repeated the intermittent sprints tests at 2-weekly intervals over the LC six weeks.

A non-probability, self-selection sampling approach was used. This sampling method is commonly used in dietary intervention studies, including LC intervention studies, in order to promote dietary adherence (Burke *et al.*, 2017; McSwiney *et al.*, 2017; Carr *et al.*, 2018). Participants visited the Sport Physiology Laboratory in the Department of Sport Science on 11 occasions, over a maximum of 10 weeks (figure 3.1).

3.1.1 Participants

A convenience sample of 23 healthy, active men and women between the ages of 18 and 40 years was recruited for the study. The required sample size was

calculated with G*Power 3 (Faul *et al.*, 2007) and based on the results of Fleming *et al.* (2003). It was calculated that 12 participants would be sufficient to detect a statistically significant change in mean power output (MPO) during the repeated sprint test, with a power of 0.95 and a 5% level of significance. Recruitment and the induction of volunteers into the study continued until 15 complete data sets were collected.

3.1.2 Inclusion criteria

Volunteers were included in the study if:

- they were between 18 and 40 years old;
- they engaged in deliberate training at least three times per week;
- they have not been on a CHO restricted eating plan for more than one month in the six months prior to the commencement of this study;
- they had no weight loss goals.

3.1.3 Exclusion criteria

Volunteers were excluded from the study if:

- they reported acute illness, chronic disease or any other medical problems during the health screening session (Appendix A);
- they answered 'yes' to one of the seven questions of the Physical Activity Readiness Questionnaire (PAR-Q+) (Appendix B);
- they had insufficient meal preparation equipment and facilities to follow a CHO restricted eating plan;
- they were habitually following a vegetarian or vegan diet;
- they had any diagnosed metabolic condition or disease (i.e. type 1 or type 2 diabetes, hypo- or hyperthyroidism) that could affect energy metabolism;
- they had a musculo-skeletal injury which could constrain their performance during the exercise tests;
- they used pharmaceutical drugs and/or ergogenic aids that could affect any measurements of physical performance or energy metabolism.

Figure 3.1 illustrates volunteer inclusion and participant completion of the study.
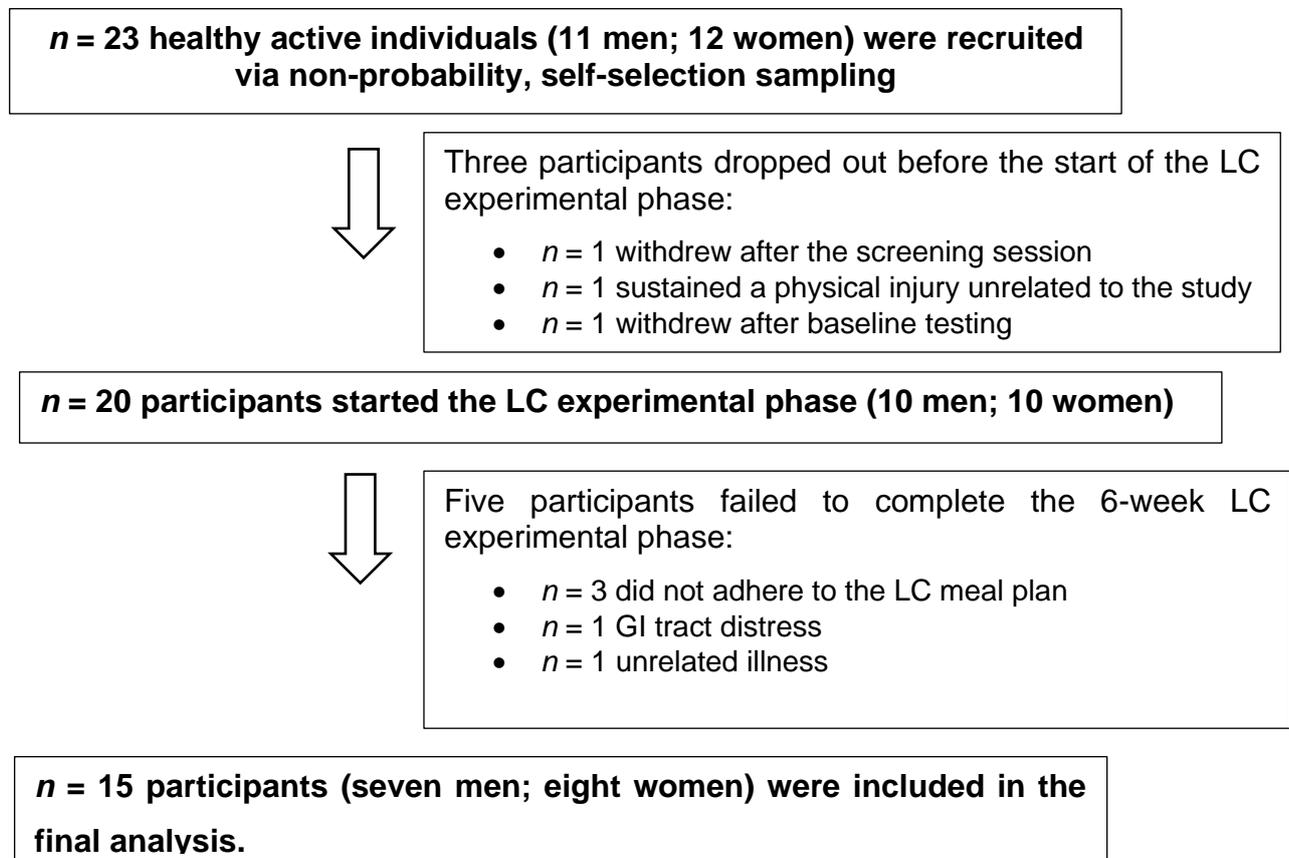
Figure 3.1: Flow diagram of recruitment and number of participants in the data analysis.

Procedures

Figure 1.2 illustrates the timeline of the study and the procedures during each laboratory session.

On their first visit to the Sport Physiology Laboratory, volunteers underwent a screening session to determine if he/she qualified according to the stipulated inclusion- and exclusion criteria. The screening process included the completion of the health screening form (Appendix A) and the physical activity readiness questionnaire (PARQ+) (Appendix B). Eligible candidates who, after the screening process and a discussion of what was expected from them, were still interested to take part in the study, were asked to read and sign the informed consent form (Appendix D). Volunteers who did not qualify for the study or chose not to participate received a voucher from the Sport Physiology Laboratory for a free fitness assessment as a token of appreciation for their interest.

Participants who signed the consent form underwent body composition measurements, a maximal aerobic capacity (VO_2max) test and a sprint familiarization session on the cycle ergometer. After the VO_2max test, participants rested for at least 15 min before performing three cycle sprints (as in the test protocol) as familiarization for the sprint test.

In order to control cycling posture for all the assessments, a standardized bike fitting procedure was performed during the familiarization session. Seat height, seat setback, handlebar height and handlebar reach were recorded and documented for consistent setup throughout the study. Participants' contact details were also collected for communication during the study.

During the following visits (HD1 and HD2), participants completed two baseline testing sessions, separated by two weeks while on their habitual diet. After HD1, they received instructions and a self-developed booklet on how they have to adapt their daily food consumption to comply with a LC eating plan.

All testing sessions followed the same exercise protocol, consisting of 6 x 10 s all-out sprints on the cycle ergometer, separated by a two min rest. Before the test, oxygen uptake (VO_2) was measured for three min while seated on a chair; thereafter VO_2 was measured throughout the five min warm up and the test until 10 min after the last sprint. Blood samples were collected after the five min warm up, as well as

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every two min after the last sprint for the measurement of blood lactate concentration ([BLa]). Blood samples were collected until a peak value was identified.

Following HD2, participants switched to a LC eating plan, which was defined as < 50 g CHO / day. Participants visited the laboratory every week during the 6-week intervention period for check-in sessions. During these check-in sessions they completed a short questionnaire to rate their state of wellness (Hooper-index, Appendix E) and blood ketone and hydration measurements were obtained. Laboratory testing were repeated on day 14 (LC week two), 28 (LC week four) and 42 (LC week six). Participants performed a post intervention VO$_2$max test while still on the low-CHO eating plan. This test was no earlier than 48 hours before, or after the last sprint test session.

Study design

2 wk habitual diet (HD)

6 wk low-CHO (LC) eating plan

Meal logging weeks

Screening & familiarization   HD 1   HD 2   Wk2   Wk4   Wk6

2wk   2wk   2wk   2wk   2wk

- Health screening questionnaire
- PAR-Q
- Hooper-index

- Anthropometry
- Body composition-Informed consent
- Bike fitting
-VO$_2$max
- [BLa]

Rest at least 15 min

- 3x10 s sprints @ 2 min recovery

- Hooper-index
- Urine osmolality
- Anthropometry

- 6x10 s sprints @ 2 min recovery; 10 min post-test
• Power output
• VO$_2$
• [BLa]
• EPOC

Receive 7-day meal plan

- Hooper-index
- Urine osmolality
- Anthropometry
- Body composition
- [ketone]
- 6x10 s; 2min recovery; 10 min post-test
• Power output
• VO$_2$
• [BLa]
• EPOC

Discuss meal plan Start LC eating plan

- Hooper-index
- Urine osmolality
- Anthropometry
- [ketone]
- 6x10 s @ 2 min recovery +10 min post-test
• Power output
• VO$_2$
• [BLa]
• EPOC

- Hooper-index
- Urine osmolality
- Anthropometry
- [ketones]
- 6x10 s @ 2 min recovery +10 min post-test
• Power output
• VO$_2$
• [BLa]
• EPOC

- Hooper-index
- Urine osmolality
- Anthropometry
-Body metrics
- RMR
- [ketones]
- 6x10 s @ 2 min recovery +10 min post-test
• Power output
• VO$_2$
• [BLa]

± 48 hours:
-VO$_2$max
• [BLa]

Weekly check-ins:
Subjective wellness, [blood ketones] and hydration levels

Figure 3.2: Study design, time line and procedures

3.2 Tests, Measures and Equipment

3.2.1 Health screening measurement

A modified version of the ACSM Medical Screening Questionnaire (Appendix A) was used to identify any contraindications that may warrant the participant's exclusion from the study. The ACSM questionnaire is in the public domain and was specifically developed for research laboratories, such as the Sport Physiology Laboratory (Riebe *et al.*, 2018).

3.2.2 Physical activity readiness measure

The Physical Activity Readiness Questionnaire for Everyone (PAR-Q+) (Appendix B) was developed by the Canadian Society of Exercise Physiology (Warbuton *et al.*, 2018). It determines whether it would be advisable for a participant to consult a medical doctor before engaging in (more) physical activity. The questionnaire consists of seven YES/NO questions. If a participant would have answered 'yes' to any of these questions, they should have answered an additional two pages of questions and they would have been referred to a medical doctor for further medical screening. However, none of our volunteers had to answer these additional 2 pages and therefore no one required clearance from their general practitioner, or the designated medical practitioner for this project.

3.2.3 Subjective wellness measure

The Hooper-index (Appendix E) (Hooper and Mackinnon, 1995) was included to monitor participants` wellness on a weekly basis throughout the course of the study. The questionnaire is a subjective self-reported wellness measure, which includes questions on perceived sleep quality and quantities of stress, muscle fatigue and muscle soreness. Each question was scored on a seven-point scale ranging from (1) "very, very good" to (7) "very, very poor".

3.2.4 Urine hydration measurement

To ensure that participants were sufficiently hydrated, they were asked to provide a clean-catch urine sample in a urine collection container before each exercise test, as well as during weekly check-in sessions. A handheld digital refractometer (Pocket Osmocheck, Vitech Scientific, UK) was calibrated between 0 to 1500 mOsmol.kg H_2O. The prism surface was cleaned and approximately 0.3 mL urine were placed on the prism surface to provide a digital osmolality reading. A reading of higher than 600 mOsmol pointed to a near-dehydration state, while a reading over 1000 mOsmol was consistent with a dehydrated state. Dehydrated participants were provided with a bottle of still water to consume immediately and instructed to increase their daily consumption of water (> 2 L per day). The testing session was subsequently postponed to the following morning.

3.2.5 Blood [ketone] measurement

Participants' blood ketone levels were measured once a week during the LC dietary intervention in order to monitor adherence and adaptation to the diet. A fingertip was cleaned with an alcohol swab and then pricked with an Accu-Chek Soft Click (Roche diagnostics, Manhein, Germany). A 1.2 µL blood sample was drawn into the capillary tube of the Keto-Mojo (Taiwan) device and the blood ketone reading was recorded.

3.2.6 Anthropometric measurements

Anthropometric measures were taken as part of body composition evaluation. These measures were also taken before each testing session in order to update participants` weight on the Veletron Wingate software and Cosmed metabolic analyzing system for accurate application of relative cycle resistance and measures of relative metabolic data.

While participants were barefoot and wearing minimal clothing, their stature and body mass was determined via the stretch stature method. Stature was defined as the perpendicular distance between the transverse planes of the vertex and the

inferior aspects of the feet, and measured with a sliding stadiometer (Seca, Germany). The participants were asked to stand barefoot on the scale and with their heels together. The heels, buttocks and upper part of the back had to touch the scale. The head of the person was held in the Frankfort position. This is when the orbital (lower edge of the eye socket) and the tragion (the notch superior to the tragus of the ear) are horizontally aligned. The participant was then asked to take a deep breath while the researcher placed the headboard firmly down the vertex and compressing the hair as much as possible. The measurement was then taken to the nearest 0.1 cm.

Body mass was measured using a calibrated electronic scale (UWE BW-150, 1997 model, Brisbane Australia) and recorded to the nearest 0.1 kg.

3.2.7 Body composition measurement

The ultrasound device, BodyMetrix BX2000 (Hosand Technologies srl, Verbania) was used to determine the participant's body composition (percentage body fat, fat mass and fat free mass). Intra-class correlations of 0.84 - 0.95 deems this device valid and reliable (Schoenfeld *et al.*, 2017; Wagner, 2013). Wagner *et al.* (2019) reported that with the exception of the suprailiac site, ultrasound provided measurements of subcutaneous fat thickness with an accuracy of <1 mm. With the exception of the abdomen (0.76), correlations between dissected measurements exceeded 0.90 at all other measurement sites. Thus, this device can provide relatively accurate and reliable measurement of body fat thickness.

The individual's standing height and body mass were entered into the computer software. The seven anthropometric sites for measurements (according to the International Society for the Advancement of Kinanthropometry) were marked using a non-permanent marker on the right side of the body. The device utilizes the Jackson and Pollock seven site protocol to estimate the body composition measures (Jackson & Pollock, 1980).

The seven sites are defined as follows:

- Scapula: located just below the bottom tip of the shoulder blade.
- Axilla: located below the armpit and level with the bottom of the sternum.

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- Waist: located 5 cm to the side of the belly button.

- Hip (Supra-iliac): located 5 cm above the front side tip of the hip bone.

- Thigh: the midpoint of the anterior thigh between the knee and the hip joint.

- Triceps: the midpoint of the posterior upper arm, between the shoulder and the elbow.

- Chest: halfway between the shoulder and the nipple.

A droplet of ultrasound gel was applied to the BodyMetrixTM probe and reapplied during the assessment, if necessary. The probe was placed on each anatomical site and scanned for 3-5 s. The device then generated an ultrasound signal travelling through the tissue and recorded the localized fat and muscle layer thickness (mm). Each site was measured two to three times for accuracy. If the first and second measurement did not differ by more than one mm, the second measurement was recorded. In the case of a larger difference, a third measurement was taken and recorded, provided that two measurements were within 1 mm.

3.2.8 Cardiometabolic measurements

The Cosmed Quark CPET (Rome, Italy) metabolic analyzer was used to measure VO_2 max, as well as selected cardio-respiratory parameters during and after the intermittent sprint test protocol. Heart rate was measured with a Garmin chest strap HR monitor and was wirelessly interfaced with the COSMED metabolic system. The gas analyzers were calibrated to 16% O_2, 4% CO_2 and the balance N_2, and the turbine flow meter was calibrated with a 3 L calibration syringe before each day of tests.

CHO and fat oxidation (%) were calculated using the Ferrannini's equations (Ferrannini, 1988). Since urinary nitrogen excretion was not measured, nitrogen was reported as 13 g / 24 h, reflecting nitrogen excretion during fasting (Thorburn *et al.*, 1991).

It is important to note that ketone bodies were likely a significant energy source during the LC intervention period. Ketones produce energy through conversion to acetyl-CoA, an intermediate of lipid metabolism. This energy source is not directly

accounted for by the stoichiometric calculations of fuel utilization. However, intermediate metabolic processes apparently do not influence overall fuel oxidation calculations (Jeukendrup & Wallis, 2005).

The anaerobic threshold (AT) was defined as the point where the V_E/ VO_2 curve, having been flat or decreasing, begins to rise as the V_E/ VCO_2 curve remained constant or decreased. If this method did not provide a clear threshold, the V_E-time curve was inspected to determine the point where a non-linear rise in V_E occurred (Santos & Giannella-Neto, 2004). The respiratory compensation point (RC) was identified as the second breakpoint on the V_E–time curve (Santos & Giannella-Neto, 2004).

3.2.9 Bike fitting

A standardized bike fitting procedure was performed at the first session, to maintain consistency in each participant's cycling posture across all exercise testing sessions. Measurements were documented for each participant for consistent set up before each subsequent test. Cycling posture is known to affect energy cost (Nordeen-Snyder, 1977). Seat height and fore / aft position were adjusted so that when the pedal surface was parallel to the ground, and the participant's pedal was at the bottom of the pedal stroke (6 o'clock), his/ her knee was in a position of 25 – 30° flexion (Bini *et al.*, 2014). Handlebar height and fore / aft position were adjusted so that when the participant placed his / her hands on the brake hoods and maintain a slight flexion in his / her elbows.

3.2.10 Pre-exercise test requirements

Measurements and testing were conducted in the morning between 06:00 and 11:00. The ambient temperature in the laboratory was kept between 19 and 22°C. To standardize the participant's metabolic state during the testing sessions, they:

1.   did not eat for at least two hours prior to testing;

2.   avoided caffeine-containing drinks and alcohol ingestion at least 12 hours before testing;

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3. avoided vigorous activities – rating of perceived exertion (RPE) above 12 on the Borg scale - or any unaccustomed exercise at least 24 hours before testing;

4. had to be sufficiently hydrated prior to testing (>2 L of water every day and refractometer measurement of < 800 mOsmols prior to test).

3.2.11 Determination of maximal aerobic capacity (VO$_2$ max)

A progressive incremental exercise test was performed on the Velotron Dynafit Pro cycle ergometer (RacerMate, Seattle, USA) to determine maximal aerobic capacity (VO$_2$ max).

The participants performed a 10 min warm up at 80 W and a cadence of their choice. They were allowed to drink water after the warm-up and then the face mask and heart rate monitor were fitted. Men started at 100 W, which increased by 1 W every 3 s. Women started at 50 W and the workload increased by 1 W every 3 s. Participants were asked to keep the cadence between 80 - 100 rpm during the entire test. The intensity increased until the participant reached exhaustion and were unable to maintain the cadence at or above 80 rpm, at which point the test was terminated.

The test was considered a true maximal effort if at least two of the following ACSM criteria were met:

(i) if the VO$_2$ reached a plateau;

(ii) if a respiratory quotient (RQ) equal or above 1.10 was reached;

(iii) if heart rate was more than 90% of the age predicted maximal heart rate;

(iv) if the RPE was above 19 on the 6-20 Borg scale (Appendix C).

(v) if the participant indicates he/ she is exhausted.

Before analysis, breath-by-breath data was smoothed to a five-point moving average. VO$_2$max was obtained as the average of the highest 5 - 15 s VO$_2$ window measured near the end of the test.

3.2.12 Intermittent sprints test

Figure 3.3 illustrates the intermittent sprints test session procedure with the time points for metabolic measurements. These metabolic measurements were used in the calculation of the energy system contributions (see Calculation of the energy systems, section 3.2.15).
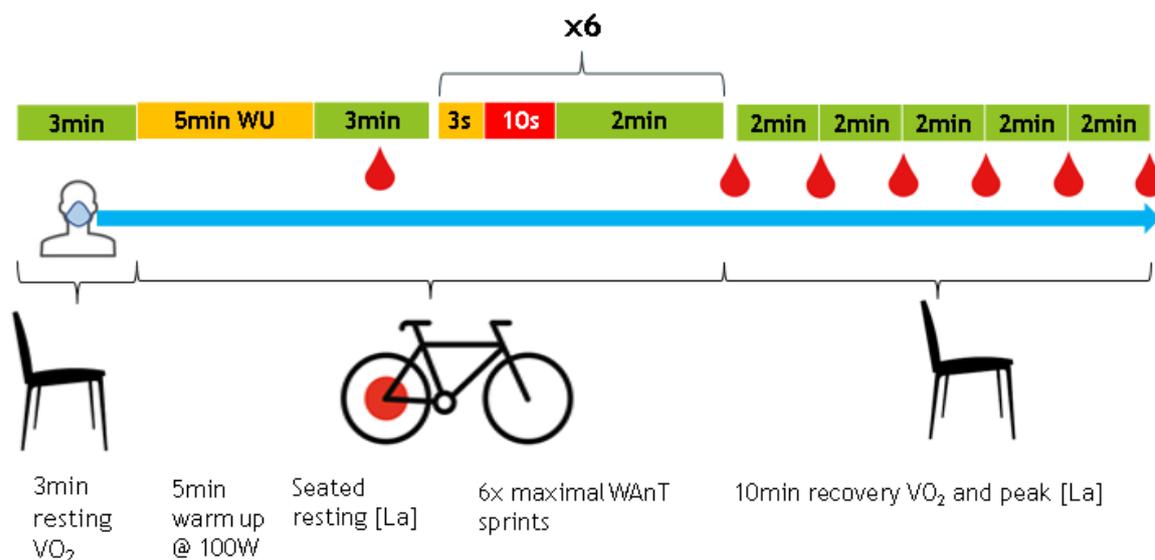
Figure 3.3: Intermittent sprints protocol and metabolic measurements. WU, warm-up; s, seconds; W, watts; [La], blood lactate measurement.

An intermittent Wingate anaerobic test (WAnT) sprint cycle testing protocol was selected consisting of 6 x 10 s all-out efforts. Testing was performed on an electronically braked Velotron Dynafit Pro cycle ergometer (RacerMate, Seattle, USA), fitted with an optical pedal counter and interfaced with its corresponding Velotron Wingate software (Racer-Mate Inc., Seattle, USA). The facemask of the metabolic system and heart rate monitor were fitted, and the participant sat on a chair for three min to measure resting VO_2. The participant then performed a five min warm-up against a resistance of 80 W (women) and 100 W (men), followed by a three min passive rest period during which the first pre-bout [BLa] measurement was taken.

To start each sprint, the researcher used a count-down, "three, two, one, GO". After the GO signal, the participant started his/her all-out effort. For the first 3 s the

participant cycled against the inertia of the flywheel, after which a resistance of 0.075 kp per kg body mass automatically kicked in for the next 10 s. The selected flywheel resistance is a commonly used relative resistive load in a number of studies (Bogdanis *et al.*, 1996; Lopez *et al.*, 2014).

Verbal encouragement was provided to all participants during all sessions and care was taken to replicate the amount of encouragement as much as possible among participants. At the end of the six sprints, the participant's RPE was recorded (Borg, 1954). While still connected to the metabolic system, the participant was asked to disembark the bike and sit on a chair next to the bike. During the 10 min recovery period, ventilatory data collection continued. [BLa] readings were taken every 2 min until the peak [BLa] was identified.

For each 10 s sprint, peak power output (PPO) and mean power output (MPO) were recorded by the Velotron Wingate Testing Software Version 1.0.2 (Racermate Inc., Seattle, USA).

3.2.13 Fatigue measurements

Fatigue was defined as the percentage decrement score (Fitzsimons *et al.*, 1993) and has been described as the most reliable measure of fatigue during intermittent sprints protocols (Glaister *et al.*, 2008).

Equation 3.1: Percentage decrement score

$$Fatigue = 100 - [(Total\ power\ output \div Ideal\ power\ output) \times 100]$$

Where:

- $Total\ power\ output$ = sum of MPO for all sprints in a session
- $Ideal\ power\ output$ = number of sprints × MPO of best sprint

3.2.14 Blood lactate concentration [BLa] measurements

Lactate is a metabolic by-product of metabolism and was measured to calculate the contributions of the different biochemical energy systems during the intermittent sprints.

A fingertip was cleaned with an alcohol swab and then pricked with an Accu-Chek Soft Click (Roche diagnostics, Manhein, Germany). The first droplet of blood was wiped away and the second was drawn into the capillary tube of the Lactate Pro 2 meter (ARKRAY, Inc. Kyoto, Japan). The reading was taken at the end of the countdown and recorded. [BLa] measurements were taken after the five min warm-up and every two min until it reached a peak value during the post-test recovery period.

3.2.15 Calculation of the energy system contributions

The contributions of the aerobic-, glycolytic- and ATP-PCr energy systems to total chemical energy output during the intermittent sprint tests were calculated using VO$_2$ and [BLa] measurements and equations based on the original theory of Margaria *et al.*, (1933).

The following equations were used:

Equation 3.2: Aerobic energy contribution

$$W_{aer}(kJ) = VO_2(mL) \times energy\ equivalent\ (kJ.mL^{-1})$$

Where:

- VO_2 was oxygen uptake above rest: $(VO_2 \times test\ time) - resting\ VO_2$ (di Prampero *et al*, 1973).
- $Energy\ equivelants\ of\ 20.92\ kJ\ for\ each\ 1\ L\ of\ utilized\ O_2$ (Gastin, 2014)

Equation 3.3: Anaerobic glycolysis energy contribution

$$W_{lactate}(kJ) = \Delta[lactate](mmol^{-1}.L) \times O_2$$
$$- lactate\ equivalent\ (mL.kg^{-1}.mmol^{-1}.L^{-1})$$
$$\times energy\ equivelant\ (kJ^{-1}.mL) \times body\ mass(kg)$$

Where:

- $\Delta[lactate]$ is the change measured between the [BLa] after the warm up, and the peak [BLa] after the last sprint.
- $O_2 - lactate\ equivalent\ =\ 3\ mL/kg/mmol/L$ (di Prampero, 1981)

- *Energy equivelants of* $20.92\ kJ$ *for each* $1\ L\ of\ O_2\ utilized$ (Gastin, 2001)

Equation 3.4: ATP-PCr energy contribution

$$W_{PCr} = VO_{2PCr}(mL) \times energy\ equivelant(kJ^{-1}.mL)$$

Where:

- *Energy equivelants of* $20.92\ kJ$ *for each* $1\ L\ of\ O2\ utilized$ (Gastin, 2001)
- VO_{2PCr} represents the fast component of post-bout oxygen uptake (Beneke *et al.*, 2002; di Prampero *et al.*, 1973). Details on the curve fitting settings for each method as well as corresponding GOF outcomes can be referred to in Appendix G. Goodness of fit statistics revealed the most accurate fitting to be the Trust-Region algorithm with set upper and lower coefficient constraints, in the Robust Bisquare setting, smoothed with a 10-point moving average.

3.3 Nutritional intervention

3.3.1 LC eating plan

Consistent with previous research (Cipryan *et al.*, 2018; Dostal *et al.*, 2019; Greene *et al.*, 2018; Kephart *et al.*, 2018; McSwiney *et al.*, 2017), the researcher did not impose a specific and uniform 6-week LC eating plan on the participants. Instead, participants were allowed to follow a self-selected eating plan with the aim to meet the macronutrient goals of > 75% fat, 10 – 15% protein and < 50 g/day CHO (or < 10% of total macronutrient intake) (Cipryan *et al.*, 2018; Dostal *et al.*, 2019; Greene *et al.*, 2018; McSwiney *et al.*, 2017). Participants were informed that the purpose of the eating plan was to become less dependent on CHO and more on fat for fuel metabolism and that they should not restrict their daily calorie intake in order to lose weight. It was stressed that the focus of the study was on the effect of the eating plan on exercise performance and not changes in body composition measures (e.g. percent body fat).

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All participants were advised to consume enough flaxseed and vegetables on their permitted food lists to minimize the possible side-effect of constipation. Participants were informed of all possible side-effects, how to prevent them and asked to inform the researcher if any of these side-effects persisted for more than two days.

The use of lemon in drinking water or non-carbohydrate electrolytes were encouraged to maintain hydration levels (Sampath *et al.*, 2007). At each test- and check-in session the participants' hydration levels were tested, and no testing commenced if the participant was not sufficiently hydrated. During the weekly testing sessions, participants were asked to report any side-effects that they may experience.

3.3.2 LC information booklet (Appendix F)

Participants received a booklet with information to assist them with their LC eating plan, as in previously employed research methods (Cipryan *et al.*, 2018; Dostal *et al.*, 2019; Greene *et al.*, 2018; McSwiney *et al.*, 2017). The booklet contained sample meal plans for seven days, food lists with macronutrient breakdown, a few recipes, as well as reputable internet resources. The latter included websites with information on the rationale behind LC eating, the physiology involved, frequently asked questions and recipes with specific amounts of CHO content.

The meal plans were developed by a certified sports nutritionist, accredited by the International Society of Sports Nutrition. Together with the study doctor they were responsible for offering advice and guidance to participants regarding their nutrition and any health problems related to the research project.

3.3.3 Standardized pre-test breakfasts

To ensure that the true metabolic state of each nutritional phase was fully present and standardized within participants at the testing sessions, the sports nutritionist prescribed a standardized pre-test breakfast. These breakfasts were consumed exactly two hours before participants arrived at the laboratory for testing.

During the baseline / control habitual diet sessions (HD1 and HD2) the aim of the breakfast was to refill glycogen stores after the overnight fast. The breakfast consisted of Kellogg's Cornflakes, low fat milk and a banana or fruit juice. The breakfast quantities were calculated to provide 2 g CHO per kg bodyweight. Participants were provided with their weighed portion of Cornflakes and asked to add the prescribed quantity of low-fat milk and fruit juice or a banana to complete the CHO requirement. As far as possible the sports nutritionist accommodated for participants` personal preferences in these prescriptions.

During the LC dietary phase, an overnight fast would not cause alterations in participants` expected metabolic state. Therefore, participants were given the choice to complete the LC sessions after an overnight fast, or two hours after a standard LC breakfast. The breakfast choice consisted of two to three eggs, fried or scrambled in coconut oil or butter, and additional leafy vegetables. If they chose the breakfast option, they had to measure exact quantities which was recorded by the researcher during their first LC test. To ensure consistency, participants were required to employ identical pre-test breakfasts on all LC testing days. Therefore, the researcher sent a reminder to the participant the day before their next test with their recorded breakfast quantities.

3.3.4 Meal logging

Participants were asked to report their daily food and drink intake in order to calculate their macronutrient consumption for two, 2-week cycles. The first 2-week meal logging cycle was the last week on the habitual diet and the first week of their LC eating plan, and the second 2-week meal logging cycle was the last two weeks on the LC eating plan. Altogether, one habitual diet week and three LC diet weeks of nutritional data was obtained for analysis. This information was also used to provide feedback to the participants during the LC intervention period and to assist them to comply with the macronutrient prescription for the intervention.

Most participants used a Google-form, however, three participants chose to keep a pen and paper logbook. The Google form asked participants to log all food items in as much detail as possible, including the quantity. Participants were provided with a kitchen scale to ensure accurate tracking of their macronutrient intake.

The Google form had a section where the participant was asked to upload an image of the food label when they logged a new item for the first time. Responses on the Google form were imported directly into a Google sheet and then to Excel for analysis. Participants who chose to use a pen and paper logbook, were asked to send a picture of newly logged food labels via Whatsapp to the sports nutritionist.

3.4 Statistical Analysis

Statistical analysis was conducted in IBM SPSS statistics version 26 and Excel (Microsoft Office 2013). Descriptive statistics are presented as means ± SD and Cohen`s effect size (ES) ± 95% confidence interval (CI). The mean of the two baseline measurements (HD1 and HD2) were calculated and reported as one baseline measurement (week 0) for all outcome variables. In order to determine if a learning-effect was present, repeatability was determined from total session MPO between HD1 and HD2. The intraclass coefficient (ICC) (0.99; 90% CI = 0.99-1.00) and the typical error (0.16; 90% CI = 0.12 – 0.24) was < 0.2, indicative of a *small* difference (Hopkins, 2000). Percentage difference in MPO between HD1 and HD2 was 1.6%, indicative of a small learning effect.

Kolmogorov-Smirnov tests revealed that all outcome measures, except blood ketones, followed a Gaussian distribution. Mauchly's Test of Sphericity revealed a statistically significant outcome for blood ketone levels alone. Greenhouse-Geisser correction was therefore applied in the analysis of the ketone data via a repeated measures ANOVA. In all cases results were considered statistically significant if $p < 0.05$.

Macronutrient composition for the HD and the LC phases were reported as mean values for the meal logging weeks (HD = one week; LC = three weeks). These values were reported as calories, kilojoules and as percentages of daily energy intake.

Energy system contributions were reported as absolute values and percentage of total chemical energy output for each intermittent sprint session (Campos *et al.*, 2012; Julio *et al.*, 2019; Julio *et al.*, 2017; Milioni *et al.*, 2017; La Monica, Fukuda *et al.*, 2020), as well as relative to body mass (Beneke *et al.*, 2004; Smith & Hill, 1991).

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Absolute outcomes consisted of the total energy contribution in Joules, while relative outcomes were reported as Joules per kg body weight. Percentage outcomes were calculated as the percentage contribution of each energy system to total chemical energy output for each session.

For the calculation of the ATP-PCr energy system contribution, R squared goodness of fit statistics were used to establish the best fitting model for the various exponential plots. R-squared outcomes of three methods are summarized in Appendix G (Table 1).

A repeated measures ANOVA was used to determine whether statistically significant changes in fuel metabolism and exercise performance occurred during the LC intervention. In case of a statistically significant result, a Bonferroni post-hoc correction was used for pairwise comparisons.

Cohen's effect sizes (ES) and 95% CI were calculated to compare the magnitude of change in outcome measures. Quantitative interpretation of the ES values was based on Cohen (1988). Effect sizes up to 0.20 is referred to as a *negligible* difference, between 0.20 and 0.50 is interpreted as *small*, between 0.50 and 0.80 reflects a *moderate* effect size, while ES between 0.80 and 1.00 is regarded a *large* difference and > 1.00 a *very large* difference*.* Threshold values of [-0.2;0.2] were representative of the smallest worthwhile change (SWC) for standardized mean differences.

A Pearson`s correlation coefficient was calculated between changes in macronutrient intake (protein, fat and CHO) and the change in absolute VO_2 max. The change in the consumption of each macronutrient (in grams) was calculated as the difference between the participant's mean intake during the one habitual diet (HD) meal logging week and the mean daily intake across the three meal logging weeks during the LC phase. The change in VO_2 max was calculated by subtracting post- values from pre- values for each participant. The effect sizes for the correlation coefficients were interpreted according to the thresholds of Hopkins *et al.* (2009), namely 0.10 (*small*), 0.30 (*moderate*), 0.50 (*large*), 0.70 (*very large*) and 0.90 (*almost perfect*). Smallest meaningful correlation threshold values were set at [-0.1, 0.1].

3.5 Ethical aspects

Institutional permission was obtained since students at the University volunteered for the study. Participants were informed that their participation was completely voluntary. Therefore, participants could withdraw from the project at any point in time. An informed consent form, in either Afrikaans or English, was completed by participants and they received a clear explanation of the protocols and procedures that were used and encouraged to ask questions. There were no serious risks involved in the study as the participants only included healthy and fit individuals. All the tests were performed according to the standards and requirements for testing healthy, active individuals and no invasive procedures were included in the protocol. Nonetheless, participants were informed that they may experience dizziness, fainting and discomfort during the repeated cycle sprint tests. The potential risks were minimized as much as possible by thoroughly explaining the procedure to the participants, carefully monitoring changes in the physiological variables during exercise testing and stopping the test immediately if the person indicated to stop, or if the researchers detected any unusual physiological responses. Participants were contacted six hours post-test for confirmation of their well-being.

Blood samples were obtained non-invasively via a finger prick and did not exceed 2 mL per test. Gloves, alcohol swabs and hermitically sterilized needles were used at all times. All consumables were sent for incineration in a biohazard collection bin.

Chapter 4

Results

4.1 Descriptive characteristics

Twenty-three healthy, active men (n = 11) and women (n = 12) volunteered to participate in the study. Eight participants (4 men and 4 women) did not complete the study, due to injury (unrelated to the study) or illness, life commitments or dietary non-compliance. Data from these individuals were excluded from the final data set. Results are reported from 15 participants (7 men and 8 women) who completed all testing sessions.

The participants were between 19 and 39 years old (Table 4.1). The women presented with significantly higher body fat percentages (23.1 ± 5.75% vs. 15.2 ± 3.59%; p = 0.008), but lower body mass (69.9 ± 11.36 kg vs. 84.5 ± 13.13 kg; p = 0.038) and fat free mass (14.5 ± 1.59 kg vs. 19.5 ± 2.67 kg; p = 0.0007) compared to the men.

Table 4.1: Descriptive characteristics of the participants

	Group (n=16)		Men (n=7)		Women (n=8)	
Variable	Mean ± SD	Min – Max	Mean ± SD	Min – Max	Mean ± SD	Min – Max
Age (years)	25.1 ± 6.42	19 – 39	27.4 ± 7.41	21 – 39	23 ± 4.99	19 – 33
Height (cm)	172.2 ± 6.02	164 – 183.5	176.2 ± 5.48	167 – 183.5	169 ± 3.97	164.0 – 175.8
Body mass (kg)	76.7 ± 13.97	57.6 – 107.2	84.5 ± 13.13	69.3 – 107.2	69.9 ± 11.36	57.6 – 88.5
Fat %	19.4 ± 6.22	10.4 – 29.8	15.2 ± 3.59	10.4 – 20	23.1 ± 5.75	14.8 – 29.8
FFM (kg)	16.8 ± 3.30	12.1 – 23.4	19.5 ± 2.67	16 – 23.4	14.5 ± 1.59	12.1 – 17.2

FFM, fat free mass

Since sex comparisons were not one of the aims of this study, only group results are reported from here on.

Table 4.2 presents body composition changes that occurred over the 6-week LC intervention. On average, the participants experienced *small,* but significant decreases in body fat percentage (2.1 ± 1.81 kg; p = 0.001; ES = -0.34; CI = -1.05; 0.39), body mass (3.4 ± 2.12 kg; p = 0.000; ES = -0.25; CI = -0.97; 0.47) and fat free

mass (0.3 ± 0.37 kg; p = 0.011; ES = -0.09; CI = -0.80; 0.63). While reductions in body mass occurred in all 15 participants (3.4 ± 2.12 kg; p = 0.000; ES = -0.25; CI = -0.97; 0.47), two women experienced an increase in body fat percentage (0.2% and 2.5%, respectively) and three women's fat free mass increased (0.1, 0.2 and 0.5 kg, respectively). On average the drop in body mass was greater in men compared with women (4.7 ± 2.15 kg vs. 2.3 ± 1.45 kg; p = 0.036; ES = -0.83; CI = -1.83; 0.27).

Table 4.2: The changes in body composition over six weeks of carbohydrate restriction.

	Group (n=15)				
Variable	Pre	Post	Change	p-value	ES (95% CI)
Body mass (kg)	77.4 ± 14.27	74.0 ± 12.57	3.4 ± 2.12	0.000	-0.25 (-0.97; 0.47)
% Body Fat	19.4 ± 6.22	17.3 ± 6.23	2.1 ± 1.81	0.001	-0.34 (-1.05; 0.39)
Fat free mass (kg)	16.8 ± 3.30	16.5 ± 3.11	0.3 ± 0.37	0.011	-0.09 (-0.80; 0.63)

ES, effect size; *CI*, confidence interval

4.1.1 Nutritional characteristics

Table 4.3 presents nutritional characteristics of participants` diets on the HD and LC phases as reported in meal-logging weeks. The participants' average carbohydrate (CHO) intake during the low CHO (LC) phase was less than the required 50 g per day (26.4 ± 9.36 g/day) and there was a *very large* and significant decrease from the HD phase (p = 0.000; ES = -2.94). Average energy intake was also *moderately* lower on the LC diet compared to the HD diet (-13.4 ± 22.48%; p < 0.05; ES > 0.5).

Table 4.3: Dietary energy and total daily macronutrient intake.

Variable	HD	LC	p-value	ES (95% CI)
Daily energy intake (kJ)	8845.3 ± 2985.91	7223.9 ± 2169.34	0.016	-0.62 (-1.3, 0.13)
Daily energy intake (Calories)	2114.1 ± 738.70	1726.5 ± 518.49	0.016	-0.61 (-1.32,0.14)
Fat (g)	96.6 ± 40.19	120.9 ± 38.61	0.018	0.62 (-0.13,1.33)
CHO (g)	172.3 ± 69.60	26.4 ± 9.36	0.000	-2.94 (-3.88, -1.84)
Protein (g)	95.1 ± 35.34	116.8 ± 33.57	0.005	0.63 (-0.12,1.34)

HD, habitual diet; *LC*, low-carbohydrate diet; *CHO*, carbohydrate; *ES*, effect size; *CI*, confidence interval

Figure 4.1 shows that mean CHO intake was restricted to 7% of the participants' daily energy intake during the LC phase, while fats made up the majority of their caloric intake (66%). Protein intake also increased by 8% from the HD to the LC phase.
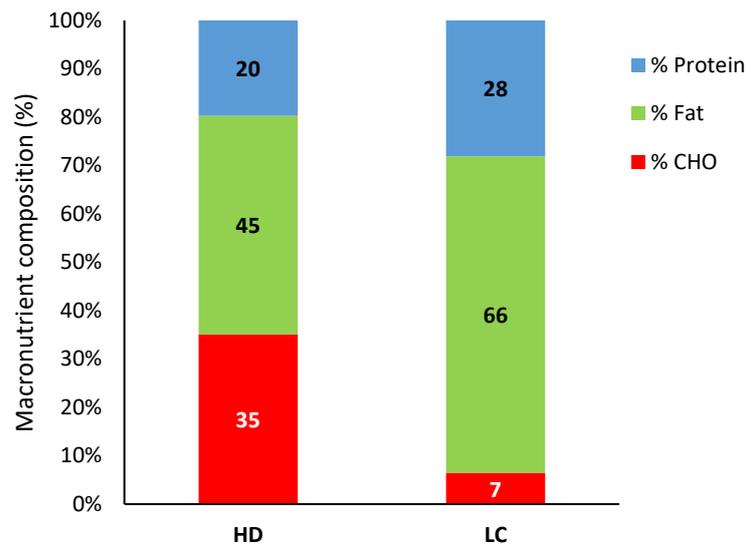
Figure 4.1: Macronutrient consumption as percentage of daily energy intake during the meal logging weeks of the HD and LC phases. HD, one week of meal logging on the habitual diet; LC, three weeks of meal logging on the low-carbohydrate diet (LC weeks 1, 5 and 6).

4.1.2 Blood ketone levels

Figure 4.2 illustrates the significant changes in mean blood ketone levels throughout the study period (p = 0.002). Mean differences indicated *very large* increases in blood [ketone] for all LC timepoints compared to the HD baseline measurement (ES > 1.29; CI = 0.47; 2.04). The average group blood [ketone] reached a peak at week three on the LC diet phase and although it dropped slightly until the end of the study period, the average value did not reach the HD average value.

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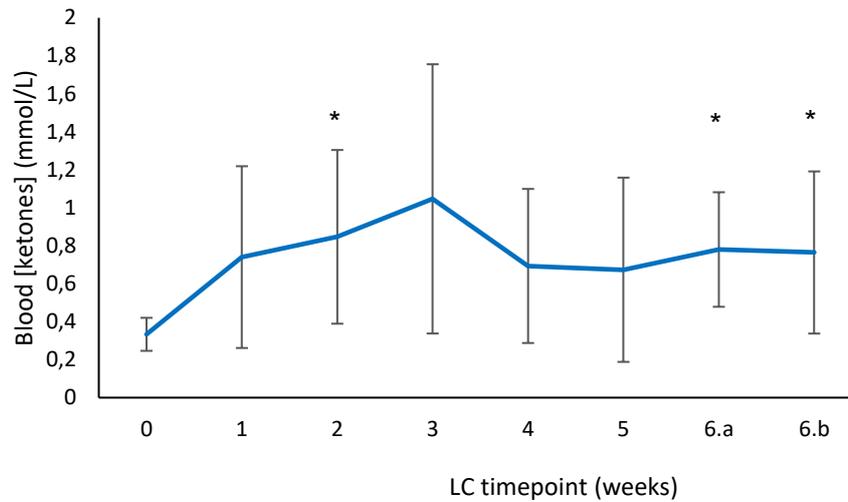
*Figure 4.2: Blood [ketone] (mean ± SD) from the HD phase and throughout the six-week LC diet phase. The last blood ketone measurement (week 6.b) was at the Post LC VO$_2$max test and occurred at least two days (48 h) before or after LC week 6, while participants maintained their LC diet. Week 0, one measurement on habitual (HD) diet; LC weeks, low-carbohydrate week number; *Significantly different from HD (p < 0.05).*

4.1.3 Subjective wellness

The Hooper scale (Hooper *et al.*, 1995) measures subjective wellness and consists of a score for fatigue, sleep quality, general muscle soreness, stress levels and mood. Hooper outcomes, where higher scores indicate lower levels of overall wellness, are presented in Figure 4.3. There was no statistically significant change in Hooper scores across the study period. However, there was a *moderate* improvement in overall wellness during the LC phase (average score over 6 weeks) compared to the HD phase (12.4 ± 1.94 vs. 11.5 ± 1.28; p = 0.062; ES = -0.54; CI = -1.25; 0.20).
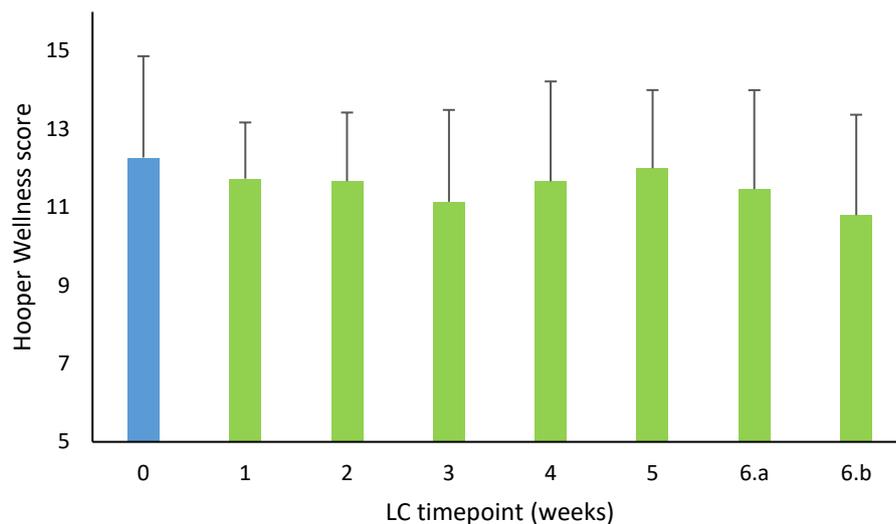
*Figure 4.3: Weekly subjective wellness scores (Hooper scale) throughout the study. Lower scores on the Hooper scale indicate higher level of subjective wellbeing. The last measurement (*week 6.b*) was taken at the post-intervention VO₂max test that occurred at least two days (48 h) before or after LC week 6, while participants maintained their LC diet.* Week 0, *constitute the mean of the baseline measurements on the habitual diet;* LC*, low-carbohydrate diet.*

4.2 Metabolism and performance during intermittent sprints

4.2.1 Absolute energy system contributions during intermittent sprints

Figure 4.4 illustrates the changes in absolute energy system contributions at 2-week time intervals throughout the study. Statistically significant changes occurred in the contributions of the ATP-PCr ($p = 0.021$) and anaerobic glycolytic ($p = 0.008$) energy systems over the study period. The contributions of the aerobic energy system remained unchanged ($p = 0.85$).

There was a *small* significant increase in the contribution of the ATP-PCr system to total energy expenditure following 6 weeks on the LC diet (+22.0 ± 43.15 Joule; $p = 0.019$; ES = 0.47; CI = -0.27; 1.18). A *moderate* statistically significant decrease in the contribution from the absolute anaerobic glycolytic system was observed after two weeks on the LC diet (69.2 ± 27.39 Joule vs. 54.8 ± 24.77 Joule; $p = 0.031$; ES = -0.51; CI = -1.22; 0.23), where after this energy system contribution remained lowered throughout the LC intervention.

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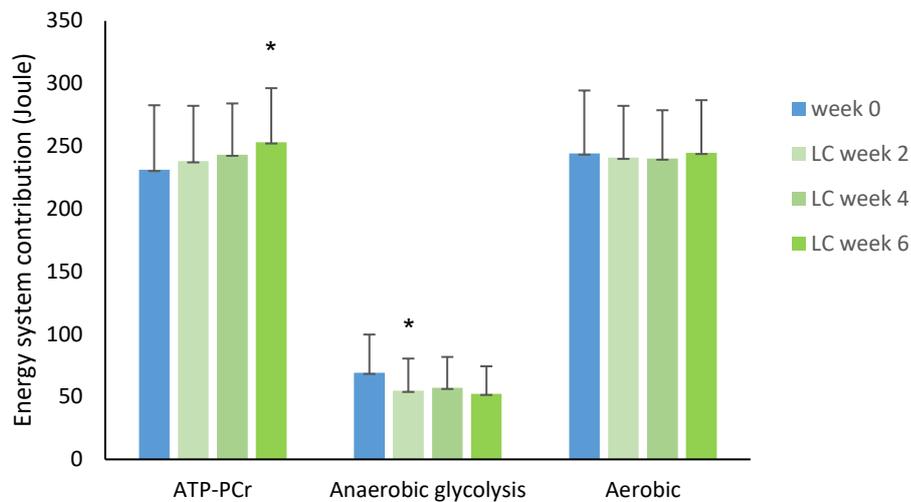
*Figure 4.4: Absolute energy system contributions (mean ± SD) in Joules. Week 0: the mean of the two baseline tests; LC week 2-6: test sessions in week 2,4 and 6 on the LC diet. * Statistically significant difference from baseline (p < 0.05).*

Figure 4.5 illustrates the magnitude of the changes in absolute energy system contributions from baseline (week 0) to LC week 2, 4 and 6.
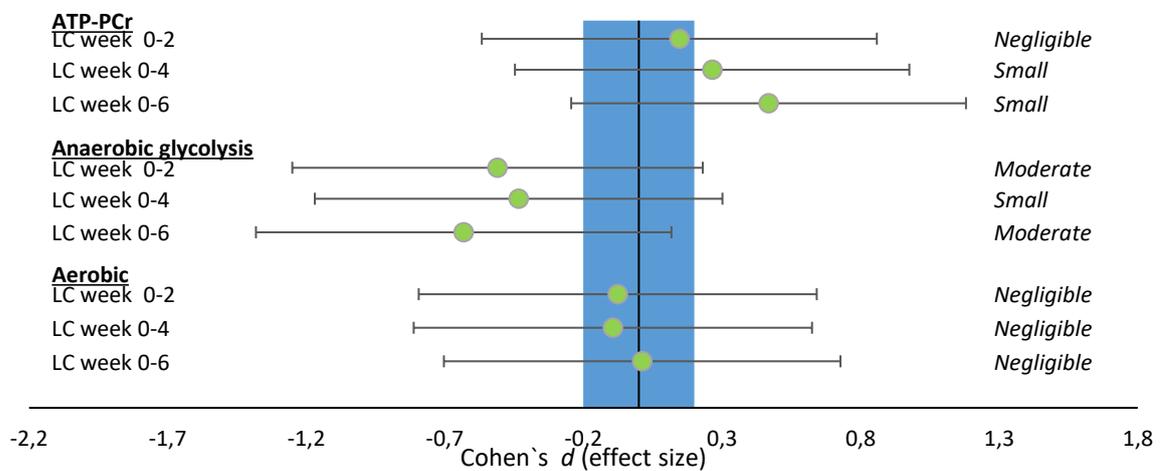
Figure 4.5: Standardized mean differences with 95% confidence intervals for absolute energy system contributions from baseline (week 0). The blue column represents the smallest worthwhile change (SWC) [-0.2, 0.2] LC, low-carbohydrate diet intervention.

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4.2.2 Percentage contributions of the energy systems during intermittent sprints

Changes in the energy system contributions in relation to total chemical energy output per repeated sprint test can be seen in Figure 4.6. Statistically significant changes over time were evident in the contributions of the ATP-PCr (p = 0.000) and anaerobic glycolytic energy systems (p = 0.005), with no significant change in the aerobic energy system (p = 0.580).

A *large* increase in the contribution of the ATP-PCr system from week 0 (baseline) was evident within the first two weeks on the LC intervention (42.6 ± 2.54% vs. 44.7 ± 2.67%; p = 0.072: ES = 0.81; CI = 0.04; 1.53). A *very large* and statistically significant increase followed after LC week 4 (42.6 ± 2.54% vs. 45.1 ± 1.20%; p = 0.011; ES = 1.10; CI = 0.31; 1.84) and LC week 6 (42.6 ± 2.54% vs. 46.1 ± 2.16%; p = 0.002; ES = 1.50; CI = 0.65; 2.27).

Compared to week 0 (baseline), a *large* decrease in the contribution of the anaerobic glycolytic energy system was evident within the first two weeks on the LC intervention (12.3 ± 3.12% vs. 10.0 ± 3.35%; p = 0.021: ES = -0.71; CI = -1.43; 0.04), as well as at LC week 4 (12.3 ± 3.12% vs. 10.3 ± 2.90%; p = 0.276; ES = -0.68; CI = -1.39; 0.08). A *very large* and statistically significant decline was observed at LC week 6, compared to baseline (12.3 ± 3.12% vs. 9.3 ± 2.70%; p = 0.028: ES = -1.04; CI = -1.77; -0.25).
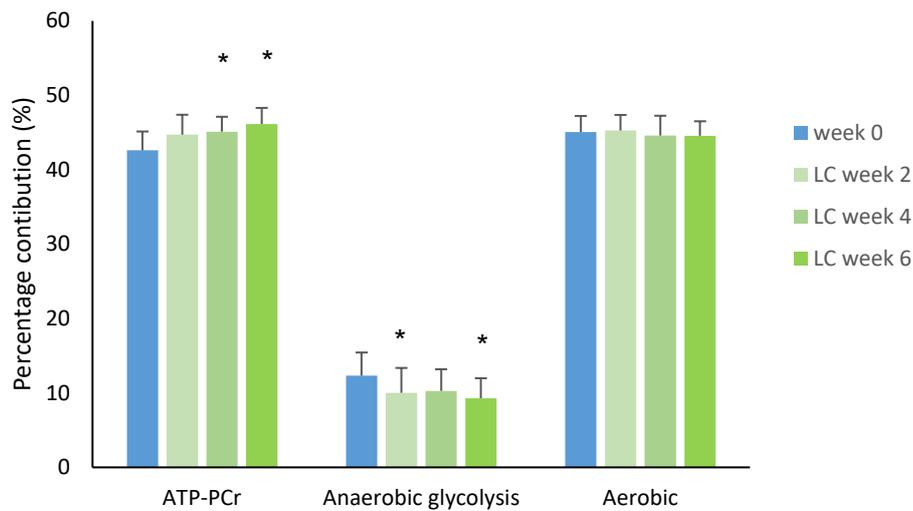
Figure 4.6: Percentage energy system contributions in relation to total chemical energy expenditure. Week 0, constitute the mean of the two baseline tests; LC week 2-6. *test sessions in week 2.4 and 6 on the LC diet. *Statistically significantly different from baseline (p < 0.05).*

Figure 4.7 illustrates the magnitude of changes in the energy system contributions from baseline (week 0) to LC week 2, 4 and 6.
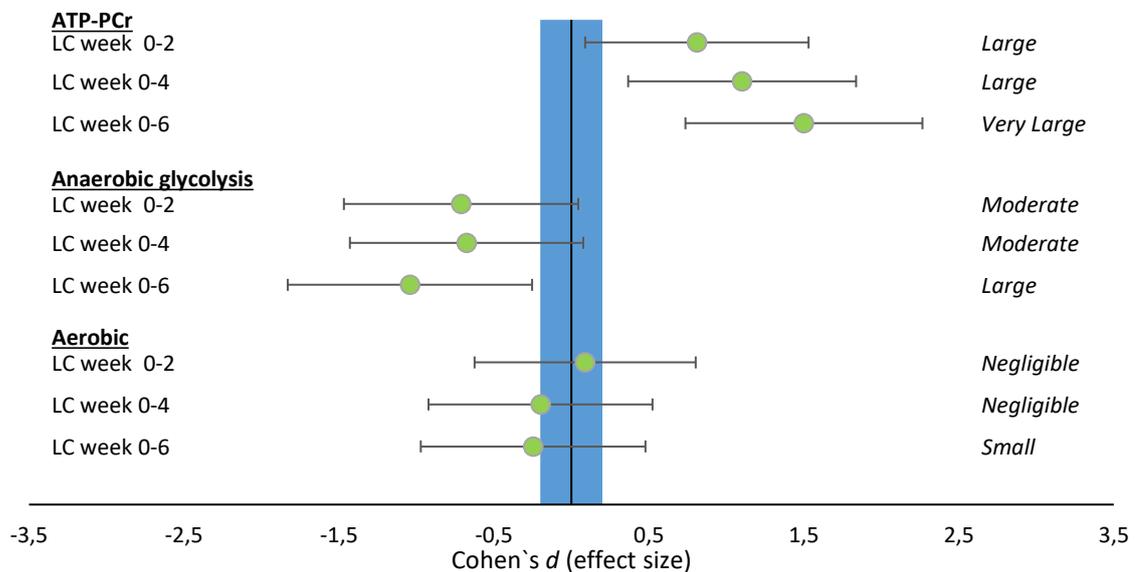
Figure 4.7: Standardized mean differences with 95% confidence intervals for percentage energy system contributions from baseline (week 0). The blue column represents the smallest worthwhile change (SWC) [-0.2, 0.2] LC, low-carbohydrate diet intervention.

4.2.3 Energy system contributions relative to body weight during intermittent sprints

The contributions of the energy systems, relative to body weight, are illustrated in Figure 4.8. Significant changes over time were observed for the ATP-PCr energy system ($p < 0.000$) and the anaerobic glycolysis system ($p = 0.037$).

The contribution from the ATP-PCr energy system increased slightly, but not statistically significantly, from week 0 (baseline) to LC week 2 ($p = 0.421$; ES = 0.46; CI = -0.28; 1.17). A *moderate* significant change was observed at LC week 4 from baseline ($p = 0.045$; ES = 0.68; CI = -0.08, 1.39) and a *large* significant change from baseline to LC week 6 ($p = 0.005$; ES = 0.99; CI = 0.21; 1.72). *Moderate* decreases in the contribution from the anaerobic glycolysis energy system occurred from week 0 to LC week 2 ($p = 0.059$; ES = -0.62; CI = -1.33; 0.13), LC week 4 ($p = 0.432$; ES = -0.50; CI = -1.21, 0.24) and LC week 6 ($p = 0.279$; ES = -0.60; CI = -1.32; 0.14).
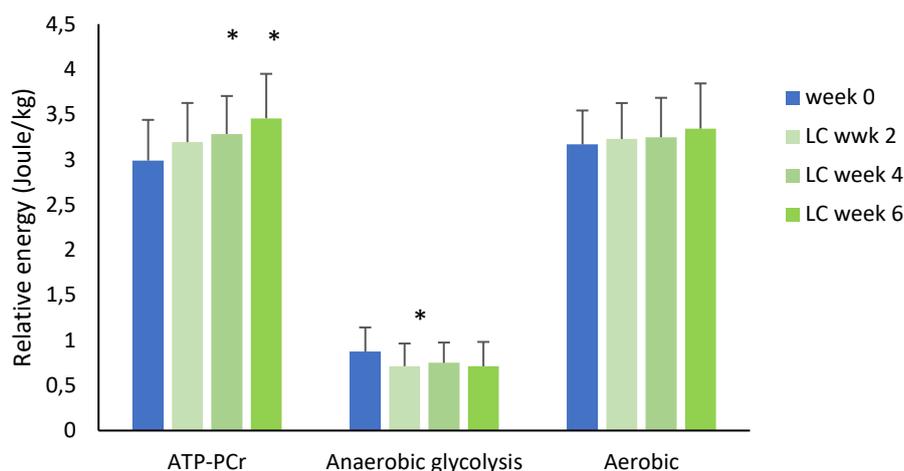

Figure 4.8: Relative energy system contributions in Joule per kg body weight (mean ± SD). Week 0: the mean of two baseline tests; LC week 2-6: test sessions in week 2, 4 and 6 on the LC diet. * Statistically significantly different from baseline (p < 0.05).

Figure 4.9 illustrates the standardized mean differences in energy system contributions relative to body weight from baseline (week 0) to LC week 2, 4 and 6.
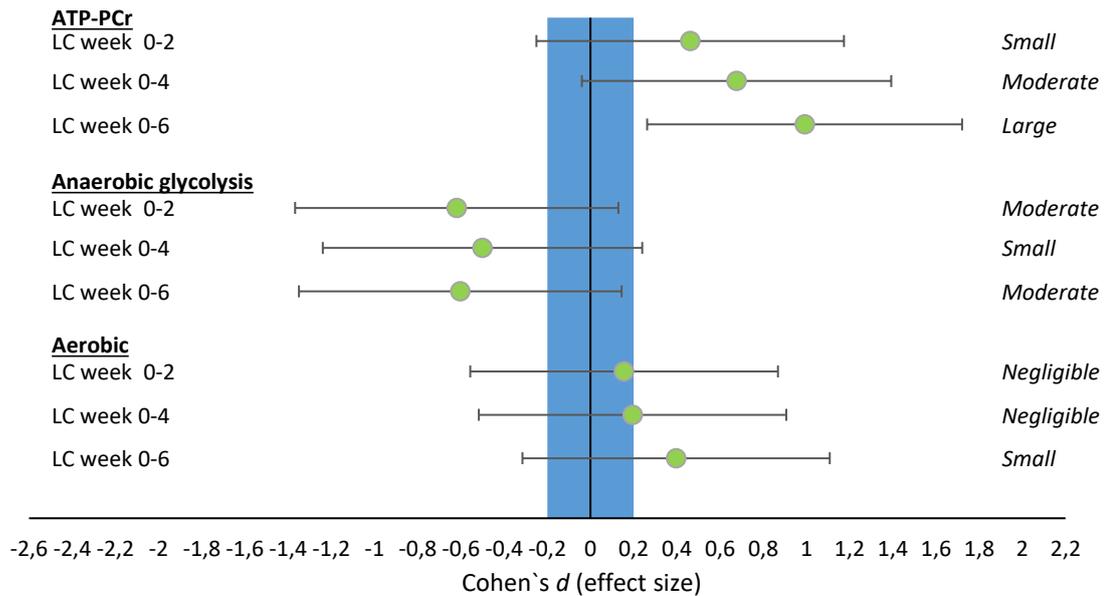
Figure 4.9: Standardized mean differences with 95% confidence intervals for energy system contributions relative to body weight, from baseline (week 0). The blue column represents the smallest worthwhile change (SWC) [-0.2, 0.2] LC, low-carbohydrate diet intervention.

4.2.4 Mean Power Output

No statistically significant changes in the mean power output (MPO) across sessions ($p = 0.917$), or across sprints ($p = 0.057$) occurred during the intermittent sprint test over the six-week LC intervention (Figure 4.10). Similarly, there was no significant sprints vs. time point (week) interaction effect ($p = 1.000$). A *moderate* decline in MPO was evident from sprint 1 to 6 (682.0 ± 176.51 W vs. 599.5 ± 137.36 W; $p = 0.091$; ES = -0.52; CI = -1.24; 0.22). No significant differences in RPE scores were recorded during the intermittent sprint tests at the different time points ($p = 0.875$).
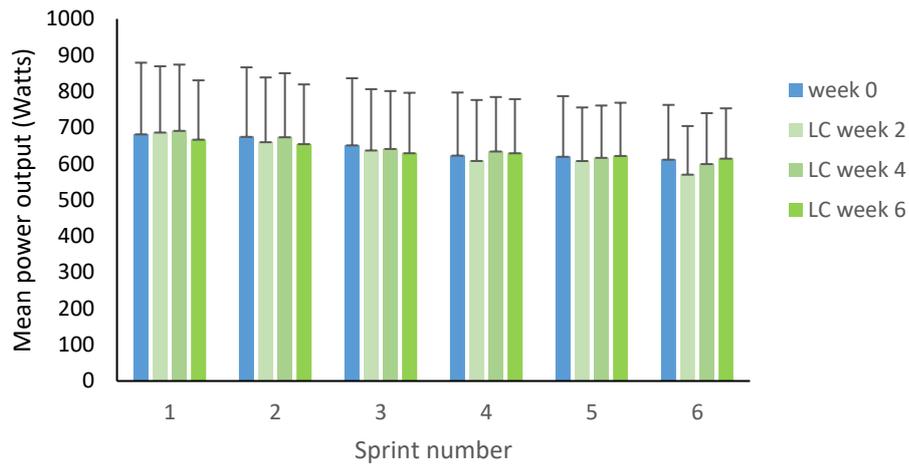
Figure 4.10: Mean power output (mean + SD) during the repeated sprints (n = 15). Week 0*, mean of two baseline tests on HD;* LC week 2-6*, test sessions at week 2, 4 and 6 on the LC diet.*

4.2.5 Intermittent sprints fatigue

Figure 4.11 depicts the percentage change in mean power output (MPO), expressed as a percentage decrement score (calculated according to Fitzsimons *et al*., 1993), for each intermittent sprint test and across the study period. The higher level of fatigue at LC week 2 was not statistically significant from baseline (week 0) (8.8 ± 5.12% vs. 7.4 ± 3.92%; p = 0.720; ES = 0.31; CI = -0.42; 1.02). However, a *very large* decrease in fatigue levels occurred from LC week 2 to LC week 6 (8.8 ± 5.12% vs. 5.7 ± 2.64; p = 0.066; ES = -1.17; CI = -1.91; -0.36). Overall, a *moderate* net decrease occurred in fatigue over the six-week LC intervention from week 0 to LC week 6 (7.4 ± 3.92% vs. 5.7 ± 2.64; p = 0.332; ES = -0.50; CI = -1.21; 0.24).
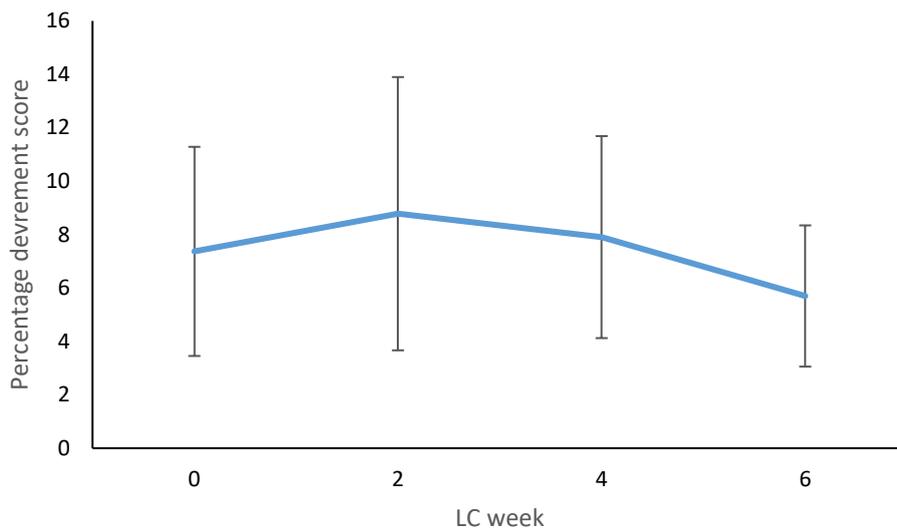
Figure 4.11: Intermittent sprint fatigue expressed as percentage decrement score (mean ± SD). Week 0 represents the mean value of the HD phase; LC week 2, 4 and 6 indicates intermittent sprints test sessions at week 2,4 and 6 on the LC diet.

Figure 4.12 summarizes the magnitude of changes in fatigue over the study period.
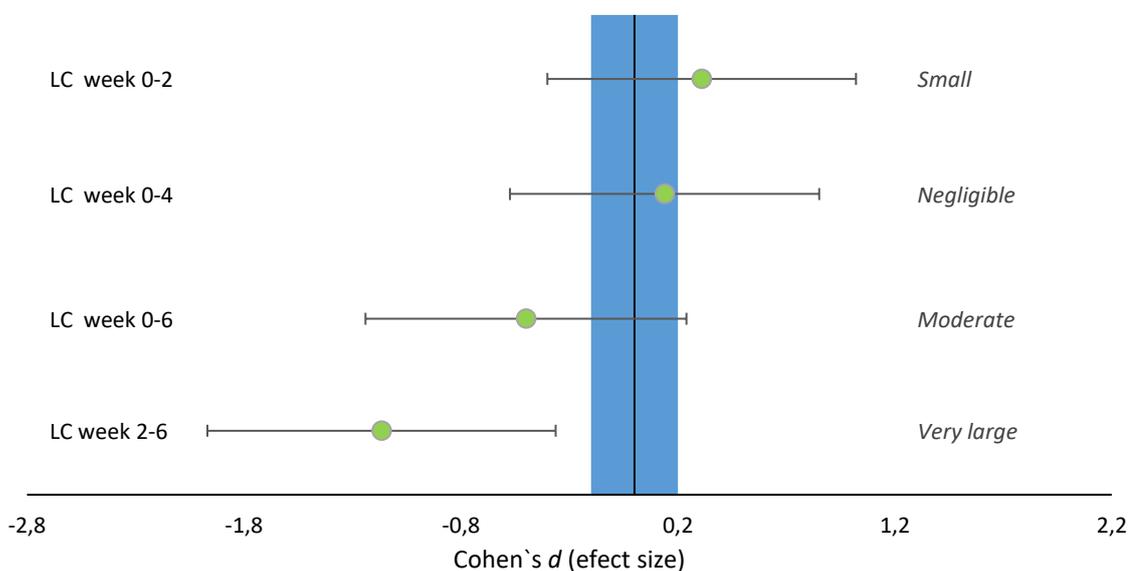
Figure 4.12: Standardized mean differences with 95% confidence intervals for changes in fatigue scores. The blue column represents the smallest worthwhile change (SWC) [-0.2, 0.2] LC, low-carbohydrate diet intervention.

4.3 Maximal aerobic capacity

Table 4.4 and Figure 4.4 illustrate the changes in maximal aerobic capacity parameters before and after the 6-week LC intervention. One participant's test did not meet the ACSM criteria for a maximal effort test and her data were excluded from this data set for all parameters. One outlier for power output @ AT (> 2 SD different from the group mean difference) was also removed, while another participant`s blood [lactate] data was excluded from the data set due to a faulty reading.

Small, but significant increases were observed in relative VO$_2$max (5.3 ± 5.66%; ES = 0.3216; CI = -0.43;1.06) and power output @ AT (12.1 ± 12.77%; ES = 0.43; CI = -0.36;1.19), while a *large* significant decrease was observed in peak blood [lactate] following the LC intervention (-19.1 ± 18.34%; ES = -0.99; CI = -1.77; 0.15).

Table 4.4: Changes in maximal aerobic exercise capacity over the 6- week LC intervention.

	Group (n=14)				
	Pre	Post	p-value	Relative change (%)	ES (95% CI)
Absolute VO$_2$max (mL.min-1)	3133.5 ± 641.55	3179.6 ± 657.32	0.302	1.5 ± 5.19	0.07 (-0.67; 0.81)
Relative VO$_2$max (ml.kg^{-1}.min^{-1})	40.5 ± 6.34	42.7 ± 7.14*	0.004	5.3 ± 5.66	0.32 (-0.43; 1.06)
Power output @ AT (Watts)	190.5 ± 47.64	211.8 ± 51.86*	0.002	12.1 ± 12.77	0.43 (-0.36; 1.19)
Power output @ RC (Watts)	243.5 ± 51.36	248.6 ± 48.12	0.304	2.7 ± 7.43	0.10 (-0.64; 0.84)
PPO (Watts)	271.6 ± 60.19	272.7 ± 54.48	0.772	1.0 ± 5.52	0.02 (-0.72; 0.76)
HR max (bpm)	191.3 ± 5.38	190.9 ± 5.38	0.965	0.1 ± 3.29	0.01 (-0.75, 0.78)
Peak [BLa] (mmol.L^{-1})	18.1 ± 4.45	14.3 ± 3.14*	0.005	-19.1 ± 18.34	-1.00 (-1.77; 0.15)
RQ max	1.10 ± 0.045	1.07 ± 0.072	0.170	-2.23 ± 5.93	-0.05 (-0.89, 0.79)
RPEmax	18 ± 3.33	17.9 ± 2.99	0.914	-0.46 ± 0.25	-0.05 (-0.89; 0.79)

VO$_2$max, maximal oxygen consumption; *AT*, aerobic threshold; *RC*, respiratory compensation point; *PPO*, peak power output; *HR*, heart rate; *bpm*, beats per minute; *[BLa]*, Peak blood lactate concentration; *RQ,* respiratory quotient; *RPE*, rating of percieved exhertion.

Figure 4.13 illustrates the group changes, as well as the individual responses in maximal aerobic capacity parameters. Eleven of the 14 participants improved their relative VO$_2$max following the 6-week LC intervention, while three participants

performed worse (Figure 4.13 A). Nine of the 14 participants showed an improvement in absolute VO_2 max, while five had lower values after the six weeks (Figure 4.13 B).

Power output at RC improved in 10 of the 14 participants (Figure 4.13 C), while power output at AT improved in 11 of 13 participants (Figure 4.13 D). Peak power output (PPO) at the end of the test improved in seven participants (Figure 4.13 E) and post-test peak blood [lactate] were lower for all participants (Figure 4.13 F).
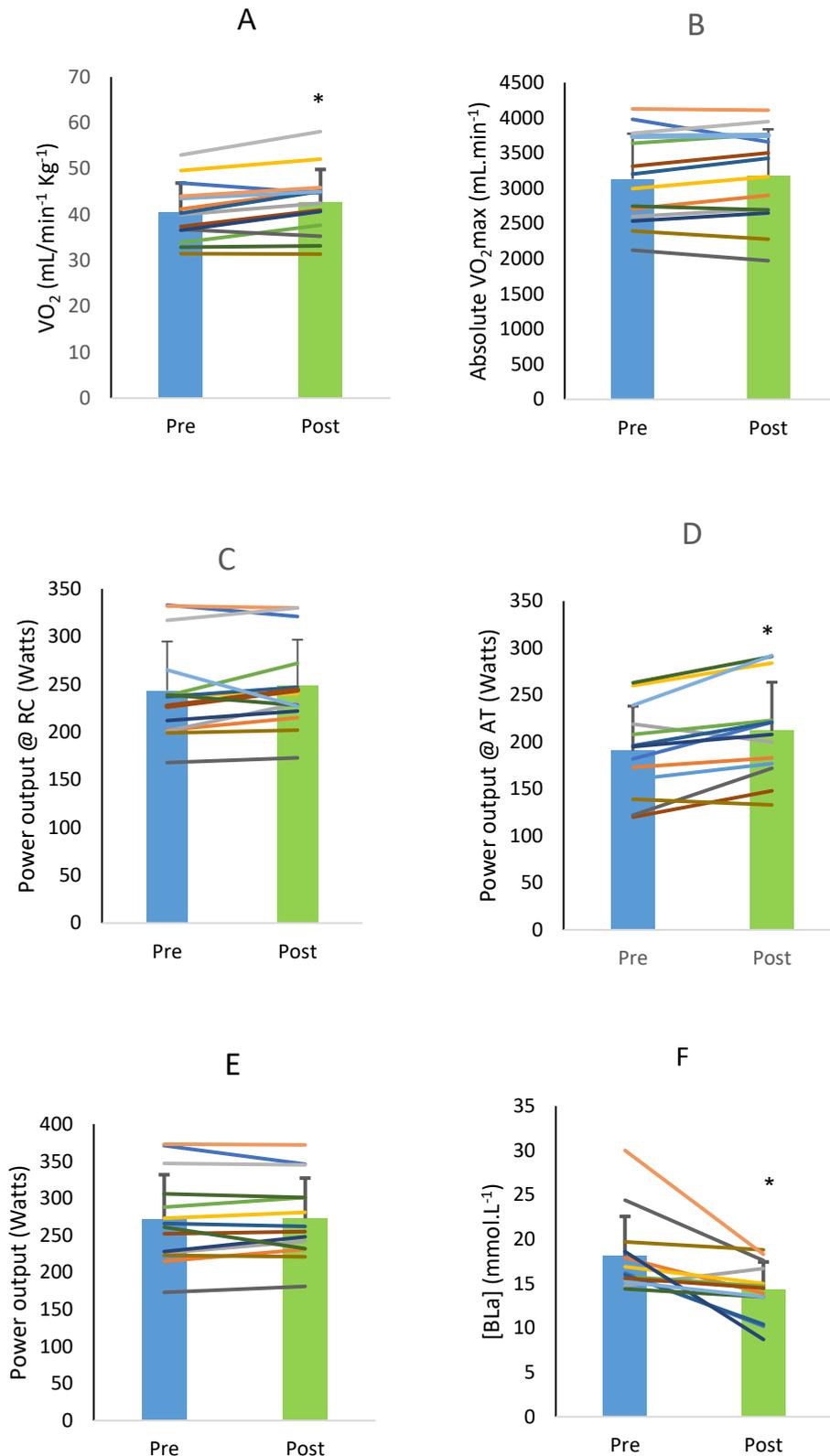
Figure 4.13: Changes in maximal aerobic capacity parameters from pre- to post six-week low-carbohydrate intervention (mean ± SD). (A) relative VO₂max, (B) absolute VO₂max, (C) power output at respiratory compensation point, (D) power output at anaerobic threshold, (E) peak power output, (F) peak blood [lactate]. *Significant changes (p< 0.01) from pre-test.

4.4 Effect of changes in diet and VO$_2$ max

There was no meaningful correlation between the change in absolute VO$_2$max from pre- to post 6-week LC intervention, and the change in CHO intake (from HD to LC) (r = -0.071; CI = -0.563; 0.458) or change in fat intake (r = 0.062; CI = -0.465; 0.557). However, a *very large* negative correlation (r = -0.702; CI = -0.893; -0.296) was found between changes in protein intake and changes in absolute VO$_2$ max.

Figure 4.14 illustrates the correlation between change in absolute VO$_2$max and changes in the three macronutrient intakes from the HD to the LC phases derived from the meal logging weeks.
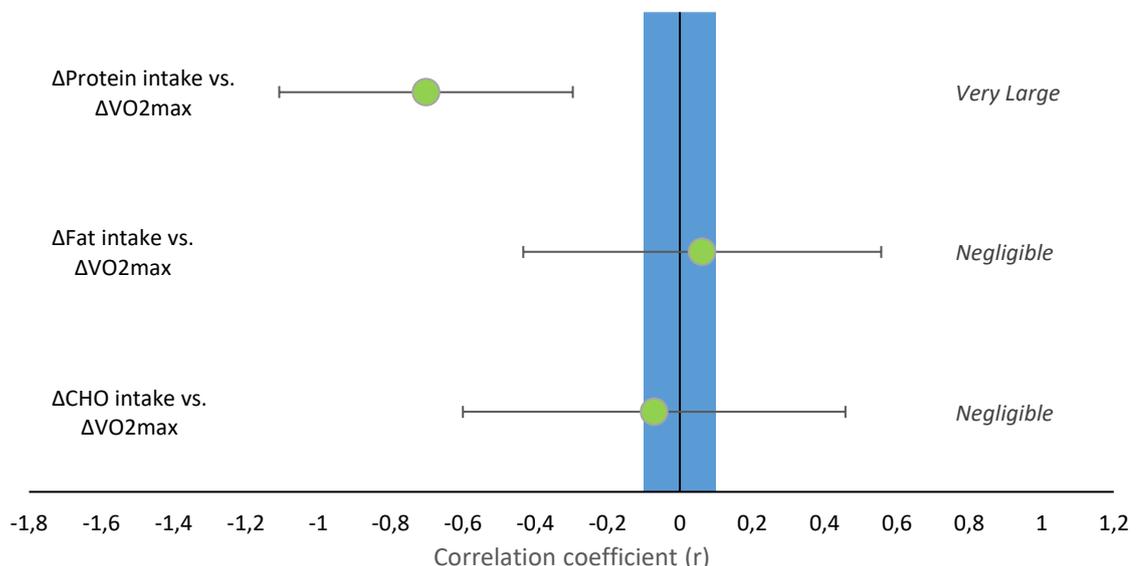
Figure 4.14: The relationships between macronutrient intake changes (Δ) from habitual diet to low-carbohydrate diet and changes in absolute VO$_2$ max from pre- to post intervention, illustrated Pearson`s r $\pm$ 95% confidence intervals. The blue column indicates the smallest meaningful correlation [-0.1, 0.1].

4.5 Overview of findings

4.5.1 Descriptive characteristics; Secondary aims

Secondary aim # 1: To quantify the nutritional intake of healthy, active individuals during the adoption of a self-selected LC dietary plan.

The participants adhered to the dietary requirements and restricted CHO intake to < 50 g/day. However, protein intake was higher than the desired 20% of daily energy intake.

Secondary aim # 2: To monitor blood [ketone] levels of the participants during their adoption of a self-selected LC dietary plan.

The significant reduction of CHO intake brought about significant elevations in blood [ketones] during the LC intervention.

Secondary aim # 3: To monitor the wellness of the participants during their adoption of a self-selected LC dietary plan.

A *moderate* improvement in overall wellness scores were observed throughout the LC intervention.

4.5.2 Outcomes of hypothesis; Primary aims

Primary aim # 1: To quantify the changes in the contributions of the metabolic energy systems in healthy active individuals during intermittent sprint cycle exercise throughout a 6-week self-selected CHO restricted eating plan (< 50 g/day).

> *H_1: It is hypothesised that adaptation in the phosphocreatine energy system will occur, so that energy production from this pathway will increase throughout the LC diet intervention during maximal intensity intermittent cycle exercise.*

H_0 for this hypothesis could be rejected, since a significant increase in ATP-PCr energy system contribution was indeed found.

> *H_2: It is hypothesised that restriction of fuel for anaerobic glycolysis on a LC diet will down-regulate this energy system during maximal intensity intermittent cycle exercise.*

H_0 for this hypothesis could be rejected, since significant down regulation occurred in the anaerobic glycolytic energy system.

> H_3: It is hypothesised that adaptation in aerobic fat metabolism will occur, so that energy production from this pathway will increase throughout the LC diet intervention during maximal intensity intermittent cycle exercise.

H_0 for this hypothesis could not be rejected, since no significant change in aerobic energy production occurred following the LC intervention.

Primary aim # 2: To determine whether 6-week CHO restriction affects the intermittent sprint cycle performance (fatigue and MPO) of healthy active individuals.

> H_4: It was hypothesised that participants' fatigue and sprint MPO performance would follow a supercompensation curve (Cole, 1998).

The net *moderate* decrease in fatigue at the 6-week timepoint was not statistically significant, thus H_0 for this hypothesis could not be rejected.

Nonetheless, although the final *moderate* improvement after the initial decline was not significant., the changes in the performance variables still reflected the hypothesised supercompensation time course.

Primary aim # 3: To determine if changes occur in maximal aerobic capacity and aerobic exercise metabolism in response to a 6-week LC diet.

> H_5: It is hypothesised that relative substrate utilization will notably shift towards greater fat oxidation, with a consequent reduced reliance on anaerobic CHO metabolism, and endurance performance (PPO) will be maintained.

H_0 for this hypothesis could be rejected. A clear metabolic shift towards greater reliance on aerobic fat metabolism and reduced reliance on CHO metabolism were observed in significantly lower peak blood lactate and increased power at AT, accompanied by a significant increase in relative VO_2 max.

However, the 6-week intervention was indeed only sufficient time to achieve maintenance of peak power output performance. Therefore, the 6-week LC

intervention still proved insufficient time for the absolute increases in rate of aerobic ATP production to exceed anaerobic glycolysis rates of the habitual diet. Thus, this metabolic shift did not yet result in a performance improvement (peak power output).

Chapter 5
Discussion

5.1 Introduction

In most field and court sports, the ability to repeatedly produce a high-power output or sprint speed is an important component of performance. Based on his review of the literature on time-motion analysis in field-based team sports, Spencer *et al.* (2005) found mean sprint distances of 10 – 20 m (2 – 3 s) and six to seven bouts best represent field-based team sports. During these intermittent sprint type activities, a unique fuel metabolism pattern was evident:

Anaerobic glycolysis has been shown to be down regulated during the later sprints in these types of protocols (Bogdanis *et al.*, 1996; Gaitanos, 1993; McCartney *et al.*, 1986; Parolin *et al.*, 1999; Trump *et al.*, 1996), to enable the athlete to sustain metabolic homeostasis and prolong the onset of fatigue (Parolin *et al.*, 1999). The present study further hypothesised that, through additional restriction of CHO, up-regulation of the ATP-PCr and aerobic metabolic pathways would be stimulated during all-out effort sprint exercise. If true, a low-carbohydrate (LC) diet poses promising potential as a sport-specific nutritional intervention for this type of exercise.

Although a high-intensity "30-15 Intermittent Fitness Test" has recently been utilized in two LC interventions (Cipryan *et al.*, 2018; Dostal *et al.*, 2019), these 30 s shuttle runs are still not representative of the proposed short sprint movement patterns in field-based sports (Spencer *et al.*, 2005). Therefore, the present study is, to my knowledge, the first to investigate the effect of an LC intervention on energy metabolism and performance during intermittent maximal intensity sprints as exercise modality.

Furthermore, although the adaptations in the aerobic- and glycolytic energy pathways with LC interventions were computed in previous research (Cipryan *et al.*, 2018; Dostal *et al.*, 2019; Fisher *et al.*, 1983; Phinney *et al.*, 1983; Vogt *et al.*, 2003; Volek *et al.*, 2016; Waldman *et al.*, 2018; Zajac *et al.*, 2014), the current study is also the first to quantify and monitor adaptation in the phosphocreatine (ATP-PCr) energy system in response to a LC intervention.

Lastly, most LC intervention studies in sport consisted of only pre- and post-intervention measurements (Carr *et al.*, 2018; Fleming *et al.*, 2003; Greene *et al.*, 2018; Lambert *et al.*, 1994; McSwiney *et al.*, 2017; Paoli *et al.*, 2012; Zajac *et al.*, 2014), while investigations on the time course of the adaptation processes through repeated measures are currently limited (Dostal *et al.*, 2019; Phinney *et al.*, 1983; Prins *et al.*, 2019; Waldman *et al.*, 2018). The current study employed a time-series design with measures at 2-weekly intervals over the 6-week LC period. Two baseline tests, while participants followed their habitual diet, were included. These baseline measurements were used as the control period.

Hence, the purpose of this study was to investigate the effect of a 6-week self-selected LC dietary eating plan on the metabolic adaptations and physical performance of healthy, active men and women during all-out effort intermittent sprint exercise.

5.2 Study design, sample size and statistical considerations

To optimize commitment and compliance to the 6-week LC diet, self-selected sampling was employed. This sampling method, however, may result in large variation in physical characteristics among participants. For instance, mean relative VO_2 max values of 40 - 42 $mL^{-1}.kg^{-1}.min$, indicative of unfit to moderately fit participants, might have been influenced by diversity in sex, body composition and genetics. In this regard, the SD of 6.34 at pre-test and 7.14 at post-testing and a range of 31.5 – 53 $mL^{-1}.kg^{-1}.min$ (additional to reported Results), is testament to the large variability in aerobic fitness levels of the participants in this study. Accordingly, it was decided that each participant would act as their own best control. Furthermore, since adaptations in the ATP-PCr system is known to be highly irreversible with detraining (Ross & Leveritt, 2001), a wash-out period may similarly not be effective to reverse these adaptations if stimulated by a LC diet. Consequently, it was decided that a crossover design would also not deem suitable for the purpose of this investigation. Therefore, a single group, time-series study design was selected. Chrzanowski-Smith *et al.* (2020) suggested repeated control condition measurements to calculate the typical error of measurement (or the within-

participant SD). Accordingly, two baseline tests, two weeks apart on their habitual diet was employed. Thus, each participant acted as his/ her own control. Moreover, due to the particular influence of individual variation in physical characteristics and unknown durations of wash-out periods of physiological adaptations, single group study designs is highly regarded in the field of exercise physiology (Chrzanowski-Smith *et al.*, 2020). Accordingly, study designs where within-participant changes are measured, are commonly employed in LC studies investigating exercise performance (Fisher *et al.*, 1983; Heatherly *et al.*, 2018; Phinney *et al.*, 1983, 1980; Urbain *et al.*, 2017; Waldman *et al.*, 2018).

In order to mitigate the possibility of a learning-effect, a familiarization session was also employed. In this regard, Glaister *et al.* (2007) showed that only one familiarization session is required to account for the influence of a learning-effect, whereafter repeated sprints results are reliable.

To my knowledge, only one investigation on the effects of a LC intervention in sport achieved a greater sample size than the current investigation (Urbain *et al.*, 2017). The final sample size (n = 15) in this study was larger than in previous sport studies which ranged from five to 14 participants (Burke *et al.*, 2017; Cipryan *et al.*, 2018; Dostal *et al.*, 2019; Fisher *et al.*, 1983; Fleming *et al.*, 2003; Greene *et al.*, 2018; Heatherly *et al.*, 2018; Kephart *et al.*, 2018; Lambert *et al.*, 1994; Langfort *et al.*, 1997; McSwiney *et al.*, 2017; Paoli *et al.*, 2012; Phinney *et al.*, 1983; Phinney *et al.*, 1980; Prins *et al.*, 2019; Vogt *et al.*, 2003; Volek *et al.*, 2016; Waldman *et al.*, 2018; Webster *et al.*, 2016; Zajac *et al.*, 2014). It is speculated that the practical challenges and the costs involved in administering LC interventions in sport research may be a common experience.

Effect size statistics were reported in conjunction with traditional p-values, to mitigate the effects of small sample sizes on inferential statistics. However, the fact that the group results were analysed for men (n = 7) and women (n = 8) combined, also affected the effect size statistics. Sex differences in performance, energy metabolism and energy expenditure measures resulted in large baseline performance variation (large SD`s). It is thus very likely that these inter-individual differences affected the magnitude of changes in outcome variables, and thus decreased the likelihood of *moderate* or *large* effect sizes. However, since the

investigation of sex differences were not one of the aims of this study, only group results were reported in this thesis.

5.3 Low-carbohydrate diet implementation

The present study aimed to obtain a "real-life" view of the effects of a practically implementable LC intervention in recreationally active men and women. Considering that meal-logging may add additional strain to everyday life, and shorter meal-logging periods were previously utilized (Greene *et al.*, 2018; Kephart *et al.*, 2018), the current participants logged their daily food and drinks intake for two, two-week cycles. Furthermore, although some previous investigations employed recall food diaries (Moshfegh *et al.*, 2008), real-time meal-logging is considered a more reliable research method (Costello *et al.*, 2017) and also commonly employed (Dostal *et al.*, 2019; Greene *et al.*, 2018). Therefore, the participants used real-time meal-logging through a convenient Google-form on their Smartphones, to optimize reliability within the real-life context. Participants were furthermore provided with an electric kitchen scale for accurate measurements of food logging. Whenever participants encountered days where prior meal preparation could not be performed at home, they carried the scale with them to still accurately measure foods. Participants were first required to maintain their personal habitual diet, without intentionally increasing CHO intake (the baseline / control phase). According to the Food-based dietary guidelines for South Africa (Vorster *et al.*, 2013) the traditional South African diet consists of higher fat and protein intake and lower CHO contents than the "traditional high-CHO" diet, where > 50% of total daily energy comes from CHO (Burke *et al.*, 2018). Accordingly, the baseline habitual diet of the participants in this study, consisting of 35% CHO, 45% fat and 20% protein (Figure 4.1) did not reflect a traditional high-CHO diet, however, is probably reflective of a typical South African diet. The nutritional intervention in this investigation can consequently be considered in line with the aim of obtaining a "real-life" view of the habitual and self-adopted LC eating plans in the South African context. However, the higher fat and lower CHO intake on the current participants` habitual diets, may have lowered the margin for adaptation, since their habitual diet may have already stimulated adaptations in fat metabolism.

LC interventions in sport have previously been safely employed in non-elite recreationally active individuals, similar to the present study population (Cipryan *et al.*, 2018; Dostal *et al.*, 2019; Fleming *et al.*, 2003; Prins *et al.*, 2019; Urbain *et al.*, 2017). In order to mimic a practically implementable "real-life" LC intervention, a quasi-experimental design was employed which did not enforce a specific meal plan on the participants. Rather, participants received clear guidelines on how to adopt the LC diet (see Methodology section 3.3). This self-selected approach to an LC dietary intervention is a commonly performed method in previous sport studies (Cipryan *et al.*, 2018; Dostal *et al.*, 2019; Greene *et al.*, 2018; Kephart *et al.*, 2018; McSwiney *et al.*, 2017).

The macronutrient targets for the participants in the present study were based on the defined criteria for a ketogenic low-carbohydrate, high-fat (k-LCHF) intervention, consisting of > 75% fat, 15 - 20% protein and < 50 g/day CHO (Burke *et al.*, 2018). These prescribed targets was consistent with other LC studies in athletes (Cipryan *et al.*, 2018; Dostal *et al.*, 2019; Greene *et al.*, 2018; McSwiney *et al.*, 2017). The participants adhered to the CHO restriction throughout the 6-week intervention (26.4 ± 9.36 g/day). However, during the LC phase the participants' diets were characterised by higher protein intake and lower fat intake; therefore this nutritional intervention does not strictly qualify as a k-LCHF diet (Burke *et al.*, 2018). Nonetheless, the higher protein intake is consistent with numerous LC interventions in previous sport studies, where the participants also did not attain the required macronutrient composition to qualify as k-LCHF interventions (Cipryan *et al.*, 2018; Fleming *et al.*, 2003; Lambert *et al.*, 1994; Langfort *et al.*, 1997; Paoli *et al.*, 2012; Prins *et al.*, 2019). Some researchers reported protein to fat ratios up to 53% fat; 15 % protein (Vogt *et al.*, 2003), 50% fat and 45% protein (Langfort *et al.*, 1997), 55% fat, 42% protein (Paoli *et al.*, 2012), 61% fat and 39% protein (Fleming *et al.*, 2003). Thus, the participants in the current study adopted an LC diet that was closer to a proper k-LCHF diet (7% CHO, 66% fat, 28% protein) (Figure 4.1).

Similar to previous studies (Cipryan *et al.*, 2018; Kephart *et al.*, 2018), the participants in this study decreased their total daily energy intake. This may probably be attributed to the higher protein and fat intake with CHO restriction, which reportedly increases satiety (Mirtschin *et al.*, 2018; Waldman *et al.*, 2018).

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Evidence also exists that ketone bodies modify ghrelin and leptin hormone levels during LCHF eating (Sumithran *et al.*, 2013), which is known to induce appetite suppression. Therefore, it is conceivable that the increase in circulating ketone bodies during the LC phase may account for the lower self-selected energy intake of the participants in this investigation.

Significant reductions in CHO intake, as in the present study, is also generally associated with lower circulating insulin levels (Langfort *et al.*, 1997; Ludwig & Ebbeling, 2018; Shimy *et al.*, 2020). Lower circulating insulin is furthermore associated with a reduction in hunger and food cravings (Ludwig & Ebbeling, 2018; Shinny *et al.*, 2020), hence posing another possible explanation for the reduced energy intake of the individuals in the current study.

Although, no clear negative impact of lowered total energy intake was evident in the present exercise performance results, this possibility can nonetheless not be excluded.

Although it was emphasised to participants that weight loss was not the aim of the study, *small* significant changes in body mass, body fat percentage and a *negligible* reduction in fat free mass, occurred during the LC intervention (Table 4.2). Likewise, body- and fat mass reductions is a common finding in self-selected LC interventions in recreationally active and well-trained populations (Cipryan *et al.*, 2018; Dostal *et al.*, 2019; Greene *et al.*, 2018; Kephart *et al.*, 2018; McSwiney *et al.*, 2017). In some of these studies, participants experienced reductions in body mass and fat mass, despite increased or unchanged total energy intake (Dostal *et al.*, 2019; Greene *et al.*, 2018; McSwiney *et al.*, 2017). It can thus be deduced that weight loss during LC interventions is not caused by lowered energy intake, but rather reduced circulating insulin levels, since insulin is an anabolic hormone and high blood insulin levels are associated with greater fat storage.

5.3.1 Blood ketone response during CHO restriction

The *very large* increases in blood [ketone] levels from baseline to the LC intervention in the present study, suggests that CHO restriction served as the metabolic stimulus that accounted for the observed metabolic and performance changes in the

participants. The participants' mean blood [ketone] of 0.8 ± 0.47 mmol.L^{-1} during the LC phase, were significantly higher than at baseline (0.3 ± 0.09 mmol.L^{-1}). These levels were similar, or higher, than eight previous sport-LCHF studies (Cipryan *et al.*, 2018; Fleming *et al.*, 2003; Greene *et al.*, 2018; Heatherly *et al.*, 2018; McSwiney *et al.*, 2017; Prins *et al.*, 2019; Webster *et al.*, 2016; Zajac *et al.*, 2014), while only three studies reported higher mean blood [ketones] (Langfort *et al.*, 1997; Phinney *et al.*, 1983, 1980). It should, however, be noted that the blood ketone levels of 2.7 ± 0.33 mM in the study by Phinney *et al.* (1980) occurred while participants followed a protein-supplemented fast (< 10 g CHO; 1.2 g of protein per kg ideal body weight). This is a severe form of CHO restriction that cannot be compared to the diets of participants in the current study (< 50 g CHO per day).

It was observed that the blood [ketone] levels of the group peaked at week three of the LC intervention, where after it dropped in the following weeks, although maintaining higher values than at baseline (Figure 4.2). Similar tendencies were reported previously (Dostal *et al.*, 2019; Kephart *et al.*, 2018). Dostal *et al.* (2019) suggested that an enhanced cellular uptake of ketones from the blood could explain the lower blood [ketone] levels during later weeks of CHO restriction. This may also be indicative of enhanced ketone utilization as a primary fuel source during rest and exercise, which would count for a positive metabolic adaptation. A greater contribution from ketones for energy metabolism may further explain a consistent finding in the literature, namely a reduction in the dependence on CHO as fuel for exercise (Fisher *et al.*, 1983; McSwiney *et al.*, 2017; Phinney *et al.*, 1983; Volek *et al.*, 2016; Webster *et al.*, 2016; Webster *et al.*, 2017).

Additionally, the direct and positive action of ketone metabolism on mitochondrial and anti-oxidant health through altered gene expression is also widely reported (Gano *et al.,* 2014; Miller *et al., 2018;* Newman & Verdin, *2014;* Rojas-Morales *et al.*, 2016; Stafford *et al.*, 2010; Veech, 2004; Veech *et al.*, 2017; Vidali *et al.*, 2015). These adaptations, in response to CHO restriction, suggest that the participants in the current study may have undergone additional metabolic changes, which could have contributed to the observed adaptations in exercise metabolism, but which was not measured.

5.4 Wellness during the LC diet phase

Participants reported higher overall wellness scores during the LC phase compared to the HD phase. This is in agreement with Zinn *et al.* (2017) who examined wellbeing qualitatively through semi-structured interviews in five New Zeeland endurance athletes (4 women, 1 man) over a 10-week LCHF intervention. All the athletes in this case-study reported improved well-being and recovery during the CHO restriction period.

There could be multiple reasons for this enhanced rating of wellness, which did not form part of the current study. It is therefore speculated whether it may be related to the significantly higher blood [ketone] that was measured during the LC phase. The positive effects of nutritional ketosis on the brain are well documented, specifically in the case of epilepsy (Bough *et al.*, 2006; Huttenlocher *et al.*, 1976; Kossoff *et al.*, 2004) and Alzheimer`s disease (Pinto *et al.*, 2018), but also in other neurodegenerative diseases and neurological disorders (Bough *et al.*, 2006; Dimitrova *et al.*, 2017; Gano *et al.*, 2014; Paoli *et al.*, 2014).

In a medical hypothesis paper, Brown (2007) stated that ketone bodies are isomers of the notorious drug of abuse Ɣ-hydroxybutyrate (GHB). It was proposed that this metabolite is responsible for the subjective feelings of euphoria reported during LC diets and fasting. Although Brown (2007) did not conduct an experiment to quantitatively support his hypothesis, it is possible that the current study lends support for his proposal.

Fasting is another nutritional intervention associated with high levels of circulating blood ketone levels. In psychiatry research, "modified fasting" has been shown to improve subjective well-being, increased vigilance and mood and it was also sometimes associated with euphoria (Fond *et al.*, 2013). The latter review stated that patients with diagnosed mood disorders reported reductions in depressive symptoms, improvement in mood, alertness and a sense of tranquillity during fasting.

Furthermore, evidence exist that increases in sympathetic hormones and neurotransmitters occur when CHO intake is restricted (Langfort *et al.*, 1997; Michalsen *et al.*, 2003). The increases in dopamine and catecholamine production

have been proposed as another mechanism behind the positive effects of fasting or CHO restriction on mood (Michalsen *et al.*, 2003).

5.5 Rationale behind the intermittent sprint protocol

An intermittent sprints protocol of 6 x 10 s WAnT sprints at 2 min recovery was selected for this study. The intermittent sprint protocol was specifically selected to optimize conditions for investigation of the energy system contributions, in a controlled laboratory setting with gold standard measurement equipment. Towards this aim, sport-specificity was somewhat compromised in that the cycle-based protocol consisted of somewhat longer sprints and longer recovery periods than movement patterns in field- and court sports. The following theoretical and practical considerations, form the basis for the specific protocol:

1. **Sprint duration: 10 s**

According to the energy continuum model, 10 s sprints are approximately the duration where the ATP-PCr energy system will be depleted during maximal intensity exercise. Since it was hypothesised that the nutritional intervention will stimulate adaptations in the ATP-PCr energy system, 10 s sprint durations were selected to utilize this energy system to its maximal capacity in order to detect possible adaptation in this parameter.

Furthermore, due to flywheel inertia, PPO was only reached at about 10 s during a WAnT sprint from a static start.

2. **Recovery duration: 2 min**

The re-phosphorylation rates of Cr during post-exercise recovery phases are still unclear. Bogdanis *et al.* (1995) reported PCr levels that were depleted to roughly 20% of resting levels after sprints, raised to only 65% following 90 s recovery and 85% following 6 min recovery, while Gaitanos (1993) reported incomplete PCr resynthesis with 30 s rest periods between 6 s sprints. Parolin *et al.* (1999) on the other hand, observed almost complete PCr resynthesis after 4 min of rest between 30 s maximal sprints.

Although precise estimations of re-phosphorylation duration cannot be derived from the literature, it could be estimated that 2 min recovery durations between 10 s WAnT sprints would allow for incomplete re-phosphorylation of Cr.

Furthermore, 2 min recovery periods were chosen to allow enough recovery to encourage participants to deliver all-out efforts at intensities that would adequately tap into the ATP-PCr energy system. Shorter recovery periods may have discouraged some participants to produce maximal efforts.

Thus, it was argued that 2 min recovery periods would result in incomplete PCr recovery, yet allow for the observation of possible adaptations, while also provide just enough motivation for participants to give their best efforts during each sprint.

3. **Number of sprints:** Six

   a. Based on his review of the literature on time-motion analysis in field-based team sports, Spencer *et al.* (2005) reported that an intermittent sprint protocol with six to seven bouts best represent field-based team sports. Even though this study did not specifically focus on team sports, using a protocol that are approximately representative of these sports may add practical relevance to the results.

   b. To avoid excessive discomfort caused by fatigue, the training status of our healthy, active but non-elite athletic study population was also taken into account. Hence, the number of repetitions was restricted.

   c. During the pilot tests, it was observed that visible drops in power output performance occurred across 6 sprint repetitions. Therefore, there was a good possibility that changes in fatigue and performance across the intervention period would be observed.

4. **Active or passive recovery:**

   In order to accurately quantify the metabolic interactions of intermittent sprint exercise, passive recovery ensures that the metabolic responses can only be attributed to the sprint exercise. For this reason, even though performance during intermittent sprints are better with active recovery (Signorile *et al.*, 1993), studies that investigated metabolism during intermittent sprints consistently utilized passive recovery protocols.

5.6 Energy system calculation methods

The equations and methods employed in the current study to quantify the three energy systems have previously been employed in various sport and exercise modalities (Beneke *et al.*, 2004; Beneke *et al.*, 2002; Davis *et al.*, 2014; Julio *et al.*, 2019; Julio *et al.*, 2017; La Monica *et al.*, 2020; Zagatto *et al.*, 2016). These calculations are based on the original investigation by Margaria et al. (1933) where direct measurement of muscle metabolites (such as muscle glycogen, PCr and Cr) were obtained through muscle biopsies before and after exercise tests. These direct measures were then mathematically integrated with measures of VO_2 and [BLa] in order to develop equations that provided energy output from each energy pathway only from VO_2 and [BLa] as input variables. These methods were later reappraised, and the mathematical calculations were revised based on experiments using more recent technologies (Di Prampero & Ferretti, 1999).

It was proposed that different phases of the exponential curve fitted to post-exercise VO_2, represents restoration of different metabolic fuel sources. It was observed that the "fast-component" of the post-exercise exponential VO_2 curve is indicative of PCr replenishment from Cr. "Fast-component" VO_2 is consequently used to indirectly calculate the ATP-PCr contribution during the preceding exercise bout.

While calculation of the aerobic- (Equation 3.2) and anaerobic glycolysis (Equation 3.3) energy systems were clear (Beneke *et al.*, 2004, 2002; Davis *et al.*, 2014; Julio *et al.*, 2019, 2017; La Monica *et al.*, 2020; Zagatto *et al.*, 2016), minimal detail on the precise mathematical procedures for the determination of the "fast-component" VO_2 are reported in previous publications. Moreover, no other investigation has, to my knowledge, employed intermittent cycle sprints as exercise modality. Based on these uncertainties, a test of concept trial was conducted to test the mathematical procedures. Data sets were collected from 10 healthy active volunteers to test various mathematical options. Mathematical modelling, using MATLAB® software was performed by a qualified engineer. Various exponential curve fitting combinations were applied and corresponding goodness of fit (GOF) statistics for each method were calculated.

The algorithm used was Trust-Region with set upper and lower coefficient constraints. Mono- and bi-exponential curves were fitted to all plots. Based on GOF outcomes (R-squared mean ± SD), bi-exponential fits proved to exhibit more accurate fits than mono-exponential models (bi-exponential vs. mono-exponential; 0.906 ± 0.057 vs. 0.876 ± 0.058; p = 0.000). It was therefore decided that the bi-exponential curves displayed more accurate fits for the post-bout VO_2 curves. Robust (Bisquare)- and Default MATLAB curve fitting settings were alternately applied. Unsmoothed and 10-point moving average options were also alternately applied and compared. GOF (R-squared) outcomes of the three best fitting methods are presented in Appendix G, **Error! Reference source not found.**.

From these various mathematical combinations, goodness of fit statistics finally revealed that the most accurate fit was the Trust-Region algorithm with set upper and lower coefficient constraints, in the Robust Bisquare setting, smoothed with a 10-point moving average. Figure 5.1 shows the exponential plot for one intermittent sprints session using these settings.
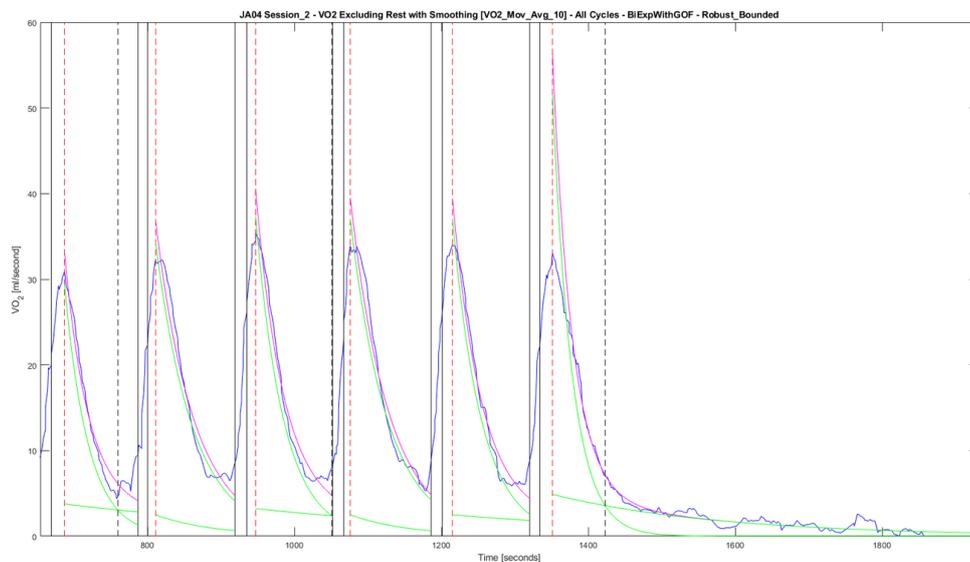
Figure 5.1: Bi-eksponential Robust_MA plots on smoothed VO_2 data during a 6 x 10 s intermittent sprint session. Black dashed lines denote the end of the fast component for VO_2 at the two exponential plots intercept.

5.7 Adaptations in the energy systems

5.7.1 Overview of outcomes

Previously, the absolute energy contributions (in Joule) and percentages of total chemical energy output were popular methods to report the energy system contributions (Campos *et al.*, 2012; Julio *et al.*, 2019, 2017; Milioni *et al.*, 2017; La Monica *et al.*, 2020). A few researchers also reported the energy system contributions relative to body mass (Beneke *et al.*, 2004; Smith & Hill, 1991). Each method highlights a different aspect of the nature of adaptations in energy systems. In this regard, absolute outcomes revealed changes in the total energy contribution across the intervention period, while relative outcomes were also reported to account for individual- and sex differences in muscle mass and consequent power / energy outputs. Finally, by reporting energy system contributions as a percentage of the total energy output per session, the effects of subconscious pacing strategies on absolute power- and energy outputs could be accounted for. Shifts in fuel metabolism could therefore be reflected, irrespective of fluctuations in total energy output.

A significant increase in absolute ATP-PCr energy system contribution was observed over the 6-week LC phase. This significant improvement is regarded as the primary and novel finding of the present investigation. The desired metabolic shift for intermittent sprints was further evident in the significant down-regulation in absolute anaerobic glycolytic energy contribution, which was observed after only two weeks of the LC intervention. These significant changes in absolute energy system contributions were evident even before applying mathematical corrections for within- or between-participant differences in energy output.

To address within-participant fluctuations in total power / energy outputs across sessions, the outcomes were also reported as percentage contributions to total energy output. Not surprisingly, these outcomes magnified the changes observed in absolute energy system shifts. In this respect, a *large* 2.1 ± 2.62% increase in percentage ATP-PCr energy system contribution was already evident after only two weeks of the LC diet, where after further adaptation in this energy system resulted in *very large* and significant increases at LC week 4 and LC week 6, compared to baseline measurements (HD control diet phase). Moreover, a *large* drop in the

percentage contribution of the anaerobic glycolytic energy system was evident at LC week 2 and 4, compared to baseline. Finally, by the end of the 6-week LC intervention, the percentage contribution of anaerobic glycolysis was down-regulated significantly by a *very large* 3.0 ± 2.91%.

To account for between-participant body mass related differences in total power- and energy output, a mathematical correction was applied by reporting energy system contributions relative to body mass. The ATP-PCr energy system increase revealed a *small* change at the 2-week LC timepoint, while a *moderate* and significant change was evident at the 4-week LC timepoint. By the end of the 6-week LC intervention, adaptation resulted in a *large* significant improvement in the ATP-PCr energy system contribution relative to body mass (Figure 4.10 - 4.11).

With regards to the contribution of the anaerobic glycolysis energy system, it should be noted that multiplication with body mass was already incorporated in the equation (Equation 3.3). Thus, a smaller effect was observed in the anaerobic glycolytic energy system, compared to the other energy systems, when expressed relative to body mass. Still, *moderate* decreases in the contribution of this energy system was evident at all LC time points compared to the habitual diet control measurements.

5.7.2 ATP-PCr energy system adaptations

> *H_1: It is hypothesised that adaptation in the phosphocreatine energy system will occur, so that energy production from this pathway will increase throughout the LC diet intervention during maximal intensity intermittent cycle exercise.*

H_0 for this hypothesis could be rejected, since a significant increase in ATP-PCr energy system contribution was indeed found.

Since the present study is, to my knowledge, the first to quantify the effects of a LC intervention on ATP-PCr energy metabolism, comparison of the results to current literature is restricted. Nonetheless, during the first few seconds (≤ 10 s) of maximal intensity exercise, the ATP-PCr energy system produces the majority of ATP from stored PCr in the muscle. In this regard, a short single bout sprint test and strength

/ power tests at this intensity and within this time-frame were employed in previous LC studies (Greene *et al.*, 2018; Kephart *et al.*, 2018; McSwiney *et al.*, 2017; Paoli *et al.*, 2012). Hence, even though the ATP-PCr energy system was not quantitatively measured in these studies, the reported performance outcomes might still provide some information for comparative purposes.

In this respect, all three studies that examined the effects of LC interventions on strength performance reported maintenance in this outcome (Greene *et al.*, 2018; Kephart *et al.*, 2018; Paoli *et al.*, 2012). One repetition maximum (1RM) bench press, squat, or deadlift performance in power-lifting athletes (Greene *et al.*, 2018) and 1RM back squat in CrossFit trainees (Kephart *et al.*, 2018) were maintained over 12-week LC interventions, while performance in various strength tests in elite male gymnasts were also maintained following a 4-week LC intervention (Paoli *et al.*, 2012). Similar to these outcomes in strength tests, power performance in Olympic lifting competition lifts (jerk, clean and snatch) in Olympic-lifting athletes (Greene *et al.*, 2018) and power-clean performance in CrossFit trainees (Kephart *et al.*, 2018) were also maintained over the 12-week nutritional interventions.

In another 12-week k-LCHF intervention, 6 s cycle sprint power output improved significantly (p = 0.025, ES: 0.263) (McSwiney *et al.*, 2017). Although no quantitative measurement of the ATP-PCr energy system was performed in this study, the authors speculated that the unchanged energy production of this pathway on the k-LCHF diet may account for this finding. Hence, quantification of the ATP-PCr energy system in the current study, where a statistically significant improvement occurred over the 6-week LC intervention, possibly affirm this speculation by McSwiney *et al.* (2017).

Collectively, current literature on strength, power and short sprint performance outcomes (where ATP-PCr is the main contributor to energy production), supports the finding in the present study that CHO restriction caused a significant adaptation in this energy pathway.

The Wingate Anaerobic Test (WAnT) is another, but longer (30 s) maximal intensity test that has been employed in previous LC interventions (Lambert *et al.*, 1994; Langfort *et al.*, 1997). In this test, peak power (PPO) reached within the first 5 - 10 s of the 30 s sprint has been reported to reflect the utilization of the ATP-PCr energy

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system to energy output, while mean power output (MPO) over the 30 s reflects the utilization of the anaerobic glycolysis energy pathway (Smith & Hill, 1991). Thus, this standardized exercise test can also indirectly estimate the contributions of these energy systems to ATP production for comparison to the quantitative outcomes of the present investigation.

Findings of unchanged (Langfort *et al.*, 1997) and a non-significant (58 W) greater PPO (Lambert *et al.*, 1994) following the LC interventions compared to the high-CHO diet phases, support the finding of a significant increase in ATP-PCr energy system contribution found in the present study. Even so, opposed to the 6-week LC intervention employed in the current investigation, these studies employed short, 3-day (Langfort *et al.*, 1997) and 2-week (Lambert *et al.*, 1994) interventions. These short interventions might not have allowed sufficient time for great margins of metabolic adaptation. Nonetheless, the non-significant improvement in PPO following the 2-week intervention by Lambert *et al.* (1994), was in line with the time course of adaptation in the ATP-PCr energy system contribution in the present study. In this regard, absolute ATP-PCr contribution at the 2-week time point in the current investigation was also already higher than at the baseline measurements. Since the present study did not obtain a measurement as early as day three, the unchanged PPO following this short LC intervention by Langfort *et al.* (1997) might provide additional time course information to the present investigation. The outcome of an unchanged PPO at day three of the LC intervention (Langfort *et al.*, 1997) might indicate that ATP-PCr metabolism was not negatively affected in the initial period, before compensation occurred.

A large body of evidence also exists that as little as five days of oral creatine supplementation (fuel source for the ATP-PCr energy system) improves the capacity of this energy system, as well as high-intensity or intermittent exercise performance (Aaserud *et al.*, 1998; Balsom *et al.*, 1993; Clarkson *et al.*, 2000; Cooper *et al.*, 2012; Crisafulli *et al.*, 2018; Deminice *et al.*, 2013; Finn *et al.*, 2001; Greenhaff *et al.*, 1993). Although it is known that creatine is naturally produced by the liver and kidneys, previous research rarely investigated methods to stimulate the body`s capacity to increase endogenous production of creatine, or to enhance the capacity of this energy system, without oral supplementation. Moreover, oral creatine

supplementation has been shown to down-regulate endogenous biosynthesis of creatine (Walker, 1960).

The present study eliminated the use of any dietary supplement for the intervention period, and participants were asked not to change their exercise routines. Hence, the *very large* (3.5 ± 2.36%) increase in ATP-PCr energy system contribution, in the absence of oral creatine supplementation, can be attributed to the 6-week LC diet intervention. Thus, the significant improvement in the ATP-PCr energy system to the current LC nutritional intervention, demonstrates the potential for adaptation in this energy system in the absence of oral creatine supplementation.

5.7.3 Anaerobic glycolysis energy system adaptations

H_2: It is hypothesised that restriction of fuel for anaerobic glycolysis on a LC diet will down-regulate this energy system during maximal intensity intermittent cycle exercise.

H_0 for this hypothesis could be rejected, since significant down regulation occurred in the anaerobic glycolytic energy system.

With regards to the anaerobic glycolytic energy system, the method whereby the contribution of this energy system during intermittent sprints was calculated (equation 3.3), has not previously been used in LC intervention studies. The investigation of the effects of a LC dietary intervention on intermittent sprint exercise metabolism is also a unique feature of the current study. Consequently, the findings of the current study can only be compared to studies where other quantitative measures of the anaerobic energy systems were utilized in exercise modalities other than intermittent sprints.

Compared to PPO achieved in the first 10 s of the WAnT sprint, which represents the ATP-PCr energy contribution, the entire 30 s duration of this test has been reported to require a large contribution of the anaerobic glycolytic pathway for ATP production (Beneke *et al.*, 2002; Julio *et al.,* 2019). As expected, MPO during this test has been reported to be representative of the anaerobic glycolysis energy system (Smith & Hill, 1991). Accordingly, the significantly lower MPO in the 30 s

WAnT tests reported in previous LC investigations (Lambert *et al.*, 1994; Langfort *et al.*, 1997), suggests lowered anaerobic glycolysis, as was found in the current investigation. In line with the time course of down-regulation in anaerobic glycolysis in the present study, where significant reductions already occurred at the 2-week time point, these lowered MPO outcomes (suggesting lowered anaerobic glycolysis) were also achieved after short 3-day (Langfort *et al.*, 1997) and 2-week (Lambert *et al.*, 1994) LC interventions.

RQ and blood [lactate] ([BLa]) metabolic measurements were two popular methods in LC studies to estimate contributions in the anaerobic glycolytic pathway. Although fuel metabolism during low intensity endurance exercise differs greatly from the intermittent sprints exercise modality of the present study, most LC interventions that measured metabolic parameters only utilized endurance tests. In these endurance tests in current LC literature, lower RQ values were consistently reported, thus reflecting lower glycolysis (Dostal *et al.*, 2019; Fisher *et al.*, 1983; Fleming *et al.*, 2003; Heatherly *et al.*, 2018; Lambert *et al.*, 1994; Phinney *et al.*, 1983; Vogt *et al.*, 2003; Zajac *et al.*, 2014). However, opposed to endurance tests, gas exchange metabolic parameters were rarely measured during high-intensity test protocols. Nonetheless, one investigation by Cipryan *et al.* (2018) measured RQ during his high-intensity interval test. Consistent with down-regulation of anaerobic glycolysis observed during the LC phase of the present study, lower RQ values were reported in the high-intensity test in their LC intervention group (Cipryan *et al.*, 2018). Although one should consider the influence of differences in exercise modality and measurement method, decreased anaerobic glycolysis during the intermittent sprint tests found in the present study is in line with these previous findings.

Consistent with gas exchange measures, lower [BLa] levels on LC interventions during aerobic endurance exercise tests (Vogt *et al.*, 2003; Zajac *et al.*, 2014) also indicated lower anaerobic glycolysis during the LC tests. During high-intensity anaerobic exercise tests however, [BLa] measurements still provided inconclusive evidence in support of decreased anaerobic glycolysis found in the present study.

In support of the significant down-regulation in anaerobic glycolytic energy system contribution on the LC phase of the current study, Langfort *et al.* (1997) observed significantly lower [BLa] (9.5 ± 0.4 vs. 10.6 ± 0.5 mmol/L; $p < 0.05$) in eight healthy

men on a three-day LC intervention following a 30 s WAnT sprint test. The lowered MPO during the LC phase of this investigation discussed earlier, in conjunction with these lowered [BLa] measurements, provide additional evidence that anaerobic energy system contribution was doubtlessly down-regulated in this investigation (Langfort *et al.*, 1997). Moreover, even though sympathetic activation is generally known to up-regulate anaerobic glycolysis, the down-regulation in this energy pathway on the LC phase of this investigation was observed even in the presence of a greater sympathetic response, evident from significantly higher adrenaline ($p < 0.01$) and noradrenaline ($p < 0.01$) on the LC phase (Langfort *et al.*, 1997). Accordingly, the reported acute metabolic response to the short, 3-day LC intervention (Langfort *et al.*, 1997), is in line with the finding of the present study, where a significant decrease in absolute anaerobic glycolytic energy system contribution also already occurred at the 2-week timepoint of LC testing ($p = 0.031$). These results may indicate that lowered anaerobic glycolytic energy system contributions occur after a short time period on a LC diet. Thus, the shift in this energy system does not seem to need a long adaptation period.

5.7.4 Aerobic energy system adaptations

> H_3: It is hypothesised that adaptation in aerobic fat metabolism will occur, so that energy production from this pathway will increase throughout the LC diet intervention during maximal intensity intermittent cycle exercise.

H_0 for this hypothesis could not be rejected, since no significant change in aerobic energy production occurred following the LC intervention.

Although the changes in the anaerobic energy systems (ATP-PCr and anaerobic glycolysis) doubtlessly confirm the desired hypothesised shift in intermittent sprints energy metabolism, no statistically significant change in absolute contributions in the aerobic energy system occurred over the 6-week LC intervention period. A *small* increase in this energy system was only evident when expressed relative to body mass at the 6-week LC timepoint. Thus, due to the lack of a significant change observed in the aerobic energy system over the 6-week LC intervention during the intermittent sprints, the aerobic shift part of the hypothesis was not confirmed by the current study results.

The proposed explanation for the lack of aerobic energy system adaptation, is the higher protein intake on the self-selected LC diet of the current participants. The measure of absolute VO_2max is considered one of the gold standard direct measures of aerobic capacity. This measure reflects the maximum rate of muscle aerobic metabolism and cardiovascular oxygen delivery during exercise, irrespective of changes in body mass. A *very large* negative correlation between change in protein intake from the HD to the LC phase and changes in absolute VO_2max emerged from the present results (Figure 4.14), and will be further discussed in the section on maximal aerobic capacity outcomes (5.12). This negative correlation may demonstrate a possible detrimental effect of excessive protein intake on the aerobic metabolic adaptations in the present study, where a *moderate* increase in protein intake of 21.7 ± 34.47 g/day was evident on the self-selected LC diet. Similarly, Hietavala *et al.* (2012) observed increased O_2 consumption during sub-maximal cycling on a low-protein, vegetarian diet.

A possible molecular-level mechanistic explanation for impaired aerobic adaptations in the presence of excessive protein intake, is that protein activates the mTOR cell-signalling pathway, which inhibits the cell process of autophagy (Hanjani & Vafa, 2018; Moscat & Diaz-Meco, 2011). Regarding this proposition, the cell-process of autophagy is associated with enhanced mitochondrial functioning and aerobic ATP production (Moscat & Diaz-Meco, 2011; Roberts & Miyamoto, 2015; Schieke *et al.*, 2006). Thus, through protein-induced activation of mTOR, autophagy is down-regulated, which might in turn impair aerobic mitochondrial ATP production during exercise.

While the anaerobic energy systems do not seem to have been negatively affected by the higher protein intake, the significant increase in protein intake might pose a plausible explanation for the lack of aerobic adaptations during the intermittent sprints, resulting in the rejection of this hypothesis. Consequently, if aerobic metabolic adaptations are desired, it might also be advisable from these findings to rather implement k-LCHF macronutrient guidelines with protein intake restricted to < 20% daily energy.

5.8 Quantification of fatigue

Since there is no gold-standard in the quantification of fatigue during intermittent sprints tests, Glaister *et al.* (2008) evaluated the validity and reliability of eight different equations to quantify fatigue in tests of multiple-sprint performance. Problems regarding the logical validity for many of the equations were observed in poor or inconsistent test-retest reliability (CV range: 0.8 – 145.7%; ICC range: 0.09 – 0.75). For other equations, inaccurate assumptions like "first sprint has highest power output and last sprint the lowest", as foundation for these formulae resulted in low logical validity. Since "Percentage Decrement" score equation (Equation 3.1) (Fitzsimon *et al.*, 1993) showed consistent reliability and good construct and logical validity through incorporation of data from each sprint, this formula is regarded the most reliable measure of fatigue during intermittent sprint tests (Glaister *et al.*, 2008).

5.9 Time course of changes in fatigue

In most field and court sports the ability to repeatedly produce a high-power output or sprint speed is an important component of performance. In practice, lower levels of fatigue during intermittent sprint sports will likely result in improved performance during real life matches and is often an objective for the players and coaches. However, performance changes over time is the eventual outcome of a complex set of adaptations including, but not limited to, metabolic adaptations (Buchheit *et al.*, 2012; Buchheit & Laursen, 2013; Mujika *et al.*, 2018).

As discussed earlier in this thesis (Chapter One, 1.3), the time course of performance change in response to a stimulus could be described by a supercompensation curve (Cole, 1998; Furnas, 2000) (Figure 1.2). According to this model, performance initially declines after the stimulus / "challenge", where after adaptation occurs by over-compensation to the "challenge" and resultant performance improvements. An integrated, multifactorial approach to performance improvements should address nutrition, in combination with the training stimulus, in order to promote optimal physiological adaptations (Mujika *et al.*, 2018). Therefore, the following hypothesis was developed:

H_4: It was hypothesised that participants' fatigue and sprint MPO performance would follow a supercompensation curve (Cole, 1998)*.*

Since the net *moderate* decrease in fatigue at the 6-week timepoint was not statistically significant, this hypothesis was rejected. Nonetheless, although the final *moderate* improvement after the initial decline was not significant, the time course of changes in fatigue in the current investigation still reflected a super-compensation curve over the 6-week adaptation period (Figure 4.12). Initially, a *small* (1.41%) increase in fatigue was observed after two weeks of CHO restriction as stimulus compared to testing on the habitual diet. This initial increase in fatigue at the two-week LC time point, corresponds to "fatigue" (phase one) on the supercompensation curve (Figure 1.2). After the 2-week LC time point, fatigue levels gradually decreased with a *negligible* drop (0.37%) from week two to week four on the LC diet, corresponding to the initial "compensation" (phase two Figure 1.2). "Compensation" finally resulted in a *moderate* decrease (3.082%) in fatigue score in the final week of LC testing after the initial *small* increase at LC week 2.

Finally, compared with baseline, a *moderate* (1.67%) net decrease in fatigue was observed at the 6-week LC test in the present study. This net improvement in fatigue reflects a non-significant supercompensation (phase three Figure 1.2) according to the model (Cole, 1998). Although the supercompensation did not reveal a statistically significant net decrease in fatigue in the present study, the *moderate* effect size of this improvement (ES = -0.52) suggests practical significance for intermittent sprint sport players. This current supercompensation time course of change in fatigue in response to a LC intervention, corresponds with the study by Phinney *et al.* (1980), where a marked supercompensation curved adaptation in endurance capacity, in response to CHO restriction, was evident.

The untrained status of the participants in the study by Phinney *et al.* (1980) was ideal to observe changes in endurance capacity in response to a nutritional intervention, as these individuals did not process training-induced adaptations that affected their ability for further adaptations in physiological systems. Since no training intervention was included in this investigation, the robust (55%) supercompensation in walking performance, demonstrated the potential of CHO restriction to enhance endurance performance (Phinney *et al.*, 1980).

Participants in the present study, however, were recreationally active, as opposed to the untrained training status of the participants in the study of Phinney *et al.* (1980). Therefore, participants in the present study had a smaller margin for improvement in response to a nutritional intervention. In a sport context, however, even small magnitudes of improvement might result in practically significant performance changes. The endurance exercise modality in the investigation by Phinney *et al.* (1980) might also have allowed for greater margins of adaptation than the measure in the present study, namely intermittent sprint fatigue (percentage decrement score).

In conclusion, irrespective of vast differences in study designs, the observation of supercompensation-curved time courses of adaptation was observed in both investigations in response to the implementation of a LC intervention. Since repeated measure designs with longer adaptation periods (at least six weeks) have not been commonly performed, these findings might provide valuable evidence in this field of research.

Besides, since the performance parameter in this study did not reach a plateau on the supercompensation curve at the 6-week time point, it is unknown if further improvements would have been evident, should the LC period continue. Therefore, the *moderate* (1.67%) supercompensation in fatigue during intermittent sprints after the 6-week intervention, may still pose a promising incentive for athletes to implement a LC nutritional intervention in combination with training as stimuli in the multifactorial approach to performance optimization.

5.10 Integration of metabolism and performance hypothesis

Figure 5.2 is an illustration of the integration of the main hypotheses in this thesis: intermittent sprint metabolism (H_{1-3}) and -fatigue (H_4). The contributions of ATP-PCr and anaerobic glycolysis towards total anaerobic energy production is illustrated by the stacked bar graphs, while fatigue during intermittent sprints, as a performance indicator, is illustrated by the line graph. Additionally, these interactions are

superimposed with the supercompensation curve as the theoretical basis of the observed adaptation process.
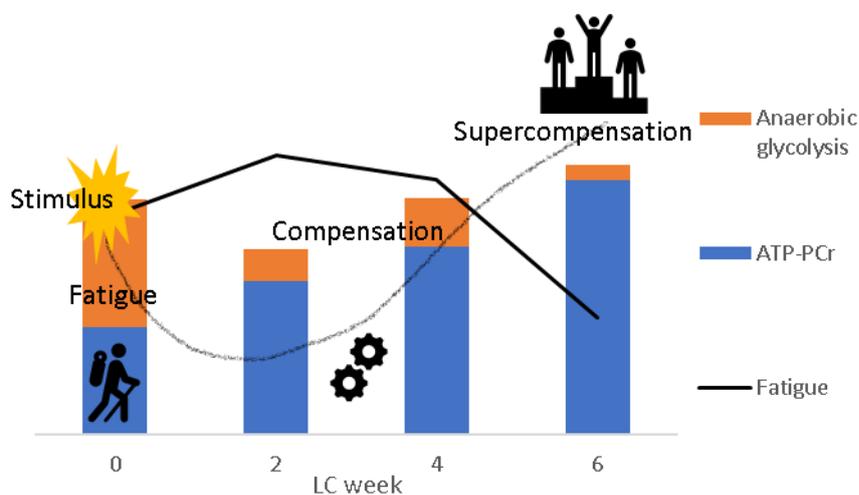
Figure 5.2: Change in fatigue related to absolute anaerobic energy output over the six-week low-carbohydrate intervention (LC1-3) compared to baseline performance on the habitual diet (Pre LC) overlapped with the Supercompensation curve of adaptation to a stimulus (Cole, 1998)

The metabolic adaptations in energy system contributions is proposed as a mechanistic explanation for the time course of supercompensation in fatigue as a performance outcome. As discussed earlier, a positive shift in fuel metabolism during intermittent sprints occurred over the LC intervention period in the present study. This observed energy system shift was two-fold:

(1) Significant down regulation in the absolute anaerobic glycolytic system that occurred after only two weeks on the LC diet, where after mean values stayed unchanged for the following four LC weeks.

(2) A linear increase in absolute ATP-PCr energy system contribution that achieved statistical significance at LC week 6 for the absolute measure.

The down-regulation of anaerobic glycolysis in the present study has previously been suggested to assist in the maintenance of homeostasis and reduce the onset of fatigue during later sprints of intermittent sprints protocols (Parolin *et al.*, 1999). However, if this energy system is down-regulated while a corresponding up-regulation of the other energy producing pathways have not yet occurred, performance will initially decrease due to lower total energy output. In this regard,

the initial increase in fatigue scores at the 2-week LC time point in the present study, was accompanied by lower total ATP production through anaerobic glycolysis. Furthermore, "compensation" through the up-regulation of the ATP-PCr energy system was still insufficient to compensate for the down-regulated glycolytic energy contribution at this time point.

This initial decline in total energy availability through down-regulated anaerobic glycolysis is proposed as the metabolic "challenge"/ stimulus which accounted for the linear up-regulation of the ATP-PCr energy system capacity throughout the 6-week LC intervention.

Finally, at the 6-week LC time point, the magnitude of adaptation in the ATP-PCr energy system, combined with improved maintenance of homeostasis through a decline in anaerobic glycolysis (Parolin *et al.*, 1999), is a plausible reason for the *moderate* (1.67%) supercompensation that took place in the performance variable (fatigue).

5.11 The possible influence of pacing strategies on sprint performance

Although changes in metabolic shifts, as reflected in this study, should be conducive to intermittent sprint performance, the positive metabolic shift did not translate into significant mechanical power output improvements. Similarly, the *moderate*, but non-significant decrease in fatigue scores, only achieved at the 6-week LC time point, did not result in statistically significant changes in mean power output, and participants only maintained performance in this outcome variable. As discussed earlier, however, performance changes in sport is the cumulative outcome of a complex set of adaptations. It is acknowledged that metabolic adaptations in energy metabolism, as investigated here, is a single component in the integrated, multifactorial adaptation process that result in performance improvements.

Perceptual and psychological factors are other influences that should be taken into account in the evaluation of performance changes. Previous investigations demonstrated that prior knowledge of the number of sprints influenced intermittent sprint performance through pacing strategies (Billaut *et al.*, 2011). The time-series

study design, employed in the current investigation, thus enabled participants to anticipate session effort based on discomfort levels, which were experienced in previous sessions.

Although pacing strategies in experienced athletes are conducive to optimal performance, the participants in this study were active, but not elite team sports players. Hence, they were largely unaccustomed to intermittent sprint activities. The psychological effect of perceived discomfort caused by this strenuous exercise modality might, therefore, have influenced centrally acting performance modifiers, and affected sprint power output during following sessions. Williams *et al.* (2014) reviewed the literature on deception as a central modification in performance. This review summarised the effects of manipulating knowledge of task end point, false performance feedback, and previous experience on intermittent sprint performance. Ten of the studies reviewed in this article reported positive performance effects of deception as a central performance modifier (Williams *et al.*, 2014). Hence, it can be derived that some pacing strategies might inhibit optimal performance when participants reserve too much energy for later sprints, and consequently not produce all out efforts. Although it is acknowledged that more research is needed in this field, it would be reasonable to consider the influence of prior knowledge of the intermittent sprint protocol on the performance outcomes in the present study.

Lastly, lower total energy intake of the participants` self-selected LC diets, may possibly be another confounding variable, hindering performance improvements in the present investigation.

5.12 Maximal aerobic capacity outcome measures

Since LC interventions in sport research mainly focused on endurance performance, graded maximal aerobic capacity tests with cardiometabolic measurements were commonly performed (Burke *et al.*, 2017; Carr *et al.*, 2018; Cipryan *et al.*, 2018; Dostal *et al.*, 2019; Fleming *et al.*, 2003; Phinney *et al.*, 1983; Prins *et al.*, 2019; Urbain *et al.*, 2017; Vogt *et al.*, 2003; Zajac *et al.*, 2014). However, these tests were mostly used to describe the study population, while pre- to post-intervention comparisons were seldom made.

Since relative VO_2max reflects the ratio between active aerobic metabolic tissue and body mass, improvements in this outcome is generally accepted as a reliable indicator of endurance capacity. There was a significant increase of 5.3% in relative VO_2 max among participants in the current study. Aerobic metabolic pathways involved in utilization of fat as fuel, necessitate higher O_2 utilization (Burke *et al.*, 2017), which might have contributed to the observed increased relative VO_2max. This finding is in agreement with a 4-week LC diet study in trained off-road cyclists (Zajac *et al.*, 2014). Other investigations, however, reported unchanged values of maximal aerobic capacity (Fleming *et al.*, 2003; Vogt *et al.*, 2003), or no significant between-group differences in changed VO_2 max values (Cipryan *et al.*, 2018; Dostal *et al.*, 2019; Prins *et al.*, 2019). This study did not involve an exercise training intervention and participants were asked not to change their habitual training routines for the duration of the study. Thus, it is unlikely that exercise training stimulated additional metabolic adaptations in the current participants.

On the other hand, a much smaller *(*1.5%) increase in absolute VO_2 max was observed in this study. Thus, it could be argued that the improvement in relative VO_2 max was largely because of the reduction in body mass, while the real improvement in maximal exercise capacity was minimal. This discrepancy between absolute and relative VO_2max is not an uncommon finding during LC intervention studies, as body mass reductions is commonly observed (Cipryan *et al.*, 2018; Dostal *et al.*, 2019; Fleming *et al.*, 2003; Zajac *et al.*, 2014).

Further analysis of the relationship between variables was conducted to shed light on the effects of body mass reductions on relative VO_2max changes. Surprisingly, mean body mass reductions in the three participants who experienced a drop in relative VO_2max were actually 0.9 kg less than the eleven participants who experienced an improvement in this parameter. Furthermore, there was also no meaningful correlation between body mass changes (pre- to post-intervention) with changes in relative VO_2max (r = -0.214; CI = -0.36; 0.67). Hence, although body mass reductions accounted for the discrepancy between absolute and relative VO_2max, no direct relationship between body mass changes and VO_2max outcomes were evident that might suggest a causal relationship between these variables.

Intriguingly, the changes in VO_2 max was more related to another variable, namely protein intake. A *very large* negative correlation between change in protein intake from the HD to the LC phase and changes in absolute VO_2max emerged (Figure 4.14). This negative correlation shows that greater increases in protein intake on the self-selected LC phase of the study, was associated with negative VO_2max changes in the current investigation.

Nonetheless, overall, the group maintained their absolute VO_2 max after the 6-week LC intervention, while overall protein intake increased from 20% to 28% from the HD to the LC phase. A similar finding was observed by Fleming *et al.* (2003) where their participants' energy derived from protein during the LC phase increased by 16% (from 15% to 31%) compared to the 8% increase in the current study. Reportedly, there was a significant decline in absolute VO_2max from 3.5 ± 0.14 L.min^{-1} to 3.27 ± 0.09 L.min^{-1}. Although these authors did not calculate a correlation statistic for their findings, in absolute terms, these outcomes may highlight the results of this study. This decline in absolute VO_2max (Fleming *et al.*, 2003) might be a magnified result of the negative correlation revealed from the present study results.

Furthermore, Hietavala *et al.* (2012) reported that VO_2 were significantly higher on a 4-day low-protein, vegetarian diet, at sub-maximal cycling workloads of 40% ($p < 0.05$), 60% and 80% ($p < 0.001$). However, at maximal endurance intensities (100% VO_2max), oxygen consumption was not significantly greater compared to the normal-protein diet. Still, the short dietary intervention (4-days) in this study, indicates that lower protein intake increased oxygen consumption. This finding is in line with the negative correlation between protein intake and VO_2max, observed in the current study.

It is furthermore well-established in physiology that protein stimulates the mammalian Target of Rapamycin (mTOR) cell signalling pathway at molecular level, whereby inhibiting the process of autophagy (Hanjani & Vafa, 2018; Moscat & Diaz-Meco, 2011). The cell-process of autophagy is associated with enhanced mitochondrial functioning and aerobic ATP production (Moscat & Diaz-Meco, 2011; Roberts & Miyamoto, 2015; Schieke *et al.*, 2006). Consequently, mTOR induced down-regulation of autophagy through excessive protein intake, might be a plausible

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mechanistic explanation for the strong negative correlation between protein intake and VO$_2$max found in this study.

The possibility that individuals may consume excessive protein when switching to a LCHF diet, may thus have been a confounding variable in previous LC research. Of the 18 studies where the effects of LC interventions on endurance capacity were investigated, only six met the k-LCHF macronutrient guidelines where protein intake is restricted to < 20% of daily energy. Therefore, it is possible that the reported results on aerobic adaptations and endurance capacity may have been affected by too high protein consumption. Although further research is needed to confirm or refute the negative effects of high protein intake on maximal aerobic capacity, it might be advisable to consider protein intake as a possible confounding variable in the interpretation of current literature.

Previous studies (Dostal *et al.*, 2019; Vogt *et al.*, 2003; Zajac *et al.*, 2014) reported similar decreases in peak blood lactate ([BLa]) following LC interventions than the current study (-19.1 ± 18.34%). Except for one participant with faulty [BLa] readings and whose data was removed from the data set, all other participants recorded lower peak [BLa] at the end of the 6-week LC intervention.

Muscle glycogen levels (fuel for anaerobic glycolysis) are known to be lower on LC diets (Lambert *et al.*, 1994; Phinney *et al.*, 1980; Vogt *et al.*, 2003). Therefore, the reduced rate of glycolysis might be, in part, the explanation for the drop in [BLa] when dietary CHO intake is restricted. However, reduced fuel source for anaerobic glycolysis on CHO-restricted diets might be an over-simplified mechanism for reduced lactate accumulation on these diets, and the complex nature of lactate kinetics should not be under-estimated, since inconclusive mechanistic evidence still exists in the current literature. For instance, when [lactate] are measured from blood samples, such as in this study, it cannot be deduced whether there was less lactate production, or improved lactate clearance.

While most investigations observed lowered rates of muscle glycogen utilization associated to lower starting muscle stores (Fisher *et al.*, 1983; Phinney *et al.*, 1983, 1980; Webster *et al.*, 2016), another study observed that lower muscle glycogen levels on a LC intervention did not alter the rate of glycogenolysis (Lambert *et al.*,

1994). A possible confounding variable that might explain the equivocal outcomes, might be related to the influence of sympathetic nervous system activation when CHO is restricted. The action of various sympathetic neurotransmitters is known to stimulate glycogenolysis. In this respect, short term CHO-restriction has been shown to increase circulating sympathetic hormones and neurotransmitters (Langfort *et al.*, 1997; Michalsen *et al.*, 2003; Waldman *et al.*, 2018). Yet, Langfort *et al.* (1997) reported elevated circulating adrenaline and noradrenaline on a 3-day LC intervention in conjunction with significantly lower [BLa] after exercise. Moreover, this initial increase in sympathetic nervous system activation seems to normalize when LC diets are sustained for longer periods (± 7 days) (Fond *et al.*, 2013; Waldman *et al.*, 2018). Hence, the changes that take place in the autonomic nervous system contributes to the complexity of lactate kinetics, specifically with CHO restriction.

Another mechanistic consideration in lactate kinetics is that, although lactate is mainly produced as an anaerobic metabolic by-product, it can also be aerobically oxidized as a fuel source. Hence, when aerobic metabolism rates are slower than anaerobic glycolysis rates, accumulation of this metabolite occurs in the muscle and blood. In this respect, previous studies have reported improved lactate clearance rates following LC interventions (Lambert *et al.*, 1994). Furthermore, a higher VO_2 at lactate threshold, as reported by Zajac *et al.* (2014), also provides evidence for enhanced lactate oxidation during CHO restriction.

In combination with the significantly lower blood lactate levels in the current LC intervention, gas exchange outcome measures further stressed the fuel metabolism shift that occurred. A significant 12.1% increase in power at aerobic threshold (AT), a 2.7% increase in power at respiratory compensation point (RC) and a 2.2% decrease in RQ were observed. Accordingly, together with the significantly lower [BLa], the gas exchange measures (RQ, AT and RC) also reflects a shift in fuel metabolism towards increased fat utilization during exercise. Higher threshold intensities is a desirable adaptation for athletes in endurance sports, since higher intensities can then be maintained throughout endurance races. To my knowledge, the metabolic thresholds (AT and RC) were not previously reported in LC-sport literature. Consequently, these outcomes cannot be directly compared to previous studies.

RQ however, is a widely reported gas exchange measure of fuel metabolism. Similar to the results of this study, lower RQ values during graded and continuous aerobic exercise tests following CHO restriction are a consistent finding (Dostal *et al.*, 2019; Fisher *et al.*, 1983; Fleming *et al.*, 2003; Heatherly *et al.*, 2018; Lambert *et al.*, 1994; Phinney *et al.*, 1983, 1980; Vogt *et al.*, 2003; Zajac *et al.*, 2014). Although the change in RQ in the present study did not reach statistical significance, most other studies report statistically significant drops in RQ (Dostal *et al.*, 2019; Fleming *et al.*, 2003; Heatherly *et al.*, 2018; Lambert *et al.*, 1994; Phinney *et al.*, 1983; Vogt *et al.*, 2003; Zajac *et al.*, 2014). A possible reason for this study's result might be that maximal values at the end of the incremental exercise protocol were reported, while some other researchers reported mean RQ's for the whole test. Nonetheless, these findings, including those of the current study, provide strong evidence for a metabolic shift in fuel metabolism during aerobic exercise following LC interventions, in support of the study hypothesis (H_5).

In further agreement with the study hypothesis (H_5), the *negligible* increase in peak power output (PPO) following the LC diet in the present study indicates that maximal endurance performance was maintained. Although a marked increase in the relative rate of ATP production from aerobic fat oxidation was evident from the metabolic measures, this shift in fuel metabolism was also accompanied by a reduction in glycolytic ATP production from CHO metabolism. In accordance with previously reported supercompensation-curved adaptation in aerobic capacity in response to CHO restriction (Phinney *et al.*, 1980), it was hypothesised that up-regulation in the rates of absolute ATP production from aerobic fat metabolism might need a longer adaptation time to exceed down-regulated glycolysis rates. Therefore, it is proposed that "unchanged" PPO in the maximal aerobic capacity test actually undergone a supercompensation-curved change, which was still in the compensation phase. Thus, it is suggested that if the LC stimulus would be sustained, further adaptation in aerobic metabolic pathways may exceed down-regulated glycolysis rates to result in improvements in PPO performance.

This result of maintained PPO performance, is supported by previous findings during graded (Dostal *et al.*, 2019; Kephart *et al.*, 2018; Prins *et al.*, 2019; Vogt *et al.*, 2003) and continuous effort endurance tests (Fisher *et al.*, 1983; Phinney *et al.*, 1983;

Prins *et al.*, 2019). Even so, results in current literature on the influence of an LC intervention on endurance performance are still equivocal. While four investigations reported an improvement in endurance PPO, or other performance indicators (Cipryan *et al.*, 2018; Lambert *et al.*, 1994; McSwiney *et al.*, 2017; Phinney *et al.*, 1980), four other studies reported decrements in endurance performance outcomes (Burke *et al.*, 2017; Fleming *et al.*, 2003; Zajac *et al.*, 2014). Yet, short LC intervention periods (3 weeks) (Burke *et al.*, 2017), lack of a wash-out period (Zajac *et al.*, 2014) and LC interventions that did not result in meaningful increases in blood ketone levels (Fleming *et al.*, 2003; Zajac *et al.*, 2014) are some of the possible reasons that might account for the findings of reductions in endurance capacity.

Lastly, in light of the *negligible* differences in maximal heart rate (HR) and rating of perceived exertion (RPE) between the pre- and post- aerobic capacity tests, it can be concluded that the participants produced maximal efforts on both occasions.

Chapter 6

Conclusion and recommendations

6.1 Conclusion

It was clear from the literature on metabolism during intermittent sprints that anaerobic glycolysis is down-regulated during later sprints (Bogdanis *et al.*, 1996; Gaitanos, 1993; McCartney *et al.*, 1986; Parolin *et al.*, 1999; Trump *et al.*, 1996), to aid in the regulation of homeostasis through the lowering of metabolic by-product production, known to counter fatigue (Parolin *et al.*, 1999). Based on intermittent sprint metabolism physiology as a theoretical framework, the present study set out to investigate the effects of dietary restriction of the fuel for anaerobic glycolysis on energy metabolism and performance in this exercise modality, through a 6-week LC intervention. Apart from the positive effects of decreased anaerobic glycolysis on fatigue reductions in later sprints, it was further hypothesised that lowered total energy availability from the anaerobic glycolytic pathway would serve as a "challenge" / -stimulus for the up-regulation of the other two main energy producing pathways, namely ATP-PCr and aerobic metabolism.

In light of this theoretical framework, the present study indeed found that CHO restriction down regulated the anaerobic glycolytic energy system contribution. This reduction in the anaerobic glycolytic pathway occurred abruptly and was already evident at the first LC test after two weeks. At the same time, the ATP-PCr energy system underwent a marked increase throughout the 6-week LC intervention. In view of these outcomes, it can be argued that the reduction in anaerobic glycolytic energy contribution may indeed have acted as a stimulus for the up-regulation of the ATP-PCr energy pathway. Hence, following the 6-week LC intervention, the findings of this study confirmed the desired hypothesised metabolic shift in the anaerobic energy systems during intermittent sprint exercise.

Collectively, the findings of the decrease in metabolic by-product production through anaerobic glycolysis in conjunction with the significant increase in ATP-PCr energy output, further resulted in a supercompensation-curved decrease in fatigue over the six-week LC intervention period. These findings reveal that the six-week LC

intervention induced positive metabolic adaptations in the energy pathways, which resulted in a *moderate* net decrease in fatigue as performance variable.

Moreover, this research is, to my knowledge, novel in terms of the quantification of the ATP-PCr energy system in response to an LC intervention. The finding of a significant increase in this energy system contribution, provides novel insight into the adaptation capacity of this energy producing pathway to a LC nutritional stimulus, and in the absence of oral creatine supplementation.

Although positive aerobic and mitochondrial metabolic adaptations to LCHF diets have been previously reported (McSwiney *et al.*, 2017; Miller *et al.*, 2018; Phinney *et al.*, 1980; Veech *et al.*, 2017; Volek *et al.*, 2016), the hypothesised up-regulation in the aerobic energy system contribution during the intermittent sprint exercise did not occur in the present investigation. However, a *very strong* negative correlation between change in protein intake and change in absolute VO_2 max found in this study, might reveal a possible negative effect of increased protein intake on aerobic adaptations. The increase from 20 to 28% energy intake from protein during the LC phase, may consequently have impaired aerobic energy system adaptations, to account for rejection of the aerobic- part of the hypothesised shift in intermittent sprints metabolism. Even so, protein intake did not seem to have a negative effect on the adaptations of the anaerobic energy system contributions (ATP-PCr and anaerobic glycolysis), since these energy systems still underwent the significant adaptations as hypothesised.

Lastly, the repeated measures study design employed in this investigation allowed for monitoring of the time course of the CHO restriction adaptation process. This study design has rarely been employed in LC interventions in sport. Thus, the supercompensation curved time course of change in fatigue as a performance indicator might shed light on some negative exercise performance outcomes of previous shorter LC interventions (≤ 3 weeks) (Burke *et al.*, 2017; Carr *et al.*, 2018; Heatherly *et al.*, 2018; Lambert *et al.*, 1994; Waldman *et al.*, 2018), since post intervention testing might have occurred in phase one or two of the supercompensation curve.

6.2 Limitations

1. Since the fatigue improvements were still in the supercompensation phase of the curve (Cole, 1998), and a plateau or decrease in this performance outcome was not yet visible at the 6-week timepoint of the current study, it must be considered that the peak of supercompensation has not yet been achieved. Similarly, the gradual linear increase in the ATP-PCr energy system contribution had also not yet plateaued at the last LC testing session. Therefore, this 6-week LC intervention might have been too short to see complete adaptations in the contribution of the ATP-PCr energy system and decrements in fatigue during intermittent sprints.

2. The habitual diet of the participants already consisted of higher fat contributions (45% daily energy) and relatively lower CHO (35% of daily energy), and therefore does not qualify as a high-CHO diet. This might have lowered the margin for fat-adaptation, and can explain the small changes in the outcome variables.

3. Although CHO was restricted to 26.4 ± 9.36 g/day, the actual protein and fat intakes on the self-selected LC intervention of the participants did not meet the target k-LCHF criteria. K-LCHF interventions might provide a more optimal metabolic stimulus for aerobic metabolic adaptation. Accordingly, the lack of adaptation in the aerobic energy system contribution during the intermittent sprints as reflected by the results of this study, might have been the consequence of this limitation.

4. Since the study population was recreationally active, but not elite, intermittent sprint sport players, participants were largely unaccustomed to this strenuous exercise modality. Thus, subconscious pacing strategies, based on the participants` perception of discomfort levels from previous sessions might have influenced their performances through centrally acting performance modification.

5. Although elite sport players and coaches might be interested in the findings of the current study, the findings cannot be extrapolated to elite-level, competitive field and court sport players.

6. It is important to note that ketone bodies were likely a significant energy source during the low-CHO intervention period. Ketones produce energy

through conversion to acetyl-CoA, an intermediate of lipid metabolism. This energy source is not directly accounted for by the stoichiometric calculations of fuel utilization. However, intermediate metabolic processes apparently do not influence overall fuel oxidation calculations (Jeukendrup & Wallis, 2005).

7. A repeated measures study design was used to monitor the adaptation process in this novel field. However, although it is generally accepted that a minimum training frequency of 2x per week is required to stimulate physiological adaptation, it is unknown if the test frequency of once every two weeks could have contributed minimal training induced adaptation in addition to the LC stimulus.

8. Due to slightly longer sprint- and recovery durations, the current protocol is not entirely representative of running-based field and court sports movement patterns. Study results are therefore not directly relevant for these sport players.

6.3 Implications in practice

1. Although it is widely acknowledged that oral creatine supplementation as the fuel source for the ATP-PCr energy system, has been shown to improve intermittent exercise performance, creatine is also naturally produced in the body by the liver and kidneys. Even so, methods of stimulating the body`s capacity to increase endogenous biosynthesis of creatine or improvements in this energy system without oral supplementation is not widely acknowledged. The present study eliminated the use of any dietary supplement for the duration of the intervention. The significant increase in ATP-PCr energy system contribution in the absence of oral creatine supplementation found in the present study, is consequently proposed to be the result of the 6-week LC diet intervention. Intermittent sprint sport players who prefer to avoid supplement use, but still want to enhance their ATP-PCr energy contributions associated with improved intermittent sprint performance, may consider implementation of a LC nutritional intervention.

2. Intermittent sprint sports in South Africa (e.g. rugby, netball, field hockey, soccer and racquet sports) at university level is becoming increasingly

competitive, and players at this level often spend many hours a week training to improve conditioning for performance improvements. The finding that a *moderate* supercompensation in fatigue decreases occurred over the six-week LC intervention, in the absence of a training intervention, might suggest that CHO restriction can be implemented as a sport-specific nutritional intervention as part of an integrated approach to periodization in performance optimization by these athletes.

3. The finding of *moderate* improvements in overall wellness in the healthy, recreationally active study population on the LC phase of the study, may pose an incentive to adopt a LC lifestyle as part of a holistic approach to optimize general vigour and wellbeing.

4. The strong negative correlation between changes in protein intake and changes in absolute VO_2max observed in the present study, might encourage players who desire to stimulate aerobic adaptations, to keep protein intake around 20% of energy intake, while increasing healthy sources of fat.

6.4 Recommendations for future research

1. Since this investigation is, to my knowledge, the first to investigate the effect of an LC intervention on adaptations in the ATP -PCr energy system, further research on this topic is needed to gain a broader understanding of the potential for adaptation in this important energy system, particularly in relation to CHO restriction.

2. Reductions in fatigue and improvements in energy metabolism on a LC nutritional intervention for maximal intensity intermittent sprints, were findings of the present study. Competitive elite-level sport players and coaches might be interested in the potential of these promising findings. However, the present study population was non-elite, recreationally active individuals. Future investigations in this field could thus consider examining these effects in elite-level sport players.

3. The role of ketone bodies on overall wellbeing in healthy, recreationally active individuals emerged from the results of the current study as another topic that could be further investigated. A review by Fond *et al.* (2013) on the positive

effects and neurobiological mechanisms for improvements in wellness in mood disorder patients, concluded that the neurobiological role of ketone bodies in mood is still unclear and that further investigation in this field is needed. Thus, the moderate improvement in overall wellness in conjunction with elevated blood [ketone] levels in the present study, might encourage further investigation on the role of blood [ketones] in wellness of healthy men and women.

4. The *very large* negative correlation between increases in protein intake and changes in absolute VO_2 max might be an explanation for not observing the hypothesised shift in aerobic energy system contributions during the intermittent sprints. Future research may examine if the hypothesised increase in aerobic energy contribution is stimulated when > 75% of energy is derived from healthy fat sources as prescribed by the K-LCHF guidelines.

5. The *strong* negative correlation between increases in protein intake and absolute VO_2 max in the present study further highlights the importance of not only placing emphasis on CHO restriction, but also emphasise implementation of the protein and fat guidelines of a k-LCHF intervention in future exercise performance studies.

6. Sex differences in metabolic and performance responses to a LC nutritional intervention during intermittent sprint exercise is another promising field of future research that emerged from the present study.

7. Future investigations may consider implementation of a sport-specific, running-based intermittent sprints protocol with shorter sprints and -recovery periods, to determine if the current results are applicable for these sport players.

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References

Aaserud, R., Gramvik, P., Olsen, S.R. & Jensen, J. 1998. Creatine supplementation delays onset of fatigue during repeated bouts of sprint running. *Scandinavian Journal of Medicine & Science in Sports*. 8(5):247–251.

Balsom, P.D., Seger, J.Y., Sjodin, B. & Ekblom, B. 1992. Maximal-intensity intermittent exercise: Effect of recovery duration. *International Journal of Sports Medicine*. 13(7):528–533.

Balsom, P.D., Seger, J.Y., Sjödin, B. & Ekblom, B. 1992. Physiological responses to maximal intensity intermittent exercise. *European Journal of Applied Physiology and Occupational Physiology*. 65(2):144–149.

Balsom, P.D., Ekblom, B., Söderlund, K., Sjödin, B. & Hultman, E. 1993. Creatine supplementation and dynamic high-intensity intermittent exercise. *Scandinavian Journal of Medicine and Science in Sports*. 3(3):143–149.

Beneke, R., Pollmann, C., Bleif, I., Leithäuser, R.M. & Hütler, H. 2002. How anaerobic is the wingate anaerobic test for humans? *European Journal of Applied Physiology*. 87(4–5):388–392.

Beneke, R., Beyer, T., Jachner, C., Erasmus, J. & Hütler, M. 2004. Energetics of karate kumite. *European Journal of Applied Physiology*. 92(4–5):518–523.

Billaut, F., Bishop, D.J., Schaerz, S. & Noakes, T.D. 2011. Influence of knowledge of sprint number on pacing during repeated-sprint exercise. *Medicine and Science in Sports and Exercise*. 43(4):665–672.

Bini, R.R., Hume, P.A. & Kilding, A.E. 2014. Saddle height effects on pedal forces, joint mechanical work and kinematics of cyclists and triathletes. *European Journal of Sport Science*. 14(1):44–52.

Bogdanis, G., Nevill, M., Boobis, L. & Lakomy, H. 1994. Recovery of Power Output and Muscle Metabolism after 10S and 20S of Maximal Sprint Exercise in Man. *Clinical Science*. 87(s1):121–122.

Bogdanis, G.C., Nevill, M.E., Boobis, L.H., Lakomy, H.K.A. & Nevill, A.M. 1995. Recovery of power output and muscle metabolites following 30 s of maximal sprint cycling in man. *Journal of Physiology*. 482(2):467–480.

Bogdanis, G.C., Nevill, M.E., Boobis, L.H. & Lakomy, H.K. 1996. Contribution of phosphocreatine and aerobic metabolism to energy supply during repeated sprint exercise. *Journal of Applied Physiology*. 80(3):876–884.

Borg, G.A.V. 1954. Psychophysical bases of perceived exertion. *Plastic and Reconstructive Surgery*. 14(5):377–381.

Bough, K.J., Wetherington, J., Hassel, B., Pare, J.F., Gawryluk, J.W., Greene, J.G., Shaw, R., Smith, Y., et al. 2006. Mitochondrial biogenesis in the anticonvulsant mechanism of the ketogenic diet. *Annals of Neurology*. 60(2):223–235.

Brown, A.J. 2007. Low-carb diets, fasting and euphoria: Is there a link between ketosis and γ-hydroxybutyrate (GHB)? *Medical Hypotheses*. 68(2):268–271.

Buchheit, M. & Laursen, P.B. 2013. High-intensity interval training, solutions to the programming puzzle: Part I: Cardiopulmonary emphasis. *Sports Medicine*. 43(5):313–338.

Buchheit, M., Abbiss, C.R., Peiffer, J.J. & Laursen, P.B. 2012. Performance and physiological responses during a sprint interval training session: Relationships with muscle oxygenation and pulmonary oxygen uptake kinetics. *European Journal of Applied Physiology*. 112(2):767–779.

Burke, L.M., Hawley, J.A., Angus, D.J., Cox, G.R., Clark, S.A., Cummings, N.K., Desbrow, B., Hargreaves, M., et al. 2002. Adaptations to short-term high-fat diet persist during exercise despite high carbohydrate availability. *Med. Sci. Sports Exerc*. 34(1):83–91.

Burke, L.M., Ross, M.L., Garvican-Lewis, L.A., Welvaert, M., Heikura, I.A., Forbes, S.G., Mirtschin, J.G., Cato, L.E., et al. 2017. Low carbohydrate, high fat diet impairs exercise economy and negates the performance benefit from intensified training in elite race walkers. *J Physiol*. 595(9):2785–2807.

Cameron-Smith, D., Tunstall, R.J., Hargreaves, M., Burke, L.M., Angus, D.J., Cox, G.R., Bonen, A. & Hawley, J.A. 2003. A short-term, high-fat diet up-regulates lipid metabolism and gene expression in human skeletal muscle. *American Journal of Clinical Nutrition*. 77(2):313–318.

Campos, F.A.D., Bertuzzi, R., Dourado, A.C., Santos, V.G.F. & Franchini, E. 2012. Energy demands in taekwondo athletes during combat simulation. *European Journal of Applied Physiology*. 112(4):1221–1228.

Carr, A.J., Sharma, A.P., Ross, M.L., Welvaert, M., Slater, G.J. & Burke, L.M. 2018. Chronic ketogenic low carbohydrate high fat diet has minimal effects on acid–base status in elite athletes. *Nutrients*. 10(2):1–13.

Casey, A., Constantin-Teodosiu, D., Howell, S., Hultman, E. & Greenhaff, P.L. 1996. Metabolic response of type I and II muscle fibers during repeated bouts of maximal exercise in humans. *American Journal of Physiology-Endocrinology and Metabolism*. 271(1):E38–E43.

Cheng, B., Karamizrak, O., Noakes, T.D., Dennis, S.C. & Lambert, E. V. 1997. Time course of the effects of a high-fat diet and voluntary exercise on muscle enzyme activity in Long-Evans rats. *Physiology and Behavior*. 61(5):701–705.

Chrzanowski-Smith, O.J., Piatrikova, E., Betts, J.A., Williams, S. & Gonzalez, J.T. 2020. Variability in exercise physiology: Can capturing intra-individual variation help better understand true inter-individual responses? *European Journal of Sport Science*. 20(4):452–460.

Cipryan, L., Plews, D.J., Ferretti, A., Maffetone, P.B. & Laursen, P.B. 2018. Effects of a 4-Week Very Low-Carbohydrate Diet on High-Intensity Interval Training Responses. *Journal of Sports Science and Medicine*. 17(June):259–267.

Clarkson, P., Eichner, E.R., Greenhaff, P.L., Hespel, P.J., Israel, R.G., Kraemer, W.J., Ronald, A., Spriet, L.L., et al. 2000. Physiological and Health Effects of Oral Creatine Supplementation. *Medicine & Science in Sports & Exercise*. 32(3):706–717.

Coffey, V.G. & Hawley, J.A. 2007.

Cohen, S. 1988. Perceived stress in a probability sample of the United States. In *The Claremont Symposium on Applied Social Psychology. The social psychology of health*. 31–67.

Cole, M. 1998. *The Theory and Methedology of Periodization of Strength Training*.

Collins, B.W., P Pearcey, G.E., Buckle, N.C., Power, K.E., Button, D.C., Collins, B. & Pearcey, G. 2018. Neuromuscular fatigue during repeated sprint exercise: underlying physiology and. *Applied Physiology, Nutrition, and Metabolism*.

Cooper, R., Naclerio, F., Allgrove, J. & Jimenez, A. 2012. Creatine supplementation with specific view to exercise/sports performance: an update. *Journal of the International Society of Sports Nutrition*. 9(33):1–11.

Costello, N., Deighton, K., Dyson, J., Mckenna, J. & Jones, B. 2017. Snap-N-Send: A valid and reliable method for assessing the energy intake of elite adolescent athletes. *European Journal of Sport Science*. 17(8):1044–1055.

Crisafulli, D.L., Buddhadev, H.H., Brilla, L.R., Chalmers, G.R., Suprak, D.N. & San Juan, J.G. 2018. Creatine-electrolyte supplementation improves repeated sprint cycling performance: A double blind randomized control study. *Journal of the International Society of Sports Nutrition*. 15(21).

Davis, P., Leithäuser, R.M. & Beneke, R. 2014. The energetics of semicontact 3 × 2-min amateur boxing. *International Journal of Sports Physiology and Performance*. 9(2):233–239.

Dawson, B., Goodman, C., Lawrence, S., Preen, D., Polglaze, T., Fitzsimons, M. & Fournier, P. 1997. Muscle phosphocreatine repletion following single and repeated short sprint efforts. *Scandinavian Journal of Medicine & Science in Sports*. 7(4):206–213.

Deminice, R., Rosa, F.T., Franco, G.S., Jordao, A.A. & de Freitas, E.C. 2013. Effects of creatine supplementation on oxidative stress and inflammatory markers after repeated-sprint exercise in humans. *Nutrition*. 29(9):1127–1132.

Dimitrova, N., Zamudio, J.R., Jong, R.M., Soukup, D., Resnick, R., Sarma, K., Ward, A.J., Raj, A., et al. 2017. Ketones inhibit mitochondrial production of reactive oxygen species production following glutamate exitotoxicity by increasing NADH oxidation. *PLoS ONE*. 32(7):736–740.

Dostal, T., Plews, D.J., Hofmann, P., Laursen, P.B. & Cipryan, L. 2019. Effects of a 12-week very-low carbohydrate high-fat diet on maximal aerobic capacity, high-intensity intermittent exercise, and cardiac autonomic regulation. *Frontiers in Pharmacology*. 10(July).

Faul, F., Erdfelder, E., Lang, A.-G. & Buchner, A. 2007. G*Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behavior Research Methods*. 39(2):175–191.

Febbraio, M.A., Steensberg, A., Walsh, R., Koukoulas, I., Van Hall, G., Saltin, B. & Pedersen, B.K. 2002. Reduced glycogen availability is associated with an elevation in HSP72 in contracting human skeletal muscle. *Journal of Physiology*. 538(3):911–917.

Ferrannini, E. 1988. The Theoretical Bases of Indirect Calorimetry: *Metabolism*. 37(3):287–301.

Finn, J.P., Ebert, T.R., Withers, R.T., Carey, M.F., Mackay, M., Phillips, J.W. & Febbraio, M.A. 2001. Effect of creatine supplementation on metabolism and performance in humans during intermittent sprint cycling. *European Journal of Applied Physiology*. 84(3):238–243.

Fisher, E.., Evans, W.., Phinney, S., Blackburn, G., Bistrian, B. & Young, V.. 1983. Changes in skeletal muscle metabolism induced by a eucaloric ketogenic diet. *Biochemistry of exercise: International Series on Sports Sciences*. 13:497–501.

Fitzsimons, M., Dawson, B., Ware, D. & Wilkinson, A. 1993. Cycling and Running Tests of Repeated Sprint Ability. *Aust J Sci Med Sport*. 25:82–87.

Fleming, J., Sharman, M.J., Avery, N.G., Love, D.M., Gómez, A.L., Scheett, T.P., Kraemer, W.J. & Volek, J.S. 2003. Endurance capacity and high-intensity exercise performance responses to a high fat diet. *International journal of sport nutrition and exercise metabolism*. 13(4):466–478.

Fond, G., Macgregor, A., Leboyer, M. & Michalsen, A. 2013. Fasting in mood disorders: Neurobiology and effectiveness. A review of the literature. *Psychiatry Research*. 209(3):253–258.

Furnas, G.W. 2000. Future design mindful of the MoRAS. *Human-Computer Interaction*. 15(2–3):205–261.

Gaitanos. 1993. Human muscle metabolism during intermittent maximal exercise.

Gano, L.B., Patel, M. & Rho, J.M. 2014. Ketogenic diets, mitochondria, and neurological diseases. *Journal of Lipid Research*. 55(11):2211–2228.

Gastin, P.B. 2001. Energy System Interaction and Relative Contribution During Maximal Exercise. *Sports Medicine*. 31(10):725–741.

Gibala, M.J., Little, J.P., van Essen, M., Wilkin, G.P., Burgomaster, K.A., Safdar, A., Raha, S. & Tarnopolsky, M.A. 2006. Short-term sprint interval versus traditional endurance training: Similar initial adaptations in human skeletal muscle and exercise performance. *Journal of Physiology*. 575(3):901–911.

Glaister, M., Howatson, G., Lockey, R.A., Abraham, C.S., Goodwin, J.E. & McInnes, G. 2007. Familiarization and reliability of multiple sprint running performance indices. *Journal of Strength and Conditioning Research*. 21(3):857–859.

Glaister, M., Howatson, G., Jpattison, O.R. & Mcinnes, G. 2008. The Reliability and validity of fatigue measures during multiple-sprint work: Andd issue revisited. *Journal of Strength and Conditioning Research*. 22(5):1597–1601.

Greene, D.A., Varley, B.J., Hartwig, T.B., Chapman, P. & Rigney, M. 2018. A low-carbohydrate ketogenic diet reduces body mass without compromising performance in powerlifting and olympic weightlifting athletes. *Journal of Strength and Conditioning Research*. 32(12):3373–3382.

Greenhaff, P.L., Casey, A., Short, A.H., Harris, R., Soderlund, K. & Hultman, E. 1993. Influence of oral creatine supplementation of muscle torque during repeated bouts of maximal voluntary exercise in man. *Clinical Science*. 84(5):565–571.

Hanjani, N.A. & Vafa, M. 2018. Protein restriction, epigenetic diet, intermittent fasting as new approaches for preventing age-associated diseases. *International Journal of Preventive Medicine*. 9(1).

Hawley, J.A., Burke, L.M., Angus, D.J., Fallon, K.E., Martin, D.T. & Febbraio, M.A. 2000. Effect of altering substrate availability on metabolism and performance during intense exercise. *British Journal of Nutrition*. 84(6):829–838.

Hawley, J.A., Tipton, K.D. & Millard-Stafford, M.L. 2006. Promoting training adaptations through nutritional interventions. *Nutrition and Football: The FIFA/FMARC Consensus on Sports Nutrition*. 0414:71–90.

Hawley, J.A., Burke, L.M., Phillips, S.M. & Spriet, L.L. 2011. Nutritional modulation of training-induced skeletal muscle adaptations. *Journal of Applied Physiology*. 110(3):834–845.

Heatherly, A.J., Killen, L.G., Smith, A.F., Waldman, H.S., Seltmann, C.L., Hollingsworth, A. & O'Neal, E.K. 2018. Effects of Ad libitum Low-Carbohydrate High-Fat Dieting in Middle-Age Male Runners. *Medicine and Science in Sports and Exercise*. 50(3):570–579.

Hietavala, E.M., Puurtinen, R., Kainulainen, H. & Mero, A.A. 2012. Low-protein vegetarian diet does not have a short-term effect on blood acid-base status but raises oxygen consumption during submaximal cycling. *Journal of the International Society of Sports Nutrition*. 9:1–9.

Hooper, S.L., Mackinnon, L.T., Howard, A., Gordon, R.D. & Bachmann, A.W. 1995. Markers for monitoring overtraining and recovery. *Medicine and Science in Sports and Exercise*. 27(1):106–112.

Hopkins, W.G. 2000. Measures of Reliability in Sports Medicine and Science. *Sports Medicine*. 30(1):1–15.

Hopkins, W.G., Marshall, S.W., Batterham, A.M. & Hanin, J. 2009. Progressive statistics for studies in sports medicine and exercise science. *Medicine and Science in Sports and Exercise*. 41(1):3–12.

Huang, Q., Ma, S., Tominaga, T., Suzuki, K. & Liu, C. 2018. An 8-week, low carbohydrate, high fat, ketogenic diet enhanced exhaustive exercise capacity in mice part 2: Effect on fatigue recovery, post-exercise biomarkers and anti-oxidation capacity. *Nutrients*. 10(10):1–16.

Huttenlocher, P.R. 1976. Ketonemia and Seizures: Metabolic and Anticonvulsant Effects of Two Ketogenic Diets in Childhood Epilepsy. *Pediat. Res*. 10:536–540. [Online], Available: https://www.nature.com/articles/pr197666.pdf [2018, May 31].

Hyatt, H.W., Kephart, W.C., Holland, A.M., Mumford, P., Brooks Mobley, C., Lowery, R.P., Roberts, M.D., Wilson, J.M., et al. 2016. A ketogenic diet in rodents elicits improved mitochondrial adaptations in response to resistance exercise training compared to an isocaloric western diet. *Frontiers in Physiology*. 7(NOV):1–9.

Jeukendrup, A.E. 2017. Periodized Nutrition for Athletes. *Sports Medicine.* 47(s1):51–63.

Jeukendrup, A.E. & Wallis, G.A. 2005. Measurement of substrate oxidation during exercise by means of gas exchange measurements. *International Journal of Sports Medicine, Supplement.* 26(1).

Julio, U.F., Panissa, V.L.G., Esteves, J. V., Cury, R.L., Agostinho, M.F. & Franchini, E. 2017. Energy-system contributions to simulated judo matches. *International Journal of Sports Physiology and Performance.* 12(5):676–683.

Julio, U.F., Panissa, V.L.G., Cury, R.L., Agostinho, M.F., Esteves, J.V.D.C. & Franchini, E. 2019. Energy System Contributions in Upper and Lower Body Wingate Tests in Highly Trained Athletes. *Research Quarterly for Exercise and Sport.* 90(2):244–250.

Keller, C., Steensberg, A., Pilegaard, H., Osada, T., Saltin, B., Pedersen, B.K. & Neufer, P.D. 2001. Transcriptional activation of the IL-6 gene in human contracting skeletal muscle: influence of muscle glycogen content. *The FASEB journal : official publication of the Federation of American Societies for Experimental Biology.* 15(14):2748–2750.

Kephart, W., Pledge, C., Roberson, P., Mumford, P., Romero, M., Mobley, C., Martin, J., Young, K., et al. 2018. The Three-Month Effects of a Ketogenic Diet on Body Composition, Blood Parameters, and Performance Metrics in CrossFit Trainees: A Pilot Study. *Sports.* 6(1):1.

Kossoff, E.H. 2004. More fat and fewer seizures: dietary therapies for epilepsy. *The Lancet Neurology.* 3(7):415–420.

Lambert, E. V, Speechly, D.P., Dennis, S.C. & Noakes, T.D. 1994. Enhanced endurance in trained cyclists during moderate intensity exercise following 2 weeks adaptation to a high fat diet. *European Journal of Applied Physiology and Occupational Physiology.* 69(4):287–293.

Lane, S.C., Camera, D.M., Lassiter, D.G., Areta, J.L., Bird, S.R., Yeo, W.K., Jeacocke, N.A., Krook, A., et al. 2015. Effects of sleeping with reduced carbohydrate availability on acute training responses. *Journal of Applied Physiology.* 119(6):643–655.

Langfort, J., Zarzeczny, R., Pilis, W., Nazar, K. & Kaciuba-Uścitko, H. 1997. The effect of a low-carbohydrate diet on performance, hormonal and metabolic responses to a 30-s bout of supramaximal exercise. *European Journal of Applied Physiology and Occupational Physiology*. 76(2):128–133.

Lopez, E.-I.D., Smoliga, J.M. & Zavorsky, G.S. 2014. The Effect of Passive Versus Active Recovery on Power Output Over Six Repeated Wingate Sprints. *Research Quarterly for Exercise and Sport*. 85:519–526.

Ludwig, D.S. & Ebbeling, C.B. 2018. The carbohydrate-insulin model of obesity: Beyond "calories in, calories out". *JAMA Internal Medicine*. 178(8):1098–1103.

MacLaren, D. & Morton, J. 2012. *Biochemistry for sport and exercise metabolism*.

Margaria, R., Edwards, H.T. & Dill, D.B. 1933. THE POSSIBLE MECHANISMS OF CONTRACTING AND PAYING THE OXYGEN DEBT AND THE RÔLE OF LACTIC ACID IN MUSCULAR CONTRACTION. *American Journal of Physiology-Legacy Content*. 106(3):689–715.

Maughan, R.J., Greenhaff, P.L., Leiper, J.B., Ball, D., Lambert, C.P. & Gleeson, M. 1997. Diet composition and the performance of high-intensity exercise. *Journal of Sports Sciences*. 15(3):265–275.

McCartney, N., Spriet, L.L., Heigenhauser, G.J., Kowalchuk, J.M., Sutton, J.R. & Jones, N.L. 1986. Muscle power and metabolism in maximal intermittent exercise. *Journal of Applied Physiology*. 60(4):1164–1169.

McSwiney, F.T., Wardrop, B., Hyde, P.N., Lafountain, R.A., Volek, J.S. & Doyle, L. 2017. Keto-adaptation enhances exercise performance and body composition responses to training in endurance athletes. *Metabolism*. 81:25–34.

Michalsen, A., Schneder, S., Rodenbeck, A., Ludtke, R., Huether, G. & Dobos, G.J. 2003. The Short-term Effects of Fasting on the Neuroendocrine System in Patients with Chronic Pain Syndromes. *Nutritional Neuroscience*. 6(1):11–18.

Milioni, F., Zagatto, A.M., Barbieri, R.A., Andrade, V.L., Dos Santos, J.W., Gobatto, C.A., Da Silva, A.S.R., Santiago, P.R.P., et al. 2017. Energy systems contribution in the running-based anaerobic sprint test. *International Journal of Sports Medicine*. 38(3):226–232.

Miller, V.J., Villamena, F.A. & Volek, J.S. 2018. Nutritional Ketosis and Mitohormesis: Potential Implications for Mitochondrial Function and Human Health. *Journal of Nutrition and Metabolism*. 2018:1–27.

Mirtschin, J.G., Forbes, S.F., Cato, L.E., Heikura, I.A., Strobel, N., Hall, R. & Burke, L.M. 2018. Organization of dietary control for nutrition-training intervention involving periodized carbohydrate availability and ketogenic low-carbohydrate high-fat diet. *International Journal of Sport Nutrition and Exercise Metabolism*. 28(5):480–489.

La Monica, M.B., Fukuda, D.H., Starling-Smith, T.M., Clark, N.W. & Panissa, V.L.G. 2020. Alterations in energy system contribution following upper body sprint interval training. *European Journal of Applied Physiology*. 120(3):643–651.

Morton, J.P., Croft, L., Bartlett, J.D., MacLaren, D.P.M., Reilly, T., Evans, L., McArdle, A. & Drust, B. 2009. Reduced carbohydrate availability does not modulate training-induced heat shock protein adaptations but does upregulate oxidative enzyme activity in human skeletal muscle. *Journal of Applied Physiology*. 106(5):1513–1521.

Moscat, J. & Diaz-Meco, M.T. 2011. Feedback on fat: P62-mTORC1-autophagy connections. *Cell*. 147(4):724–727.

Moshfegh, A.J., Rhodes, D.G., Baer, D.J., Murayi, T., Clemens, J.C., Rumpler, W. V., Paul, D.R., Sebastian, R.S., et al. 2008. The US Department of Agriculture Automated Multiple-Pass Method reduces bias in the collection of energy intakes. *American Journal of Clinical Nutrition*. 88(2):324–332.

Mujika, I., Halson, S., Burke, L.M., Balagué, G. & Farrow, D. 2018. An integrated, multifactorial approach to periodization for optimal performance in individual and team sports. *International Journal of Sports Physiology and Performance*. 13(5):538–561.

Newman, J.C. & Verdin, E. 2014. Ketone bodies as signaling metabolites. *Trends in Endocrinology and Metabolism*. 25(1):42–52.

Nordeen-Snyder, K.S. 1977. The effect of bicycle seat height variation upon oxygen consumption and lower limb kinematics. *Medicine and science in sports*. 9(2):113–7. [Online], Available: http://www.ncbi.nlm.nih.gov/pubmed/895427 [2018, May 22].

Olivier, G., Mendez-Villanueva, A. & Bishop, D. 2011. Repeated-Sprint Ability – Part I Factors Contributing to Fatigue. *Sports Med*. 41(8):673–694.

Paoli, A., Grimaldi, K., D'Agostino, D., Cenci, L., Moro, T., Bianco, A. & Palma, A. 2012. Ketogenic diet does not affect strength performance in elite artistic gymnasts. *Journal of the International Society of Sports Nutrition*. 9.

Paoli, A., Bianco, A., Damiani, E. & Bosco, G. 2014. Ketogenic diet in neuromuscular and neurodegenerative diseases. *BioMed Research International*. 2014.

Parolin, M.L., Chesley, A., Matsos, M.P., Spriet, L.L., Jones, N.L. & Heigenhauser, G.J.F. 1999. Regulation of skeletal muscle glycogen phosphorylase and PDH during maximal intermittent exercise endurance capacity in humans Regulation of skeletal muscle glycogen phosphorylase and PDH during maximal intermittent exercise. *Am. J. Physiol.* 277:E890–E900.

Pearcey, G.E.P., Murphy, J.R., Behm, D.G., Hay, D.C., Power, K.E. & Button, D.C. 2015. Neuromuscular fatigue of the knee extensors during repeated maximal intensity intermittent-sprints on a cycle ergometer. *Muscle and Nerve*. 51(4):569–579.

Phinney, S.D., Horton, E.S., Sims, E.A.H., Hanson, J.S., Danforth, E. & LaGrange, B.M. 1980. Capacity for moderate exercise in obese subjects after adaptation to a hypocaloric, ketogenic diet. *Journal of Clinical Investigation*. 66(5):1152–1161.

Phinney, S.D., Bistrian, B.R., Evans, W.J., Gervino, E. & Blackburn, G.L. 1983. The human metabolic response to chronic ketosis without caloric restriction: Preservation of submaximal exercise capability with reduced carbohydrate oxidation. *Metabolism*. 32(8):769–776.

Pilegaard, H., Keller, C., Steensberg, A., Helge, J.W., Pedersen, B.K., Saltin, B. & Darrell Neufer, P. 2002. Influence of pre-exercise muscle glycogen content on exercise-induced transcriptional regulation of metabolic genes. *Journal of Physiology*. 541(1):261–271.

Pinto, A., Bonucci, A., Maggi, E., Corsi, M. & Businaro, R. 2018. Anti-Oxidant and Anti-Inflammatory Activity of Ketogenic Diet: New Perspectives for Neuroprotection in Alzheimer's Disease. *Antioxidants*. 7(5).

Powers, S.K. & Howley, E.T. 2012. *Exercise physiology: Theory and application to fitness and performance*. Eight ed. New York: McGraw-Hill.

di Prampero, P.E. 1981. Energetics of Muscular Exercise. *Rev. Physiol. Biochem. Pharmacol*. 89:144–222.

di Prampero, P.E., Peeters, L. & Margaria, R. 1973. Alactic O2 debt and lactic acid production after exhausting exercise in man. *Journal of applied physiology*. 34(5):623–632.

Di Prampero, P.E. & Ferretti, G. 1999. The energetics of anaerobic muscle metabolism: a reappraisal of older and recent concepts. *Respiration Physiology*. 118:103–115.

Prins, P.J., Noakes, T.D., Welton, G.L., Haley, S.J., Esbenshade, N.J., Atwell, A.D., Scott, K.E., Abraham, J., et al. 2019. High rates of fat oxidation induced by a low-carbohydrate, high-fat diet, do not impair 5-km running performance in competitive recreational athletes. *Journal of Sports Science and Medicine*. 18(4):738–750.

Riebe, D., Ehrman, J.K., Liguori, G. & Magal, M. 2018. *ACSM's Guidelines for Exercise Testing and Prescription*. Tenth ed. K. Wolters (ed.).

Roberts, D.J. & Miyamoto, S. 2015. Hexokinase II integrates energy metabolism and cellular protection: Akting on mitochondria and TORCing to autophagy. *Cell Death and Differentiation*. 22(2):248–257.

Rodriguez, N., DiMarco, N. & Langley, S. 2009. Position of the American Dietetic Association, Dietitians of Canada, and the American College of Sports Medicine: Nutrition and Athletic Performance. *Journal of the American Dietetic Association*. 109(3):509–527.

Rojas-Morales, P., Tapia, E. & Pedraza-Chaverri, J. 2016. β-Hydroxybutyrate: A signaling metabolite in starvation response? *Cellular Signalling*. 28(8):917–923.

Ross, A. & Leveritt, M. 2001. Long-term metabolic and skeletal muscle adaptations to short-sprint training: Implications for sprint training and tapering. *Sports Medicine*. 31(15):1063–1082.

Rubini, A., Bosco, G., Lodi, A., Cenci, L., Parmagnani, A., Grimaldi, K., Zhongjin, Y. & Paoli, A. 2015. Effects of Twenty Days of the Ketogenic Diet on Metabolic and Respiratory Parameters in Healthy Subjects. *Lung*. 193(6):939–945.

Sahlin, K., Harris, R.C. & Hultman, E. 1979. Resynthesis of creatine phosphate in human muscle after exercise in relation to intramuscular ph and availability of oxygen. *Scandinavian Journal of Clinical and Laboratory Investigation*. 39(6):551–557.

Sampath, A., Kossoff, E.H., Furth, S.L., Pyzik, P.L. & Vining, E.P. 2007. Kidney stones and the ketogenic diet: risk factors and prevention. *J Child Neurol*. 22(4):375–378.

Santos, E.L. & Giannella-Neto, A. 2004. Comparison of computerized methods for detecting the ventilatory thresholds. *European Journal of Applied Physiology*. 93(3):315–324.

Schieke, S.M., Phillips, D., McCoy, J.P., Aponte, A.M., Shen, R.F., Balaban, R.S. & Finkel, T. 2006. The mammalian target of rapamycin (mTOR) pathway regulates mitochondrial oxygen consumption and oxidative capacity. *Journal of Biological Chemistry*. 281(37):27643–27652.

Schoenfeld, B.J., Aragon, A.A., Moon, J., Krieger, J.W. & Tiryaki-Sonmez, G. 2017. Comparison of amplitude-mode ultrasound versus air displacement plethysmography for assessing body composition changes following participation in a structured weight-loss programme in women. *Clinical Physiology and Functional Imaging*. 37(6):663–668.

Sherrier, M. & Li, H. 2019. The impact of keto-adaptation on exercise performance and the role of metabolic-regulating cytokines. *The American Journal of Clinical Nutrition*. 1–12.

Shinny, K.J., Feldman, H.A., Klein, G.L., Bielak, L., Ebbeling, C.B., Ludwig, D.S., Shimy, K.J., Feldman, H.A., et al. 2020. Effects of Dietary Carbohydrate Content on Circulating Metabolic Fuel Availability in the Postprandial State. *Journal of the Endocrine Society*. 4(7):1–13.

Signorile, J.F., Tremblay, L.M. & Ingalls, C. 1993. The Effects of Active and Passive Recovery on Short-Term, High Intensity Power Output. *Canadian Journal of Applied Physiology*. 18(1):31–42.

Smith, J.C. & Hill, D.W. 1991. Contribution of energy systems during a Wingate power test. *British Journal of Sports Medicine*. 25(4):196–199.

Spencer, M., Lawrence, S., Rechichi, C., Bishop, D., Dawson, B. & Goodman, C. 2004. Time-motion analysis of elite field hockey, with special reference to repeated-sprint activity. *Journal of Sports Sciences*. 22(9):843–850.

Spencer, M., Bishop, D., Dawson, B. & Goodman, C. 2005. Physiological and metabolic responses of repeated-sprint activities. *Sports Medicine*. 35(12):1025–1044.

Stafford, P., Abdelwahab, M.G., Kim, D.Y., Preul, M.C., Rho, J.M. & Scheck, A.C. 2010. The ketogenic diet reverses gene expression patterns and reduces reactive oxygen species levels when used as an adjuvant therapy for glioma. *Nutrition and Metabolism*. 7:1–11.

Stathis, C.G., Zhao, S., Carey, M.F. & Snow, R.J. 1999. Purine loss after repeated sprint bouts in humans. *Journal of Applied Physiology*. 87(6):2037–42. [Online], Available: http://www.ncbi.nlm.nih.gov/pubmed/10601147.

Stellingwerff, T. 2005. Decreased PDH activation and glycogenolysis during exercise following fat adaptation with carbohydrate restoration. *AJP: Endocrinology and Metabolism*. 290(2):E380–E388.

Sumithran, P., Prendergast, L.A., Delbridge, E., Purcell, K., Shulkes, A., Kriketos, A. & Proietto, J. 2013. Ketosis and appetite-mediating nutrients and hormones after weight loss. *European Journal of Clinical Nutrition*. 67(7):759–764.

Thorburn, A.W., Gumbiner, B., Flynn, T. & Henry, R.R. 1991. Substrate oxidation errors during combined indirect calorimetry-hyperinsulinemic glucose clamp studies. *Metabolism*. 40(4):391–398.

Trump, M.E., Heigenhauser, G.J., Putman, C.T. & Spriet, L.L. 1996. Importance of muscle phosphocreatine during intermittent maximal cycling. *Journal of Applied Physiology*. 80(5):1574–1580.

Tunstall, R.J., Mehan, K.A., Wadley, G.D., Collier, G.R., Bonen, A., Hargreaves, M. & Cameron-Smith, D. 2002. Exercise training increases lipid metabolism gene expression in human skeletal muscle. *American Journal of Physiology - Endocrinology And Metabolism*. 283(1):E66–E72.

Urbain, P., Strom, L., Morawski, L., Wehrle, A., Deibert, P. & Bertz, H. 2017. Impact of a 6-week non-energy-restricted ketogenic diet on physical fitness, body composition and biochemical parameters in healthy adults. *Nutrition and Metabolism*. 14(1):1–11.

Veech, R.L. 2004. The therapeutic implications of ketone bodies: the effects of ketone bodies in pathological conditions: ketosis, ketogenic diet, redox states, insulin resistance, and mitochondrial metabolism. *Prostaglandins, Leukotrienes and Essential Fatty Acids*. 70:309–319.

Veech, R.L., Bradshaw, P.C., Clarke, K., Curtis, W., Pawlosky, R. & King, M.T. 2017.

Vidali, S., Aminzadeh, S., Lambert, B., Rutherford, T., Sperl, W., Kofler, B. & Feichtinger, R.G. 2015. Mitochondria: The ketogenic diet - A metabolism-based therapy. *International Journal of Biochemistry and Cell Biology*. 63:55–59.

Vogt, M., Puntschart, A., Howald, H., Mueller, B., Mannhart, C., Gfeller-Tuescher, L., Mullis, P. & Hoppeler, H. 2003. Effects of dietary fat on muscle substrates, metabolism, and performance in athletes. *Medicine and Science in Sports and Exercise*. 35(6):952–960.

Volek, J.S., Freidenreich, D.J., Saenz, C., Kunces, L.J., Creighton, B.C., Bartley, J.M., Davitt, P.M., Munoz, C.X., et al. 2016. Metabolic characteristics of keto-adapted ultra-endurance runners. *Metabolism*. 65:100–110.

Vorster, H., Badham, J. & Venter, C. 2013. Food-Based Dietary Guidelines for South Africa FBDG- SA 2013 Food-Based Dietary Guidelines for South Africa. *South African Journal of Clinical Nutrition*. 26(3):S1–S164.

Wagner, D.R. 2013. Review Article Ultrasound as a Tool to Assess Body Fat. *Journal of Obesity*. 2:1–9. [Online], Available: http://www.hindawi.com/journals/jobe/2013/280713/.

Wagner, D.R., Thompson, B.J., Anderson, D.A. & Schwartz, S. 2018. A-mode and B-mode ultrasound measurement of fat thickness: a cadaver validation study. *European Journal of Clinical Nutrition*. 73(4):518–523.

Waldman, H.S., Krings, B.M., Smith, J.E.W. & McAllister, M.J. 2018. A shift toward a high-fat diet in the current metabolic paradigm: A new perspective. *Nutrition*. 46:33–35.

Waldman, H.S.H., Krings, B.M.B., Basham, S., Smith, J.E.W.J., Fountain, B. & McAllister, M.J.M. 2018. Effects of a 15-day low carbohydrate, high-fat diet in resistance trained men. *J Strength Cond Res*. 32(11):3103–3111.

Walker, J.B. 1960. Metabolic Control of Creatine Biosynthesis. *The journal of biological chemistry*. 235(8):19–38.

Warbuton, D., Jamnik, V., Bredin, S. & Gledhill, N. 2018. 2018 PAR-Q +| The physical activity readiness questionnaire for everyone. *Health and Fitness Journal of Canada*. 4(2):4–7.

Webster, C.C., Noakes, T.D., Chacko, S.K., Swart, J., Kohn, T.A. & Smith, J.A.H. 2016. Gluconeogenesis during endurance exercise in cyclists habituated to a long-term low carbohydrate high-fat diet. *The Journal of Physiology*. 594(15):4389–4405.

Webster, C.C., Swart, J., Noakes, T.D. & Smith, J.A. 2017. A Carbohydrate Ingestion Intervention in an Elite Athlete Who Follows a LCHF Diet. *International Journal of Sports Physiology and Performance*. 1–12.

Williams, E.L., Jones, H.S., Sparks, S., Marchant, D.C., Micklewright, D. & McNaughton, L.R. 2014. Deception studies manipulating centrally acting performance modifiers: A review. *Medicine and Science in Sports and Exercise*. 46(7):1441–1451.

World Medical Association. 2013. Declaration of Helsinki, Ethical Principles for Scientific Requirements and Research Protocols. *Bulletin of the World Health Organization.* 79(4):373. [Online], Available: https://www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for-medical-research-involving-human-subjects/.

Yeo, W., Lessard, S.J., Chen, Z.-P., Garnham, A.P., Burke, L.M., Rivas, D.A., Kemp, B.E. & Hawley, J.A. 2011. Fat adaptation followed by carbohydrate restoration increases AMPK activity in skeletal muscle from trained humans. *J Appl Physiol.* 105:1519–1526.

Yeo, W.K., Carey, A.L., Burke, L., Spriet, L.L., Hawley, J.A. & Carey, A. 2011. Fat adaptation in well-trained athletes: effects on cell metabolism. *Appl. Physiol. Nutr. Metab.* 36:12–22.

Yoshida, T. & Watari, H. 1997. Effect of circulatory occlusion on human muscle metabolism during exercise and recovery. *European Journal of Applied Physiology and Occupational Physiology.* 75(3):200–205.

Zagatto, A.M., de Mello Leite, J.V., Papoti, M. & Beneke, R. 2016. Energetics of Table Tennis and Table Tennis Specific Exercise Testing. *International Journal of Sports Physiology and Performance.* 1–44.

Zajac, A., Poprzecki, S., Maszczyk, A., Czuba, M., Michalczyk, M. & Zydek, G. 2014. The Effects of a Ketogenic Diet on Exercise Metabolism and Physical Performance in Off-Road Cyclists. *Nutrients.* 6:2493–2508.

Zinn, C., Wood, M., Williden, M., Chatterton, S. & Maunder, E. 2017. Ketogenic diet benefits body composition and well-being but not performance in a pilot case study of New Zealand endurance athletes. *Journal of the International Society of Sports Nutrition.* 14(1):1–9.

Appendix A:

Modified ACSM Medical Screening Questionnaire taken from *ACSM's guidelines for exercise testing and prescription* 9th ed. (Philadelphia: Wolters Kluwer/Lippincott Williams & Wilkins Health, pp.25.)

Health Status Questionnaire

On this questionnaire, a number of questions regarding your physical health are to be answered. Please answer every question as accurately as possible so that a correct assessment can be made. Please mark the space to the left of the question to answer "yes". Leave blank if your answer is "no". Please ask if you have any questions. Your response will be treated in a confidential manner.

Name:_____   Date:_____

Medical Screening – ACSM Medical Screening Questionnaire

- Do you have any personal history of heart disease?

- Do you suffer from chronic migraines?

- Do you suffer from any anxiety disorders?
- Do you have any personal history of metabolic disease (thyroid, renal, liver)?
- Do you know of any traumatic brain injury you have obtained? Are you using medication to treat a previous incident?
- Have you suffered from a severe head injury/concussion within 6 months of this study?
- Have you had diabetes for less than 15 years?
- Have you had diabetes for 15 years or more?
- Have you experienced pain or discomfort in your chest apparently due to blood flow deficiency?
- Any unaccustomed shortness of breath (perhaps during light exercise)?
- Have you had any problems with dizziness or fainting?
- Do you have difficulty breathing while standing or sudden breathing problems at night?
- Do you suffer from ankle oedema (swelling of the ankles)?
- Have you experienced a rapid throbbing or fluttering of the heart?
- Have you experienced severe pain in leg muscles during walking?
- Do you have a known heart murmur?
- Do you have any family history of cardiac or pulmonary disease prior to age 55?
- Have you been assessed as hypertensive on at least 2 occasions?
- Has your serum cholesterol been measured at greater than 5.4mmol/l?
- Are you a cigarette smoker?
- Would you characterize your lifestyle as "sedentary"?

Medical History

__ Are you currently being treated for high blood pressure?
If you know your average blood pressure, please enter: _____/_____
Please Check All That Apply.

_ has doctor ever found an abnormal ECG?	_ Limited Range of Motion?	_ Stroke?
_ Abnormal Chest X-Ray?	_ Recently Broken Bones? / _ Arthritis?	_ Epilepsy or Seizures?
_ Rheumatic Fever?	_ Bursitis?	_ chronic Headaches or Migraines?
_ Low Blood Pressure?	_ Swollen or Painful Joints?	_ Persistent Fatigue?
_ Asthma?	_ Foot Problems?	_ Stomach Problems?
_ Bronchitis?	_ Knee Problems?	_ Hernia?
_ Emphysema?	_ Back Problems?	_ Anemia?
_ Other Lung Problems?	_ Shoulder Problems?	_ Are You Pregnant?

＿ Has a doctor imposed any activity restrictions? If so, please describe:

_____

_____

_____

Family History

Have your mother, father, or siblings suffered from (please select all that apply):

＿＿ Heart attack or surgery prior to age 55
＿＿ Stroke prior to age 50
＿＿ Congenital heart disease or left ventricular hypertrophy
＿＿ High cholesterol
＿＿ Diabetes
＿＿ Obesity
＿＿ Hypertension
＿＿ Osteoporosis
＿＿ Asthma
＿＿ Leukemia or cancer prior to age 60

Medications

Please Select Any Medications You Are Currently Using

＿ Diuretics   ＿ Other Cardiovascular

＿ Beta Blockers   ＿ NSAIDS/Anti-inflammatories (Motrin, Advil)

＿ Vasodilators   ＿ Cholesterol

＿ Alpha Blockers   ＿ Diabetes/Insulin

＿ Calcium Channel Blockers   ＿ Other Drugs (record below).

Please list the specific medications that you currently take:

_____

_____

Emergency Contacts

Please list your general practitioner and person to be contacted in case of emergency

Doctor:_____   Phone:_____

Contact:_____   Phone:_____

Activities and Goals

On average, how many times do you exercise per week? _____

On average, how long do you exercise? _____ minutes

On a scale from 1 to 10, how intense is your typical workout (circle one):
Very Easy **1   2   3   4   5   6   7   8   9   10** Very Intense

For each activity that you participate in, indicate your typical exercise time in minutes per session:

Running/Jogging:	_____	Weight Training:	_____	Skiing/Boarding:	_____
Walking:	_____	Aerobics Classes:	_____	Yoga/Martial Arts:	_____
Stair Climbing:	_____	Swimming:	_____	Other:	_____
Bicycle/Spinning:	_____	Racquet Sports:	_____		

Lifestyle

Are you a cigarette smoker? _____   If so, how many per day? _____

Previously a cigarette smoker? _____   If so, when did you quit? _____

How many years have you smoked or did you smoke before quitting? _____

Do you/did you smoke: cigarettes?   cigars?   pipe?

Please rate your daily stress levels (select one):

Low   Moderate   High: I enjoy   High: sometimes   High: often difficult
                        the challenge   difficult to handle   to handle

Do you drink alcoholic beverages? _____
How many units of alcohol do you consume per week: _____ (see Alcohol Units Calculator below)

Alcohol Units Calculator

Type of Drink	Units

1 glass of wine

1

1 pub measure of spirits (Gin, Vodka etc.)

1

[ ]

1 can of beer

1.5

[ ]

1 bottle of strong lager

2.5

[ ]

1 can of strong lager

4

[ ]

1 bottle of wine

7

[ ]

1 litre bottle of wine

10

[ ]

1 bottle of fortified wine (port, sherry etc.)

14

[ ]

1 bottle of spirits

30

[ ]

Dietary Habits: Please select all that apply

__   I seldom consume red or high fat meats
__   I pursue a low-fat diet
__   I eat at least 5 servings of fruits/vegetables per day

__   I almost always eat a full, healthy breakfast
__   My diet includes many high-fiber foods
__   I rarely eat sugar of high-fat dessert

Other

Please indicate any other medical conditions or activity restrictions that you may have. It is important that this information be as accurate and complete as possible.

_____

_____

_____

Is any of this information critical to understanding your readiness for exercise? Are there any other restrictions on activity that we should know about?

_____

_____

Thank you for taking the time to complete this questionnaire!

Appendix B

2018 PAR-Q+

The Physical Activity Readiness Questionnaire for Everyone

The health benefits of regular physical activity are clear; more people should engage in physical activity every day of the week. Participating in physical activity is very safe for MOST people. This questionnaire will tell you whether it is necessary for you to seek further advice from your doctor OR a qualified exercise professional before becoming more physically active.

GENERAL HEALTH QUESTIONS

Please read the 7 questions below carefully and answer each one honestly: check YES or NO.	YES	NO
1) Has your doctor ever said that you have a heart condition ☐ OR high blood pressure ☐?	☐	☐
2) Do you feel pain in your chest at rest, during your daily activities of living, **OR** when you do physical activity?	☐	☐
3) Do you lose balance because of dizziness **OR** have you lost consciousness in the last 12 months? Please answer **NO** if your dizziness was associated with over-breathing (including during vigorous exercise).	☐	☐
4) Have you ever been diagnosed with another chronic medical condition (other than heart disease or high blood pressure)? **PLEASE LIST CONDITION(S) HERE:** _____	☐	☐
5) Are you currently taking prescribed medications for a chronic medical condition? **PLEASE LIST CONDITION(S) AND MEDICATIONS HERE:** _____	☐	☐
6) Do you currently have (or have had within the past 12 months) a bone, joint, or soft tissue (muscle, ligament, or tendon) problem that could be made worse by becoming more physically active? Please answer **NO** if you had a problem in the past, but it *does not limit your current ability* to be physically active. **PLEASE LIST CONDITION(S) HERE:** _____	☐	☐
7) Has your doctor ever said that you should only do medically supervised physical activity?	☐	☐

✓ **If you answered NO to all of the questions above, you are cleared for physical activity.**
Please sign the PARTICIPANT DECLARATION. You do not need to complete Pages 2 and 3.

- ▶ Start becoming much more physically active – start slowly and build up gradually.
- ▶ Follow International Physical Activity Guidelines for your age (www.who.int/dietphysicalactivity/en/).
- ▶ You may take part in a health and fitness appraisal.
- ▶ If you are over the age of 45 yr and NOT accustomed to regular vigorous to maximal effort exercise, consult a qualified exercise professional before engaging in this intensity of exercise.
- ▶ If you have any further questions, contact a qualified exercise professional.

PARTICIPANT DECLARATION
If you are less than the legal age required for consent or require the assent of a care provider, your parent, guardian or care provider must also sign this form.

I, the undersigned, have read, understood to my full satisfaction and completed this questionnaire. I acknowledge that this physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if my condition changes. I also acknowledge that the community/fitness centre may retain a copy of this form for records. In these instances, it will maintain the confidentiality of the same, complying with applicable law.

NAME _____ DATE _____

SIGNATURE _____ WITNESS _____

SIGNATURE OF PARENT/GUARDIAN/CARE PROVIDER _____

🛑 **If you answered YES to one or more of the questions above, COMPLETE PAGES 2 AND 3.**

⚠️ **Delay becoming more active if:**

- ✓ You have a temporary illness such as a cold or fever; it is best to wait until you feel better.
- ✓ You are pregnant - talk to your health care practitioner, your physician, a qualified exercise professional, and/or complete the ePARmed-X+ at **www.eparmedx.com** before becoming more physically active.
- ✓ Your health changes - answer the questions on Pages 2 and 3 of this document and/or talk to your doctor or a qualified exercise professional before continuing with any physical activity program.

2018 PAR-Q+

FOLLOW-UP QUESTIONS ABOUT YOUR MEDICAL CONDITION(S)

1. Do you have Arthritis, Osteoporosis, or Back Problems?

If the above condition(s) is/are present, answer questions 1a-1c   If **NO** ☐ go to question 2

1a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
1b.	Do you have joint problems causing pain, a recent fracture or fracture caused by osteoporosis or cancer, displaced vertebra (e.g., spondylolisthesis), and/or spondylolysis/pars defect (a crack in the bony ring on the back of the spinal column)?	YES ☐ NO ☐
1c.	Have you had steroid injections or taken steroid tablets regularly for more than 3 months?	YES ☐ NO ☐

2. Do you currently have Cancer of any kind?

If the above condition(s) is/are present, answer questions 2a-2b   If **NO** ☐ go to question 3

2a.	Does your cancer diagnosis include any of the following types: lung/bronchogenic, multiple myeloma (cancer of plasma cells), head, and/or neck?	YES ☐ NO ☐
2b.	Are you currently receiving cancer therapy (such as chemotheraphy or radiotherapy)?	YES ☐ NO ☐

3. Do you have a Heart or Cardiovascular Condition? *This includes Coronary Artery Disease, Heart Failure, Diagnosed Abnormality of Heart Rhythm*

If the above condition(s) is/are present, answer questions 3a-3d   If **NO** ☐ go to question 4

3a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
3b.	Do you have an irregular heart beat that requires medical management? (e.g., atrial fibrillation, premature ventricular contraction)	YES ☐ NO ☐
3c.	Do you have chronic heart failure?	YES ☐ NO ☐
3d.	Do you have diagnosed coronary artery (cardiovascular) disease and have not participated in regular physical activity in the last 2 months?	YES ☐ NO ☐

4. Do you have High Blood Pressure?

If the above condition(s) is/are present, answer questions 4a-4b   If **NO** ☐ go to question 5

4a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
4b.	Do you have a resting blood pressure equal to or greater than 160/90 mmHg with or without medication? (Answer **YES** if you do not know your resting blood pressure)	YES ☐ NO ☐

5. Do you have any Metabolic Conditions? *This includes Type 1 Diabetes, Type 2 Diabetes, Pre-Diabetes*

If the above condition(s) is/are present, answer questions 5a-5e   If **NO** ☐ go to question 6

5a.	Do you often have difficulty controlling your blood sugar levels with foods, medications, or other physician-prescribed therapies?	YES ☐ NO ☐
5b.	Do you often suffer from signs and symptoms of low blood sugar (hypoglycemia) following exercise and/or during activities of daily living? Signs of hypoglycemia may include shakiness, nervousness, unusual irritability, abnormal sweating, dizziness or light-headedness, mental confusion, difficulty speaking, weakness, or sleepiness.	YES ☐ NO ☐
5c.	Do you have any signs or symptoms of diabetes complications such as heart or vascular disease and/or complications affecting your eyes, kidneys, **OR** the sensation in your toes and feet?	YES ☐ NO ☐
5d.	Do you have other metabolic conditions (such as current pregnancy-related diabetes, chronic kidney disease, or liver problems)?	YES ☐ NO ☐
5e.	Are you planning to engage in what for you is unusually high (or vigorous) intensity exercise in the near future?	YES ☐ NO ☐

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6. **Do you have any Mental Health Problems or Learning Difficulties?** *This includes Alzheimer's, Dementia, Depression, Anxiety Disorder, Eating Disorder, Psychotic Disorder, Intellectual Disability, Down Syndrome*

If the above condition(s) is/are present, answer questions 6a-6b   If **NO** ☐ go to question 7

6a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
6b.	Do you have Down Syndrome **AND** back problems affecting nerves or muscles?	YES ☐ NO ☐

7. **Do you have a Respiratory Disease?** *This includes Chronic Obstructive Pulmonary Disease, Asthma, Pulmonary High Blood Pressure*

If the above condition(s) is/are present, answer questions 7a-7d   If **NO** ☐ go to question 8

7a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
7b.	Has your doctor ever said your blood oxygen level is low at rest or during exercise and/or that you require supplemental oxygen therapy?	YES ☐ NO ☐
7c.	If asthmatic, do you currently have symptoms of chest tightness, wheezing, laboured breathing, consistent cough (more than 2 days/week), or have you used your rescue medication more than twice in the last week?	YES ☐ NO ☐
7d.	Has your doctor ever said you have high blood pressure in the blood vessels of your lungs?	YES ☐ NO ☐

8. **Do you have a Spinal Cord Injury?** *This includes Tetraplegia and Paraplegia*

If the above condition(s) is/are present, answer questions 8a-8c   If **NO** ☐ go to question 9

8a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
8b.	Do you commonly exhibit low resting blood pressure significant enough to cause dizziness, light-headedness, and/or fainting?	YES ☐ NO ☐
8c.	Has your physician indicated that you exhibit sudden bouts of high blood pressure (known as Autonomic Dysreflexia)?	YES ☐ NO ☐

9. **Have you had a Stroke?** *This includes Transient Ischemic Attack (TIA) or Cerebrovascular Event*

If the above condition(s) is/are present, answer questions 9a-9c   If **NO** ☐ go to question 10

9a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
9b.	Do you have any impairment in walking or mobility?	YES ☐ NO ☐
9c.	Have you experienced a stroke or impairment in nerves or muscles in the past 6 months?	YES ☐ NO ☐

10. **Do you have any other medical condition not listed above or do you have two or more medical conditions?**

If you have other medical conditions, answer questions 10a-10c   If **NO** ☐ read the Page 4 recommendations

10a.	Have you experienced a blackout, fainted, or lost consciousness as a result of a head injury within the last 12 months **OR** have you had a diagnosed concussion within the last 12 months?	YES ☐ NO ☐
10b.	Do you have a medical condition that is not listed (such as epilepsy, neurological conditions, kidney problems)?	YES ☐ NO ☐
10c.	Do you currently live with two or more medical conditions?	YES ☐ NO ☐

PLEASE LIST YOUR MEDICAL CONDITION(S) AND ANY RELATED MEDICATIONS HERE: _____

_____

GO to Page 4 for recommendations about your current medical condition(s) and sign the PARTICIPANT DECLARATION.

2018 PAR-Q+

☑ **If you answered NO to all of the FOLLOW-UP questions (pgs. 2-3) about your medical condition, you are ready to become more physically active - sign the PARTICIPANT DECLARATION below:**

▶ It is advised that you consult a qualified exercise professional to help you develop a safe and effective physical activity plan to meet your health needs.

▶ You are encouraged to start slowly and build up gradually - 20 to 60 minutes of low to moderate intensity exercise, 3-5 days per week including aerobic and muscle strengthening exercises.

▶ As you progress, you should aim to accumulate 150 minutes or more of moderate intensity physical activity per week.

▶ If you are over the age of 45 yr and **NOT** accustomed to regular vigorous to maximal effort exercise, consult a qualified exercise professional before engaging in this intensity of exercise.

🛑 If you answered **YES** to **one or more of the follow-up questions** about your medical condition:

You should seek further information before becoming more physically active or engaging in a fitness appraisal. You should complete the specially designed online screening and exercise recommendations program - the **ePARmed-X+ at www.eparmedx.com** and/or visit a qualified exercise professional to work through the ePARmed-X+ and for further information.

⚠️ **Delay becoming more active if:**

✓ You have a temporary illness such as a cold or fever; it is best to wait until you feel better.

✓ You are pregnant - talk to your health care practitioner, your physician, a qualified exercise professional, and/or complete the ePARmed-X+ **at www.eparmedx.com** before becoming more physically active.

✓ Your health changes - talk to your doctor or qualified exercise professional before continuing with any physical activity program.

● You are encouraged to photocopy the PAR-Q+. You must use the entire questionnaire and NO changes are permitted.
● The authors, the PAR-Q+ Collaboration, partner organizations, and their agents assume no liability for persons who undertake physical activity and/or make use of the PAR-Q+ or ePARmed-X+. If in doubt after completing the questionnaire, consult your doctor prior to physical activity.

PARTICIPANT DECLARATION

● All persons who have completed the PAR-Q+ please read and sign the declaration below.

● If you are less than the legal age required for consent or require the assent of a care provider, your parent, guardian or care provider must also sign this form.

I, the undersigned, have read, understood to my full satisfaction and completed this questionnaire. I acknowledge that this physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if my condition changes. I also acknowledge that the community/fitness center may retain a copy of this form for records. In these instances, it will maintain the confidentiality of the same, complying with applicable law.

NAME _____   DATE _____

SIGNATURE _____   WITNESS _____

SIGNATURE OF PARENT/GUARDIAN/CARE PROVIDER _____

For more information, please contact
www.eparmedx.com
Email: eparmedx@gmail.com

The PAR-Q+ was created using the evidence-based AGREE process (1) by the PAR-Q+ Collaboration chaired by Dr. Darren E. R. Warburton with Dr. Norman Gledhill, Dr. Veronica Jamnik, and Dr. Donald C. McKenzie (2). Production of this document has been made possible through financial contributions from the Public Health Agency of Canada and the BC Ministry of Health Services. The views expressed herein do not necessarily represent the views of the Public Health Agency of Canada or the BC Ministry of Health Services.

Key References

1. Jamnik VK, Warburton DER, Makarski J, McKenzie DC, Shephard RJ, Stone J, and Gledhill N. Enhancing the effectiveness of clearance for physical activity participation; background and overall process. APNM 36(S1):S3-S13, 2011.

2. Warburton DER, Gledhill N, Jamnik VK, Bredin SSD, McKenzie DC, Stone J, Charlesworth S, and Shephard RJ. Evidence-based risk assessment and recommendations for physical activity clearance; Consensus Document. APNM 36(S1):S266-s298, 2011.

3. Chisholm DM, Collis ML, Kulak LL, Davenport W, and Gruber N. Physical activity readiness. British Columbia Medical Journal. 1975;17:375-378.

4. Thomas S, Reading J, and Shephard RJ. Revision of the Physical Activity Readiness Questionnaire (PAR-Q). Canadian Journal of Sport Science 1992;17:4 338-345.

Appendix C: **RPE scale** (Borg,1982)

RPE Scale

6	
7	Very, Very Light
8	
9	Very Light
10	
11	Fairly Light
12	
13	Somewhat Hard
14	
15	Hard
16	
17	Very Hard
18	
19	Very, Very Hard
20	

Appendix D
PARTICIPANT INFORMATION LEAFLET AND CONSENT FORM

TITLE OF RESEARCH PROJECT:	
Changes in energy metabolism and intermittent sprint performance in healthy active individuals following a 6-week low carbohydrate eating plan	
DETAILS OF PRINCIPAL INVESTIGATOR (PI):	
Title, first name, surname: Ms Anika Pretorius	**Ethics reference number:**
Full postal address: Department Sport Science, Stellenbosch University, Private Bag X1, Matieland, Stellenbosch, 7601	**PI Contact number:** 0764635283

We would like to invite you to take part in a research project. Please take some time to read the information presented here, which will explain the details of this project. Please ask the study staff any questions about any part of this project that you do not fully understand. It is very important that you are completely satisfied that you clearly understand what this research entails and how you could be involved. Also, your participation is **entirely voluntary** and you are free to decline to participate. In other words, you may choose to take part, or you may choose not to take part. Nothing bad will come of it if you say no: it will not affect you negatively in any way whatsoever. Refusal to participate will involve no penalty or loss of benefits or reduction in the level of care to which you are otherwise entitled to. You are also free to withdraw from the study at any point, even if you do agree to take part initially.

This study has been approved by the **Health Research Ethics Committee at Stellenbosch University.** The study will be conducted according to the ethical guidelines and principles of the international Declaration of Helsinki, the South African Guidelines for Good Clinical Practice (2006), the Medical Research Council (MRC) Ethical Guidelines for Research (2002), and the Department of Health Ethics in Health Research: Principles, Processes and Studies (2015).

What is this research study all about?

The study will be conducted in the Sport Physiology Laboratory in the Department of Sport Science. We will invite 15 men and women to participate in the study and recruitment will continue until we achieve this number.

The aims of the study are to investigate how dietary carbohydrate restriction affects your performance during short, high intensity interval exercise, as well as how the change in diet affects the way your body fuels your body during exercise.

You will be expected to adapt your diet and follow a low carbohydrate high fat (LCHF) eating plan for 6 consecutive weeks. You will be allowed to construct your own meal plans as long as it meets the requirements for the study namely, consisting of less than 50 g carbohydrates per day, combined with fats (> 75% of total calorie intake) and protein (10-15% protein). You will receive a booklet with information on LCHF eating and you will be assisted and guided by Dr Schoombee, the designated project doctor and Ms Louise Engelbrecht, a qualified sport nutritionist.

If you agree to take part in the study and the screening procedure indicate that you are eligible, you will be invited to attend a familiarization session in the laboratory, which will last at least an hour. Your height, weight, % body fat and resting metabolic rate will be measured. The latter test requires you to lie down on an examination bed for 15 minutes while breathing through a face mask that is connected to a machine; the machine will analyse the air that your breath out. We will then ask you to sit on the cycle ergometer so that we can determine your most comfortable position on the bike. Measurements will be taken and recorded so that we can adjust the bike to your specifications for all the tests.

You will then perform a maximal exercise test on the cycle ergometer. We will fit you with a heart rate monitor around your chest and a face mask (as above). You will be required to cycle at a constant pedal rate while the resistance on the bike is adjusted every 2.5 min. You will be asked to cycle until you are exhausted. The data that we gather from this test will allow us to calculate your maximal aerobic capacity.

After a 15 minute rest period, you will be asked to mount the cycle ergometer again and then we will explain how the sprint tests will be done which you will perform every 2 weeks while you are on the LCHF eating plan. You will be allowed to try out three sprint intervals so that you will know exactly what to expect in the following testing sessions.

During the following two weeks you will be required to visit the laboratory on two occasions, at least 7 days apart, for baseline testing. You will be asked to perform various tests and measurements. These include the measurement of body weight and % body fat, your hydration level via a urine sample, your ketone concentration in your blood via a small finger prick and the intermittent cycle sprint test. The latter consists of 6 x 10 sec all-out sprints, with 2 min recovery in between sprints. During the test you will wear the heart rate monitor and face mask as before to measure and analyse the air that you breathe out. We will also take small blood samples via finger pricks to measure the lactate concentration in your blood. In total about 2 mL of blood will be collected. Following the six sprints, you will be asked to sit quietly for 15 minutes while your breathing and heart rate are still measured. These laboratory sessions will last one hour.

Following the second baseline tests, you will be asked to change your diet to fit the LCHF meal plan requirements for six consecutive weeks. As mentioned above, you will be guided through this process by two professionals and you will also receive a booklet with information which you may take home. During the six weeks we ask that you check in to the laboratory once per week (appointments will be made). During these quick visits we will measure your hydration and ketone levels as before and you may consult with the researchers if you have any questions or need assistance with your meals. You will also perform the intermittent sprint tests and all other tests and measurements, as during the baseline testing sessions, during week 2, 4 and 6 of your carbohydrate restricted eating period. After the final tests you are welcome to revert to your traditional diet if you wish. We will put all your results in a report and we will discuss the findings with you at a time and place that is convenient for you.

Why do we invite you to participate?

You are invited to take part in this study because you have indicated your interest in the research project by responding voluntarily to the invitation asking for participants and you meet the inclusion criteria for the study. The inclusion criteria stipulate that you are a young and healthy individual, aged between 18 and 40 years and actively participate in deliberate exercise and training at least three times per week.

What will your responsibilities be?

We ask that you complete all questionnaires as honestly as possible and that you follow the instructions of the researchers before, during and after the testing sessions as best you can. You will also be asked to give your best efforts during the cycle ergometer tests. In case of any deviations from the instructions (see below), we ask that you inform the researchers.

Prior to each testing session, we ask that you do the following:
1.   not eat for at least four hours prior to testing;
2.   avoid caffeine-containing drinks and alcohol ingestion at least 12 hours before testing;
3.   avoid vigorous activities – RPE above 12 on the Borg scale - or any unaccustomed exercise at least 24 hours before testing;
4.   stay hydrated prior to testing.

You are also required to follow the LCHF eating plan as best you can, report your daily food intake honestly and as accurately as possible and that you notify the researchers immediately if you experience any debilitating side-effects.

Will you benefit from taking part in this research?

Through your participation in this project you will have the opportunity to try out a carbohydrate restricted eating plan under supervision and while having access to professionals for guidance and advice. You may also benefit from the many positive health effects associated with carbohydrate restricted eating plans. These include decreased abdominal fat (shown as a lifestyle disease risk), positive anti-inflammatory and anti-oxidant effects, as well as a lowered risk for cardiovascular disease and insulin resistance. You will receive a personal report on all your test results and these findings will be discussed with you in person.

Are there any risks involved in your taking part in this research?

There will be no serious risks involved in the study; nonetheless, you may experience dizziness, physical discomfort, muscle fatigue and in rare instances, fainting, during the intermittent sprint tests. In the case that you experience any of these symptoms the exercise test will be stopped immediately and the researchers will take the necessary steps to make you comfortable. All the staff in the Sport Physiology Laboratory is trained in first aid. Should any serious emergency arise, you will be stabilized and transported to the emergency room of Stellenbosch Medi-Clinic.

During the first few days and weeks after the initiation of the low carbohydrate high fat (LCHF) eating plan you may experience mild side effects such as dehydration, headaches, fatigue constipation or diarrhoea; these symptoms usually subside within a few days. Serious adverse events, i.e. electrolyte alteration or heart arrhythmias are uncommon. If you experience any of these symptoms, you may stop the eating plan immediately and you may consult with the medical doctor overseeing the study, Dr Schoombee for medical support.

Even though it is unlikely, what will happen if you get injured somehow because you took part in this research study?

Stellenbosch University will provide comprehensive no-fault insurance and will pay for any medical costs that came about because participants took part in the research, either as a result of the LCHF eating plan or the exercise testing in the laboratory. The participant will not need to prove that the researchers were at fault.

Will you be paid to take part in this study and are there any costs involved?

You will not receive any payment or compensation for your participation in this study. You will also not have to pay for anything, if you do take part.

Is there anything else that you should know or do?

If you have any questions or concerns about this study, please feel free to contact Anika Pretorius [0764635283; 16961269@sun.ac.za] and/or the supervisor Prof Elmarie Terblanche [082 7076501; et2@sun.ac.za].

You may phone the Health Research Ethics Committee at 021 938 9677/9819 if there still is something that the researchers have not explained to you, or if you have a complaint.

You will receive a copy of this information and consent form for you to keep safe.

Declaration by participant

By signing below, I ……………………………..………… agree to take part in a research study entitled *"Changes in energy metabolism and intermittent sprint performance in healthy active individuals following a 6-week low carbohydrate eating plan".*

I declare that:

- I have read this information and consent form, or it was read to me, and it is written in a language in which I am fluent and with which I am comfortable.

- I have had a chance to ask questions and I am satisfied that all my questions have been answered.

- I understand that taking part in this study is **voluntary,** and I have not been pressurised to take part.

- I may choose to leave the study at any time and nothing bad will come of it – I will not be penalised or prejudiced in any way.

- I may be asked to leave the study before it has finished, if the study doctor or researcher feels it is in my best interests, or if I do not follow the study plan that we have agreed on.

Signed at (*place*) ....….......……………... on (*date*) …………...……….. 2019.

....
Signature of participant   **Signature of witness**

Declaration by investigator

I (*name*) …………………………………………..……… declare that:

- I explained the information in this document in a simple and clear manner to …………………………………..

- I encouraged him/her to ask questions and took enough time to answer them.

- I am satisfied that he/she completely understands all aspects of the research, as discussed above.

- I did/did not use an interpreter. (*If an interpreter is used then the interpreter must sign the declaration below.*)

Signed at (*place*) ....….......……………... on (*date*) …………...……….. 2015.

....
Signature of investigator   **Signature of witness**

Appendix E:

Wellness questionnaire (Hooper *et al.,* 2010)

	1	2	3	4	5	Record score
Fatigue	Very fresh	Fresh	Normal	More tired than normal	Always tired	
Sleep quality	Very restful	Good	Difficulty falling asleep	Restless sleep	Insomnia	
General muscle soreness	Fellng great	Feeling good	Normal	Increase in soreness/ tightness	Very sore	
Stress levels	Very relaxed	Relaxed	Normal	Feeling stressed	Highly stressed	
Mood	Very positive mood	A generally good mood	Less interested in others &/ activities than usual	Snappiness at team mates, family or co-workers	Highly annoyed/ irritable/ down	

Wellness questionnaire developed by McLean et al (2010) on the recommendations of Hooper & Mackinnon (1995).

Please provide reasons for high scores (>4) in any category:

Appendix F

Participant LCHF Guide
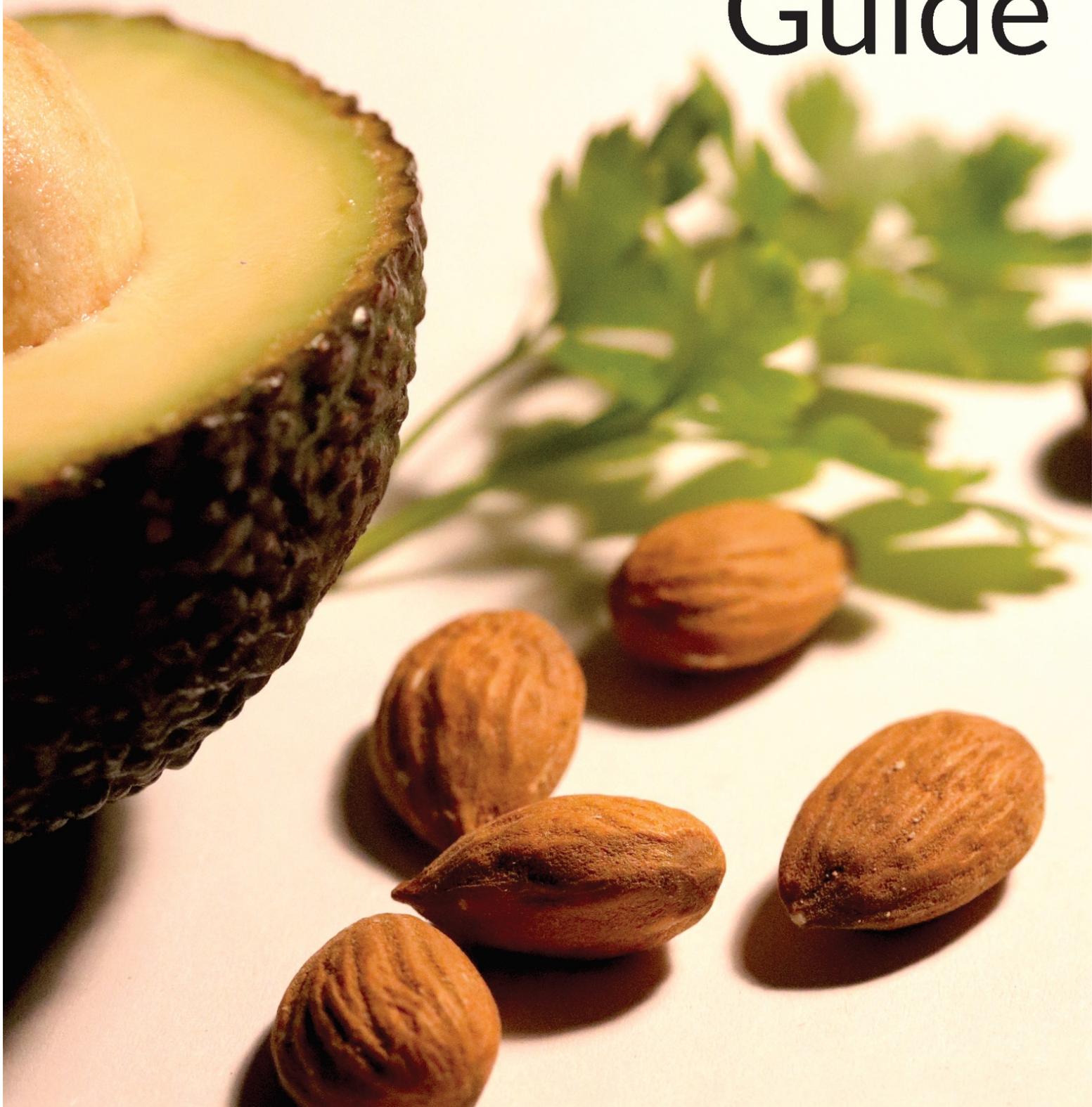
Welcome

Firstly, thank you for taking part in this exciting 6-week research study. You are in for a possible lifestyle-changing adventure!

You will be introduced to a Low-carb, High-fat (LCHF) lifestyle during this study and this booklet was designed to assist you in this new journey. We are also going to check-in with you on a weekly basis to guide you through the process. We trust that you will enjoy this experience with us.

If you have any questions please feel free to contact us:

Sport Physiology Laboratory: 021 808 2818
Louise Engelbrecht (nutritionist): louisee@sun.ac.za
Anika Pretorius (researcher): 16961269@sun.ac.za ; 076 463 5283
Dr Schoombee (general practitioner in private practice): 021 883 9090
Prof Terblanche (supervisor): 082 707 6501

How to get started

Rule number 1: you may not consume more than 50 g of carbohydrates per day

Macronutrient targets:

70% fat, 20% protein, and <50g or <10% carbohydrates

Use your myfitnesspal app to monitor if you are hitting these targets. Alternatively, consult with the researchers.

Rule number 2: Read ALL food labels. You will soon learn what you can eat and what to avoid.

A low carb diet may require more planning than what you are used to, but Tupperware is a great invention. Be organised, make grocery lists and prep several meals in advance.

Rule number 3: Only eat when you are hungry

- Enjoy lots of vegetables from the green list provided in this guide
- Make sure you eat enough healthy fat (extra-virgin olive oil (use cold), coconut oil, avocado, butter and seeds) and don't trim fat from meat; this will help keep you fuller for longer. Good fats also help curb the sugar cravings.
- Select meat sources with minimum processing, fillers and sugar. You can still enjoy bacon, ham and salami, but choose wisely.
- Non-calorie drinks include water, coffee and teas without milk and sugar
- See the food list for low-energy, nutrient dense fruit, such as berries.
- Craving a treat? Opt for a block of dark chocolate

- Eliminate junk and processed food from your diet and replace it with nutrient-dense food, such as quality meat and fish, non-starchy vegetables, full-fat dairy, nuts and seeds.
- Try to select the least processed foods for each meal
- Don't drink your calories. Soda, fruit juice and flavoured milk are packed with sugar. Beer is pretty much liquid bread, and avoid sweet wines, sweet liqueurs and sweet mixers.
- Avoid all sugar and flour-based foods (pastries, cakes, pasta, etc.)
- Fats to avoid, margarine, canola oil, sunflower oil, and processed vegetable oils such as corn oil and don`t cook with olive oil; use it cold over salads.

Tips to ensure a low-carb meal when eating out
- Order meat -/ fish-based dish
- Ask for vegetables (non-starch) or salad with your meal instead of bread, potatoes or rice
- Drink plain water instead of sugar soda or fruit juice. Or limit yourself to a single glass of dry wine
- Avoid breaded, battered or crumbled dishes, pasta, rice or anything with sweet sauces.
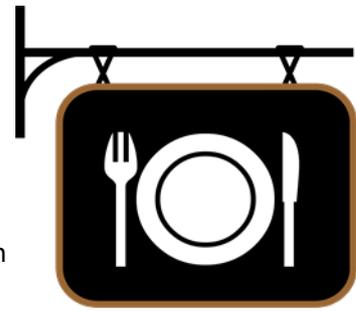
Low-Carb shopping list

Shopping tip: Shop in outer aisles of the shop (vegetable and meat sections). Avoid the inner aisles with the processed food, junk food and sugary drinks

Focus on whole foods. Organic and grass-fed foods are considered healthier options, but typically more expensive.

Try to select the least processed option that still fits into your price range.

- Meat (beef, lamb, pork, poultry)
- Fish
- Eggs (pastured eggs if you can)
- Butter
- Coconut oil
- Olive oil
- Cheese
- Cream
- Sour cream
- Yogurt (double thick, unsweetened)
- Mixed berries (fresh or frozen)
- Nuts (macadamia and Brazil nuts are best)
- Avocado
- Vegetables like broccoli, spinach, tomatoes, mushrooms etc. (refer to green list)
- Lemon (for water)
- Condiments (sea salt, pepper, garlic)
- Super food fats:
    - Flaxseed (use ground)
    - Chia seeds
    - Sunflower seeds

Green (Eat to hunger)

Fruit & vegetables
Leavy greens
Artichoke hearts
Asparagus
Avocado
Bean sprouts
Beans
Broccoli
Cabbage
Cauliflower
Celery
Chard
Courgettes
Cucumber
Fennel
Garlic
Gem squash
Kale
Leeks
Lemon & Limes
Lettuce
Mushrooms
Olives
Onions
Peppers (all kinds)
Radishes
Rhubarb
Rocket
Spinach
Spring onions
Sugar-snaps
Tomatoes
Watercress

Drinks
Caffeine-free
herbal teas
Water (still or
sparkling)

Proteins
**As natural and
little processed as
possible**
All meats, poultry
and game
All naturally cured
meats
All offal
All seafood
Eggs

Condiments
All vinegars are
okay that are sugar
gluten and
preservative free
Tamari/ fermented
soy sauce

Fertilizers
All homemade
bone broths
Coconut yogurt
Coconut kefir
Kefir
Kimchi
Naturally
fermented pickles
Sauerkraut

Fats
Avocado oil
Butter
Coconut oil
Firm cheese
Hard cheese
Macadamia oil
Nut oils
Olive oils
seeds

Orange (Exercise self-control)

Nuts (palmful)
All raw nuts
Homemade/
unprocessed
sugar-free nut
butters

Dairy
**Unpasteurised is
better (1/4 cup)**
Cottage cheese,
cream cheese, full-
fat yogurt
Sour cream
Full-fat cheeses
like brie,
camembert
Milk
Milk substitutes:
almond milk,
coconut milk, rice
milk and hemp
milk
Soft cheeses like
mozzarella, feta

Fruit & Veg
**No more than half
a palmful**
Beetroot and
golden beets
Berries
Butternut, squash
Calabash
Carrots
Celeriac
Corm, baby corn
Papaya
Parsnips
Peas
Pineapple
Pumpkin
Sweet potatoes

Drinks
Tea (caffeinated).
Coffee

Dried legumes
All legumes
Alfalfa sprouts
Beans (cannellini,
kidney & black-eyed)
Chickpeas
Lentils
Peanuts

Fertilizers
Water kefir
Kombucha

Fruits & Veg
Apples (p)
Apricots
Bananas
Cherries
Edamame
Figs (only fresh)
Granadilla
Grapes
Jackfruit
Kiwi fruit
Kumquats
Litchis
Mangoes
Oranges,
clementines and
tangerines
Peaches and
nectarines
Pears and prickly
pears
Persimmon
Plums
Pomegranates
Potatoes
Quinces
Watermelon

Light Red (Hardly ever)

Vegetable juices/smoothies
Fruit/yogurt
smoothies without
frozen yogurt or ice
cream
Vegetable juices

Treats and chocolate
Dates
Dark chocolate, ≥80%
Dried fruit
Honey
Prunes
Pure maple syrup

Gluten-free grains
Buckwheat
Bran
Gluten-free pasta
Millet
Oats
Popcorn
Quinoa
Rices: whole grain,
sushi, jasmine, Thai
and rice noodles
Tapioca

Flours
**Non-GMO and gluten
free should be a
standard rule**
Almond flour
Coconut flour
Chickpea flour
Maize meal
Pea flour
Polenta
Rice flour

Really Red (Never ever)

General
Any food with
added sugar
Crisps
Fast food
Sugary condiments
(unless homemade
and sugar free)

Sweet things
All confectionary
and (non-dark)
chocolate,
Artificial
Sweeteners
Agave
Canned fruit
Cordials
Fructose
Glucose
Jam
Malt, rice malt
syrup
Sugar: white,
caster, icing, light
brown, dark brown
Sugar-cured or
commercially
pickled foods
Golden syrup

Foods containing gluten
All flours and all
breads made from
grains containing
gluten
Barley
Bulgur
Couscous
Rye
Wheat

Other grain-based products
All commercial
breaded or
battered foods
All commercial
breakfast cereals
All crackers and
cracker breads

Drinks
All energy drinks
Soft drinks,
including diet
Commercial fruit
juices
Commercial iced
teas
Flavoured milk

Dairy-related
Cheese spread
Coffee creamers
Condensed milk
Ice cream and
frozen yogurt

Fats
All industrial seed
and vegetable oils
Butter spreads
Canola oil
Corn oil
Margarine
Sunflower oil
Safflower oil

Proteins
Highly processed
sausages and
luncheon meats
like polony
Meats cured with
excessive sugar

Grey (It's a grey area)

Treats
Banting baked
goods
Sugar-free ice
cream

Sweeteners
Erythritol
Isomalt
Stevia powder
Sucralose
Xylitol

Drinks
All alcoholic
beverages
Protein shakes
Supplements

Vegetarian proteins
Naturally
fermented tofu
Pea protein
Processed soy

FOOD LISTS

7-day Meal Plan (this plan is an example and to provide you with a variety of meal ideas)

Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
Breakfast	**Breakfast**	**Breakfast**	**Breakfast**	**Breakfast**	**Breakfast**	**Breakfast**
2 scrambled eggs with ¼ avo and 5 cherry tomatoes **CHO: 7g**	75g cold meat or left-over meat, ½ avocado, 40g cream cheese, 30g lettuce, 30ml olive oil and salt and pepper **CHO:9g**	Omelette with mushrooms **CHO: 4g**	125ml coconut cream, 50g mixed berries, 1 pinch of vanilla extract **CHO: 9g**	Frittata with fresh spinach **CHO: 4g**	Eggs and bacon **CHO:1g**	Low-carb coconut pancakes **CHO:3g**
Lunch	**Lunch**	**Lunch**	**Lunch**	**Lunch**	**Lunch**	**Lunch**
Poached coconut chicken salad **CHO: 16g**	Crunchy tuna salad with dressing **CHO: 8g**	Bun-less burger with mushroom, cheese and avocado **CHO: 9g**	Chicken (100-120g) salad with cucumber, cherry tomato, sunflower seeds, ¼ avo, feta, tbsp olive oil and 1 tbsp lemon juice **CHO: 8g**	Lamb chops with creamy lemon green beans CHO: 5g and caprese salad **CHO: 3g**	Keto quesadillas **CHO: 5g**	Low-carb beef stroganoff **CHO:09g** With salad or vegetables of choice **CHO: 8-15g** (depending on sides)
Dinner	**Dinner**	**Dinner**	**Dinner**	**Dinner**	**Dinner**	**Dinner**
Spinach and cream cheese chicken **CHO: 0.8g**	Beef stirfry with cabbage, baby marrow, peppers, broccoli and green beans **CHO: 16g**	Chicken stirfry with cabbage, baby marrow, peppers, broccoli and green beans **CHO: 10g**	Fish fillet of choice, with cup of cooked vegetables (ex. Broccoli/spinach/ gem squash) **CHO:~10g**	Oriental chicken satay stir-fry **CHO: 9g**	Steak/ braai vleis with roasted veggies **CHO: 8g**	Chicken and broccoli bake **CHO: 6.5g**

Snack options

Easy options

- Hard boiled eggs
- Nuts (macadamia or Brazil)
- Cheese
- Vegetable slices, etc, cucumber, carrots, baby tomatoes and have with a source of fat, like creamed cottage cheese or olives
- Berries with yogurt or coconut cream
- Dark chocolate, but limit yourself to 1-2 blocks and only once a week
- Biltong and droëwors
- Olives

Requires slightly more effort

- Make your own hummus and have with vegetable sticks
- Stuffed peppers
- Small smoothie with mixed berries and nut butter
- Baked cheese (halloumi, camembert, feta)
- Egg muffins
- Zucchini chips
- Salad sandwich

Recipes

Breakfast

Keto tuna salad with capers

Ingredients (serves 1)

- 30g tuna in olive oil
- 2 tbsp mayonnaise
- ½ tbps cream
- ¼ tbsp capers
- 1/8 leek,finely chopped
- 1/8 tsp chili flakes
- Salt and pepper to taste

Method

1. Drain the tuna
2. Mix ingredients together, and season with salt, pepper and chilli flakes
3. Optional to serve with boiled eggs

Mushroom omelette

Ingredients (serves 1)

- 3 eggs
- 30g butter for frying
- 30g shredded cheese
- 1/5 onion
- 3 mushrooms
- Salt and pepper to taste

Methods

1. Crack the eggs into a mixing bowl and add salt and pepper. Whisk the eggs until smooth and frothy
2. Added spices to taste
3. Melt butter in frying pan. Once butter has melted, pour the eggs into the pan
4. When the omelette begins to cook and get firm, but still a little bit raw, sprinkle the cheese, mushroom and onion on top.
5. Use a spatula to carefully ease around the edges of the omelette, fold it over in half. When it starts to turn golden brown underneath, remove the pan from the heat and slide the omelette on to a plate.

Other filling ideas:

- Avo, spinach, creamed cottage cheese/other cheese, leftover veggies / meat

Frittata with fresh spinach

Ingredients (serve 1)

- 35g diced bacon or chorizo
- ½ tbsp butter for frying
- 50g fresh spinach
- 2 eggs
- 60ml cream
- 35g (75ml) shredded cheese
- Salt and pepper to taste

Methods

1. Preheat oven to 175°C
2. On medium heat fry the bacon in butter until crispy. Add the spinach and stir until wilted. Remove the pan from the heat and set aside.
3. Whisk the eggs and cream together and pour into a greased baking dish.
4. Add the bacon, spinach and cheese on top and place in the middle of the oven. Bake for 20-25minutes or until set in the middle and golden brown on top.

Low-carb coconut pancakes

Ingredients (serve 4)

- 6 eggs
- 125ml coconut flour
- 180ml coconut milk
- 2 tbsp melted coconut oil
- Pinch of salt
- 1 tsp baking powder
- Butter/ coconut oil for frying

Method

1. Separate the egg yolks from the egg whites and whop the egg whites and pinch of salt vigorously with a hand mixer. Continue whipping until stiff peaks form and then set aside
2. In a separate bowl, whisk together yolks, oil and coconut milk
3. Add coconut flour and baking powder. Mix into a smooth batter
4. Ever so gently fold the egg whites into the batter. Let batter rest for 5 minutes
5. Fry in butter for a couple of minutes or so on each side on low to medium heat
6. Serve with melted butter and fresh berries

Lunch / Dinner

Dinner tips:

1. Make enough for leftovers for next day`s lunch-box
2. General dinner outline: meat with either:
   a. Low carb vegetables from list: steamed/ oven grilled, or pan fried with enough coconut oil/ butter
   b. Baby spinach salad (avo, feta, cucumber, tomatoes, sunflower seed) Add olive oil to all salads; balsamic vinegar optional

Crunchy Tuna salad

Ingredients (serves 4)

Salad
- 2x ~150g cans tuna in olive oil
- 1 small carrot, diced
- 2 stalks of celery, diced
- ¼ cup coarsely chopped celery leaves, or parsley
- ¼ cup diced yellow bell pepper
- 2 tbsp minced red onion
- Lettuce of choice

Dressing
- ¼ cup plain Greek yogurt
- ¼ cup mayonnaise
- 1 tbsp whole-grain mustard
- 1 tsp lemon juice
- 1 tsp chopped fresh dill/ ¼ tsp dried
- ¼ tsp kosher salt
- Pepper to taste

Methods

1. Place tuna in bowl and break up with fork into bite-size chunks. Add rest of ingredients and mix together
2. For dressing: Whisk yogurt, mayonnaise, lemon juice, dill, salt and pepper in medium bowl.

Spinach and cream cheese chicken

Ingredients (serves 6)

- 6 chicken breast/fillets
- 6 tbsp creamed cottage cheese
- 2 slice bacon diced (raw/cooked)
- 1 handful of spinach (raw/cooked)

Methods

1. Preheat oven to 180°C
2. Slice each chicken breast along the centre
3. Place chopped spinach, cream cheese, and bacon along the centre of each chicken breast
4. Fold over, and use a toothpick to secure the chicken and its fillings
5. Place the chicken in an oven dish and pour some olive oil over each one.
6. Bake for 30minutes, or until cooked

Poached coconut chicken salad

Ingredients

- 1 chicken breast
- 100ml coconut milk
- ¼ lemon, juice
- 3ml fish sauce
- 10g ginger
- 1 garlic clove
- 10g coconut flakes

- 30g baby spinach
- 15g watercress
- 80g cucumber, diced
- 80g baby tomatoes, quartered
- 20g sugar snap peas, roughly chopped
- 5g coriander, roughly chopped
- 1 spring onion, finely sliced

Methods

1. Put a pot (that has a lit) on low-medium heat. Add a drizzle of oil/coconut water and the spring onion. Cook until it starts to turn golden, about 3-5 minutes. Remove spring onion from pot and set aside.
2. Peel and finely slice the ginger and garlic. Place it in the pot along with the coconut milk, fish sauce, a squeeze of lemon juice and a sprinkle of salt. Bring to a simmer. When simmering add the chicken, pop the lid on and poach the chicken until cooked, ~10-12 minutes. Add water if the sauce dries up. Remove the chicken from the pot when cooked, and set aside to rest. You can use the sauce in the pot as a salad dressing, return to heat for few minutes if it needs to thicken.
3. Dice and chopped the remaining ingredients and plate up.
4. Slice the chicken and place on top of the salad. Then drizzle over the salad dressing, and scatter over the spring onion, coconut flakes and coriander.

Oriental chicken satay stir-fry

Ingredients (serves 3-4)

Stir-fry
- 2 tbsp coconut oil
- 300g chicken breast
- 1 bunch of large spring onion, halve lengthways
- 1 yellow bell pepper
- 1 head pak choi, leaves separated

Satay sauce
- 150ml boiling water
- 4 tbsp smooth peanut butter
- 2 tbsp soy sauce
- 1 tbsp tabasco sauce

Method

1. Heat the oil in large wok or frying pan, add the chicken strips and stir-fry over a high heat for 5 minutes until golden in places.
2. Add the spring onions and stir-fry for a further 3 minutes
3. Add the orange pepper and continue to stir-fry for 3 minutes until chicken and vegetables are cooked through.
4. Add the pak choi and cook for 1 minute. Remove from the heat
5. For the sauce, place the water in small sauce pan with remaining ingredients and bring to the boil, stirring with a balloon whisk until smooth. Pour into the wok with the chicken and vegetables and toss to coat

Chicken Bounty bowl

Ingredients

- 25g Chevin goat's cheese
- Chicken breast
- 5g sunflower seeds
- 5g pumpkin seeds
- 40g kale

- 20ml apple cider vinegar
- 8ml Dijon mustard
- 5ml honey

Methods

1. Place a pan on medium heat. Toast the seeds for 3-5 minutes. Remove from heat and set aside
2. Prepare the kale, Rinse and chop into bite-sized pieces. Place it in a bowl, add half of the apple cider vinegar, season with salt and massage for 2-3 minutes.
3. Make the sweet Dijon dressing by mixing the mustard, honey and other half of the apple cider vinegar with 15ml oil, 15ml water, and some salt, whisk together.
4. When the beetroot has 5 minutes to go, cook the chicken in a pan over medium-high heat.

Plate beetroot with kale and chicken, on top crumble the goat's cheese, add seeds and Dijon dressing.

Bun-less burger with mushroom, cheese and avocado

Ingredients (serve 1)

- 150g ground beef
- 1 garlic clove
- 3g parsley, roughly chopped
- ¼ avocado
- 2 brown mushrooms
- 15g cheese of choice
- 15g rocket/lettuce of choice

Method

1. Preheat oven to 200ºC, prepare a baking tray.
2. Remove the stems from the mushrooms using a teaspoon, scrape out the dark brown fluffy "gills" around the stem. Place on tray and cook in the oven for ~10 minutes, until softened.
3. In a bowl, combine the beef mince, garlic and parsley (can swop for fresh herb of choice), season well. Using your hands, mix well and form into a burger patty, about 2cm thick.
4. Place a pan over a medium heat. Add a splash of cooking oil and when hot, cook the patty until golden on both sides, ~3-4 minutes. Remove from the heat and let rest of 1 minute.
5. You can use the mushrooms as a bun, add cheese, rocket, and avocado.

Chicken and broccoli bake

Ingredients (serve 5-6)

- 200g broccoli
- 200g greek yogurt
- 250ml cream
- 2 eggs
- ½ cup feta crummed
- 1 cooked chicken
- 60g chorizo/bacon/ham cut in small blocks

Method

1. Preheat oven to 200ºC
2. Steam the broccoli for 3 minutes in the microwave
3. Mix the wet ingredients in mixing bowl (yogurt, cream, eggs, cheese) and season with salt and pepper
4. Cut the chicken in pieces and add with the broccoli to the wet ingredients
5. Place in oven dish
6. Fry the chorizo in over hot heat till golden brown and mix into other ingredients, top with extra feta
7. Place in the oven for 25mintes or until cooked.

Keto quesadillas

Ingredients (serve 3)

Tortillas

- 2 eggs
- 2 egg whites
- 170g creamed cottage cheese
- 1 ½ tsp (4g) ground psyllium husk powder
- 1 tbsp coconut flour
- ½ tsp salt

Filling

- 150g grated cheese
- 30g baby spinach
- Protein of choice (bacon, chicken, etc.)
- 1 tbsp coconut oil, for frying

Method
Tortillas

1. Preheat oven to 200ºC
2. Beat the eggs and egg whites together until fluffy. Add the cream cheese and continue to beat until the batter is smooth.
3. Combine the salt, psyllium husk powder and coconut flour in a small bowl and mix well. Add the flour mixture into the batter while beating. When combined, let the batter sit for a few minutes. It should be thick like pancake batter. Your brand of psyllium husk powder affects this step (be patient), if it doesn't get thick enough, add some more.
4. Place baking paper on tray. Use a spatula to spread the batter over the parchment paper into a big square (or fry in pan like pancake if you want round tortillas)
5. Bake on the upper rack for about 5-7 minutes. The tortilla turns a little brown around the edges when done. Remove and cut into smaller pieces.

Quesadillas

1. Heat a small, non-stick skillet. Add oil (or butter) if desired. Put a tortilla in the frying pan and sprinkle with cheese, a handful of leafy greens, cooked protein, and sprinkle with some more cheese, and top with another tortilla.
2. Fry each quesadilla for about a minute on each side. You'll know it's done when the cheese melts.

Beef stroganoff

Ingredients (serve 4)

- 2 tbsp oil
- 350g stew beef in bite-size chunks
- 1 small onion, chopped
- 1 garlic clove, minced
- 1 cup mushrooms, sliced

- 1 cup beef stock
- 1 cup double cream
- ½ cup sour cream
- 1 tbsp parsley, fresh and chopped
- 1 tsp salt and pepper

Method

1. Heat the avocado oil in a large pan over medium-high heat
2. Add the beef and saute it for about 2 minutes until lightly browned.
3. Remove the beef from the pan and set aside on a plate
4. Using the same pan, saute the onion, mushroom, and garlic until they are cooked, ~ 5 minutes.
5. Add the beef back to the pan
6. Pour the beef stock and double cream and bring to a boil
7. Turn the heat to a low and shimmer until the beef is very tender, ~ 15minutes
8. Stir in the sour cream with salt and pepper, sprinkle parsley on top and serve.

Sesame butter fried chicken
Ingredients (serve 2)

- 2Tbs butter
- 2 Chicken breasts/ any other chicken pieces
- 1Tbs sesame seeds
- Salt and herbs to taste *(no spices with MSG; check labels)*

Methods

1. Melt butter in pan
2. Seal chicken by frying at high heat on both sides
3. Remove hot chicken breasts covered with butter from the pan and role in sesame seeds so that sesame seeds cleave to the chicken pieces.
4. Season with salt and herbs to taste
5. Fry again until ready to eat.

Serve with Cabbage-feta *(recipe below)* steamed broccoli or any other vegetables from the list with <2g carbs indicated

Braai

Ingredients

- Steak/ lamb chops/ chicken pieces or anything you like to braai
- Salt and pepper to taste

You are South African; you know how to braai 😉

Enjoy with baby spinach salad (avo, feta, cucumber, tomatoes and sunflower seed). Add olive oil and balsamic vinegar

Lemon and garlic butter fried fish

Ingredients (serve 2)

- 2 servings of any fish (eg. Hake, yellow tail, snoek…)
- 3Tbs butter
- 1-2 cloves Garlic
- 1 Lemon
- Salt, pepper and herbs to taste

Method

1. Melt butter in pan at high heat
2. Place fish in pan when pan is really hot and seal on both sides
3. Chop garlic and place half on fish in the pan
4. Turn fish over and add other half of the garlic
5. Squeeze half a lemon over the fish in the pan
6. Add salt, pepper and herbs to taste
7. Remove fish from pan and serve with more lemon

Green mince

Ingredients (serve 4)

- 1Tbs coconut oil
- 500g beef mince
- 1 Head broccoli
- 1 cup spinach
- Celery
- 1-2 cloves garlic
- 3Tbs turmeric (optional)
- Salt, pepper and herbs to taste

Method

Optional: Cook/ steam broccoli before if you want it softer

1. Melt coconut oil in pan and cover the bottom of the pan with the oil
2. Fry chopped celery and garlic for 3 min
3. Add mince and spread through pan and cover with lid for 5 min
4. Add chopped broccoli, green beans and spinach and stir mince.
5. Cover with lid and stir every 2min until ready to eat

Sides

Caprese salad

Ingredients (serve 1)

- 50g cherry tomatoes
- 50g mozzarella, mini cheese balls
- ½ tbsp green pesto
- Salt and pepper

Method

Cut the tomatoes and mozzarella balls in half. Add pesto and stir. Salt and pepper to taste

Creamy lemon green beans

Ingredients (serve 1)

- 75g green beans
- 25g butter
- Pinch of sea salt and pepper
- 60ml heavy whipping cream
- Lemon zest

Method

1. Trim and rinse the green beans
2. Heat butter in frying pan
3. Sauté the beans for 3-4 minutes over medium-high heat until they begin to brown. Lower the heat towards the end. Salt and pepper to taste
4. Add cream and let simmer for 1-2 minutes. Grate the lemon zest finely and sprinkle on top of the green beans for serving.

Cabbage-Feta
Ingredients

- 1Cup shredded cabbage and / or spinach
- 2 tsp coconut oil / butter
- Feta to taste

Method

1. Fry shredded vegetables till desired consistency.
2. Take off heat, and immediately add crumbled feta, stir and close the lid.
3. Enjoy after 2 min

Helpful resources

If you want to learn more about the low-carb lifestyle and need further recipe ideas the following resources gets the quality approval from us.

- Banting 7 Day Mela plans and health Public Facebook group
- Real Meal Revolution book
- Dietdocter.com
- Ditchtehcarbs.com
- Healthline.com/nutrition/ketogenic-diet-foods
- Idmprogram.com
- Low Carb is lekker – recipe book

How to combat the most common Side-effects when starting a low-carb diet

- Induction Flu
- Muscle cramps
- Constipation or diarrhea
- Bad breath
- Heart palpitations
- Reduced physical performance
- Skin rash
- Reduced tolerance to alcohol

Induction Flu: Headaches, lethargy, nausea, confusion, brain fog, irritability, dizziness

The "induction flu", is the most common side-effect on a low carb diet that most people experience in the first week, often on day 2-4.

Headaches are especially common during the transition, and feeling tired, lethargic and unmotivated. Nausea is also common, while it's possible to feel confused or "brain fog". Finally, it's common to feel irritable.

Luckily these symptoms don't last long and disappear within a few days. However, you can avoid them all together, by drinking enough water and consuming salt. The main cause for these symptoms are typically dehydration and/salt deficiency, due to a temporarily increased urine production.

Drink a glass of water with half a teaspoon of salt, symptoms will reduce or disappear with-in 15-30 minutes. Alternatively, you can drink some chicken, beef or bone broth.

To counter the weakness and fatigue make sure to up your fat intake. Going low carb and low fat is a recipe for feeling hungry and fatigued. You should eat enough fat to make you feel satisfied and energetic. If you struggle to increase fat intake, add some butter/ coconut oil/ olive oil to your food.

If adding water, salt and fat doesn't completely work, just hang in there, symptoms with subside within a few days.

Muscle cramps

This side-effect is also due to increased urination, resulting in loss of minerals, specifically magnesium. Typically not a big issue if it occurs, but can be painful.

Tips to avoid muscle cramps:

- Drink plenty of water and consume enough salt
- If needed, take a magnesium supplement

Constipation

Your digestive system may need some time to adapt to the low-carb diet, which may lead to constipation or diarrhea.

Tips to solve the problem:

- Drink plenty of water and consume enough salt.
- Make sure to eat enough non-starchy vegetables and other sources of fiber, like chia seeds and flax seeds.

Bad breath

Unfortunately, some people develop a characteristic fruity smell in their breath that can remind you of nail polish remover. This is the smell of ketone bodies, which means your body is burning fat. This smell can also occur in your sweat.

Not everyone on a low-carb diet develops this smell in their breath and it is often only a temporary thing (1-2 weeks). However, for some people it doesn't go away.

Tips to deal with the bad breath:

- Drink enough water and consume salt
- Maintain good oral hygiene
- Use breath freshener regularly
- Reduce the degree of ketosis

Heart palpitations

In the first few weeks you may experience a slightly higher heart rate. This is normal and nothing to worry about. Dehydration and lack of salt is the most common cause. This side-effect can once again be lowered by drinking plenty of water and consuming enough salt. If this does not work the cause may be due to elevated stress hormones in the blood but should go away within 2 weeks.

Reduced physical performance

Your body is used to burning sugar for energy and it takes time (weeks) to start using fat as your primary source of energy. Make sure to drink enough fluids and salt in the 30-60minutes leading up to training session.

Appendix G:

VO$_{2PCr}$ curve fitting

Margaria et al. (1933) originally proposed the phenomena that different phases of the exponential curve fitted to post-exercise VO$_2$, represents restoration of different metabolic fuel sources. This investigation obtained direct measurement of muscle metabolites (like muscle glycogen, PCr and Cr) through muscle biopsies to mathematically integrate these direct measures with measures of VO$_2$ and [BLa]. This phenomena was later reappraised, confirming these mathematical calculations on experiments conducted with more recent technologies (Pietro Enrico Di Prampero & Ferretti, 1999). The phenomena suggest that the "fast-component" of the post-exercise exponential VO$_2$ curve, is responsible for PCr replenishment from Cr. "Fast-component" VO$_2$ are consequently used to indirectly calculate ATP-PCr contribution during the preceding exercise bout. Various investigations employed calculation methods based on these equations, to determine the contributions of these three energy systems from VO$_2$ and [BLa] measurements in various sports and exercise modalities (R. Beneke et al., 2002; Ralph Beneke et al., 2004; Davis et al., 2014; Julio et al., 2017, 2019; La Monica et al., 2020; Zagatto et al., 2016). However, minimal detail on precise mathematical procedures on determination of the "fast-component" VO$_2$ is defined were reported in these publications. Due to this minimal detail obtained from the literature on mathematical procedures and, to my knowledge no other investigation employed intermittent cycle sprints, a trial was conducted to test the mathematical procedures. N=10 healthy active volunteers provided 14 intermittent sprint protocol VO$_2$ and [BLa] datasets to test various mathematical options. Modulation were performed by a qualified engineer and analyst (C. Mills; cmills@sun.ac.za) in MATLAB® software. Various exponential model curve fitting combinations with corresponding goodness of fit (GOF) statistics outcomes for each method were conducted.

The algorithm used was Trust-Region with set upper and lower coefficient constraints. Mono- and bi-exponential curves were fitted to all plots. Based on GOF outcomes (R-squared mean ± SD), bi-exponential fits proved to exhibit more accurate fits than mono-exponential fits (bi-exp vs. mono-exp; 0,906 ± 0,0568 vs. 0,876 ± 0,0577; p = 0.000004). Robust (Bisquare)- and Default MATLAB curve fitting settings

respectively were alternately applied. Unsmoothed and 10-point moving average options were also alternately used and compared. GOF (R-squared) outcomes of the three best fitted methods are presented in Table1.

Table1: R-squared Goodness of fit outcomes for various curve fitting methods (mean ± SD):

Method	Bi-ekspDefault_MA		Bi-ekspRobust		Bi-ekspRobust_MA		CV% w.	CV% w.o.
Session	R^2	min-max	R^2	min-max	R^2	min-max	outliers	outliers
1	0.950 ± 0.014	0.927 ± 0.980	0.866 ± 0.058	0.704 ± 0.924	0.958 ± 0.012	0.943 ± 0.982	5.64 ± 3.556	4.41 ± 1.617
2	0.956 ± 0.030	0.861 ± 0.981	0.866 ± 0.061	0.747 ± 0.936	0.964 ± 0.019	0.920 ± 0.985	5.96 ± 3.267	4.66 ± 1.974
3	0.954 ± 0.023	0.896 ± 0.976	0.852 ± 0.089	0.646 ± 0.935	0.963 ± 0.019	0.913 ± 0.984	6.92 ± 5.245	5.07 ± 2.038
4	0.961 ± 0.012	0.942 ± 0.983	0.868 ± 0.057	0.777 ± 0.937	0.964 ± 0.016	0.936 ± 0.986	5.93 ± 3.255	5.09 ± 2.575
5	0.957 ± 0.016	0.921 ± 0.984	0.866 ± 0.090	0.615 ± 0.945	0.960 ± 0.017	0.927 ± 0.984	6.03 ± 5.711	4.06 ± 2.223
Mean	0.956 ± 0.019	0.909 ± 0.981	0.864 ± 0.071	0.698 ± 0.935	0.962 ± 0.016	0.928 ± 0.984	6.10 ± 4.207	4.66 ± 2.085

Bi-ekspDefault_MA. bi-exponential curve fitted in the default MATLAB settings. smoothed with a 10-point moving average; *Bi-ekspRobust.* Bi-exponential curve fitted in the Robust Bisquare setting without smoothing; *Bi-ekspRobust_MA.* Bi-exponential curve fitted in the Robust Bisquare setting. smoothed with a 10-point moving average

Plots were generated for all curve fitting methods. Figure 1. 2 & 3 shows plots generated for one participant during the same session for the three methods represented in **Table1**.
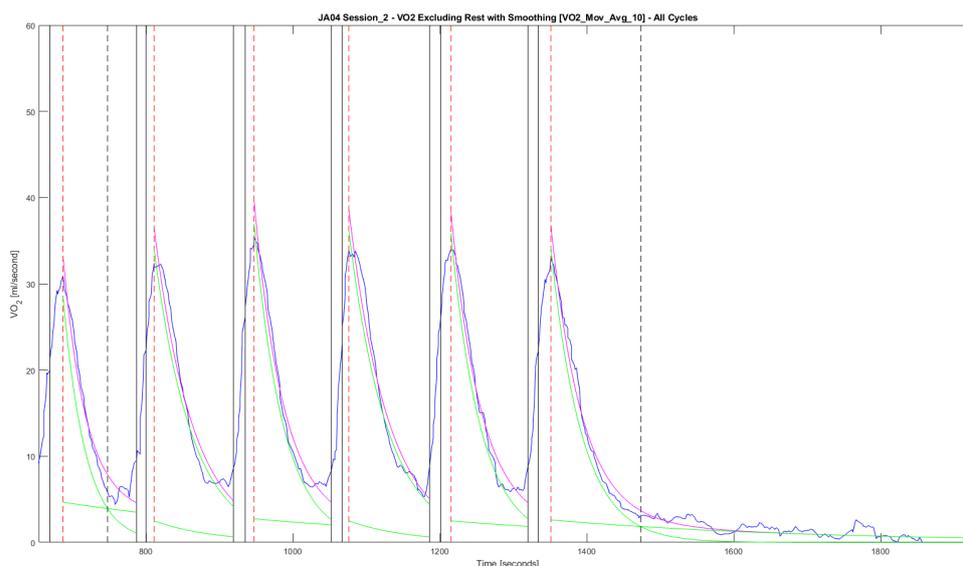

Figure 1: Bi-ekspDefault_MA plots on smoothed VO_2 data during a 6x 10 sec intermittent sprints session. Black dashed lines denote the end of fast component VO_2 uptake at the two exponential plots intercept.
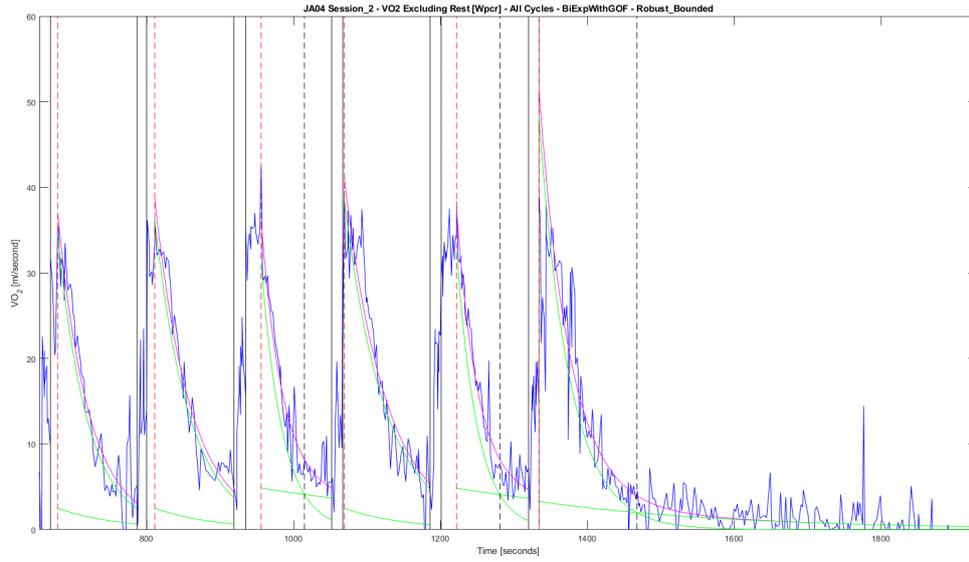
Figure 2: Bi-ekspRobust plots on unsmoothed VO₂ data during a 6x 10 sec intermittent sprints session. Black dashed lines denote the end of fast component VO₂ uptake at the two exponential plots intercept.
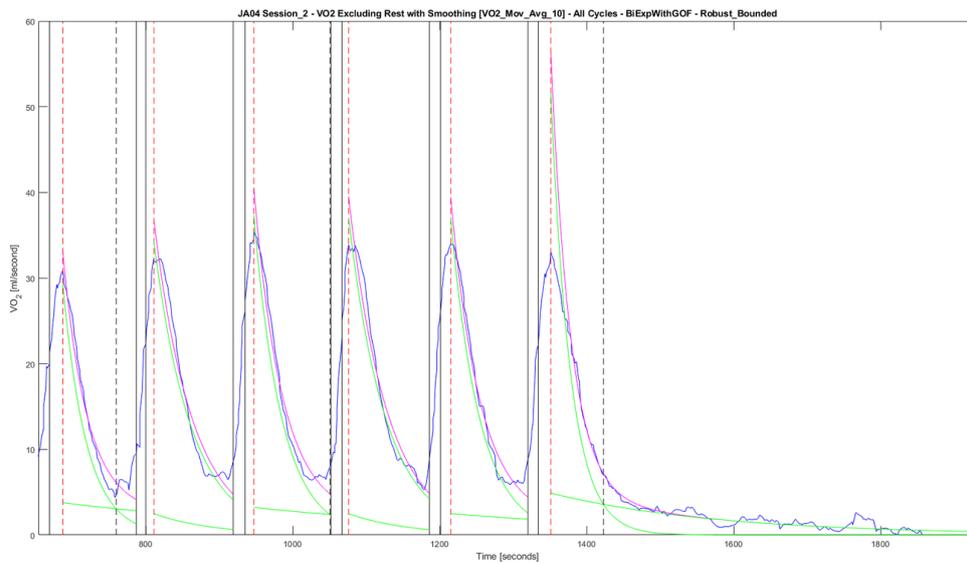
Figure 3: Bi-ekspRobust_MA plots on smoothed VO₂ data during a 6x 10 sec intermittent sprints session. Black dashed lines denote the end of fast component VO₂ uptake at the two exponential plots intercept.

Appendix H; Tuurnitin report

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