EFFECT OF SHORT-TERM MACRONUTRIENT MANIPULATION ON ENDURANCE CAPACITY OF LONG-DISTANCE RUNNERS

by

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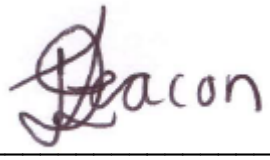

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ABSTRACT

Introduction: The influence of specific nutrition programmes on optimal endurance performance enjoys wide interest. However, limited knowledge in this regard accentuates the need for further research on optimal nutrition for individual endurance performance optimisation.

Objectives: (i) To investigate differences in the effects of a short-term (48-hour) high-carbohydrate (high-CHO) versus a high-FAT diet on indirect respiratory indices of long-distance runners, namely maximal oxygen consumption ($\dot{V}O_2max$), oxygen consumption ($\dot{V}O_2$), carbon dioxide output ($\dot{V}CO_2$), respiratory exchange ratio (RER), minute ventilation ($\dot{V}_E$), and substrate utilisation (CHO oxidation and fat oxidation), as well as on physiological and perceptual measurements such as time to exhaustion, absolute (W) and relative power output (W/kg) and work output (kJ), during a treadmill graded exercise test (GXT) to exhaustion. (ii) To determine certain threshold points that occurred during the GXT, including ventilatory threshold 1 (VT_1), ventilatory threshold 2 (VT_2), lactate lhreshold (LT), peak oxygen uptake ($\dot{V}O_2$peak) and maximal oxygen consumption ($\dot{V}O_2$max) after the high-CHO and high-FAT trials, respectively. (iii) To explore individual preferential fuel source use over a short-term period to enhance performance.

Methods: This was a randomised controlled cross-over trial assessing the effects of a 48-hour high-CHO (67%CHO, 17%fat, 16%Prot) or 48-hour high-FAT (65%fat, 21%CHO, 14%prot) diet amongst 24 well-trained male endurance runners. After each 48-hour diet period and an overnight fast, the participants completed a GXT consisting of 3-minute stages with 1 km/h increments until exhaustion. The two dietary treatment periods were parted by a two-week washout period. The study treatments were compared with respect to the various measurements using ANOVA with diet, participant and period as fixed effects. From these ANOVAs, the mean values for each study treatment (high-FAT and high-CHO diets) were calculated, including a point estimate and 95% confidence interval (CI) for the mean difference "high-FAT – high-CHO", the p-value associated with a test of the null-hypothesis of no difference between treatment means, and the effect size calculated as the ratio of the point

estimate of the mean difference divided by the residual standard deviation from the ANOVA.

Results: No statistically significant differences were observed between the diets with regard to any of the indirect indices measured [$\dot{V}O_2$max, $\dot{V}O_2$, $\dot{V}CO_2$, RER and $\dot{V}_E$ and carbohydrate oxidation (CHOox) and fat oxidation (FATox] as well as LT. Furthermore, no statistically significant differences were observed with regard to the physiological and perceptual responses (RPE, HR, time to exhaustion, work and absolute and relative power output). Moderate effect sizes were observed for $\dot{V}O_2$ at VT_1 (d = 0.58) and at VT_2 (d = 0.41), and for $\dot{V}O_2$max at VT_1 (d = 0.61) and VT_2 (d = 0.47). Otherwise, moderate effect sizes were observed for speed at VT_1 (d = 0.48) and HR at $\dot{V}O_2$max (d = 0.41). For fat contribution, moderate effect sizes were observed at both VT_1 (d = 0.40) and VT_2 (d = 0.43), and a medium effect size at $\dot{V}O_2$max (d = 0.56).

Conclusion: No statistically significant differences were seen between the effects of the short-term high-CHO and high-FAT diets on any of the respiratory and other indices measured in endurance runners during a GTX to exhaustion. However, some moderate effects sizes observed for some of the indices either favouring high-CHO or high-FAT depending on the individual, suggest that further research is justified, possibly involving longer-term diets.

Key words: nutrition, endurance performance, graded exercise test, dietary strategies, individualised approach, high-carbohydrate, high-FAT, indirect respiratory indices, physiological measures, longer-term diets

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LIST OF ABBREVIATIONS, ACRONYMS, AND UNITS

am	ante meridiem (before midday)
ANOVA	analysis of variance
APSS	Adult Pre-Exercise Screening System
AT	anaerobic threshold
BASS	British Association of Sport Sciences
BMI	body mass index
BMR	basal metabolic rate
°C	degrees Celsius
CHOox	Carbohydrate oxidation
CI	confidence interval
cm	centimetre
CO_2	carbon dioxide
CPET	Maximum Flow Volume Test
CV	coefficient of variation
F	female
FATox	Fat oxidation
FAT_{max}	Maximal fat oxidation
$FECO_2$	fraction of end-tidal carbon dioxide
FEO_2	fraction of end-tidal oxygen
G	gram
g/h	gram per hour
g/kg/day	gram per kilogram per day
g/min	gram per minute
High-CHO	High carbohydrate
High-FAT	High fat
HIIT	high intensity interval training
HR	heart rate
HR/min	heart rate per minute
HR_{max}	maximum heart rate
HSREC	Health Sciences Research Ethics Committee

kg/m^2	kilogram per meter squared
kg/mL/min	kilogram per millilitre per minute
kJ	kilojoules
kJ/min	kilojoules per minute
km	kilometre
km/h	kilometre per hour
L/min	litre per minute
L/sec	litre per second
M	male
L/min	millilitre per minute
mm	millimetre
mmol/L	millimoles per litre
N/A	not applicable
O_2	oxygen
PSQI	Pittsburgh Sleep Quality Index
RCP	respiratory compensation point
REE	resting energy expenditure
RER	respiratory exchange ration
RESTQ-sport	Recovery-Stress Questionnaire for Athletes
RHR	resting heart rate
RMR	resting metabolic rate
RPE	rating of perceived exertion
RPE_{peak}	rate of perceived exertion peak
SPO_2	estimate of arterial oxygen saturation
TEM	technical error of measurement
TEM%	percentage of technical error of measurement
$\dot{V}$	refers to mixed venous blood
$\dot{V}_E$ L/min	ventilatory exchange per litre per minute
$\dot{V}CO_2$	carbon dioxide output per unit of time
$\dot{V}CO_2$ L/min	ventilatory carbon dioxide per litre per minute
$\dot{V}E$	expired volume
$\dot{V}O_2$	oxygen uptake
$\dot{V}O_2$ kg/mL/min	ventilatory oxygen per kilogram per millilitre per minute

$\dot{V}O_2$ L/min	ventilatory oxygen per litre per minute
$\dot{V}O_2$max	maximal oxygen uptake
VT_1	ventilatory threshold 1
VT_2	ventilatory threshold 2
W	Absolute power output in Watts
W/kg	Relative power output in Watts per kilogram
y	years

CHAPTER 1

OVERVIEW

CHAPTER 1 - OVERVIEW

1.1 Introduction

In a world bursting with competitive sports, whether a team sport or individual sport, the least improvement in performance can mean the difference between winning or losing a championship or race, making a team or being cut, or missing or breaking a standing record. One of the variables used most by athletes is nutrition manipulation to try and find the competitive edge between even the smallest increases in performance (Hurst, 2016:9).

Contemporary elite sports involve the combination of several psychological, biomechanical, and physiological aspects within the checks of the specific sport's competition regulations, as well as under the fundamental environmental conditions to excite the best performance possible. The champion structure, dating back to the 1900s, concerning the physiological aspects that relate to endurance performance ability is still applicable today and for endurance athletes that includes striving for maximal adaptation of which the goal is to build up capacity to tolerate the highest power output or speed for the specified time or distance (Hawley *et al.,* 2018:962).

The nutrition protocol of the athlete is a key component or factor that is highly reliant on the amount and effectiveness of the shift from chemical energy into power-driven energy to ensure the best possible performance of the working muscles, as the adaptation of several contraction-induced events in the muscle can be modified through the correct nutrient availability (Hawley *et al.,* 2018:963).

Ericsson (1996:1) stated that there are "many starts on the road to excellence, but only a few reach the highest levels of achievement and performance". In his book, *The Road to Excellence*, it is clear that, since the early 1900s, the quest to find the optimal sequence for achieving the highest level of performance and achievement in sport, games, arts and science, has been a topic of interest (Ericsson, 1996:1). Luckily, there has been a growing field of interest to better understand the direct impact of nutrition on the molecular basis of training adaptation, which helps somewhat to gain an understanding of the importance of sports nutrition. Scientists have studied these empirical marvels within theoretical frameworks in depth and, based on the emerging findings on the relation between athletes' training and performance, and also the

genome-wide mapping studies, the integration of these two bodies of knowledge is beginning to form a new understanding of the complexity of elite performance development (Brewer, 2014:12; Ericsson, 2013:533; Hawley *et al.,* 2018:963).

Sports nutrition is termed as the specialisation in the field of human nourishment regarding exercise science. In basic terms, it involves the application of knowledge on human nutrition to make daily eating plans practical in order to provide fuel for physical activity (Fink & Mikesky, 2015:4). In human nutrition, the most important basic daily requirement is an energy source provided by the metabolic fuel macronutrients: carbohydrates (CHO), fats and protein and other metabolic fuels, including fluids and micronutrients (Bender, 2014:4). When investigating nutrition in association with sport, one of the most important determinants that lead to either an increase or a decrease in athletic performance, is an optimal dietary intake to maximise exercise ability and performance during competition, and also to encourage physiological adaptation to training, assistance in recovery and general health (Devlin & Belski, 2015:225; Stohs & Kitchens, 2013:3). The metabolism of fuel during exercise can be improved through modifications in macronutrient consumption which modifies the absorption of hormones and blood-borne substrates which are mainly responsible for the noticeable agitations in the storage capacity outline of the skeletal muscle (Hawley *et al.,* 2018:964).

The importance of recognising sports nutrition as a specialty area developed due to the daily challenges athletes face, including physical training, preparation for competition and competition itself, and to help these athletes keep up with the extreme demands of their specific sport by fuelling their bodies adequately and making nutrition a high-priority demand on a daily basis (Devlin & Belski, 2015:226; Fink & Mikesky, 2015:4).

1.2 Aims and objectives

The aim of this study was to investigate the effects of short-term macronutrient manipulation on the endurance capacity of long-distance runners, using indirect measures.

In order to reach the aim of this study, the following objectives were set:

- To investigate the effects of a short-term (48-hour) high-carbohydrate (high-CHO) diet on a long-distance runner's indirect respiratory indices, as well as physiological and perceptual measures during a maximal graded exercise test to exhaustion, using a treadmill protocol, including $\dot{V}O_2$ (ml/kg/min), $\dot{V}O_2$ (L/min), $\dot{V}CO_2$ (L/min), RER and $\dot{V}_E$ (L/min), substrate utilisation (CHO oxidation and fat oxidation), time to exhaustion, absolute power output (W), relative power output (W/kg) and work output (kJ).
- To use these indirect respiratory indices' values to determine certain threshold points that occurred during the test, including Ventilatory threshold 1 (VT_1), Ventilatory threshold 2 (VT_2) or Respiratory Coefficient Point (RCP), Lactate Threshold (LT), $\dot{V}O_2$peak and $\dot{V}O_2$max following the high-CHO intervention period.
- To investigate the effects of a short-term (48-hour), high-FAT diet on a long-distance runner's indirect respiratory indices, as well as physiological and perceptual measures during a maximal graded exercise test to exhaustion, using a treadmill protocol, including $\dot{V}O_2$ (ml/kg/min), $\dot{V}O_2$ (L/min), $\dot{V}CO_2$ (L/min), RER and $\dot{V}_E$ (L/min), substrate utilisation (CHO oxidation and fat oxidation), time to exhaustion, absolute power output (W), relative power output (W/kg) and work output (kJ).
- To use these indirect respiratory indices' values to determine certain changing points that occurred during the test including Ventilatory threshold 1 (VT_1), Ventilatory threshold 2 (VT_2) or Respiratory Coefficient Point (RCP), Lactate Threshold (LT), $\dot{V}O_2$peak and following the high-FAT intervention period.
- To explore whether there is a difference in indirect measures measured when comparing results of the high-CHO and high-FAT trials.
- To use the results of the study as a possible indication for individual preferential use of a particular fuel source (high-CHO or high-FAT) over a short period of time to enhance a distance runner's performance.

1.3 Rationale of study

Sport performance, recovery and training have evolved due to the transformative effects of sports nutrition, focused research in the discipline of nutrition in sport and its

successive relevance to athletic performance (Brewer, 2014:4; Close *et al.,* 2016:1; Valenta & Dorofeeva, 2018:404). Research over the past 50 years has considered strategies to prepare athletes for all these demands (Close *et al.,* 2016:1). "Good food choices will not transform a mediocre athlete into a champion, but poor food choices may prevent the potential champion from realising his/her potential" (Maughan & Shirreffs, 2012:112).

The influence of nutrition on optimal endurance performance and the individual performance-based difference in these individuals regarding specific macronutrients is a field of study that enjoys wide interest. Also, the limitations of current studies in the discipline of specific sport nutrition principles, for example strategies to improve performance, accentuate the need for further research with regard to optimal nutrition for sports performance (Burke *et al.,* 2000:2413; Carey *et al.,* 2001:115; Couto *et al.,* 2014:380; Erlenbusch *et al.,* 2005:11; Havemann *et al.,* 2006:194; Hawley, 2014:5; Lambert *et al.,* 2001:209; Phinney *et al.,* 1983:769; Pitsiladis & Maughan, 1999:919; Rowlands & Hopkins, 2002:689; Starling *et al.,* 1997:1185). Burke and Hawley (2002:1497) furthermore recommend that potential studies regarding this topic should focus on assessing the prospect that there are "responders" and "non-responders" to different nutrition approaches, as nutrition adaptation diets may be advantageous for some individuals and not for others.

The principle of CHO loading has long been a foundation to all athletes, independent of the type of activity, as a way to increase and super compensate muscle glycogen stores hours or days before an endurance event to optimise endurance capacity and/or performance (Bartlett *et al.,* 2015:3; Hammond, 2019:2; Havemann & Goedecke, 2008:551; Jeukendrup, 2014:s26; Rauch *et al.,* 1995:25). The initial protocol for CHO loading is comprised of the classic seven-day model which includes three days of extreme training with low-CHO consumption (depletion phase), followed by reduced training with high-CHO consumption for another three days (loading phase) (Beck *et al.,* 2015:260; Burke, 2007:344; Havemann & Goedecke, 2008:551; Rauch *et al.,* 1995:25; Williams *et al.,* 1992:18). Conversely, Burke (2007:345) developed a modified protocol which included three days of high-CHO intake with a training taper. As liver glycogen stores are reduced substantially during an overnight fast – to as much as 0.0-0.3 m/moll glucose units per minute – it implies that glycogen will reduce by approximately 80% during an overnight fast (Ormsbee *et al.,*

2014:1786). For this reason, the common CHO-loading strategy before, during and after exercise is still widely used as literature points out that a rise in glycogen stores in the liver and skeletal muscle leads to improved endurance and performance (Bartlett *et al.,* 2015:3; Beck *et al.,* 2015:260; Byars *et al.,* 2010:1; Moore *et al.,* 2013:294). Coleman writes in her review that all these methods to increase CHO in the body might be limited to the athlete's opportunity to consume additional energy when needed and may therefore decrease energy stores (Coleman, 2010:1). She also points out that these CHO loading methods do nothing to slow the rate of CHO utilisation and thus the justification for fat loading generated by utilising fat as alternative fuel source in such conditions is supported (Coleman, 2010:1).

Recent investigations lean more towards the "train-low, compete high" model that comprises of CHO periodisation which entails the deliberate reduction in CHO intake during specific training sessions to augment muscle adaptive responses (increased lipid oxidation and free fatty acid (FFA) availability). However, clear facts regarding whether it is the optimisation due to muscle adaptations and/or energy restriction which is a result from restriction in CHO that bring on the adaptation and improved performance are still raising some questions (Hammond, 2019:3).

The above findings are noteworthy and are in agreement with Burke and Hawley's (2002:1497) review study where they instructed that future studies of this fat-loading, fat-adaptation/CHO-loading strategy should be focused on fine-tuning the aforementioned. The proposition, by Burke and Hawley (2002:1497), for the fine-tuning of this fat-loading phenomenon is to simplify (i) the minimal amount of time required to adhere to whatever dietary protocol will be performed, (ii) to achieve the ideal intensity and volume of exercise required to accomplish such metabolic changes after a dietary manipulation strategy, and (iii) to identify the number of times such a protocol needs to be completed in the athlete's macro cycle to achieve the desired effect. All the above will consequently require a series of independent studies to properly identify the optimal protocol benefit. Burke *et al.* (2019) further address this topic in their recent paper that focusses on modern-day dietary approaches to improve performance in endurance athletes, regardless of whether it is runners or walkers, and again advises that rigorous study concerning this subject is needed in spite of the calculations made around $\dot{V}O_2max$, O_2 cost of oxidising fat and/or CHO and the energy cost of a race (Burke *et al.,* 2019:117).

In summarising studies with dietary manipulation of three days or less, studies by Galbo *et al*. (1979:19) and Guimaraes Couto *et al*. (2014:380) are shown to be the only studies that investigate the outcome of short-term dietary manipulation strategies on endurance runners, while the other short-term studies focus on cycling performance (Burke *et al.,* 2002:83; Goedecke *et al.,* 1999:1509; Pitsiladis & Maughan, 1999:920; Starling *et al.,* 1997:1185; Stepto *et al.,* 2002:449; Støa *et al.,* 2016:399). Guimaraes Couto *et al*. (2014:381) tested the outcome of a two-day high-FAT diet on running performance in trained adolescent boys. Each athlete had to finish a ten-minute run on a treadmill at 65% of calculated $\dot{V}O_{2peak}$ to estimate substrate utilisation as well as gas exchange measurements. After the treadmill run, each participant had to run a ten kilometre self-paced performance test on an athletic track to determine each subject's ten kilometre completion, gas exchange, as well as substrate utilisation which were not measured again after the ten kilometre run was complete. In the other study performed on endurance runners, Galbo *et al*. (1979:19) tested the outcome of insulin infusion on hormonal reactions during continued exercise after the ingestion of different diets. Dietary periods in the above-mentioned study lasted four days and consisted of a low-fat/high-CHO and high-FAT/low-CHO intervention. Each dietary period finished with an overnight fast, followed by a unique testing method where athletes ran for 30-minute intervals, separated by ten-minute rest periods and had to continue this sequence until exhaustion. Upon exhaustion, a glucose infusion was administered, and participants again had to run until exhaustion The focus of their study was solely on hormonal responses to prolonged exercise and did not focus on running performance or substrate utilisation outcomes, therefore, conclusions around running performance, time to fatigue, and the effect of these four-day dietary interventions on different substrates were not made. One can also observe the different methods used by authors that tested the outcome of short-term dietary manipulation on performance by looking at the studies of Starling *et al*. (1997:1185), Støa *et al*. (2016:399) and Pitsiladis and Maughan (1999:920).

In contrast with the aforementioned studies, the current study is a cross-over design study, with experimental trials separated by two weeks, where subjects (adult male long-distance runners with an average marathon time of four hours and 20 minutes) did a graded exercise test (GXT) to exhaustion on a treadmill after each 48-hour dietary intervention period using the protocol of Peserico *et al.* (2015:732). The

protocol of Peserico *et al*. (2015:732) was also found to have the highest correlation between a GXT and a one-hour running time-trial performance. On both testing occasions, indirect indices' measures [$\dot{V}E$ (L/min), $\dot{V}O_2$ (L/min), RER, $\dot{V}CO_2$ (L/min) and $\dot{V}O_2$ (ml/kg/min), FATox and CHOox], as well as physiological and perceptual measures (time to exhaustion, capillary blood lactate (CBL), W, W/kg, kJ and CHO and fat contribution were measured throughout the GXT, as well as for five minutes during recovery time. These measurements were then used to determine certain changing points that occurred during the test including Ventilatory threshold 1 (VT_1), Ventilatory threshold 2 (VT_2) or Respiratory compensation point (RCP), $\dot{V}O_2$peak and $\dot{V}O_2$max.

Alongside the studies mentioned, in which major performance outcomes were based on correspondingly time-trial performance, time to fatigue, substrate utilisation at a fixed intensity (% of $\dot{V}O_{2peak}$) and hormonal responses, it seem clear that research which includes all aspects of performance, as mentioned in the objectives of the study, will be of great benefit to prove whether a linear relationship between these different outcomes after a short-term dietary intervention exist.

The present study's separate intervention periods (high-FAT and high-CHO) were only taking place over a 48-hour timeframe with a washout phase of two weeks separating the interventions. In order to substantiate the 48-hour dietary period for this study, the researcher took the lead from studies done by Burke *et al.* (Burke *et al.,* 2004:26; Burke *et al.,* 2001:297; Burke *et al.,* 2000:2421) that five days on a high-FAT low-CHO diet already showed some adaptation and could not have been reversed entirely with replenishing muscle glycogen stores. Also, a study done by Støa *et al.* (2016:402) which tested the day-to-day adaptability in FATox after only one day of adjustment in diet structure, demonstrated changes in substrate use during aerobic exercise. Additionally, the observations made by Ormsbee *et al*. (2014:1786) that liver glycogen stores are reduced substantially during an overnight fast – to as much as 0.0-0.3 m/moll glucose units per minute – imply that glycogen will decrease by approximately 80% during an overnight fast (Ormsbee *et al.,* 2014:1786), along with the all the above-mentioned short-term study results around indirect indices and physiological and perceptual measures' differences which occurred during a period of four days or less. Sensibly, another motive for this short-term approach was that athletes do not always have the time to comply with longer-term dietary approaches and also the

aforementioned might not be in favour of trying out such an extreme lifestyle change during their training schedule. In addition, as athletes' body size, training intensity, training volume and energy needs differ individually, a longer-term research approach may not always be practical and/or ethical (Burke, 2015:s48).

Even though current studies have failed to observe any noteworthy performance-based increases or decreases through short-term dietary manipulation strategies, the researcher observed Burke and Hawley's effort in their review (Burke & Hawley, 2002:1497), that future studies regarding the fat-loading phenomenon should be undertaken as the studies currently done comprised of a variety of methods, outcomes, purposes and interventions. Also, Burke and Hawley's (2002:1497) suggestion that future studies should be directed as such to fine tuning the most appropriate individual loading strategy, sporting situation or exercise testing protocol to use when testing the benefit of different loading strategies fuelled the need for this study. In addition, outlining the particular mechanism supporting already observed metabolic perturbations in reaction to fat-adaptation, directed the current researcher to fill this range of possibilities currently existing in literature.

In conclusion, the present study investigated this field of short-term macronutrient/dietary manipulation and its effect on the overall endurance capacity of long-distance runners with the application of acute (48-hour) dietary interventions, as it can clearly be identified as a shortcoming in the knowledge available from the above-mentioned studies.

1.4 Structure of the thesis

Chapter two comprises the literature study that focuses on endurance performance and all physiological aspects regarding endurance ability during exercise.

After a brief introduction, the reader is familiarised with the energy systems associated with endurance performance, followed by adaptations that succeed during endurance training. This is followed by a discussion on the factors associated with endurance performance, followed by a clarification on the cardiorespiratory and non-invasive responses occurring during a maximal $\dot{V}O_2MAX$ test. This is followed by an overview of previous studies done on this topic, ending with the important question as to whether such a dietary strategy as explored in the current study will be beneficial or not.

Chapter three describes the research methodology undertaken in this study. The research design is highlighted. The study participants, the recruitment and inclusion and exclusion criteria are clarified. The chapter offers a detailed explanation of the pre-exercise testing procedures, as well as the exercise testing procedures to be undertaken during both dietary trials during this study. The statistical analysis is expounded upon. Mention is made of the pilot study that was undertaken to ensure that all pre-exercise testing and exercise testing procedures proceed smoothly. Ethical considerations are then clarified along with methodological measurement errors that were considered. Finally, the limitations of the study are referred to.

Chapter four reports the research results. The discussion concludes with recommendations and conclusions. A reflection on the research process from a personal perspective is provided in the final section. The reference list incorporates all resources for all chapters in one comprehensive list. Referencing is done according to the regulations of the Department of Exercise and Sports Sciences at the University of the Free State, making use of the Harvard referencing system throughout.

CHAPTER 2

LITERATURE REVIEW

CHAPTER 2 – LITERATURE REVIEW

2.1 Introduction

One of the fundamental components for optimal sports performance, health and fitness is the nutritional status of an athlete which forms an integral and important part of their lifestyle. The nutritional status of an athlete cannot be overemphasised as it plays a central role in adaptation, rehydration, refuelling and recovery from injury which is important for optimal sports performance (Bagchi *et al.,* 2019:3).

The evolution of sports performance, recovery and training with the practice and science of sports nutrition, developed quickly and can be seen over a broad spectrum of literature with focused research and its subsequent application to human performance. Therefore, it is indispensable that athletes be in the best possible nutritional adequacy to reach their best possible performance goals (Bagchi *et al.,* 2019:3; Beck *et al.,* 2015:259; Brewer, 2014:4; Close *et al.,* 2016:144).

A variety of dietary strategies for improving endurance performance are supported by literature (Hammond, 2019:2), where the use of an isolated strategy is discouraged and the use of a combination of strategies encouraged as more beneficial. These strategies include intake of macronutrients, micronutrients, and fluids, as well as the composition of each as well as the spacing throughout the day or weeks. Also, once again the importance of individualised and personalised nutrition advice is accentuated as individuals differ in many aspects (gender, strength, endurance) and consequently one specific strategy should not be assumed overall (Beck *et al.,* 2015:260). Therefore, the likelihood of "responders" and "non-responders" to nutritional strategic approaches must be accounted for as different individuals might find it either beneficial or inconvenient or sometimes even be willing to withstand inconvenience due to performance benefit.

During exercise, the breaking down of glycogen, glucose or phosphocreatine (PCr) to lactate or else pyruvate along with the oxidation of CHO and fats start to replenish Adenosine triphosphate (ATP) that is associated with fatigue and impaired performance (Coleman, 2010:1: Jeukendrup, 2012:41). It is important for optimal sports performance that an athlete has a mixture of sufficient power or energy stores relative to the pressures of the specific sport, together with ‘metabolic flexibility’ to be

able to rapidly switch between fat as fuel in the fasted state to glucose in the fed state (Burke, 2015:s36; Moro *et al.,* 2014:584).

It is well known that during distance running (considered in athletics as ranging from three kilometres through ten to 30 kilometres and up to the marathon (42.195 kilometres), fat deposits in adipose as well as muscle tissue and CHO in the working muscle and liver are the major fuel suppliers to the body (Rapoport, 2010:2; Spriet, 2007:332; Williamson, 2016:2). During long constant load exercise that relies mainly on the aerobic metabolic pathway for optimal performance, high levels of stress can be placed on the human body together with excessive metabolism of CHO (muscle and liver glycogen) (Byars *et al.,* 2010:1; Moore, 2015:294; Valenta & Dorofeeva, 2018:404). Muscle glycogen can provide enough energy for 90 to 120 minutes of continuous, vigorous exercise signifying that the tempo of glycogen depletion is mainly dependent on the exercise intensity, time, and exogenous CHO intake; the higher the intensity the higher the muscle glycogen depletion rate (Murray & Rosenbloom, 2018:243).

As such, one of the key roles of nutrition in sport is to find a strategy to optimise the function of the central nervous system and muscles through increasing the body's capacity to store fuel (Burke, 2015:s34). As CHO are stored energy, this depletion in muscle and glycogen stores can lead to a decrease in performance resulting from fatigue (Coleman, 2010:1). As previously mentioned, this forms the main ideal behind "CHO-loading" as literature points out that a rise in glycogen stores in the skeletal muscle and liver leads to improved endurance performance (Bartlett *et al.,* 2015:3; Beck *et al.,* 2015:260; Byars *et al.,* 2010:1; Jeffers *et al.,* 2015:1; Moore, 2015:294).

Together with the above, Coleman also pointed out that these CHO loading methods do far less than originally thought to have in order to slow the rate of CHO utilisation, therefore, the justification for fat loading generated by utilising fat as alternative fuel source in such conditions (Coleman, 2010:1). Generally it should then be recognised that by means of using less CHO and more fat during endurance exercise that the rate of lactate production should be slower and thus enhance the aerobic performance of the individual as endurance training improves an athlete's capacity for fat oxidation even in the leanest of athletes (Burke, 2015:s36; Byars *et al.,* 2010:1).

Burke (2015:s36) again reports that the fuel requirements of several sports, including distance running, are more complex than one might think. In her 2015 review, she reported that marathon, distance running or cycling are categorised as endurance events performed at sub-maximal intensity, but that even these sports require times of tactical strategies during the important parts of the race which are run at more intense and often maximal pace which, in general, require a high level of metabolic shift ability. Exercise intensities can vary from 80-90% of $\dot{V}O_2$max in elite runners, to 70-75% $\dot{V}O_2$max or even 60-65% $\dot{V}O_2$max in less elite runners and it is known that CHO oxidation increases as exercise intensity escalates (Cermak & van Loon, 2013:1140: De Souza Silveira *et al.,* 2016:8). Small quantities of substrate phosphorylation are needed during bouts where an increase in speed or intensity is needed, whereas most of the energy during sub-maximal exercise is met via oxidative phosphorylation in the mitochondria (Spriet, 2007:332).

As an athlete's training and race speed progresses, it has been shown that whole body $\dot{V}O_2$max, running economy, skeletal muscle mitochondrial size, as well as carbohydrate oxidation (CHOox) and fat oxidation (FATox) maximum rates increase severalfold (Barnes *et al.,* 2013:642; Galbraith *et al.,* 2014:1023; Hansen *et al.,* 2005:94; Hawley & Leckey, 2015:s5; Morton *et al.,* 2009:1520; Spriet, 2007:333; Stangier *et al.,* 2016:45). This surge in the above mentioned also causes the skeletal muscles' ability to absorb FFA from the plasma to increase, as well as the capability to oxidise and store intramuscular triacylglycerol (IMTG) which is said to be a very beneficial adjustment for racing and training (Hammond, 2019:3; Spriet, 2007:333). Continuous training performed at the same absolute running speed causes an increase in the reliance of FATox together with the conserving of CHO. However, for an athlete to be capable of racing at a higher speed than their trained running pace, the need to oxidise CHO must equal the amount as expended during training, together with a higher FATox rate during competition (Spriet, 2007:333).

Exercise intensity, substrate availability (from both outside and inside the muscle) and training length are the principal determining factors when it comes to the interaction between the amount of CHO and fats to be metabolised during distance running (Spriet, 2014:s87).

From the literature investigated above, it seems that the most applicable dietary strategy for top performance relies on numerous physiological adaptations taking

place during exercise/race in the human body and should therefore be accounted for and perhaps verified by athletes to improve their running performance. These adaptations are described later in this Chapter.

To better understand endurance performance, a grasp of the key factors to performance is crucial. Once these factors are identified, the most relevant variable to this study, namely nutrition, can be further elaborated upon. As previously mentioned, distance or endurance running refers to distances ranging from three kilometres through to ten kilometres, to 30 kilometres and up to the marathon (42.195 kilometres).

2.2 Energy systems associated with $\dot{V}O_2$max and endurance exercise

2.2.1 Overview of energy systems associated with exercise: Applicability to human skeletal muscle

The interaction between nutrition and exercise is one of the main characteristics that strengthen the success of an athlete, independent of the complexity of the sport (Burke, 2015:s34). Well established in literature is that fuel substrates oxidised by contracting muscles during prolonged exercise are derived from equally intra- and extra-muscular CHO and lipids with only minimal contribution from amino acids (Burke, 2017:2786; Cermak & van Loon, 2013:1139; Coyle, 1995:68; De Souza Silveira *et al.,* 2016:7; Hawley & Leckey, 2015:s12). To optimise endurance performance, it is crucial that the metabolic regulation of these two main substrates be on point for the recruitment of muscle and other bodily systems to adapt during training (Aoi *et al.,* 2006:2; Burke, 2015:s34).

Even at rest, both CHO and fats are oxidised in the body to deliver energy for basic basal metabolic processes which shows the reciprocal relationship that exists between the utilisation of fats and CHO (Spriet, 2014:s87). During exercise, each of these two substrates have their exclusive function and follow a clear pattern with regard to energy production (Bitel, 2017:4; De Souza Silveira *et al.,* 2016:7). Once exercise commences, both CHOox and FATox metabolic pathways are activated simultaneously since the metabolic tempo and the need for energy rises exponentially on top of the resting rate (Spriet, 2014:s87; Van Hall, 2015:s23).

Human or mammalian cells produce energy from both the aerobic (with O_2) and anaerobic (without O_2) energy systems with the anaerobic energy system further

divided into an alactic (no lactate formation) and lactic (lactate forming) energy system (Bitel, 2017:4). Reiterating, in short, the energy systems pathways during exercise, it is well known that whether you are running a marathon or do an explosive movement, skeletal muscle is powered by either a single source or compound called adenosine triphosphate (ATP) (Jeukendrup, 2012:40; Williams & Rollo, 2015:s14).

However, as there is only a partial reserve of ATP in the body, for enough energy to produce power for only a few seconds (80 – 100 grams of ATP), the body must resynthesise ATP in the mitochondria constantly and this is done through three inputs: (1) Oxygen, (2) inorganic phosphate (Pi) and free adenosine diphosphate (ADP), and (3) nicotinamide adenine dinucleotide phosphate (NADH) (Haff & Triplett, 2016:46; Spriet, 2007:332). As mentioned, aerobic (oxidative phosphorylation) and anaerobic metabolism are the two main systems that generate ATP in the skeletal muscles with anaerobic metabolism for the manufacture of ATP, powered by the two separate systems – the intramuscular stores of phosphocreatine (PCr) and glycogen (Williams & Rollo, 2015:s14).

The three basic energy systems are at work in human muscle cells to replenish ATP: (1) the Phosphagen system, (2) the Lactic anaerobic system/Glycolysis, and (3) the Oxidative system/Aerobic system (Haff & Triplett, 2016:44). These three systems operate simultaneously with their relative contribution to produce ATP dependent primarily on the exercise intensity and secondarily on the extent of the exercise (Abernethy *et al.,* 2013:161; Haff & Triplett, 2016:45).

2.2.1.1 Anaerobic energy system

The anaerobic energy system includes two separate systems, namely the phosphagen system and the glycolytic system. These two systems are discussed as follows.

i) The phosphagen system

The phosphagen system or adenosine triphosphate phosphocreatine (ATP-PCr) system is also recognised as the alactic energy system, as mentioned above. It is the major supplier of energy needed for rapid all-out exercise occurring during the early stages or the transition from a lower to a more intense power output, as well as when exercise intensity escalates from moderate to very hard exercise as PCr breakdown facilitates ATP synthesis (Bitel, 2017:4). The phosphagen system depends on ATP

hydrolysis as well as creatine phosphate (CP) breakdown or phosphocreatine (PCr) to supply energy. Creatine kinase is the enzyme that catalyses the ATP synthesis from ADP and CP via the following reaction: ADP + CP ⇦creatine kinease⇨ ATP + Creatine (Haff & Triplett, 2016:46). The phosphagen system serves as an energy storing area that supplies the instant energy needed for ATP until the lactic- and aerobic system, with greater abilities for generating ATP, can take over and enhance the production of ATP (Fink & Mikesky, 2015:46). As ATP concentrations can decrease by up to 60% in comparison to pre-exercise levels, the ATP stores are then maintained by the aforementioned creatine kinase reaction equation to provide reserved energy to quickly refill ATP. Furthermore, Type II muscle fibres contain higher concentrations of CP than Type I muscle fibres, and therefore it is said that individuals with greater percentages of Type II fibres can replenish ATP faster through this system. The adenylate kinase reaction system also forms a significant alternative single-enzyme effect to quickly refill ATP (equation: 2ADP ⇦adenylate kinase⇨ ATP + AMP), which makes this reaction predominantly important because AMP is a product from the adenylate kinase reaction and also a powerful stimulant of glycolysis (Haff & Triplett, 2016:46). Once the body's PCr stores are depleted, the "next" energy system will be resorted to for energy, thus the lactic anaerobic system or glycolysis system (Haff & Triplett, 2016:46).

ii) Glycolytic system

The glycolytic system is another method that replenishes ATP through glucose breakdown delivered to the blood and glycogen kept in the muscle and liver via a process called glycolysis (breakdown of glucose) or glycogenolysis (breakdown of glycogen) by means of substrate level phosphorylation (Bitel, 2017:5; Haff & Triplett, 2016:46; Spano *et al.,* 2018:34). Both glycolysis and glycogenolysis occur in the cytoplasm of the myocytes, also called the sarcoplasm, and are associated with both anaerobic and aerobic energy systems (Haff & Triplett, 2016:46).

The entire glycolysis process involves a sequence of reactions that ultimately bring about pyruvate production which can later be changed to lactate or shuttled into the mitochondria (Dunford & Doyle, 2015:76; Haff & Triplett, 2016:46). The distinct difference between the glycolysis and glycogenolysis processes lies in whether a glucose molecule or glycogen molecule is broken down (Dunford & Doyle, 2015:76;

Spano *et al.,* 2018:35). Although both glycogenolysis and glycolysis yield equal numbers of pyruvate, the distinctive disparity lies in the fact that glycolysis is coupled with the net release of 2^+ H ions, while glycogenolysis produces both H^+ and ATP, but only one of each ion (Hall *et al.,* 2016:s9). When the reaction starts with glucose, two of the three reactions require ATP for the reaction to continue, with the first step involving energy and a phosphate group being added to ATP to form glucose-6-phosphate (G-6-P). In the fourth step of this process, the 6-carbon glucose molecule is divided into two 3-carbon molecules, which results in the glycolysis reaction being duplicated and leading to enough energy released to phosphorylate ADP to ATP. In the ninth step of this reaction, the same process repeats where four ATP's are now produced, leading to the production of pyruvate along with other electron carriers, NADH and $FADH_2$ (Dunford & Doyle, 2015:76; Spano *et al.,* 2018:35). When the starting point of the entire glycolysis process is glycogen, the process is the same, except that the first step is omitted (Dunford & Doyle, 2015:77).

With increasing exercise intensity, the mitochondria are incapable of oxidising all the accessible pyruvate which was shuttled into the aforementioned via the abovementioned processes where it underwent oxidative phosphorylation. This results in pyruvate being triggered to convert pyruvate into lactate via the enzyme lactate dehydrogenase and act as a buffer against acidosis (Hall *et al.,* 2016:s9).

Examples of types of exercise that use the anaerobic glycolysis energy system as the predominant energy system are summarised in Table 2.1. This usually includes activity that lasts for one to two minutes or longer, on condition the activity is intermittent (Dunford & Doyle, 2015:78).

Table 2.1: Exercises using the anaerobic glycolysis energy system

Exercise	Example
Long sprints	400 meter in track
High-intensity repeated sprints	Alternating sprints by a soccer player
High-force repeated activities	10 to 15 repetitions of weightlifting
Regular repeated intervals	50 – 100-meter swimming intervals

(Dunford & Doyle, 2015:78)

2.2.1.2 Aerobic energy system

i) Oxidative system

As already discussed in this chapter, distinction can be made between glycolysis in the anaerobic and aerobic systems by the presence of oxygen in the muscles (Haff & Triplett, 2016:47). The final system of the cellular energy generation systems is the oxidative system which involves the process where substrates are broken down by the body via O_2 to produce energy (Kenney *et al.,* 2015:61). This process is also called aerobic because of the presence of O_2 which means that the end product of this oxidative system's metabolism is not changed to lactate but moved into the mitochondria where the production of ATP takes place (Haff & Triplett, 2016:51; Kenney *et al.,* 2015:61).

When energy must be delivered at a fast rate and glycolysis takes place without adequate O_2 delivery to the mitochondria, it results in pyruvate being converted to lactate as mentioned above (this development is also called anaerobic glycolysis). However, when the rate of energy supply is not that fast, meaning exercise intensity is low enough and O_2 is present in the cells, pyruvate can further be oxidised into the mitochondria via metabolic pathways involving the aerobic energy system (Dunford & Doyle, 2015:78; Haff & Triplett, 2016:47; Spano *et al.,* 2018:35).

Both CHO and fats are used to fuel this oxidative energy production with protein not playing such a significant role in healthy subjects, but only in cases where long-term starvation with long bouts of exercise takes place (Haff & Triplett, 2016:51; Kenney *et al.,* 2015:61). Roughly 70% of ATP generated during rest comes from FATox, but as

the exercise intensity increases, reliance starts to creep in on more CHOox to provide energy. At the start of endurance exercise, some athletes starts with a fast pace and set back on the pace after a lead is set, while other athletes starts with a slow and steady pace and up the intensity as the race progresses. Although CHO and fats are both sources of energy during endurance exercise, it will depend on the athlete's strategy and fuel availability as to which source will be used more as energy reliance builds up from fats (low-intensity, long duration) to almost 100% reliance on CHO at high-intensity exercise (Haff & Triplett, 2016:51; Kenney *et al.,* 2015:61). Given the above, it is clear why nutrition strategies form a very important aspect to ensure adequate fuel availability at any stage of an endurance race.

2.3 Endurance performance

What permits an endurance/distance runner to run as fast as they do or what permits them to endure such long distances at high speeds? These are both questions that have fascinated scientists since the nineteenth century and have since generated a substantial amount of research and evidence describing the physical factors that might effect endurance performance (Basset & Howley, 2000:72; Basset & Howley, 1997: 591; Burke *et al.,* 2019:117; Conley & Krahenbuhl, 1980:357; Costill *et al.,* 1973:381; Coyle, 1999:183; Gabriel & Zierath, 2017:1004; Joyner & Coyle, 2008:37; McLaughlin *et al.,* 2010:991; Midgley *et al.,* 2007:858; Thompson, 2017:296).

Fatigue is usually the most common term used to describe "failure during endurance performance" in the athletic setting and is believed to be the limiting factor in endurance sports, with the term mostly defined according to the specific sports division's individual discipline (Abiss & Laursen, 2005:867; Rivera, 2017:2). Observing fatigue during aerobic training or racing, one must consider the factors that limit a competitor's all-out performance capability during such events (Haff & Triplett, 2016:56). Two areas of fatigue during endurance exercise have been generally accepted and include (1) peripheral and (2) central fatigue, with the latter including all models associated with the brain and spinal cord and peripheral fatigue comprising all other systems including cardiovascular, nervous and muscular areas (Rivera, 2017:2). Numerous models of fatigue that may affect performance have also been built and are discussed by Abiss and Laursen (2005:868) in their review, however, it is beyond the extent of this literature review to assess as they are not relevant to the current study.

Pertinent to this study are the Cardiovascular/Anaerobic Model and the Energy Depletion model which will be further described in this review (Abiss & Laursen, 2005:868). The above, together with the amount of adaptive responses to intense or continuous training that has been clarified, makes the understanding of the so-called "mystery of human performance" somewhat clearer (Gabriel & Zierath, 2017:1000).

2.3.1 Status of adaptations during endurance training

Successful endurance performance is highly dependent on several factors and it is imperative to understand that in spite of the bodily adaptations seen through chronic aerobic exercise, even a single session of training causes the body to undergo several physiological adaptations that adjust its cardiovascular, respiratory and muscular functions in such a way that the energy demand to the working muscles or actively contracting muscle is efficient (Haff & Triplett, 2016:116; Hawley *et al.,* 2018:963; Kenney *et al.,* 2015:262; Kenney *et al.,* 2012:248). When these physiological functions are repeatedly challenged or undergoing continuous training-induced stress, as seen in regular aerobic training, it forms the stimulus for adaptations and exerts profound effects on endurance capacity (Gibala *et al.,* 2006:901; Hawley *et al.,* 2018:962; Midgley *et al.,* 2007:858).

The term endurance (or stamina) refers to a notion that can be mentioned by two distinct but intricately linked concepts which are muscular endurance and cardiorespiratory endurance (Kenney *et al.,* 2015:262). Muscular endurance is characterised by the capability of a group of muscles or a single muscle to preserve recurring, high-intensity contractions which allow the athlete to tolerate a very fast speed throughout the maximum specific distance such as, for example, a 100-meter or 200-meter sprint, whereas cardiorespiratory endurance correlates to the capacity of large muscle groups to tolerate continued, vigorous training such as distance running (Kenney *et al.,* 2012:248).

Cardiorespiratory endurance can further be distinguished into cardiovascular and respiratory system development assisting it to sustain oxygen transport to working muscles during extended exercise and enhancing the muscles' capacity to use energy aerobically, therefore enhancing endurance capacity (Haff & Triplett, 2016:116; Kenney *et al.,* 2012:249).

The abovementioned cardiovascular, respiratory and muscular functional adaptations to aerobic training will be discussed in the next section of this review as they all form part of the factors related to endurance performance (Basset & Howley, 2000:72; Coyle, 1999:183; Joyner & Coyle, 2008:37; Wilmore & Costill, 2004:300). This will assist understanding of their particular value in the development of a conditioning program, as well as for providing information for proven assessment including the variety of considerations to be incorporated during the assessment. When the main goal is improved endurance performance, the worth of understanding these concepts about the basic science behind human body endurance training adaptations and its relevance will help athletic trainers or coaches consider this importance when designing training profiles.

2.3.2 Factors related to endurance performance

Unarguably, endurance performance has been researched extensively, where the main focus has largely been on the probable limiting aspects that might affect such performance (Nalbandian *et al.,* 2017:322). Note that endurance performance refers to exercise that can be continued for more than 30 minutes, up to four hours (either maximal or submaximal), and can also be assessed by the completion of a given amount of work in a certain timeframe (Burke *et al.,* 2019:117; Coyle, 1999:181).

Temesi *et al*. (2017:970) state that the main elements of endurance performance are reliant on the activity nature or the exercise performed. This links to Joyner and Coyle's' review from 2008 where they state that research focuses more on endurance performance (distance running and cycling) because of (1) the immense amount of data available on the physiological adaptations that add to endurance performance; (2) the outstanding records and standard events taking place all over the world, and (3) the option to use treadmills and cycle ergometers in laboratory settings where the actual racing or training environment can be simulated (Joyner & Coyle, 2008:37).

Considering the "classical" determinants of endurance performance in running, the three major physiological contributors (>70%) are mostly reflected in the maximal oxygen consumption ($\dot{V}O_2max$), lactate threshold (LT) or fractional utilisation of $\dot{V}O_2max$ ($\%\dot{V}O_2max$), and economy of running which has been well supported (Earnest *et al.,* 2019:134; Hawley *et al.,* 2018:962; Joyner & Coyle, 2008:37; Midgley *et al.,* 2007:858; Nalbandian *et al.,* 2017:322). These three contributors work in

conjunction with several other morphological components to ultimately predict performance velocity or aerobic power (Joyner & Coyle, 2008:38; Kenney *et al.,* 2012:300). The average speed maintained during an endurance event (performance velocity) will be influenced by the average work performed during the specific task (aerobic or performance power) (Bentley *et al.,* 2007:575). These morphological components all have their own stimulus on certain individual functional abilities, all of which lead to a greater overall performance ability in the end. These components and abilities are captured in a well-designed model by Dr Coyle and show a perfect example of the integrated work between the different physiological determinants of endurance performance (Coyle, 1999:183; Joyner & Coyle, 2008:37; Wilmore & Costill, 2004:300).

Keeping the above mentioned in mind, one should consider the different viewpoints of a certain philosophy or theory, which always finds its place in any science; the same goes for the philosophies or theories concerning human science and endurance performance. McLaughlin *et al*. (2010:991) compare the two major viewpoints concerning this theory, namely (1) the so-called "classical" physiological variables that are related to endurance running performance prediction with Prof. Noakes and his colleagues' concept that peak treadmill running velocity (PTV) must be nearly as good a predictor of endurance performance as the three "classical" variables. Firstly, they looked at the link involving oxygen consumption ($\dot{V}O_2$) and running speed with the notion of a superior bound in $\dot{V}O_2$ as proposed in the 1920s by Hill (McLaughlin *et al.,* 2010:991). The meaning behind Hill's notion is that as the speed during a run increases, the O_2 requirements increase unceasingly, which leads to vast values attained at very high speeds, but that real $\dot{V}O_2$ achieves an upper limit which means that no effort can drive it any further (Basset & Howley, 2000:70; McLaughlin *et al.,* 2010:991). Hill also recommended that $\dot{V}O_2$max was only regulated by the circulatory-respiratory system because the upper limit capability of the system has been reached (McLaughlin *et al.,* 2010:991). However, in 1973, Costill and his fellow colleagues speculated that other factors have to be taken into account as well after they found a negative correlation between $\dot{V}O_2$max and a ten mile time-trial in participants with a variety of $\dot{V}O_2$max values. Their argument holds that not all races can or are run at 100% $\dot{V}O_2$max and that the athlete that can run at a better %$\dot{V}O_2$max will outperform his/her counterpart if similar $\dot{V}O_2$max values were observed (Costill *et al.,* 1973:248).

Therefore, the amount of oxygen an athlete uses during a race can be described as a function of both $\dot{V}O_2$max and the % of $\dot{V}O_2$max ($\dot{V}O_2$ at LT) that can be continued during the run (McLaughlin *et al.,* 2010:992). Also, $\dot{V}O_2$ at LT and velocity at LT have been identified as better predictors of running performance than $\dot{V}O_2$max (Thompson, 2017:297).

Running economy (RE) is another factor that has been taken into account since the early 1900s when determining running performance and can be described as the $\dot{V}O_2$ at a submaximal running speed or the effectiveness to transfer skeletal muscle chemical energy into movement (Gabriel & Zierath, 2017:1006; Thompson, 2017:297).

To conclude the "classical" physiological variables, the model below has been used to estimate endurance performance during distance races (Figure 2.1). The figure exemplifies that the performance $\dot{V}O_2$ is an artefact of the %$\dot{V}O_2$max at LT and the $\dot{V}O_2$max and the speed at $\dot{V}O_2$max is a product of the $\dot{V}O_2$max and RE (McLaughlin *et al.,* 2010:992).

% $\dot{V}O_2$max at LT X $\dot{V}O_2$max X RE = Running velocity in distance races

$\dot{V}O_2$ at LT Velocity at $\dot{V}O_2$max

(performance $\dot{V}O_2$) (v $\dot{V}O_2$max)

Figure 2.1: Performance predicting model in endurance races on the basis of physiological variables

Professor Tim Noakes and his colleagues came with a strong difference in opinion as to the factors which have an influence on endurance running performance in two of his studies (Noakes *et al.,* 1990:35; Noakes, 1988:329). Their argument was that the "classical" physiological variables cannot be the only determining factors predicting running endurance performance and that the peak treadmill velocity (PTV) is just as beneficial to use when predicting such performance since LT and the limitations to perform maximally include muscle features correlated to maximal strength generation and not the cardiovascular system (Noakes *et al.,* 1990:35; Noakes, 1988:329). Overall, the two main dissimilarities in opinion according to these above mentioned authors "models" of endurance performance prediction are, firstly, the proposal that

muscle elements commonly linked to maximal strength generation are related to endurance performance and in conflict with the central oxidative energy generation model, and secondly, PTV's quantification is not a model that physiologically predicts endurance performance but rather a running assessment to predict endurance performance and might be a good predictor of endurance performance as the possibility exist that PTV is promptly linked to oxidative progressions (McLaughlin *et al.,* 2010:992). For this reason, Mclaughlin *et al.* found it appropriate to assess the classic physiological variables related to endurance performance ($\dot{V}O_2$max, LT, running economy) with PTV as a separate forecaster of running endurance performance (McLaughlin *et al.,* 2010:991). Their method included laboratory tests to determine $\dot{V}O_2$max, LT, running economy, velocity at PTV and LT during a 16 kilometre time-trial as well as a GXT to exhaustion. Results of their study showed that the classical model which incorporates the three physiological variables to explain inter individual variations in distance running ability ($\dot{V}O_2$max, LT, running economy) meticulously predicts performance of a 16 kilometre time-trial. However, they also did not find much variability in the %$\dot{V}O_2$max at LT which left $\dot{V}O_2$max and RE clarifying most of the variables in performance. Lastly, they concluded as PTV was also highly associated with endurance running performance that it might be because both highest speeds attained during a graded exercise test as well as a 16 kilometre time-trial might be linked to the same physiological variables (McLaughlin *et al.,* 2010:996).

Although different viewpoints around the major determinants of endurance performance have emerged (Basset & Howley, 2000:591), it seems that the classical theory of Hill in the 1920s has served as an excellent framework in theory to the understanding of the determining factor of endurance performance, but that many other scientists over the years have also contributed and assisted with the expanding of views around the performance philosophy (Basset & Howley, 2000:82).

2.3.3 Oxygen consumption/uptake

A person's ability to inhale oxygen through the respiratory system and subsequently transport it to working muscles through the cardiorespiratory system, as well as the skill of the operating muscles to use the O_2 can be termed $\dot{V}O_2$ (Beck *et al.,* 2018:672; Haff & Triplett, 2016:56 ; Lee *et al.,* 2020:1). An important consideration in assessing the multi-systemic reactions the body undergoes during training stress is through $\dot{V}O_2$

where the proficiency of $\dot{V}O_2$ kinetics depends on two components: O_2 supply and O_2 extraction (Drescher *et al.,* 2018:156). $\dot{V}O_2$ kinetics from the beginning of moderate intensity exercise are usually measured through breath-by-breath analysis and categorised into three phases, namely (1) the 'cardio-dynamic' phase which mirrors the sudden increase in $\dot{V}O_2$ at the onset of training, facilitated by an increased ventricular output and an increase in venous return; (2) The 'fast' component which reflects the kinetics of muscle $\dot{V}O_2$ and (3) where the steady state of $\dot{V}O_2$ is reproduced. Through the above, it can be further distinguished that the O_2 extraction is influenced by muscle fibre distribution, the size and number of mitochondria and enzyme activities, while O_2 supply depends on the capillary concentration and perfusion capacity of muscles (Drescher *et al.,* 2018:166). During an intense session of aerobic exercise, the oxygen demand of the working muscles surges and is completely associated to the metabolic efficiency, exercise intensity and muscle mass, meaning that aerobic exercise that involves the use of larger muscle mass or a harder level of work to put out will likely lead to a higher total oxygen uptake (Gløersen *et al.,* 2020:983; Haff & Triplett, 2016:117).

The speed $\dot{V}O_2$ kinetic response controls the extent of the oxygen deficit seen and/or experienced at the onset of exercise. This proposes that faster $\dot{V}O_2$ kinetics leads to a couple of important adaptations, including (1) an earlier attainment of the essential steady state $\dot{V}O_2$ which in turn leads to the reduced speed of fatigue development and a decrease in metabolic perturbations, plus (2) a rise in the power input from aerobic metabolism during high intensity running bouts which forms a very important part of any distance running event. As anaerobic energy supply is limited, the increase from aerobic energy contribution could reduce the depletion force of anaerobic conditions and thus increase performance, particularly in middle-distance compared to long-distance events (Shaw, 2016:9).

2.3.4 Maximal aerobic capacity/power

Maximal oxygen consumption ($\dot{V}O_2max$), sometimes referred to as maximal aerobic power or maximal aerobic capacity, is considered to be one of the key indicators of the degree of physical fitness and therefore forms a suitable measure of cardiorespiratory endurance or aerobic fitness assessments (Kenney *et al.,* 2015:277; St Clair Gibson *et al.,* 1999:1226; Truong *et al.,* 2018:304). $\dot{V}O_2max$ is also classified

as one of the oldest assessments in exercise physiology, developed and well-defined by Hill *et al*. in the 1920s and regarded as the 'gold standard' physiological factor to express the aerobic capability of an athlete (Basset & Howley, 2000:70; Cooper *et al.,* 2005:152). It includes four major classifications: (1) an upper threshold to $\dot{V}O_2$; (2) inter-individual variances in $\dot{V}O_2$max that occur; (3) an above average $\dot{V}O_2$max is a precondition for achievement in endurance running, and (4) $\dot{V}O_2$max is restricted by the capacity of the cardiorespiratory system to carry O_2 to working muscles and has been reviewed and established by numerous authors (Basset & Howley, 2000:71; Burke *et al.,* 2019:117; Hawley *et al.,* 2018:963; Lundby & Montero, 2019:3; Schaun, 2017:2; Sperlich, *et al.,* 2015:386). The presence of this upper threshold to consume O_2 is still agreed upon today (Haff & Triplett, 2016:261; McLaughlin *et al.,* 2010:991) and has been shown in several studies that included discontinuous test protocols that continually tried to push the $\dot{V}O_2$ to above average through increasing the working tempo, but this modus ceased to be effective (Mooses *et al.,* 2015:136; Arrese *et al.,* 2005:435).

Endurance events are usually accompanied by long periods of aerobic endurance, with the portion of overall energy requirements increasing as the length of the event intensifies to meet the demands of the aerobic metabolic system (Gløersen *et al.,* 2020:983; Haff & Triplett, 2016:560). Therefore, $\dot{V}O_2$max can be explained as the greatest amount of $\dot{V}O_2$ transportation and utilisation at the cellular level during exhaustive exercise, and is classified as the result of the amount of O_2 inhaled minus the amount of O_2 exhaled during physical exercise (Attipoe & Deuster, 2015:265; Davis, 2006:9; Kolkhorst & Buono, 2004:53; Rivera, 2017:13). This is also the precise explanation of the Cardiovascular/Anaerobic Model of fatigue by Abiss and Laursen that illustrates fatigue to appear when the heart muscle is unable to provide O_2 to the working muscles or get rid of waste products from the working muscle (Abiss & Laursen, 2005:868; Rivera, 2017:13). Another definition explains it as the maximum tempo at which energy can be produced by the athlete through oxidation of CHO, fats and proteins or the upper threshold set for production of ATP by means of oxidative phosphorylation and is generally stated as a volume of $\dot{V}O_2$ per kilogram of bodyweight per minute (ml/kg/min) (Haff & Triplett, 2016:261; McLaughlin *et al.,* 2010:991). The measurement of maximal aerobic capacity ($\dot{V}O_2$max) serves multiple roles that include (i) the classification of functional ability of the oxygen transport system, (ii) a directory

of maximal cardiovascular and pulmonary function, and (iii) significant reflection of endurance performance (Attipoe & Deuster, 2015:265).

Considering the above, it is also important to understand that sometimes an athlete reaches volitional fatigue as the exercise intensity increases before a plateau occurs in the $\dot{V}O_2$ response (which forms the criterion for an accurate $\dot{V}O_2$max reached); in such circumstances the $\dot{V}O_2$max that is achieved will then be referred to as peak oxygen uptake ($\dot{V}O_{2peak}$) (Beltz *et al.,* 2016:2; Kenney *et al.,* 2015:128; Schaun, 2017:2). In literature, these two terms are used interchangeably, but it must be noted that there is indeed a difference. VO_{2peak} is the highest rate achieved upon an incremental or high intensity test and is designed to bring an athlete to the limit of endurance to exercise while $\dot{V}O_2$max represents the utmost physiologically realistic rate achieved during such a test (Beltz *et al.,* 2016:1). What happens most of the time is that the subjects merely reach $\dot{V}O_2$max sooner as the work rate increases, making this the reason $\dot{V}O_2$ does not continue to increase open-endedly (Basset & Howley, 2000:71). It is also important to note that $\dot{V}O_{2peak}$ is not a maximum value but $\dot{V}O_2$max is always a peak, and that the distinction between $\dot{V}O_2$max and $\dot{V}O_{2peak}$ can be determined by the occurrence of an area of stability in $\dot{V}O_2$ (Beltz *et al.,* 2016:2). Furthermore, it is not guaranteed that all subjects reach a plateau in $\dot{V}O_2$ at exhaustion level of a GXT when graphed against work rate, however, failure of this does not indicate a failed attempt to reach "true" $\dot{V}O_2$max since, firstly, with a GXT to exhaustion, a subject may perhaps fatigue just as $\dot{V}O_2$max is attained and, secondly, most researchers require that subjects complete three to five minute intervals during a discontinuous GXT, thus meaning if a subject reaches $\dot{V}O_2$max in two minutes at a supramaximal intensity and then cannot continue due to overall fatigue, the point will not be graphed and in such case the $\dot{V}O_2$ plateau will not seem apparent in the final graph of work rate versus O_2, even though $\dot{V}O_2$max has been reached (Basset & Howley, 2000:71). This means that a plateau reached cannot be used as the sole determinant of achievement of $\dot{V}O_2$max and why secondary criteria are also included to verify maximal effort.

Secondary criteria include the British Association of Sport Sciences (BASS) guidelines, namely: (1) a flattening of $\dot{V}O_2$ with an increased work output intensity, which is described as a rise in $\dot{V}O_2$ of less than two ml/kg/min or 5% with an increase in exercise intensity; (2) when the RER exceeds 1.15; (3) when there is an onset of

extreme exhaustion; (4) when blood lactate levels approach or exceed eight mmol/L; (5) when a exertion rate (RPE) of 19 or 20 out of 20 is reached; and (6) when the HR is within ten beats per minute (bpm) of the age predicted maximum (Beltz *et al.,* 2016:5; Christie & Lock, 2001:19; St Clair Gibson *et al.,* 1999:1226).

The above also calls for another reason why Noakes and his colleagues presented the notion that PTV must be as good a predictor as $\dot{V}O_2$max for endurance performance (Noakes *et al.,* 1990:35). Similarly, Sedano *et al*. (2013:2433) also stated $\dot{V}O_2$max not to be the sole predictor of endurance performance in elite athletes since neuromuscular and anaerobic qualities must form part of the factors that determine such performances. Along with Noakes' study, Sedano *et al.* also stated that during a GXT, the PTV is a better indicator of running performance as performance is not only influenced by aerobic power but also via neuromuscular qualities linked to reflex and voluntary neural activation, force of the muscles along with muscle elasticity, anaerobic features and running mechanics (Sedano *et al.,* 2013:2433).

Marathon runners generally have a varying range from 70 – 85% $\dot{V}O_2$max and can tolerate running speeds that require a mere 85 – 90% $\dot{V}O_2$max for more than an hour (Burke *et al.,* 2019:118; Hawley *et al.,* 2018:963; Joyner *et al.,* 2011:275). These athletes will likely always reach a higher $\dot{V}O_2$max on a treadmill when being assessed to volitional exhaustion as when tested on a cycle ergometer since quadriceps muscle fatigue will most probably prevent testing to continue to $\dot{V}O_2$max (Kenney *et al.,* 2015:128). Bearing the above in mind, one can understand that although a higher $\dot{V}O_2$max is associated with better endurance performance and definitely necessary for elite performance, a stellar endurance athlete requires more than a high $\dot{V}O_2$max to elicit outstanding endurance performances (Haff & Triplett, 2016:560; Hawley *et al.,* 2018:963; Kenney *et al.,* 2015:128). The $\dot{V}O_2$max values of endurance athletes have been shown to improve with maximal, supramaximal and submaximal training intensities (Midgley *et al.,* 2007:866). Literature has also indicated that $\dot{V}O_2$max increases to a certain amount in each individual when following a programme for approximately 8 – 12 weeks but then plateaus off and discontinues to improve despite more or higher intensity training. However, athletes are still capable of improving their performance regardless of a plateau reach in $\dot{V}O_2$max which signifies that they start to work at a better %$\dot{V}O_2$max (Kenney *et al.,* 2015:128). It also indicates that there is more to endurance performance than the "gold standard" $\dot{V}O_2$max measurement and

that one must consider the overall factors related to aerobic performance when designing a program.

As $\dot{V}O_2$max still plays such an enormous role in predicting aerobic power and endurance performance (Kenney *et al.,* 2015:277; St Clair Gibson *et al.,* 1999:1226), scientists have different opinions on the physiological factors that may have an influence or limit on $\dot{V}O_2$max itself (Burke *et al.,* 2019:118; Kenney *et al.,* 2015:265).

2.3.4.1 Physiological limiting factors for maximal aerobic capacity ($\dot{V}O_2$max)

Endurance exercise powers adaptations that are related to kinetics and capacities, where capacities refer to the maximum bounds of the physiological system involved during endurance training [$\dot{V}O_2$max, $\dot{V}O_{2peak}$, maximal cardiac output (Q) and maximal heart rate (HRmax)] and kinetics denotes the speed adaptation to training stresses to comparative changes like the dynamic response of $\dot{V}O_2$, HR and Q in reaction to shifting metabolic stresses during provisional training (Drescher *et al.,* 2018:1). A series of steps form the pathway for O_2 from the environment to the mitochondria in the body, of which each could signify a potential barrier to O_2 flux (Basset & Howley, 2000:72). It is already known that $\dot{V}O_2$ kinetics play a pivotal role in evaluating the body's multi-systemic reactions to training stress and that the efficiency of $\dot{V}O_2$ kinetics is dependent on the O_2 inhaled and O_2 removed (which is also influenced by enzyme activities, the distribution of muscle fibres and via the mitochondria's size and numbers), while the O_2 delivery relies on the amount of muscle perfusion as well as the capillary density (Drescher *et al.,* 2018:156). Both kinetic and capacities should be considered when evaluating the complexities of endurance training as both capacities and kinetic reactions of the respiratory and cardiopulmonary system's role in the optimisation of endurance performance is of utmost importance and should therefore not be split into independent physiological variables (Drescher *et al.,* 2018:2).

Since the 1970s, the whole debate concerning the issue of central versus peripheral factors that limit $\dot{V}O_2$max has continued and has resulted in different outcomes and conclusions. In the 1980s, Saltin and his colleagues did two separate experiments which resulted in completely opposing conclusions (Saltin & Calbet, 1985:7445; Saltin *et al.,* 1976:289). Their first experiment involved the measurement of $\dot{V}O_2$max during one-legged cycle training after one leg underwent training and the other leg served as control. A 23% increase in $\dot{V}O_2$max was seen in the trained versus the untrained leg

which showed an increase of only 7% in $\dot{V}O_2$max. The results argued the inconsistency between legs due to peripheral changes that occurred within the trained leg's muscles and, therefore, Saltin and his fellow colleagues concluded that the dominant factor limiting $\dot{V}O_2$max was peripheral factors (Saltin *et al.,* 1976:303). In contrast to the above experiment, Saltin did another experiment in 1985 which resulted in the opposite conclusion that cardiac output (Q) is primarily the limiting $\dot{V}O_2$max factor with whole body exercise but not for small, isolated muscle efforts (Saltin & Calbet, 1985:7445). Furthermore, it was documented that the limiting factor of $\dot{V}O_2$max depends on the experimental model used to address the problem, because when looking at whole body exercise in humans, the cardiorespiratory system forms the limiting factor, but when looking at isolated dog hindlimbs, the peripheral diffusion gradient forms the limiting factor (Basset & Howley, 2000:77). Therefore, when looking at the limiting factors across different species, one will observe that both mitochondrial content and O_2 transport capacity are both important and that there is no single limiting component to $\dot{V}O_2$max (Basset & Howley, 2000:77).

According to Beltz, *et al*. (2016:2), the fundamental basis for enumerating O_2 transport, utilisation and mitochondrial energy production still remains as quantified a century ago and therefore the works of Hill and his fellow researchers investigations in the previous century are yet of much importance and should still be considered today.

i) Central factors

Cardiac output (Q) refers to the heart's pumped blood volume and is controlled by the amount of blood ejected by each heart beat (stroke volume(SV)), as well as the pumping rate of the heart (Haff & Triplett, 2016:118). Q is also explained by the following equation, Q = stroke volume X heart rate. This indicates that Q increases linearly with exercise intensity but reaches an area of stability when exercise metabolic requirements are met through the blood flow (Kenney *et al.,* 2015:200; McArdle *et al.,* 2010:342). The most noteworthy modification in cardiovascular function that comes from aerobic training is an improvement in maximum Q (McArdle *et al.,* 2010:465; Kenney *et al.,* 2015:269). The primary goal of a rise in Q is to link the increased demand for O_2 by the working muscles which shows the importance of Q in achieving an elevated level of aerobic metabolism (Kenney *et al.,* 2015:200). The maximum HR of the athlete generally decreases with endurance training while the Q increases as a

direct outcome of a rise in SV. This increase in maximal Q or SV is what distinguishes an elite endurance athlete from an amateur endurance athlete (McArdle *et al.,* 2010:465; Kenney *et al.,* 2015:268). However, Q just as $\dot{V}O_2$max, only increases to a certain level even with an incredible load of endurance training and can sometimes even show a decline in Q after weeks of submaximal endurance training. This is the consequence of a surge in the pulmonary oxygen difference or a reduction in the rate of $\dot{V}O_2$ required for the level of exercise intensity (Kenney *et al.,* 2015:269). However, the increases seen in Q as a reaction to endurance training is what is mainly accountable for the increase in $\dot{V}O_2$max to a certain point with exercise, while the rise in Q is again seen as an outcome of the increasing maximal SV (McArdle *et al.,* 2010:467; Kenney *et al.,* 2015:269). Generally, the statement is provided that $\dot{V}O_2$max is ultimately regulated by the inability of the Q to increase any further (Kenney *et al.,* 2015:202).

ii) Peripheral factors

More recent literature forgoes that, as exercise intensity rises, the degree of fatigue starts to heighten as well because O_2 demand starts to exceed O_2 delivery. This forms the point at which energy supply from glycolysis and phosphagens becomes progressively greater, causing even more energy depletion, electrolyte disturbances, accumulation of metabolites and increased free radical production, all of which can provoke fatigue at almost $\dot{V}O_2$max by mostly peripheral mechanisms (Morales-Alamo *et al.,* 2015:4632). Known to be the most prominent adaptation during endurance training, a change in muscle substrate metabolism can be provoked in as little as five to seven days of endurance training and causes an increase in availability of glycogen while decreasing the tempo of glycogen catabolism during training, all leading to improved $\dot{V}O_2$max or endurance performance ability (Gibala *et al.,* 2006:901). An interesting observation is that, in evaluating aerobic substrate metabolism during endurance training, the evaluation takes place on the muscle site because altered micro-cellular and mitochondrial changes are the main clarifications for an increase in endurance tolerance. Furthermore, endurance training with a non-steady state increase in effort is said to induce variances in the pulmonary quantity of $\dot{V}O_2$ at the mouth and the $\dot{V}O_2$ at exercising muscles that can be quite prominent (Drescher *et al.,* 2018:157). Training induced alterations in substrate metabolism can be credited to the

enhanced sensitivity of respiratory control due to a rise in mitochondrial mass, as imitated by alterations in the protein content of enzymes and the maximum activity in the electron transport chain and tricarboxylic acid cycle (Gibala *et al.,* 2006:901). Although measured at the muscle site, the probable mismatch of values given between measurement of substrate metabolism and $\dot{V}O_2$max at the mouth and mitochondria is ascribed to the distortive effects and time-delaying of venous return that is highlighted by the periods of non-linear transport of deoxygenated blood pumping from the exercising muscles to the lungs (Drescher *et al.,* 2018:157).

Whether $\dot{V}O_2$max is influenced by central (pulmonary) factors or by muscle (peripheral), $\dot{V}O_2$ kinetics still remain a debate and show that both these systems must be taken into account and must be of relevance and therefore focus needs to be placed on the systemic (changes in whole body perfusion and venous return) and peripheral (alterations in muscle perfusion, mitochondria cells) points of interest during $\dot{V}O_2$max experiments (Drescher *et al.,* 2018:157). Each and every step in the O_2 pathway during endurance training contributes in its own unique and integrated way to determine $\dot{V}O_2$max, and reduction in any of the steps in the transport ability of the body will predictably reduce $\dot{V}O_2$max (Basset & Howley, 2000:77).

2.3.5 Running economy

The ability to perform the same amount of work with lower O_2 and energy use is termed economy of movement, or running economy, and plays a significant role in long endurance events and racing (Madden *et al.,* 2018:962). An experienced or highly trained athlete can use less O_2 while running at submaximal pace, thus using less energy but producing the same amount of power than a non-experienced or untrained counterpart. Sport specific training is particularly important when opting to increase economy, therefore, to increase running economy, athletes should focus on running and not put emphasis on other forms of training to increase economy (Madden *et al.,* 2018:129). Although economy of movement can be improved, it is vital to know that this is only fractional and unlikely through any single physiological characteristic (Hawley *et al.,* 2018:963).

Performance velocity and/or power are highly affected by economy of movement, and is seen more in runners than cyclists, with an almost 40% difference between elite and untrained athletes. An athlete can compensate for “poor” running economy at exercise

intensities at or near the fractional utilisation of the athlete's aerobic capacity, however, the moment exercise intensity increases to a level at or around LT, compensation for the "poor" economy will not be possible (Hawley *et al.,* 2018:963).

As this study did not evaluate running economy, the section will not be discussed in depth.

2.3.6 Fractional consumption of $\dot{V}O_2max$ and blood lactate

Another influential factor for endurance performance is the proportion of $\dot{V}O_2max$ that can be uninterrupted over a given distance with sturdy correlations seen between running performance and $\dot{V}O_2max$. This proportion of $\dot{V}O_2max$ has also been closely related with accumulation markers of blood lactate, with the LT and lactate turning point being the most outstanding points (Hawley *et al.,* 2018:963; Shaw, 2016:6).

For decades, lactate has been a major subject of discussion among the athletic populations about the important role it plays in the old-fashioned concept of muscle exhaustion and whether an increase in the LT would result in improved endurance performance and how it can complement a training programme (Fernandes *et al.,* 2016:193; Hall *et al.,* 2016:s8; Midgley *et al.,* 2007:866; Poole *et al.,* 2016:2320). The long standing theory that lactate is a waste product of anaerobic metabolism causing metabolic acidosis has now been overshadowed by notions that it is in actual fact not a metabolic waste product causing weakened muscle contractibility, extreme exhaustion and the uncomfortable cessation felt at almost maximal exercise (Brooks, 2018:757; Hall *et al.,* 2016:s8). Brooks *et al.* (2018:757) described lactate formulation as a non-stop process, even under aerobic conditions, and that it can be described as the transport between producer and consumer cells. They concluded that lactate metabolism has three purposes during whole body exercise: (1) it is a key energy source, (2) it is a gesturing molecule with endocrine, autocrine and paracrine effects, and (3) it is a main gluconeogenic precursor (Brooks, 2018:757).

Fuel demands of many sports, including distance running, are more complex than one might think. Marathon or distance running or cycling are grouped as endurance events performed at sub-maximal intensity (Burke, 2015:s36: Moore, 2015:294) and sensibly these sports require times of tactical strategies during the vital parts of the race which are led at more intense and often maximal pace which in general requires a high level

of metabolic shift ability (Burke, 2015:s36). Exercise intensities can vary from 80 – 90% of $\dot{V}O_2$max in elite runners, to 70 – 75% $\dot{V}O_2$max or even 60 – 65% $\dot{V}O_2$max in less elite runners and it is known that CHOox increases as exercise intensity increases (Burke *et al.,* 2019:118; Cermak & van Loon, 2013:1140; De Souza Silveira *et al.,* 2016:2).

A well-established phenomenon is the curvilinear relationship that exists between increases in exercise intensity and blood lactate accumulation, which leads to small amounts of substrate phosphorylation required during bouts where an increase in speed or intensity is needed, whereas most of the energy during submaximal exercise is met via oxidative phosphorylation in the mitochondria (Shaw, 2016:7; Spriet, 2007:332). Therefore, even with endurance running as exercise duration or intensity increase, phosphocreatine stores decline which lead to circulating blood glucose or muscle glycogen to be shuttled through the glycolytic pathway leading to the formation of ATP (which is necessary for muscle contraction) and pyruvate (Hall *et al.,* 2016:s9). When pyruvate is oxidised in the anaerobic system, two molecules of lactate are formed, the reason being that the "participation" of the mitochondria during this anaerobic glycolysis is essentially non-existing. This means that no oxygen is present to produce ATP, and when anaerobic glycolysis is the only manner to produce ATP to fuel intense muscle contraction, lactate must be produced from pyruvate (Spano *et al.,* 2018:35). Both glycogenolysis and glycolysis produce the same amount of pyruvate, but it is the number of hydrogen ions released that differentiate the processes. Glycolysis yields the net discharge of two H^+ while glycogenolysis harvests only one H^+ with a supplementary ATP molecule (Hall *et al.,* 2016:s9). The continuous use of the anaerobic system with higher intensity exercise will eventually result in glucose and glycogen concentrations to deteriorate and being broken down by glycogenolysis, in turn, resulting in increases in lactate in the cells via the catalytic enzyme activity of lactate dehydrogenase (LDH) which results from the oxidation and recycling of NAHD (also a product produced from glycolysis) to NAD^+. This process allows for continuous breakdown of glucose to pyruvate to continuously yield ATP (Bitel, 2017:6; Dunford & Doyle, 2015:76; Spano *et al.,* 2018:36). The end result of this reaction (increase in lactate formation within the cells) is the accumulation or formation of lactic acid, but clear-cut differentiation must be drawn between the forming of a lactate molecule and the acidity that is associated with high-intensity exercise when glucose is the

predominant fuel used due to the cells' physiological pH (near 7) and previous steps in the glycolysis reaction that consume protons (Dunford & Doyle, 2015:78; Haff & Triplett, 2016:48).

When H^+ are produced as a result of anaerobic glycolysis being used at a higher rate, the intracellular pH level lowers, glycolytic reactions are inhibited and this directly hinders muscle excitation-contraction coupling, conceivably because calcium (Ca^+) is inhibited from connecting to troponin or because of the interference with cross bridge recycling, which results in increased acidity of the muscle tissue, causing muscular fatigue to be felt with exercise intensities of moderately-high to high (Dunford & Doyle, 2015:77; Madden *et al.,* 2018:127; Spano *et al.,* 2018:36). This is where the term, Metabolic Acidosis, formerly originated from as this entire lowering in muscle tissue acidity means that a very high rate of anaerobic metabolism is taking place which might be liable for the peripheral fatigue felt during exercise of such intensity (Dunford & Doyle, 2015:77; Hall *et al.,* 2016:s9; Haff & Triplett, 2016:48).

The drop in pH-levels in the cells is said to result in key metabolic enzyme activity being decreased, which interferes directly with the processes of force production which results in muscle fatigue (Dunford & Doyle, 2015:77). Overall, the "burning" sensations felt or blood lactate accumulation in the muscle cells when exercise continues to or at a higher-intensity, occurs when the production of lactate starts to exceed the rate of lactate removal from the cells (Bitel, 2017:6). Although it is believed that the acidosis that occurs overwhelms the body's ability to buffer the acidity and in return leads to detrimental decrease in performance levels, the opposite is actually true with bicarbonate (HCO_3) buffering which lessens the unsettling effect of the H+ on pH levels by taking the proton (H_2CO_3) leading to blood concentrations returning to homeostatic range (Dunford & Doyle, 2015:78; Haff & Triplett, 2016:48). It is important to notice that the simple hydrolysis of ATP outside of the mitochondria are accountable for the most part of H^+ build-up and that lactate functions to reduce acidosis which results in lactate being used as energy substrate rather than declining energy levels (Haff & Triplett, 2016:48; Hall *et al.,* 2016:s8). The reaction that occurs between pyruvate and lactate works in both directions as pyruvate can be altered to lactate, but the moment the lactate concentrations become very high and the result are the movement of lactate molecules out of the cells and into the blood, lactate can be converted back to pyruvate which can be transported via monocarboxylate

transporters into the mitochondria and metabolised aerobically by highly aerobic cells (for example, the liver, cardiac muscle fibres, Type I muscle fibres and kidneys) to produce glucose via ATP hydrolysis (Bitel, 2017:7; Dunford & Doyle, 2015:78; Haff & Triplett, 2016:49; Hoff *et al.,* 2016:1373).

Evidently, the presence of blood lactate is a by-product of an enhanced amount of glycolysis during exercise, where the intensity of the exercise leads to the inability of the muscles to generate enough energy through mitochondrial oxidation. Hence, it is important to know that a rise in oxidative ability of the working muscles through training can aid in glycogen sparing along with other mechanisms, including improved regulation of H^+, improved release function of Ca^{2+} and enhancements in muscle ion controlling (lower interstitial strengths of K^+ during intense exercise), which can all play a major role in preventing or decreasing peripheral fatigue felt during intense exercise (Gabriel & Zierath, 2017:1005).

Through the abovementioned literature, it is apparent that just as the creatine phosphate system does not operate anaerobically in its entirety, the glycolysis/lactate system also works anaerobically and aerobically to provide energy for working muscles as anaerobic glycolysis relies upon the aerobic energy system to metabolise lactate back to ATP (Dunford & Doyle, 2015:78; Haff & Triplett, 2016:49).

2.4 Fuel substrates for endurance exercise

The second fatigue model relevant to this study is the energy depletion model by Abiss and Laursen which suggests that a decrease in endurance performance can also be related to the inability of the working muscles to properly contract because of the lack of fuel substrates available (Abiss & Laursen, 2005:867; Rivera, 2017:14). During exercise, the breakdown of glucose, glycogen and PCr to lactate or pyruvate along with the oxidation of CHO and fats start to replenish ATP that is associated with fatigue and impaired performance (Jeukendrup, 2012:41; Coleman, 2010:1). The energy depletion model by Abiss and Laursen (2005:869) was termed after the authors cited a decrease in glycogen content of up to 86% during a 90-minute time to exhaustion trial. It is important for optimal sports performance that an athlete has a mixture of sufficient fuel supplies relative to the stresses of the specific sport in addition to ‘metabolic flexibility’ to be able to rapidly switch between fat as fuel while in a fasting state to glucose in the nourished state (Moro *et al.,* 2014:584; Burke, 2015:s36).

During endurance running, CHO in the working muscle and the liver along with stored fat in muscle and adipose tissue are the major fuel sources used by the body (Rapoport, 2010:2; Spriet, 2007:332; Williamson, 2016:2). During long constant load exercise that relies mainly on the aerobic metabolic pathway for optimal performance, high levels of stress can be placed on the human body and, in turn, go together with the excessive metabolism of CHO (muscle and liver glycogen) (Moore, 2015:294; Byars *et al.,* 2010:1). As such, one of the key roles of nutrition in sport is to find a strategy to optimise the purpose of the muscle and central nervous system through increasing the body's capacity to store fuel (Burke, 2015:s34). As CHO is stored energy, this depletion in muscle and glycogen stores has been seen to decrease performance as a result of fatigue (Coleman, 2010:1). For this reason, the common "CHO-loading" strategy before, during and after exercise and competition was and is still used as literature ranging back to the 1900s points out that the increase in muscle and liver glycogen stores lead to improved endurance performance (Bartlett *et al.,* 2015:3; Beck *et al.,* 2015:260; Byars *et al.,* 2010:1; Jeffers *et al.,* 2015:1; Moore, 2015:294). In contrast, Coleman (2010:1) wrote in her review that all these methods to increase CHO in the body might be limited to the athlete's opportunity to consume additional "energy" when needed and therefore decreases energy stores. Coleman also pointed out that these methods do nothing to slow the rate of CHO utilisation and therefore the justification for fat loading generated by utilising fat as alternative fuel source in such conditions (Coleman, 2010:1). It is generally excepted then that it must be by means of using less CHO and more fat during endurance exercise that the rate of lactate production should be slower and therefore enhance the aerobic performance of the individuals as athletes (Burke, 2015:s36; Byars *et al.,* 2010:1).

In summary, the foremost metabolic significance of the changes in skeletal muscle during endurance training is a slower use of liver and muscle glycogen, liver and blood lactate and blood glucose and a bigger dependence on intramuscular triglycerides (IMTG) and adipose tissue, blood-borne free fatty acids (FFA) and a lesser amount of production of lactate during low to moderate (45-70% $\dot{V}O_2$max) intensity exercise (Hawley & Leckey, 2015:s5).

Marathon or distance running or cycling are classified as endurance events performed at submaximal intensity (Burke, 2015:s36; Moore, 2015:294) and require times of tactical strategies during the key parts of the race which are led at more intense and

often maximal pace which, in general, require a high level of metabolic shift ability (Burke, 2015:s36). Exercise intensities can vary from 80 – 90% of $\dot{V}O_2$max in elite runners, to 70 – 75% $\dot{V}O_2$max or even 60 – 65% $\dot{V}O_2$max in less elite runners and it is known that CHOox increases as exercise intensity rises (Cermak & van Loon, 2013:1140; De Souza Silveira *et al.,* 2016:2). Small quantities of substrate phosphorylation are required during bouts where an increase in speed or intensity is needed, whereas most of the energy during sub-maximal exercise is generated via oxidative phosphorylation in the mitochondria (Spriet, 2007:332).

As an athlete's training and race speed progresses, it has been shown that whole body $\dot{V}O_2$max, running economy, skeletal muscle mitochondrial volume, as well as maximal CHOox and FATox rates increase severalfold (Barnes *et al.,* 2013:642; Galbraith *et al.,* 2014:1023; Hansen *et al.,* 2005:94; Hawley & Leckey, 2015:s5; Morton *et al.,* 2009:1520; Spriet, 2007:333; Stangier *et al.,* 2016:45). This surge also causes the skeletal muscles' ability to use plasma FFA to increase, as well as to oxidise and store IMTG which is an incredibly valuable adjustment for endurance capacity, assuming the small amount of CHO stored (Rapoport, 2010:2; Spriet, 2007:333; Williamson, 2016:2). Continuous training done at the same absolute running speed cause an increase in FATox reliance, together with the sparing of CHO, however, for an athlete to race faster than trained running speed, the need to oxidise CHO must account for the same proportion as during training combined with a higher FATox rate during competition (Spriet, 2007:333).

Exercise intensity, substrate availability, from both outside and inside the muscle, along with exercise length is the principal determining factor when it comes to the interaction between the amount of CHO and fats to be metabolised during distance running (Burke *et al.,* 2018:452; Spriet, 2014:s87).

From the literature investigated above, it seems that the most applicable dietary strategy for top performance relies on numerous metabolic characteristics and adaptations taking place during exercise/race in the human body and should therefore be accounted for and perhaps verified by athletes to improve their running performance. Also, in a recent review by Burke et al. (2018:452), it was stated that nutrition approaches rely on the nature, time and exercise intensity of the training session or race of the particular day and therefore has day-to-day as well as athlete

to athlete inter-individual variability, hence, fuel availability through nutrition intake needs to be advised correctly and accordingly.

2.4.1 Carbohydrate metabolism

Carbohydrates are stowed as glycogen in the muscles (approximately 500 g) (80%) and in the liver (approximately 100 g per liver glycogen storage site) (15%) and, therefore, the goal of any CHO nutritional strategy should be to optimise these liver and muscle glycogen stores leading up to endurance races (Bartlett *et al.,* 2015:3; Brown, 2002:222; Cermak & van Loon, 2013:1140; Hargreaves, 1992:4; Madden *et al.,* 2018:126).

Glycogen and blood glucose are considered particularly important sources of energy for endurance exercise (Murakami *et al.,* 2012:626; Jeukendrup, 2011:s91).

From the start of a training session, the blood glucose uptake increases exponentially as a result of contracting muscles and causes the activation of insulin and liver glycogenolysis by the actions of glucagon and epinephrine (Baker *et al.,* 2015:739).

Oxidative production of ATP is a process involving three structures: (1) Aerobic glycolysis, (2) the Krebs cycle, and (3) the electron transport chain (ETC) and, as mentioned, aerobic glycolysis plays a valuable role in CHO metabolism in both anaerobic and aerobic ATP production.

The process of glycolysis is similar in the presence and absence of O_2, however, in the presence of O_2 , pyruvic acid (the end product) is changed into acetyl coenzyme A (CoA) via oxidation for ATP synthesis through the citric cycle (Madden *et al.,* 2018:126). Anaerobic glycolysis generates lactic acid along with three molecules of ATP per glycogen molecule or two molecules of ATP per glucose molecule. CoA enters the next system, the Krebs cycle, which ends the oxidation of CoA and produces two additional ATP molecules and the original substrate, CHO, gets broken down into H^+ and CO_2. When glucose is changed to pyruvic acid during glycolysis and when it enters the Krebs cycle, H^+ is removed. If the H^+ remained free, the cell would become too acidic. Consequently, the Krebs cycle and ETC combines and forms the final system whereafter the released hydrogen merges with NAD and FAD, two coenzymes which take the hydrogen atoms to the ETC where it gets divided into

protons and electrons. Lastly, H^+ merges with O_2 and forms water (H_20), which inhibits acid formation.

A series of reactions is crossed by the electrons from where energy is delivered for the phosphorylation of ADP which, in turn, leads to the formation of ATP where one molecule of muscle glycogen can generate 37 – 39 molecules of ATP (Hargreaves & Spriet, 2006:29; Wilmore & Costill, 2004:52). CHO becomes the main energy source that is used by the muscles – up to 100% of $\dot{V}O_2$max at high-intensity exercise. Although, at exercise intensities which rank close to maximum, not CHO nor fat as fuel are used but the anaerobic system is relied upon to take over through the PC system to mobilise ATP which is stored in skeletal muscles (Madden *et al.,* 2018:126).

2.4.1.1 Carbohydrates and exercise

Carbohydrates are set to be the key energy factor when it comes to aerobic and anaerobic pathways of metabolism and, in order to optimise these fuel stores, CHO loading before an endurance event has been sought to form the foundation of athletes and coaches to minimise the potential ergolytic effects of CHO depletion (Bartlett *et al.,* 2015:3; Cermak & van Loon, 2013:1140; Hargreaves *et al.,* 2004:31; Valenta & Dorofeeva, 2018:406). However, it is essential to note that a standardised high-CHO diet for all athletes should not be promoted, but rather a sliding scale of intake that predicts the cost of fuel of the athletes' training and recovery and specific sport (Burke *et al.,* 2018:452; Hawley & Burke, 2010:152). Over time, the search for optimal nutrition strategies to optimise sports performance has been well studied. Through this vast amount of research, it is well-known that adequate CHO availability promotes exercise performance because of better preservation of blood glucose levels, CHOox increases and/or sparing of muscle glycogen (Ali *et al.,* 2016:1), as well as the relationship between increased storage of muscle glycogen and the onset of fatigue delay (Bartlett *et al.,* 2015:3; Close *et al.,* 2016:5; De Bock *et al.,* 2008:1045).

The Position paper from the Thomas *et al.* (2016:507) identified three special features detailing why CHO has received such a great deal of attention when it comes to its role in endurance performance. Firstly, the amount of CHO stored in the muscles is in essence very limited, representing less than 5% of the total energy storage (Cermak & van Loon, 2013:1140), and can be manipulated through dietary intake or even just a single exercise session on a daily basis (Thomas *et al.,* 2016:507). Secondly, as

CHO provides energy to the central nervous system and brain, coupled with substrate muscular work, even exercise over a wide variety of intensities is fuelled due to both anaerobic and oxidative pathway use. This means that CHO offers an advantage over fat as fuel during exercise even at maximum intensities because of the better production of ATP per volume of O_2 provided to the mitochondria (Thomas *et al.,* 2016:507). Thirdly, strategies to maintain high CHO availability (Cermak & van Loon, 2013:1140; Murakami *et al.,* 2012:626) are used to ensure prolonged and/or sustained endurance performance, whereas the depletion of these stores result in exertion in the appearance of decreased work rates, compromised concentration and skill and elevated sensitivity of work effort (Thomas *et al.,* 2016:507).

Over the last century, research has presented that the most useful nutritional plan is one that can supplement and sustain CHO fuel stores. Exploring the early history of research done on the aforementioned, the study by Christensen and Hansen was among the first and probably one of the primary studies to investigate the relationship between CHO consumption and performance, as early as 1939, which demonstrated the critical role of CHO availability for endurance performance (Christensen & Hansen, 1939:160). Along with their results on CHO and endurance performance, it was also apparent that exhaustion escalated after low blood glucose levels were reached and for that reason, the probable link between endurance capacity and hypoglycaemia was also established in their paper. The results of this study led Bergström *et al.* (1967:140) and Hermansen *et al.* (1967:129) to further investigate this so-called link between low blood glucose levels and fatigue. Both their individual studies made use of the muscle biopsy procedure introduced by Bergström in 1962 that permits more accurate purpose of the utilisation of glycogen throughout training (Hermansen *et al.,* 1967:130). In an effort to reinvestigate the notion that CHO is the major fuel for vigorous muscular exercise, Hermansen *et al.* (1967) assessed the accurate amount of glycogen used by the working muscle, with the determined CHO use, grounded on the respiratory sufficiency and $\dot{V}O_2$. Ten well-trained athletes and ten recreational athletes had to cycle on an ergometer until exhaustion on a pedal frequency of 50 revolutions per minute (RPM) at a 70% $\dot{V}O_2$max workload. Subjects could rest for 15 minutes after each 20 minute interval. After the cycle trials, glycogen averaged 0.06 g/100 g wet muscle in the recreational athletes and 0.12 g/100 g wet muscle in the trained athletes. It was concluded that limited glycogen stores in the working muscles

reduce the ability for continuous vigorous work during high-level comparative workloads (Hermansen *et al.,* 1967:135). After measuring glycogen stores during various dietary and exercise interventions, Bergström *et al.* (1967) published a paper with the conclusion that the decrease in glycogen availability of the working muscle was a determining factor of the ability to complete ongoing intense exercise, after their results showed that the use of CHO during work periods was well associated with the glycogen content diminution. Firstly, results showed the glycogen content after a high-FAT-protein diet (1500 kilocalories protein and 1300 kilocaloreis fat) and a high-CHO diet (2300 kilocalories CHO and 500 kilocalories protein) to vary greatly with values ranging from 0.6 g/100 g of wet muscle to 4.7 g/100 g of wet muscle. And secondly, after each diet period, subjects had to cycle at 75% $\dot{V}O_2$max until exhaustion on an ergometer whereafter results showed a total reliance on CHO as fuel during the work periods with amounts ranging from 54-798 g/h, which showed that the results were well associated with the drop in glycogen content. In view with Hermansen, Bergström *et al.* also assumed that the glycogen present in the working muscle is a contributing factor for endurance capacity and that it can be significantly altered by different diets (Bergstrom *et al.,* 1967:148).

Another collaborative position statement by the ACSM and the American Dietetic Association Canada accentuated the importance of enough CHO as part of an athlete's diet (Thomas *et al.,* 2016:519). CHO proves to be an important fuel source for skeletal muscles both during and following exercise and therefore athletes in heavy training should ensure that their CHO intake is ample enough to enable restoring of liver and muscle glycogen between sessions, as well as to prevent a depletion of glycogen during continuous exercise which is related to exhaustion experienced, ultimately leading to impaired endurance performance (Brown, 2002:222; Hargreaves *et al.,* 2004:31; Jeffers *et al.,* 2015:1; Jensen *et al.,* 2015:1).

2.4.1.2 Recommended CHO intakes prior to endurance exercise

Specific nutritional recommendations for elite athletes are to consume at least between 5 – 12 g/kg body mass of CHO in the 24 to 36 hours prior to an event as it is considered essential for replacing glycogen stores in the liver and muscles and thus considered an optimal "CHO load" for competition (Beis *et al.,* 2011:2; Close *et al.,* 2016:6; Valenta & Dorofeeva, 2018:406). The above recommendation also varies in

literature with ranges from 7 – 10 g/kg per day for endurance athletes doing one to three hours of moderate to high intensity exercise, to 10 – 12 g/kg of body mass per day over the 36 to 48 hours window before an event for endurance athletes undertaking extreme exercise programmes or competitions (Burke, 2007:347; Brown, 2002:223). The International Society of Sports Nutrition proposes a relative lower CHO loading span from 8 – 10 g/kg of body mass ingested one to three days before an endurance event, while the International Olympic Committee (IOC) recommends 7 – 12 g/kg of body mass for 36 to 48 hours prior to an endurance event (Potgieter, 2013:10). Potgieter also concludes that the most recent evidence was used by the IOC when they formulated their recommendations as super compensation of glycogen stores which is to be reached by increasing CHO intake 24 to 36 hours prior to an event in well trained athletes, guarding they combine the strategy with adequate tapering and rest (Potgieter, 2013:10).

2.4.2 Fat metabolism

Intra- and extracellular fats offer the most prevalent chemical nutrient in the body to power biological function during rest and also during exercise reliant on duration and intensity, as well as the athlete's nutritional status (Burke, 2015:s34; Fink & Mikesky, 2015:118; Jeukendrup & Burke, 2011:397; McArdle *et al.,* 2010:161; Summerfield, 2012:87). During rest, most energy is derived from the oxidisation of fatty acids from adipose tissue triacylglycerol's and hormonal equilibrium between hormones that stimulate (epinephrine) and those that hamper (insulin and lactic acid) hormone-sensitive lipase (Coleman, 2010:2; Horowitz & Klein, 2000:558s; Jeukendrup & Burke, 2011:397).

A process called lipolysis that occurs during exercise causes triacylglycerols to be broken down and hydrolysed into components (1) non-esterified fatty acid (NEFA) or free fatty acids and (2) glycerol as they cannot be oxidised by the skeletal muscles directly (Jeukendrup & Burke, 2011:395). NEFA forms the major determinant of the fatty acid uptake rate by the exercising muscle (Moro *et al.,* 2014:584). This dissolving or breaking down of the triacylglycerols is stimulated through an enzyme, hormone sensitive lipase, which is driven by epinephrine, norepinephrine, glucagon, and growth hormone (Coyle, 1995:2; McArdle *et al.,* 2010:161). The glycerol released from lipolysis is water soluble and therefore freely circulated into the blood from where it

returns to the liver to be re-esterified back into triacylglycerol all because the amount of FFA delivered from the adipose tissue surpasses the quantity oxidised. In other words, the FFA rate of appearance into plasma stands roughly at double the proportion of oxidation rate of fatty acids and consequently needs to be re-esterified or changed to dihydroxyacetone and, from there, move into the gluconeogenic or glycolytic pathways (Horowitz & Klein, 2000:558S; Jeukendrup & Burke, 2011:397).

The FFA is not water soluble and therefore requires to first be bound to a protein carrier before it can be moved across the cells and into the blood stream (Coyle, 1995:2; Jeukendrup & Burke, 2011:396; Summerfield, 2012:205; Wildman & Miller, 2004:184). Serum albumin is the protein carrier which the free fatty acids is attached to in order to be transported to tissues for oxidation and production of ATP (Cook & Haub, 2007:226; Jeukendrup & Burke, 2011:396).

It is essential to mention that the blood flow rate across the adipose tissue and the level of albumin in the blood form the two most noteworthy factors that determine the transport of FFA in the blood (Wildman & Miller, 2004:184). This is because the binding similarity of albumin for FFA decreases as the molar ratio or FFA/albumin increases, which in turn allows for more FFA's to become detached from albumin in the muscle blood vessels, thus increasing the availability of FFA to the working muscles (Coyle, 1995:2; Wildman & Miller, 2004:184).

Fatty acids are derived from either inside (intracellular fat stores) or outside the muscle fibres (peripheral adipose tissue) and, therefore, as roughly 5 – 25% of total body weight is in the form of triacylglycerol in athletes, it means it can serve as a significant resource of fatty acids during exercise, depending on the degree of hormonal stimulation as well as the availability of albumin and the circulatory flow through adipose tissue (Rivera, 2017:14; Wildman & Miller, 2004:185).

When aerobic energy is needed during exercise, fatty acids, just like CHO, enter the Krebs cycle where these fatty acids are metabolised for energy, but before it can enter the Krebs cycle, a process called beta oxidation must come about where pairs of carbon atoms are detached from the fatty acid chain to permit fats to enter the Krebs cycle as acetyl-coenzyme A (acetyl-CoA). Hydrogen atoms are released during the carbon atom removal method (Purdom *et al.,* 2018:2) and conveyed to the electron transport system where energy is then generated from oxidative phosphorylation

(Jeukendrup & Burke, 2011:395; Summerfield, 2012:205). The interstitial area between the muscle cell and endothelium, the membranes of the vascular endothelial cells, and the muscle cell membrane are all processes that occur during the transportation of FFA from the blood to the working muscle and can potentially reduce FFA uptake by the working muscle. Fortunately, the long-chain fatty acid chain transport system eases the transport process across the sarcolemma into the muscle fibres via FFA binding proteins (FABP), FFA translocases (FAT), and FFA transport proteins (FATP) (Jeukendrup & Burke, 2011:397; Rivera, 2017:15).

Triaglycerols have more than a few advantages over CHO to provide energy to the working muscles of endurance athletes of which (1) energy concentration of lipids is higher, while the comparative mass as stored energy is lower, (2) it provides more ATP per molecule than glucose, and (3) the oxidation of these FFA are O_2 dependant and consequently ensure more FATox during endurance exercise (Jeukendrup & Burke, 2011:395; Wildman & Miller, 2004:184). When the body needs aerobic energy, fats are mobilised from adipocytes making more fatty acids available to the working muscles due to increased blood flow that occurs during exercise via the pumping action of skeletal muscle that is modulated by several neurological and metabolic factors (Fink *et al.,* 2012:118; Wildman & Miller, 2004:184).

The biochemical characteristics of the muscle fibres and their recruitment pattern is largely dictated by the metabolic responses to the type of exercise that is performed sporadically, especially when trained frequently, and forms a powerful stimulus for physiological muscle adaptation (Egan & Zierath, 2013:162; Maughan & Shirreffs, 2015:71).

Skeletal muscles can be defined as either Type I (“slow” twitch) or Type II (“fast” twitch) fibres, depending on the histochemical staining for myosin ATPase (Egan & Zierath, 2013:163; Jeukendrup & Burke, 2011:397; Wildman & Miller, 2004:189). During low-moderate intensity exercise/work, the predominant muscle fibre used is Type I, as only a few motor units of the muscles are activated for such type of activities (Jeukendrup & Burke, 2011:397; Maughan & Shirreffs, 2015:71; Wildman & Miller, 2004:189). Type I muscle fibres are red in appearance, which signifies the abundance of the oxygen carrying protein myoglobin that is strongly related to mitochondrial density (Egan & Zierath, 2013:162). Also, Type I muscle fibres have a higher oxidative ability, minimal glycolytic ability and a comparative worthy supply of O_2 and, along with Type IIa fibres,

have the ability to adapt more to endurance type activities (Maughan & Shirreffs, 2015:71). Now, the fatty acid binding proteins (FABP) mentioned previously are higher in Type I muscle fibres and also increase with endurance type activities, which suggests that the degree of oxidative metabolism and FFA binding capacity forms a well-functioning relationship, and once the FFA enters the cytoplasm of the muscle cell, it can either be re-esterified and deposited as IMTG or attached to FABP for moving to the spot of oxidation and activated to a fatty acyl-CoA (co-enzyme-A) by the enzyme acyl-CoA synthase and, through this entire process, provide energy via fat oxidation (Jeukendrup & Burke, 2011:397; Rivera, 2017:15).

2.4.2.1 Recommended fat intakes for endurance athletes

Acceptable fat intake is essential for many metabolic actions that encourage optimum health. Vitamins A, E and D needs fat for appropriate absorption. It is said that fat intake for athletes should vary between 20 – 35% of overall day-to-day energy (calories/kilojoules). Modern dietary recommendations are that 10% of fat intake must be from monounsaturated and polyunsaturated sources, with less than 10% from saturated fat (Schek *et al.,* 2019:182; Williamson, 2016:6; Thomas *et al.,* 2016:523).

2.5 Cardiorespiratory and non-invasive responses to endurance exercise

Physiological evaluations that are related to endurance performance include $\dot{V}O_2$max, heart rate deflection (HRD), substrate utilisation, ventilatory thresholds (VT), LT and maximum lactate steady state (MLSS), which all predict endurance capacity to some extent, although its reproducibility, accuracy and affordability varies (Pallarés *et al.,* 2016:2). Blood lactate as well as respiratory exchange data or gas exchange data sampled during a GXT are often used to enumerate certain submaximal, physiological “inflection” points or “thresholds” (Bentley *et al.,* 2007:577; Jamnick *et al.,* 2018:1; Truong *et al.,* 2018:305). Gas exchange data tests form a typical use in laboratories by means of indirect calorimetry during a GXT to efficiently design a successful training programme through the individual aerobic-anaerobic transition workloads or individual metabolic responses measured during the test (Cerezuela-Espejo *et al.,* 2018:2; Pallarés *et al.,* 2016:2). Of these indices measured are the Anaerobic Threshold (AT) or Ventilatory Threshold 1 (VT_1), respiratory compensation point (RCP) or Ventilatory Threshold 2 (VT_2), LT, RER, substrate utilisation, $\dot{V}O_2$peak and $\dot{V}O_2$max, which all

serve a role in the athletic community when it comes to the prescription of individualised exercise programmes to optimise training stimuli, distinguishing cardiorespiratory fitness and the differentiation of exercise areas (Jamnick *et al.,* 2018:2).

Concerns regarding the use of a standardised GXT have come to light in the literature with apprehensions such as the duration being unknown to the athlete, as well as the athlete not having the ability to indicate or alter the progressive fixed intensity set out by the test which lead to the effort in such protocols being vastly different from the athlete's outside sport specific conditions (Truong *et al.,* 2018:305). When an athlete is able to apply a pacing strategy, it allows the brain to recruit the suitable quantity of muscle motor units to be able to perform the activity without homeostatic let-down and the onset of muscle fatigue, and for this reason, the use of the GXT has also been discouraged as the athletes are unable to replicate the exact sport specific conditions (Truong *et al.,* 2018:305; Noakes, 2008:554).

Nevertheless, GXT appears to be the more relevant test to use for a comprehensive gas analysis in endurance or trained runners. Peserico *et al*. (2015:732) found in their study that a GXT with one km/h increments and with a three-minute stage duration should be applied as a norm to determine V_{peak} to predict endurance performance and to assess aerobic fitness, even though the study was done on recreational runners. Subsequently, Machado *et al*. (2013:577) also showed a GXT comprising of three-minute test intervals to be a superior testing protocol when accurately trying to predict a 5 km and 10 km running performance.

2.5.1 Oxygen consumption and carbon dioxide production

During a GXT test, $\dot{V}O_2$ and carbon dioxide output ($\dot{V}CO_2$) can be used to assess changes in FATox and CHOox as an outcome of endurance training, and from the collection of data from these two variables, it can be estimated how much energy is provided from CHOox versus FATox (Bitel, 2017:10). It is assumed that metabolic processes such as gluconeogenesis from protein and ketone body development is quantitatively negligible when compared to FATox and glucose during the production and use of $\dot{V}O_2$ and $\dot{V}CO_2$ and therefore a better or more stable $\dot{V}O_2$ and lower VCO_2 can be associated with an increased influence from FATox, whereas a reduction in

CHO derived substrate utilisation can be observed in trained and untrained subjects during low to moderate intensity exercise (<65% $\dot{V}O_2$max) (Bitel, 2017:10).

2.5.2 Respiratory exchange ratio

The Respiratory Exchange Ratio (RER) is defined as the swap over of O_2 and carbon dioxide (CO_2) measured at the lungs which indicates the gas exchange from macronutrient catabolism in the cells and can be measured during a $\dot{V}O_2$max test (McArdle *et al.,* 2010:190). Knowing the type of food substrate that is being oxidised during specific times of a $\dot{V}O_2$max test can help determine the body's energy usage. The volume of O_2 used during metabolism differs on the fuel substrate being oxidised as the quantity of carbon (C) and $\dot{O}_2$ differs with glucose, free fatty acids and protein. The amount of C in CHO or fat molecules is relative to the amount of O_2 needed to completely oxidise that fuel and therefore the RER value will vary completely dependent on the fuel substrate used (Kenney *et al.,* 2015:123). RER values of 0.78 – 0.80 are usually seen at rest with an increase seen as exercise intensity increases. At exercise intensities which start to reflect anaerobic metabolism, the muscles start to rely on CHO as fuel source which causes the RER level to approach near 1.00 (Kenney *et al.,* 2015:124; Storm, 2017:13). However, apart from energy or fuel from food, there are other factors also playing a role in the increase of CO_2 that also influence the RER value. During an all-out maximal $\dot{V}O_2$max test, it is likely that hyperventilation sets in which means the CO_2 removal increases as respiration increases to disproportionately greater levels when compared to the metabolic demands of the body. With hyperventilation, the normal level of CO_2 decreases without a matching increase in $\dot{V}O_2$ which leads to an increase in the RER value of more than 1.00, but does not precisely reflect macronutrient oxidation (McArdle *et al.,* 2010:190). Once hyperventilation sets in at near exhaustion levels, lactate begins to build up in the blood, and as is known physiologically, once lactate starts to form, the body naturally starts to buffer and reverse the acidification through releasing more CO_2. This excess release of CO_2 in the blood is then diffused into the lungs for exhalation which naturally increases the release of CO_2, hence RER cannot accurately estimate the type of macronutrients used by the muscles for fuel (Kenney *et al.,* 2015:124; McArdle, *et al.,* 2010:190). Another possible complication that can lead to the misinterpretation of RER values is when glucose production from the catabolism of fats and amino acids

in the liver produces a RER of less than 0.70, which results in CHO oxidation calculated to be underestimated with regard to energy usage. However, despite the above mentioned cobblestones, RER nonetheless offers the best estimation of energy expenditure at rest and during exercise (Kenney *et al.,* 2015:125; McArdle *et al.,* 2010:190; Storm, 2017:13).

2.5.3 Minute ventilation

Minute ventilation (V_E) can be described as the volume of inhaled or exhaled gas from one's lungs in one minute and is dependent on either the increased rate of breathing or depth of breathing or both (McArdle *et al.,* 2010:263; Storm, 2017:14). An endurance athlete can breathe as rapidly as 60 to 70 times per minute and ventilation can increase to 160 L/min during maximal exercise during strenuous exercise. The rapid breathing during near exhaustive exercise is most commonly due to a compensatory effect of metabolic acidosis as the progressive increase in CO_2 production shows the body's attempt to counteract the effect of acidification (Storm, 2017:13; Valli *et al.,* 2013:1). Until about 60% of $\dot{V}O_2$max, V_E and HR increases linearly, but with continuous increasing exercise intensity the V_E starts to increase at a higher rate than HR (Storm, 2017:13).

2.5.4 Heart rate

Compared to other invasive and non-invasive methods available to measure SV and Q, HR undeniable forms a very easy cardiovascular measurement and has been used for centuries to evaluate an athlete or non-athlete's response to exercise, recovery from exercise, as well as when it comes to the prescription of exercise programmes (Bhat & Shaw, 2017:1037).

A reflex or anticipatory stimulation of sympathetic nervous system just before exercise commences or just at the beginning of exercise, causes an increase in HR, also known as the cardiovascular drift, followed by a relatively linear increase in the HR as the exercise intensity rises (Haff & Triplett, 2016:116; Rivera, 2017:28). As maximal exercise intensity approaches, a starting plateau can be seen in HR which is also an indication that HR is nearing maximal value, meaning the highest value that can be achieved during a maximum effort to volitional fatigue (Kenney *et al.,* 2015:196). The reason behind HR being the most commonly used technique when recommending

aerobic training intensity is the proximity between HR and $\dot{V}O_2$ and can be seen particularly at intensities between 50% and 90% of $\dot{V}O_2$max (Haff & Triplett, 2016:563). Although HR and $\dot{V}O_2$max form a close relationship, Swain and Leutholtz propose that one should pay close attention when prescribing an exercise programme with the assumption that the percentage heart rate reserve (%HRR) (percentage of the difference between maximal and resting heart rate) falls at the same intensity than %$\dot{V}O_2$max which is usually used to prescribe exercise training intensities. In their study, they investigated the correlation between %HRR and %$\dot{V}O_2$max and found it rather better to use the association between %HRR and %$\dot{V}O_2$max reserve (%VO_2maxR) (percentage of the difference between resting $\dot{V}O_2$max and maximal $\dot{V}O_2$max) when prescribing exercise programme intensities. It was concluded that Karvonen, failed to test $\dot{V}O_2$ in his experiment where the equality between HRR and $\dot{V}O_2$max started, and therefore such direct relationships cannot be established in literature (Swain & Leutholtz, 1997:410).

Heart rate measured during training sessions can be used to extrapolate race pace, which is of vital importance for endurance performance to ensure that athletes do not “hit the wall” during the later stages of an important race (Rivera, 2017:26). The relationship between HR and $\dot{V}O_2$max and LT is quite often used to determine an athlete’s level of fitness, and is usually done in a meticulous environment where temperature and humidity are controlled. Also, the athletes will try to perform the test under the best conditions possible leading up to the test (enough sleep, nutritional status, fluids) (Achten & Jeukendrup, 2003a:529). However, this does not mean that there are no other aspects that also play a part in HR during testing or performance. Factors that can also influence HR include but are not limited to (1) age, (2) gender, (3) environmental factors, (4) hydration status, and (5) exercise intensity (Haff & Triplett, 2016:117; Rivera, 2017:28). Of these, the exercise intensity, hydration status and environmental factors all affect the HR in a similar manner, with the linking factor – Core Temperature. Once exercise has commenced, core temperature starts to increase as the exercise intensity escalates, which also leads to the HR increasing due to a rise in O_2 needed by the working muscles (Rivera, 2017:28).

An athlete’s hydration status can also have a vital influence on endurance performance as blood volume decreases while exercising because of the loss of sweat. Exercising in a dehydrated state due to loss of sweat or not enough fluids

before and during the race causes plasma volume and stroke volume to increase, which in turn can cause an increase in HR by up to 7.5% which can have detrimental implications for endurance athletes training or racing in specific heart rate zones whilst also making exercise intensity monitoring more unreliable if making use of HR (Achten & Jeukendrup, 2003a:531; Rivera, 2017:29). Achten and Jeukendrup summarise the factors influencing HR in their article in three different categories, namely (1) day-to-day variability, (2) physiological factors which include hydration status and cardiovascular drift, and (3) environmental factors which includes altitude and temperature (Achten & Jeukendrup, 2003a:531).

While the test-retest reliability of HR has been confirmed as high (r = 0.87), it must be mentioned that there can still be a small day-to-day difference that occurs even with controlled environments. A change of two to four beats per minute is normal variation for any individual on a day-to-day basis (Achten & Jeukendrup, 2003a:531; Rivera, 2017:28).

Literature regarding the effect which nutritional manipulation has on HR during exercise testing also clearly shows significant interactions between the diet versus HR for low-CHO diets, suggesting a higher metabolic cost and perceived effort which has a strong influence on performance outcomes for endurance athletes (Burke *et al.,* 2017:2795; Burke, 2015:s45; Burke & Hawley, 2002:1496; Rosenkranz *et al.,* 2007:306).

2.5.5 Rate of perceived exertion

Rating of perceived exertion (RPE) is defined as the individual intensity of strain, exertion, fatigue or discomfort experienced by athletes during exercise and has been proposed to be a useful tool in prescribing exercise intensity in athletes (Goss *et al.,* 2011:539; Kenney *et al.,* 2015:520). The BORG RPE scale is a 20-point intermediate interval tool where participants have to verbally give descriptions of total body effort from RPE 6 (no exertion at all) to RPE 20 (maximal exertion). The rationale underpinning the range of RPE from RPE 6 (‘no exertion at all’, i.e. corresponding to rest) to RPE 20 (‘maximal exertion’, i.e. the rating resembling the theoretic maximal possible) is found on the HR reaction to exercise (Kenney *et al.,* 2015:520). RPE is confirmed against heart rate (r = 0.80-0.90). The relationship between physiological measures of exercise intensity and RPE was found to be high with the strength of the

relationship during production mode with HR, r = 0.62; CBL, r = 0.66; $\dot{V}O_2max$, r = 0.85 (Ritchie, 2012:62). Kenney *et al.* also tabulated three different methods to compare exercise intensity based on 20 to 60 minutes of endurance exercise and suggested it very beneficial to use RPE with target heart rate (THR) and HRR during non-invasive exercise intensity tests as all showed very close relation in comparing exercise intensity (Kenney *et al.,* 2015:522).

2.5.6 Submaximal lactate and ventilatory markers

Amann, along with his fellow researchers, proposed that ventilation is more sensitive to muscle hydrogen ion build-up than blood lactate measures and also respond in a different way as it is under the effect of central and peripheral chemoreflex (Amann *et al.,* 2006:27). However, the use of ventilatory threshold (indirect calorimetry) through a GXT requires expensive equipment and is usually not easily resourced for trainers and coaches, therefore the recommendation to use blood lactate concentration changes during a GXT for performance assessment and training prescription (Pallarés *et al.,* 2016:2). Whether or not these two variables (VT and LT) are related to each other or form a strong physiological linkage is still debated as some researchers believe in this correlation while others see it as co-incidental (Pallarés *et al.,* 2016:2; Amann *et al.,* 2006:27; Cerezuela-Espejo *et al.,* 2018:6).

Reviewing the literature regarding these two concepts, (1) Lactate Threshold (LT) and (2) Ventilatory Threshold, reap outcomes that are fairly thought-provoking and somewhat challenging to apply, hence, clearly more research regarding the relation is necessary.

Firstly, looking at the LT concept, one will find through the entire research network that of the intensity of exercise, muscle fibre type, duration of exercise, training status and primary glycogen levels which all can influence lactate build-up, exercise intensity is the primary contributor of blood lactate accumulation (Dunford & Doyle, 2015:79; Haff & Triplett, 2016:48). One of the biggest obstacles around understanding the lactate concept or the cause of much confusion over the years have been the numerous terms surrounding LT (Garcia-Tabar & Gorostiaga, 2018:1034; Hall *et al.,* 2016:s12; Stanula *et al.,* 2013:12). As exercise intensity increases, the amount of lactate concentration in the blood is at first very little, but as the exercise intensity continues to surge to a point that the amount of lactate in the blood increases exponentially, or if there is a

rapid rise in capillary blood lactate (CBL) above the baseline concentration level, this point has been named the LT (Dunford & Doyle, 2015:79; Haff & Triplett, 2016:50).

Over the years, the evaluation of blood lactate concentration to identify the LT through means of a short stage GXT has become the more popular means of measuring and establishing workloads and/or setting individual intensities for training programmes for endurance optimisation. Progress with numerous methods suggested including a rise in CBL of more than 0.5, 1.0 or 1.5 mmol/L from baseline, the onset of blood lactate accumulation (OBLA) ranging from 2.0 – 4.0 mmol/L, curve fitting measures used such as the Dmax method or the current maximal lactate steady state (MLSS) (Faude *et al.,* 2009:469; Fernandes *et al.,* 2016:194; Jamnick *et al.,* 2018:1).

Low intensity exercise usually causes blood lactate concentration to remain at resting value (1 mmol/L) with increases seen above the baseline as exercise intensity progresses, defined as the LT (Shaw, 2016:8). Moreover, LT can be articulated as a percentage of maximal oxygen consumption (%$\dot{V}O_2$max), usually between 50 – 60% for the general population and more than 75 – 85% for distance runners, and also important to know is that it reflects the exercise intensity or relative intensity where blood lactate concentration starts to steadily increase during incessant exercise, thus representing stability in lactate production and lactate removal (Hoff *et al.,* 2016:1373; Støren *et al.,* 2014:622). This point can also be associated with the exercise intensity that allows for longer periods of endurance exercise if sustained; it forms high correlation between race pace of a distance runner, with a pace too much faster resulting in fatigue and poor results or a pace too much slower resulting in less than optimum performance (Dunford & Doyle, 2015:79; Pennington & Kinesiology, 2015:228). In other words, LT can also be determined by the portion of maximum aerobic power sustained over an extensive period (Hoff *et al.,* 2016:1373).

Since the lactate threshold is so closely linked to aerobic endurance performance and actually portrays a physiological marker, it is also important for trainers and athletes themselves to know that with endurance training, the lactate threshold can be increased which means that one will be able to work at a higher work rate (for the same level or rate of work done previously, the lactate concentration now will be lower). It also means that running velocity as well as the benefit of blood lactate levels will remain stable at resting level while working at a higher rate of absolute oxygen

consumption (Shaw, 2016:8; Wilmore & Costill, 2004:288). A higher lactate threshold means improved aerobic or endurance performance (Wilmore & Costill, 2004:198).

Some literature signifies this as the Anaerobic Threshold (AT), because it represents the point where a significant reliance starts to creep in on the anaerobic processes for energy production to achieve energy demand. It also signifies the point of exercise intensity where the aerobic metabolism is insufficient to generate energy and reliance on the anaerobic glycolysis becomes active, which, in turn, leads to the formation of lactate. However, the overall lactate concentration in the blood does not increase since it is oxidised as fast as it is being produced by highly aerobic tissue and, therefore, the term AT is substituted with the term LT or, according to some researchers, the Ventilation threshold 1 (VT_1) [breaking point in the relationship between ventilation and $\dot{V}O_2$] (Pallarés *et al.,* 2016:2; Haff & Triplett, 2016:50; Dunford & Doyle, 2015:79; Støren *et al.,* 2014:622).

Ventilatory threshold 2 (VT_2) is the second inflection point that can be seen during a GXT test and is usually measured through respiratory exchange data or gas exchange data using indirect calorimetry (Bentley *et al.,* 2007:577; Cerezuela-Espejo *et al.,* 2018:2; Pallarés *et al.,* 2016:2). Two methods have been identified which can be used to determine the VT_1, namely (1) nonlinear increase in $\dot{V}E$ or (2) a systematic increase in $\dot{V}E/\dot{V}O_2$ without a simultaneous increase in $\dot{V}E/\dot{V}CO_2$ (Pennington & Kinesiology, 2015:22). Researchers also identified two VT points, VT_1 and VT_2 or Respiratory Compensation Point (RCP), to more easily and effectively establish and set individual exercise intensities in endurance sports because of the individual metabolic responses obtained during the test (Bentley et al., 2007:577; Cerezuela-Espejo et al., 2018:2). Wasserman and McIlroy were the first to suggest the term AT or VT_1 as they thought the sudden non-linear increase in CO_2 production seen during a GXT reflects a shift toward more anaerobic metabolism and generally occur at the same point as LT (Wasserman & McIlroy, 1964:844). VT_1 is identified at the point where $\dot{V}O_2$ and $\dot{V}$ CO_2 production increase equivalently, while HCO_2 proceeds to buffer lactic acid concentration in the blood as VT_2 or the RCP marks the point of hyperventilation and a loss in linearity can be seen between VE and VCO_2 (Cerezuela-Espejo *et al.,* 2018:2; Wasserman & McIlroy, 1964:844). VT_1 also sets the tone for low intensity exercise while VT_2 or RCP groups the critical limit for high intensity interval training (Cerezuela-Espejo *et al.,* 2018:2). Although the debate is still going on, it is seen through the

literature that researchers still use the terms AT and VT_1 interchangeably with LT and, according to some, Performance Threshold (PT), despite objection to the use of the term (Amann *et al.,* 2006:27; Jakobsson & Malm, 2019:57; Kenney *et al.,* 2015:199; Pennington & Kinesiology, 2015:227). Pennington and his colleague's biggest motive for not using the term AT when it comes to LT testing was that individuals that are incapable of increasing lactate levels due to genetic enzyme deficiency or an athlete's glycogen stores prior to exercise will change the relationship between AT and LT and one will always be able to see a clear breakpoint in ventilation of these individuals (Pennington & Kinesiology, 2015:227).

2.6 Reaching consensus on cardiorespiratory and non-invasive responses during GXT

Both FATox and CHOox are known to meet the energy demands necessary for exercise over a broad range of exercise intensities and durations while endurance training shifts energy dependence more towards FATox when exercise is performed at a continuous submaximal level and decreases again when exercise intensity falls between the 60 – 75% $\dot{V}O_2$max category and practically becomes insignificant when exercise intensity exceeds 85% $\dot{V}O_2$max (Hetelid *et al.,* 2015:1). Also reported is that periods of endurance exercise training lead to a decrease in RER, a reduction in muscle glycogenolysis and, again, greater FFA oxidation rates when compared with levels of the above before an endurance training programme (Cox & Clarke, 2014:2; Hawley & Leckey, 2015:s5).

One of the foremost goals of endurance training is to induce a number of adaptations in the skeletal muscles, including a preferment of metabolic and physiological adaptations that function to improve consequent exercise capacity (Bartlett *et al.,* 2015:5; Cox & Clarke, 2014:2; Hawley & Leckey, 2015:s5). Of these adaptations, the most important is to increase the mitochondrial mass which is the factor that leads to the ability to train at higher intensities for longer periods (Bartlett *et al.,* 2015:5; Hawley & Leckey, 2015:s5; O'Brien *et al.,* 1993:1009). In other words, it is important to know that the physiological and metabolic adaptations form part of the main purpose of endurance training as it adds the athlete's capability to tolerate the highest mean power output or velocity of movement for a certain distance or for a certain length of

time, while also reducing the $\dot{V}O_2$ of locomotion and helps to preserve a better fractional use of $\dot{V}O_2$max during competition and training (Leckey *et al.,* 2016:107).

Another goal of endurance training is to maximise power or velocity at LT since changes in acid-base equilibrium occur after the exercise intensity surpasses the athlete's MLSS, signifying that during a rise in glycolytic flux, lactate accumulation in the working muscles move to the extracellular fluid and increase Hydrogen (H^+) which is protected by bicarbonate (HCO^3), causing additional non oxidative CO_2 to be eliminated through hyperpnoea while raising the $\dot{V}CO_2$. Therefore, with an increase in LT with endurance training, $\dot{V}CO_2$ will not be elevated to an extreme, which means FATox is utilised as energy substrate (Hetelid *et al.,* 2015:2; Baar, 2014:s5).

The increase in mitochondrial mass that accompanies exercise training correspondingly insures a smaller decrease in ATP, muscle glycogen utilisation and phosphocreatine and less significant increases in ADP and adenosine monophosphate (AMP), inorganic phosphate and muscle lactate (Bartlett *et al.,* 2015:5). O'Brien *et al.* explain the aforementioned in that although this metabolic adaptation lead to bigger capabilities to oxidise all fuel substrates, it causes greater capability of the muscles to oxidise lipids due to the increase in mitochondrial adaptations that lead to CHO sparing which, in turn, helps the working muscle to tolerate continued exercise (O'Brien *et al.,* 1993:1009). This stability of substrate oxidation during any given time during training is a purpose of training-induced alterations which encourages CHOox and adaptation to endurance training which, in turn, encourages FATox, and therefore CHO based fuels become the major energy source for trained muscles when intensities are at approximately 60% of $\dot{V}O_2$ peak (Hawley & Leckey, 2015:s5).

Both CHO and fat are central substrates for energy production in the body at both rest and exercise (Spriet, 2014:s87). Cox and Clarke state in their review, "Acute nutritional ketosis", that the high performance or highly trained athlete makes ideal candidates to study fuel metabolism in endurance sports activities because it involves large muscular recruitment and high degrees of aerobic fitness that induces muscular and mitochondrial adaptations (Cox & Clarke, 2014:1). Also, aerobic type training (ATT) causes the body to be able to sustain the functional and metabolic demands that are placed on the body as a result of the exercise via a shift to rather use Type I muscle fibres while reducing the reliance on Type IIb muscle fibres that primarily rely upon

glycolytic metabolism. ATT also increases the fibre cross sectional area, causing an enhancement in oxidative capacity as well as promoting capillary angiogenesis (Dubé *et al.,* 2016:473). The need for energy and the metabolic rate starts to increase exponentially at the beginning of exercise and, therefore, both FATox and CHOox must be activated simultaneously as both these macronutrients are central foundations of energy that sustain oxidative metabolism (Croci *et al.,* 2014:1).

Although both these substrate oxidation rates increase, the interaction between FATox and CHOox is reliant on the extracellular and intracellular metabolic settings inside and outside of the muscle along with the exercise duration and intensity (Spriet, 2014:s87). Mitochondrial oxidative volume and increases in the preference for rather fat as fuel at certain given intensities are increased during high levels of aerobic fitness during endurance training (Cox & Clarke, 2014:2; Fink & Mikesky, 2015:118; Jeukendrup & Burke, 2011:397; Horowitz & Klein, 2000:558S; McArdle *et al.,* 2010:161).

Mild or moderate intensity exercise is performed at 25 – 65% of an athlete's $\dot{V}O_2$max and can also be referred to as aerobic exercise and consequently needs aerobic energy supply from the different energy sources in the body (Fink & Mikesky, 2015:118; Horowitz & Klein, 2000:558S; Jeukendrup & Burke, 2011:399). Aerobic type exercises that fall in the 25 – 65% $\dot{V}O_2$max category (for example recreational marathon running, cycling and swimming), cause a several-fold (5 – 10 times) increase in metabolism compared to resting conditions (Horowitz & Klein, 2000:558S; Jeukendrup & Burke, 2011:397).

During very low-intensity exercise (25% $\dot{V}O_2$max), most energy is produced from plasma fatty acid oxidation with only a slight amount of energy from plasma glucose, which means that even if this type of exercise is continued for one or more hours, substrate utilisation pattern will stay the same so that energy necessities can be met solely from FATox from the large adipose triacylglycerol's and because lipolysis is not stalled by blood-flow to the active muscle (Jeukendrup & Burke, 2011:400; Maughan & Shirreffs, 2015:72; McArdle *et al.,* 2010:161). In other words, whole body CHOox rate increases with the exercise work output while total body FATox rate increases from low to moderate intensity exercise and noticeably declines when exercise intensity exceeds 75% $\dot{V}O_2$max.

Although numerous studies dating back to 1939 (Christensen & Hansen, 1939:171), including Bergström *et al.* (1967:141), showed that a prescribed high-CHO diet does indeed increase endurance performance and has a confident connection between pre-exercise muscle glycogen strengths and consequent submaximal exercise, Volek *et al.* (2016:107) highlighted that none of these studies used a placebo test and can therefore not be stated as the top and only strategy. Endurance training causes muscle adaptations that start to utilise CHO based fuels at a slower rate and rely more on fat-based fuel during submaximal exercise (60 – 75% $\dot{V}O_2$max), and therefore many coaches and athletes believe that a fat adaptation diet does indeed play an enormous role in optimising endurance performance in order to "spare" CHO-based fuels and oxidise fats for energy at a greater rate which will improve endurance capacity (Hawley & Leckey, 2015:s5). However, when endurance exercise intensity starts to exceed the 75% $\dot{V}O_2$max intensity, greater emphasis is placed on CHO oxidation again as it puts the exercise intensity in the high-intensity range which relies on the use of glucose as energy substrate (Hetelid *et al.,* 2015:1).

The observation that both intra- and extracellular CHO and fat are used to fuel skeletal muscles during exercise with merely a minimal contribution by amino acids was made over a century ago (in 1901) when Zuntz and Schumburg controlled the amounts of CHO and fat in the diet for some days, which resulted in changes in the RER during successive submaximal exercise of which the results promoted the possible benefit of CHO as a fuel substrate for energy metabolism by means of 8% higher energy that is yielded per litre of O_2 consumed when CHO was the primary fuel oxidised (Zuntz & Schumburg cited in Burke *et al.,* 2017:2786). In 1920, these findings were confirmed by yet another group of researchers, Krogh and Linhard, who also reported a 5.5% greater energy yield with CHO dependence rather than fat dependence in endurance cyclists (Krogh & Linhard cited in Burke *et al.,* 2017:2786).

Since endurance training causes muscle adaptations that start to utilise CHO based fuels at a slower rate and rely better on fat-based fuel during submaximal exercise (60-75% $\dot{V}O_2$max), many coaches and athletes believe that a fat adaptation diet does indeed play an enormous role in optimising endurance performance in order to "spare" CHO-based fuels and oxidise fats for energy at a greater rate which will improve endurance capacity (Hawley & Leckey, 2015:s5). However, when endurance exercise intensity starts to exceed the 75% $\dot{V}O_2$max intensity, greater emphasis is placed on

CHO oxidation again as it puts the exercise intensity in the high-intensity range which relies on the use of glucose as energy substrate (Hetelid *et al.,* 2015:1).

2.7 Fat loading or CHO loading for endurance performance?

The so-called “classic period” of exercise biochemistry extended from the 1960s until mid-1980s where all our insights on exercise promotion through, for example, biosynthesis of the mitochondrial skeletal muscle, the impact of diets on performance and the fate of lactate and recovery was recognised (Hawley *et al.,* 2015:12; Bergstrom *et al.,* 1967:140). This notion arose after the influential work of Krogh and Lindhard in the 1920s who also discovered the negative influence that a high-FAT diet had on fatigue during endurance events in the days before endurance exercise (Krogh & Lindhard, 1920:290). It is well-established that a diet high in CHO promotes prolonged exercise performance, as well as shorter recovery time when competition and/or exercise sessions are scheduled close together, through maximising liver and muscle glycogen stores while simultaneously delaying the beginning of muscle fatigue (Bartlett *et al.,* 2015:3; Beck *et al.,* 2015:260; Brown, 2002:223; Colombani *et al.,* 2013:16; Hammond, 2019:24).

Early history of studies done on the outcome of a high-CHO diet on running endurance performance goes back to date from the late 1960s (Bergstrom, *et al.,* 1967:140; Brewer, 2014:14; Brewer *et al.,* 1988:698; Williams *et al.,* 1992:18). Along with the above-mentioned investigations, Brewer referred to another two studies on this topic. The first was by Karlssson and Saltin in 1971 (cited in Brewer, 2014:14) which concluded that high-CHO diets will possibly improve endurance performance even if their data exposed some errors in the report due to the subjects being either recreational or elite runners who conjoined their high-CHO diet with a planned race. The elite group consumed their normal daily diets preceding a time-trial exclusively planned for the study and resulted in their time-trial times dropping. Consequently, it is difficult to conclude if the affecting factor was the elite group’s diet or rather the competitive 30 km race. The second study cited was by Goforth *et al.* in 1980 (cited in Brewer, 2014:14) which discovered a 9% enhancement in time to fatigue when running at 80% $\dot{V}O_2$max. Diets involved a high-CHO diet with the consumption of approximately 550 grams of CHO per day for two days, followed by the consumption of 350 grams of CHO per day for the remaining three days.

Carbohydrates for muscle metabolism and central nervous system function play a vital role in exercise performed for long periods at submaximal level and form a critical part of elite performance, therefore, the importance of sufficient CHO to be consumed by endurance athletes that conduct training at lactate-threshold zones (Burke *et al.,* 2011:s17; Burke, 2010:48).

Given the already discussed notion that once endurance exercise intensity starts to exceed the 75% $\dot{V}O_2$max intensity, greater emphasis is placed on CHOox as it puts the exercise intensity in the high-intensity range which relies on the use of glucose as energy substrate (Hetelid *et al.,* 2015:1). One can therefore assume that muscle glycogen accessibility has been recognised as the major determining factor of optimal endurance capacity (Hammond, 2019:22). All the above mentioned studies have also promoted a high-CHO diet, whether it is for the maintenance of blood glucose concentration in the advanced exercise stages or to restore liver glycogen content which has also shown to increase muscle glycogen concentrations (Hammond, 2019:22; Hetelid *et al.,* 2015:1).

Definite guidelines for CHO loading leading up to an endurance event turn out to be more complex than just providing the generally studied guidelines to ingestion, because athletes are different in body size and the training intensity and volume of each athlete varies, therefor it is necessary to incorporate flexibility and individual differences when it comes to CHO intake guidelines (Bartlett *et al.,* 2015:4; Thomas *et al.,* 2016:510).

However, Noakes *et al*. (2014:1077) questioned the CHO-loading phenomenon by a simple question: "Is it really logical?" The main focus in their tutorial was to overcome the universally recommended CHO contribution of at least 40 – 60% of daily energy. In their opinion, our bodies function in such a way that body fat stores can provide most, if not all the energy necessary to fuel submaximal exercise activities. Therefore, they assessed the current state of knowledge on high-FAT diets (Noakes *et al.,* 2014:1077) and referred to Phinney *et al.* (1983:775), who recommended that humans can indeed adapt to a CHO-free diet without any impairment in submaximal exercise performance. Noakes *et al*. (2014:1078) made it clear that evidence-based research has shortcomings when it comes to different dietary prescriptions for athletes to promote performance. Therefore, the need to investigate high-CHO and low-CHO diets for athletes is a field of study that requires more focus.

The effects of CHO and fat during training and recovery with the criticism on high or moderate CHO intake for optimal training can be attributed to the let-down of longitudinal studies to indicate strong evidence and reliable benefits for higher CHO intakes to training alterations and performance, compared to moderate CHO intakes (Burke *et al.,* 2004:15). Studies reviewed by Burke *et al*. (2004:15) with the argument above reported an increasing interest in the findings that a higher CHO intake reduces the overreaching syndrome, but that the true benefits from higher versus lower CHO intakes have not been a common result of research studies.

Previous studies on the influence of high-FAT/low-CHO diets on training alterations and performance (Bergstrom *et al.,* 1967:140; Pitsiladis & Maughan, 1999:919; Starling *et al.,* 1997:1185) found that the effect of a high-FAT/low-CHO diet consumed for one to three days while enduring to exercise, lowered resting liver and muscle glycogen stores that resulted in decreased exercise ability and endurance. However, a high-FAT/low-CHO diet for a period longer than seven days caused metabolic adaptations that considerably enhanced FATox during exercise, resulting in enhanced endurance and ultra-endurance performance in athletes (Burke *et al.,* 2004:24).

In a review article, Brown (2002:225) concluded that CHO supplementation prior to exercise results in higher rates of CHOox by maintaining insulin concentrations and plasma glucose levels, but that a transient blood glucose level decrease is then often seen. This decrease in blood glucose levels can be credited to a rise in plasma glucose uptake that results from higher muscle contractions and plasma insulin levels, and furthermore causes suppression in hepatic glucose production. Important to note is that individuals who are sensitive to blood glucose reductions may experience hypoglycaemia and are therefore advised to experiment with a high-CHO diet beforehand, while individuals not sensitive to blood glucose reductions often do not feel any functionally significant difference in performance (Brown, 2002:225). With regard to the effect on plasma substrate concentration and physical performance after ingestion of a high-FAT energy bar compared with results of ingesting a high-CHO energy bar, however, Brown (2002:226) found no significant difference in performance and that the higher concentration of free fatty acids might possibly lead to increased time to muscle fatigue by sparing muscle glycogen stores.

Longitudinal studies by various researchers (Phinney *et al.,* 1983:769; Lambert *et al.,* 1994:288; Goedecke *et al.,* 2013:5195; Rowlands & Hopkins, 2002:679) all had a fat

adaptation protocol lasting between 7 – 28 days, and a performance protocol of cycling at 60 – 80% of $\dot{V}O_2$max with meals ingested at different times before the start of the individual cycling protocol. However, these high-FAT/low-CHO (HFLC) studies all found different results and outcomes in time to exhaustion and performance on a cycling or running time-trial.

Summarised in Table 2.2 are studies on the muscle adaptation and exercise performance reactions to short-term exposure to high-FAT and/or high-CHO protocols of which short-term was expressed as adjustment periods of less than five days (<5 days) and all had a fat content contribution of more than 40% of the total energy provided through the diet.

Despite the above literature propositions, together with the belief that trained athletes train at exercise intensities that are CHO dependent, Hawley and Leckey (2015:s7) still maintain that it is supported by inadequate laboratory-based trials of substrate utilisation collected from elite runners and more studies regarding this phenomenon are needed.

Table 2.2: Exercise performance- and muscle adaptive responses to short-term high-FAT, low-CHO protocols

SUBJECT CHARACTERISTICS	FAT ADAPTATION PROTOCOL	PERFORMANCE PROTOCOL	PERFORMANCE OUTCOME & PERFORMANCE REFERENCE MEASURE (MIN)	REFERENCE
Crossover design 7 ET M cyclists	1 day high-fat (68%, 16% CHO, 1.9 g/kg body mass) or Control (93% CHO, 9.6 g/kg body mass)	1600 kJ TT Cycling in fasted state; no CHO ingestion during exercise	16% reduction in TT performance on Fat trial: 139.3 vs. 117.1 min for high-Fat and Control ($P < 0.05$). Fat oxidation increase on fat trial (31 ± 13 to 61 ± 25 $\mu.mol \cdot kg^{1} \cdot min^{1}$)	(Stepto *et al.,* 2002)
Crossover design 6 ET M cyclists	3 days high-Fat (65% fat, 9% CHO, 1.1 g/kg body mass) or Control (82% CHO, 9.9 g/kg body mass)	70% VO_2max exhaustion cycle, temp 10°C in fasted state; no CHO ingestion during exercise	44% reduction in time to fatigue on high-FAT diet: 89.2 min vs. 158 min high-Fat group and Control group	(Pitsiladis & Maughan, 1999)
Crossover design 8 WT cyclists, M	6 d Fat (68% energy from fat) with 1 d CHO (90% energy from CHO) or CON (68% energy from CHO) with 1 d CHO (90% energy from CHO)	Testing on day 1,3,5,8. Day 3 testing: steady-state cycle for 60 minutes @ 70% VO_2peak, with cadence at 90 rpm.	Testing d 3: 6 out of 8 subjects objected tiredness and trouble continuing the described load during the steady-state cycle. 2 subjects did not finish the 60-min session after high-FAT trial.	(Havemann *et al.,* 2006)
Randomly assigned 16 ET cyclists, M	5/10/15 d Fat (69% ± 1%)	Cycling constant load for 2.5 hours @ 70%VO_2peak + 40 km cycling TT	Higher fat oxidation after 5 d on Fat trial (0.67 --- 0.16 to 0.91 --- 0.20 g/min) No difference in performance	(Goedecke *et al.,* 1999)

Randomly assigned 6 WT runners, M, F	3 d CHO or HAB (50% CHO & 70% CHO)	Run to exhaustion @ 75%-80% of $\dot{V}O_2$max	Steady state RER higher in CHO vs. CON group (0.92, SEM 0.01 vs. 0.89, SEM 0.01; P<0.05) No difference in performance	(Madsen *et al.,* 1990)
Crossover design 20 WT runners, M	2 days high-fat (24.2% CHO, 60.4% fat, and 15.5% protein), high-CHO (69.3% CHO, 15.9% fat, and 15.1% protein), or habitual (56.1% CHO, 27.5% fat, and 16.5% protein) diet	10 min steady run @ 65% VO_2peak and a 10kilometre outdoor run as fast as possible	Throughout the steady run: CHO and RER role to energy output lower, higher fat contribution, in the high-FAT diet versus the high-CHO diet (P < 0.05). 10kilometre run was comparable to performance as after habitual diet (50± 7.0, 51± 8.3 and 50± 7.4 minutes, respectively). High-CHO diet improved performance compared to a high-FAT diet (P < 0.03).	(Guimaraes Couto *et al.,* 2014)
Crossover design 7 ET cyclists M	12 hour high-CHO [83% CHO, 5% fat, 12% protein] or a high-FAT 16% CHO, 68% fat, 16% protein) diet	120-min cycling @65% $\dot{V}O_2$max before 12-h dietary period. After 12-h overnight fast test 1600 kilojoule (kJ) approx. 2-h cycling @max effort	120 min cycling test: Muscle triglyceride significantly higher 24-hours after 120 min cycling for the Fat vs. CHO trial. Muscle glycogen significantly lower 24-hours after 120 min cycling in Fat vs. CHO trials. 1600 kJ time-trial: Time to complete significantly greater in Fat trial (139.3±7.1 min) compared to CHO trial (117.1±3.2 min) High fat: 139 ± 7 ǂ High CHO: 117 ± 3* ǂ	(Starling *et al.,* 1997)

Crossover design 8 WT cyclists & triathletes M	5 days high-FAT (65% fat, 20% CHO) or control group (70–75% CHO,15% fat) followed by 1 d high-CHO for both groups	120 min performance trial @70% $\dot{V}O_2$max (SS) + 7 kJ/kg Time-trial	Fat diet: reduction in RER during 70% $\dot{V}O_2$max cycling; only partly re-established by 1 day of high CHO [0.90 vs. 0.82 (*P*<0.05) high-CHO trial RER values [0.91 vs. 0.88) ($p < 0.05$) FATox increased [94 ± 6 vs. 61 ± 5 g ($p < 0.05$)], CHOox decreased [271 ± 16 vs. 342 ± 14 g ($p < 0.05$)] for high-fat compared to high-CHO. No performance difference after high-FAT diet. High fat: 31 ± 7 ǂ High CHO: 34 ± 3 ǂ	(Burke *et al.,* 2000)
Crossover design 9 subjects	3 days high-FAT (6 subjects), followed by 3 days high-CHO. Remaining 3 subjects served as controls.	high-FAT day 3: cycling to exhaustion @75% $\dot{V}O_2$max. 3 day high-CHO diet: cycling to exhaustion @75% $\dot{V}O_2$max	Fat diet: Average work time & muscle-glycogen: 59-min & glycogen 0.63g/100g wet muscle with; CHO diet: Average work time & muscle-glycogen: 189-min & glycogen 3.31g/100g wet muscle High fat: 57 ± 2* High CHO: 167 ± 18*	(Bergstrom *et al.,* 1967)
Crossover design 4 subjects M	3 days high-FAT/normal mixed/high-CHO diets.	Walked @70% $\dot{V}O_2$max to exhaustion	CHO use during initial 1 / 2 –hours reduced in fat-diet (38.2%) compared to mixed diet (69.9%) and high-CHO diet (71.9%). Work time to exhaustion in correlation with CHO use above (r = 0.86. P < 0.001)	(Martin *et al.,* 1978)

			High fat: 33 ± 3* High CHO: 78 ± 5	
Crossover design 7 WT cyclists M	6 days High-FAT diet: (69% of energy, 16% CHO); Control treatment: (70% CHO, 15% fat)	4-h @65% peak $\dot{V}O_2$ followed by 1-h TT.	6 days: reduced RER with cycling @65% peak $\dot{V}O_2$[0.78 ± 0.01 (mean) vs. 0.85 ± 0.02; P < 0.05)] 1-d CHO restoration & pre-exercise meal & CHO ingestion: restored RER (0.88 ± 0.01; *P* < 0.05) RER higher after control treatment than high-fat (0.85 ± 0.01, 0.89 ± 0.01, and 0.93 ± 0.01 for days 2, 8, and 9, respectively; *P* < 0.05). Greater fat oxidation during 4-h ride (171 ± 32 vs. 119 ± 38 g; *P* < 0.05) & lower CHO oxidation (597 ± 41 vs. 719 ± 46 g; *P* < 0.05) after high-fat. 11% higher power output during the TT after high-fat than after control treatment (312 6 15 vs. 279 6 20 W; *P* = 0.11). High-fat: 44 ± 1 km High-CHO: 42 ± 1 km	(Carey *et al.,* 2001)
Crossover design 7 subjects M		Glycogen depletion work: 45-minutes @80% $\dot{V}O_2$max	Time to exhaustion longer in high-CHO vs. high fat group	(Galbo *et al.,* 1979)

	4 days high-FAT or high-CHO with glucose infusion upon exhaustion	Test trial: 30 min bouts with 10-min rest periods @70% $\dot{V}O_2$max, 3% incline, until exhaustion	High fat: 64 ± 6 High fat: 59 ± 6 High CHO: 106 ± 5*	
Crossover design 9 MT athletes M	7 days: at day-4 half of subjects assigned to high-FAT (50% fat, 10% CHO) and other half high-CHO (10% fat, 50% CHO). Day-6 and day-7 normal diets and day-8 either high-FAT or high-CHO for 1 day	Testing on day-5, day-9. Fat oxidation test: @60% $\dot{V}O_2$max	Higher RER (0.87±0.04 vs. 0.83±0.04, p<0.05) & lower fat oxidation in high-CHO vs. high-FAT diet (0.87±0.04 vs. 0.83±0.04, p<0.05)	(Støa *et al.,* 2016)
Crossover design 18 ET runners M & F	7 days (different increases and decreases in CHO over 7-days): CHO group = 35% fat; 56% CHO Fat group = 48% fat; 37% CHO	30 km treadmill Time-Trial run (@70% $\dot{V}O_2$max) [trial 1 before dietary manipulation; trial 2 after dietary manipulation]	No difference in performance times during trial 2 Faster times for last 5 km for CHO-group from trial 1 to trial 2 [3.64 vs. 3.44; p < 0.05]. CHO-group ran 30 km faster after CHO increases in diet (day 4) [131.0 (5.4) min vs. 127.4 (4.9) min; P<0.05] High fat: 135 ± 5ǂ High CHO: 127 ± 5 ǂ	(Williams *et al.,* 1992)

Crossover design 8 WT male endurance cyclists	3 days (high CHO 10.52 ± 0.57g/kg body mass/day or normal CHO 6.15 ± 0.23g/kg body mass/day)	2-hour cycle ride	Muscle glycogen concentration higher after high-CHO diet Greater cycling distance in higher CHO diet Higher power outputs with high-CHO diet (233 ± 15 vs. 219 ± 17 W; $p < 0.05$)	(Rauch *et al.,* 1995)
Crossover design 8 well-trained male cyclists	Either 6 days control treatment, or 5 days high-FAT + 1 day high-CHO	2-hours steady state cycling + TT	Increased FATox and reduced muscle glycogenolysis following high-fat + 1-day high-CHO. No difference in cycling time-trial performance.	(Burke *et al.,* 2002)
Crossover design 7 well-trained male cyclists	Either 6 days high-CHO or 5 days high-FAT + 1-day high-CHO.	20-minutes cycling @70% $\dot{V}O_2$max	FATox higher following high-FAT + 1-day high-CHO.	(Cameron-smith *et al.,* 2003)
Crossover design 14 well-trained male cyclists	5 days either high-FAT or high-CHO diet.	20-minutes cycling + 1-minute sprint at 150% Power Output	Greater expression of CD36 and β-HAD following high-FAT. Fat oxidation was greater following high-FAT.	(Stellingwerf *et al.,* 2006)
Crossover design 8 WT male cyclists	6-days HCHO, or 5-days high-FAT + 1-day high--CHO	1-hour cycling @70% $\dot{V}O_2$max	Whole body fat oxidation higher and muscle glycogenolysis lower	(Yeo *et al.,* 2008)

Crossover design 6 WT runners	7 days high-FAT (38/50/12%)/Normal (61/24/14%)/high-CHO diets (73/15/12%).	Prolonged treadmill run to maximal VO_2	Running time to fatigue more after high-FAT diet (91.2 ± 9.5 min, P < 0.05) vs. high-CHO (75.8 ± 7.6 min, P < 0.05) and Normal (69.3 ± 7.2 min, P < 0.05) diets. $\dot{V}O_2$max higher on the Fat- diet (66.4 ±2.7 ml/kg/min, P < 0.05) vs. CHO (59.6 ± 2.8 ml/kg/min, P < 0.05) and Normal (63.7 ± 2.6 ml/kg/min, P < 0.05) diets High CHO: 91 ± 10* High CHO: 69 ± 7 High CHO: 76 ± 8	(Muoio *et al.,* 1994)

*p < 0.05 different from other diets (same exercise intensity); ŧPerformance measure is time (min) to exhaustion unless otherwise noted; ŧŧPerformance measure is time (min) to complete task (time-trial); Mean and range; ET, endurance trained; WT, well trained; M, male; F, female; VO_2max, maximal oxygen uptake; TT, time-trial; high-CHO, high carbohydrate; FATox, fat oxidation; All values are mean SEM

2.8 Fat adaptation and CHO restoration diets and endurance performance

Studies on dietary periodisation have also emerged recently after initial research clearly did not show any noteworthy performance enhancement with a low-CHO/high-FAT diet. This dietary periodisation strategy entails a short-term low-CHO/high-FAT diet (5-12 days), followed by CHO loading or CHO restoration for one to three days thereafter (Burke *et al.,* 2000:2414; Carey *et al.,* 2001:115; Havemann *et al.,* 2006:194). The reasoning behind this dietary periodisation strategy comes from the notion that it forms the ideal scenario for optimising endurance performance as the short-term LCHF diet would increase fat as fuel source during exercise while the CHO loading or CHO restoration phase would promote glycogen resynthesis as well as increase the availability of exogenous CHO as fuel source for the high intensity demands of the race or competition to follow (Burke *et al.,* 2000:1288; Hammond, 2019:58).

This dietary periodisation approach was studied by Burke *et al.* (2000:2414) in their five-day high-FAT/low-CHO diet experiment. Improved FATox was observed with these adjustments being separate of CHO availability. It was concluded that adaptations that lead to an increase in FATox during training continued despite the repair of muscle glycogen levels and that it was correlated with sparing of muscle glycogen. However, regardless of these findings on modifications in fuel utilisation during training, no significant benefit in time-trial performance was seen by the end of two hours of cycling (Burke *et al.,* 2000:2421).

Again, Carey *et al.* (2001:115) also carried out an interesting experiment concerning this dietary periodisation strategy. Participants in their experiment ate a typical CHO diet on day one, after which some participants had to consume either a high-CHO or high-FAT diet for six days. The eighth day consisted of a high-CHO diet for all the participants, again followed by a testing day (Day 9) where all the participants were fed a high-CHO meal pre- and mid-testing (four hours of cycling at 65% of $\dot{V}O_2$max, followed by a one-hour time-trial). Results presented an 11% greater power output on the high-FAT diet and a lower RER after six days on a high-FAT diet, which was restored again after only one day of high-CHO refeeding. However, a higher RER during testing on a high-CHO diet remained. Results demonstrated that six days of ingesting a high-FAT diet, followed by one day of CHO restoration, led to an increase

in FATox during prolonged submaximal exercise with large sparing of CHO after fat adaptation. However, regardless of these noticeable changes in fuel utilisation patterns that favour a significant increase in rates of FATox during the initial four hours of exercise, no statistically significant advantage in performance of a one-hour time-trial was noted after four hours of continuous cycling (Carey *et al.,* 2001:121).

Havemann *et al*. (2006:199) also investigated this particular strategy and also found no statistical difference in performance among groups in their study that investigated the effect of a high-FAT diet, followed by a one day CHO-reloading phase on fuel substrate utilisation, heart rate, electromyography and performance during an exhaustive 100 kilometre cycling time-trial. Participants completed two trials of which the first contained a high-FAT diet for six days, followed by a high-CHO reload on day seven. The second trial consisted of a high-CHO diet throughout the full six days with the same high-CHO reload as the first trial on the seventh day. Participants completed a 100 km time-trial on day one of the study, followed by a one hour cycle at 70% $\dot{V}O_{2peak}$ on days three, five and seven, and again a 100 km time-trial on day eight. Results showed that no diet favoured performance enhancement, but that the high-FAT diet reduced RER at both rest and throughout exercise and increased plasma free fatty acid levels, which served as an indication of increased fat utilisation.

From the above studies, it seems evident that FATox remains higher after following a high-FAT, low-CHO diet whilst CHO restoration, muscle glycogen and CHOox are reduced during successive training despite having been returned to a high level of glycogen stores. Along with the above, there is also no noteworthy performance benefit that can be stated with the use of this particular dietary periodisation strategy (Burke *et al.,* 2000:2421; Carey *et al.,* 2001:115; Havemann *et al.,* 2006:199).

2.9 Conclusion

Information can be confusing when it comes to pre-exercise meals or loading strategies to properly fuel the body to consequently obtain the paramount possible energy supply for the best potential performance outcome, whether it is obtained from textbooks, academic literature or popular media. Advice ranges from ingesting CHO an hour before exercise to avoiding CHO in the hour before exercise; ingesting CHO during exercise; ingesting a high-FAT meal preceding exercise, and so forth, and it is

therefore understandable why so much confusion exists regarding optimal exercise nutrition for sport performance.

Literature findings are inconsistent with results supporting different "loading" strategies either favouring high-CHO intake, low-CHO intake or "the train low, compete high" plan to optimise performance (Burke, 2015:s47; Burke & Hawley, 2002:90; Burke *et al.,* 2000:224; Carey *et al.,* 2001:122; Cox *et al.,* 2016:256; Helge *et al.,* 1996:306; Kiens & Helge, 2000:202; Lambert *et al.,* 2001:225; Murakami *et al.,* 2012:635; Ormsbee *et al.,* 2014:1795; Zajac *et al.,* 2014:2505).

The limitations of current studies accentuate the need for further research with regard to optimal nutrition for sports performance to enhance endurance performance, particularly the manipulation of macronutrient composition (Erlenbusch *et al.,* 2005:11; Hawley, 2014:5; Rowlands & Hopkins, 2002:689). Burke and Hawley (2002:1497) also recommend that upcoming studies in this research field should place emphasis on studying the likelihood that there are "responders" and "non-responders" to dietary fat-adaptation strategies, as dietary adaptations may be advantageous for some individuals and not for others.

Athletic trainers, nutritionists, sport scientists and athletes themselves usually have a specific idea regarding the optimal strategies to enhance performance during high-intensity endurance exercise through hearsay. Furthermore, non-evidence-based beliefs form a large and common part of trainers', athletes' and even professionals' beliefs.

CHAPTER 3

RESEARCH METHODOLOGY

CHAPTER 3 – RESEARCH METHODOLOGY

3.1 Introduction

The protocol that was followed to collect data is described in this chapter. A description of the experimental approach, participants, dietary trial, instruments, and methods used, as well as more detailed information on data processing and analysis will be provided. An extensive literature review was conducted to provide background for the methodological considerations.

3.2 Location and Health Sciences Research Ethics Committee ethical approval

All assessments were done in the $\dot{V}O_2$max testing room of the Department of Sport and Exercise Sciences on the campus of the University of the Free State (UFS) in Bloemfontein, South Africa. Each of the trial procedures were accepted by the Health Sciences Research Ethics Committee (HSREC) (FS-HSD2017/0033) of the Faculty of Health Sciences, University of the Free State (see Appendix A.14).

3.3 Study design

According to Welman and Kruger (2004:46) as well as Thomas *et al.* (2011:324), research is an orderly process of gathering data and sensibly analysing the data to solve problems and to make valuable contributions in the field of science. The research includes the use of several methods and techniques to produce scientifically gained knowledge, employing objective procedures and methods (Thomas *et al.,* 2011:324).

In the first step of this research study, a comprehensive literature review was conducted to collect information for the identification of the most appropriate study methodology. Both primary and secondary resources such as journals, scientific papers, books, scholarly articles, and internet resources were used to obtain information appropriate to the study. The design of the study was a quantitative study in which a randomised, cross-over, repeated-measures experimental design was followed.

Each participant completed three tests followed by a two-week washout period in-between testing sessions. It was required of participants to be present at the laboratory

on three separate occasions for: (i) initial baseline testing; (ii) a high-FAT experimental period, and (iii) a high-CHO experimental period. The three experimental periods were executed in a randomised order across the entire participant group. Circadian variations in physiological reactions were avoided by testing participants at exactly the same time of day during the two testing sessions. Furthermore, room temperature (19 – 21°C) as well as humidity (45 – 50% relative humidity) were maintained at the same levels for each testing session. Participants were required to only consume the specific foods provided by the researcher and were also requested to abstain from any vigorous exercise or activities and to avoid caffeine and alcohol intake for at least 48 hours before each test. Participants were informed and aware of the dietary regime that they received on each occasion as it was not possible to completely hide the diet type from them. However, the researcher who was responsible for the sampling of data, was not aware of the type of diet that each participant received.

3.3.1 Randomisation and blinding

The randomisation schedule for the trial was provided by the study statistician to a person other than the researcher, to maintain blinding of the researcher and all personnel involved in data collection.

3.4 Subjects

3.4.1 Sample size calculation

Rowlands and Hopkins (2002, Table 3) reported that the within-subject percentage change in $\dot{V}O_2$max is 6% (rounded to a full integer value) if the width of a confidence interval (CI) is 95%. From this information, the within-subject standard deviation of the percent change in the $\dot{V}O_2$max can approximately be determined as 5.6%. Assuming a within-subject standard deviation of 5.6% for $\dot{V}O_2$max and a two-sided significance level of α=0.05, a sample size of eleven subjects per sequence group of the cross-over trial (total of n=22) yields a power of 80% to detect a difference of 5% in mean $\dot{V}O_2$max due to the treatment (diet). However, due to the uncertainty and approximations involved in the assumptions underlying this sample size calculation, twelve subjects per sequence group of the cross-over trial (total of N = 24) were identified.

All subjects were long-distance runners who, at that time, were able to complete a marathon (42 km) in an average time of at least 4 hours and 20 min. Subjects were recruited from the Kovsie Athletic Club of the UFS after ethical approval (Appendix A.14) was obtained and consent was given from the Vice-Rector for Academic Affairs (Dr G Vinger)(Appendix A.12) and the Director of Kovsie Sport (Mr DB Prinsloo)(Appendix A.13). Participant characteristics are presented in Table 3.1 below:

Table 3.1: Subject characteristics (N = 24)

	Mean	SD	Min	Q1	Median	Q3	Max
Age (years)	27.4	2.65	25.00	25.00	26.00	28.00	33.00
Body mass (kg)	78.84	8.72	5.60	73.90	78.10	84.50	97.40
Body height (m)	1.81	0.07	1.69	1.76	1.80	1.87	1.93
BMI	24.08	2.18	19.13	23.05	24.23	25.72	28.06

Note: N: Number of athletes. **SD:** Standard Deviation. **Min:** The smallest value in the data set. **Q1:** The median of the lower half of the data. **Q3:** The median of the upper half of the data. **Max:** The largest value in the data set.

3.4.2 Inclusion criteria

In order to take part in the study, participants had to meet the following inclusion criteria:

They had to be:

(1) male.

(2) seemingly healthy with no previous injuries during the past year.

(3) between the ages of 20 and 35 years at the time of data collection.

(4) willing to comply with the dietary trial on both dietary trial occasions.

(5) able to and have run a marathon in an average time of at least 4 hours 20 minutes during the past year.

(6) willing and able to provide consent in English.

3.4.3 Exclusion criteria

If a prospective participant displayed any of the following criteria, the participant was excluded from the study:

(1) any illness at the time of the experimental trials.

(2) outside of the age category at the time of the experimental trials.

(3) suffered an injury or busy rehabilitating an injury.

(4) not willing or unable to give consent.

3.5 Procedure before the study commenced

3.5.1 Research participant information

Before the commencement of the study, each participant had to read an Information Document (Appendix A.1) and sign an Informed Consent form (Appendix A.2). The information sheet (Appendix A.1) included information on the objectives of the study, the necessity of this type of research on the athletic population, the exact experimental protocol that was to be followed so that participants would know what is expected of them concerning dietary trials and testing days, information on food collection on each day before the commencement of the study and the overall study time, as well as the risks, benefits, and requirements of study participation to the participants. The informed consent form (Appendix A.2) was signed by each athlete who agreed to participate in the study after thoroughly reading and understanding the information sheet.

3.5.1.1 Measurement tools for data collection

The following measurement tools were used to collect data:

- The Pittsburgh Sleep Quality Index (PSQI) Questionnaire (Juliff *et al.,* 2015:14) (Appendix A.8)
- The Short-form McGill Pain Questionnaire (Melzack, 1987:196) (Appendix A.9)
- A urine colour chart (Armstrong, 2005:s45) (Appendix A.11)
- A Seca 813 electronic flat scale for body weight measurements
- A Seca 217 stable stadiometer for height measurements

- A Polar Heart Rate Monitor set (H10 heart rate sensor) (e.g. chest strap, receiver, transmitter)
- A stopwatch
- Water and Milton solution (for sterilisation of apparatus)
- A Lactate Scout Plus lactate analyser (SN: 0045301175), lancets, and lactate strips (Lactate Scout Code 57 Strips)
- Alcohol swabs
- Borg Rating of Perceived Exertion (RPE) scale (Borg, 1982:378)(Appendix A.10)
- Gauzes
- The Cortex Metamax 3B Portable Spiroergometry System (Model MPU31-105; SN: ML3 223441522) with accessories and a calibration kit

3.6 Dietary analysis

A prediction formula was used instead of food intake records to estimate a person's resting energy expenditure (REE), as research showed that the latter method is unreliable and inaccurate with people underreporting food intake, leading to an underestimation of energy expenditure (Whybrow *et al.,* 2016a:266; Whybrow *et al.,* 2016b:32). The Harris-Benedict prediction formula (Harris & Benedict, 1918:370) was used to determine each participant's REE. Harris and Benedict (1918:370) produced a regression equation from information obtained on men of various ages and body weight, that incorporated age, weight, height, and gender to predict REE. An additional activity factor was then added to modify the calculated REE to predict the total, free-living energy requirements of each participant. REE is described as the required amount of energy specifically needed for vital body functions at rest and is the biggest contributor to total daily energy expenditure (Ten Haaf & Weijs, 2014:1). Activity factors are reviewed in Table 3.2.

Table 3.2: Activity factor adjustments for healthy adults (Harris-Benedict formula)

Categories of activity	Factor
Little to no exercise	REE* x 1.20

Light exercise (1–3 days per week)	REE x 1.38
Reasonable exercise (3–5 days per week)	REE x 1.55
Hard exercise (6–7 days per week)	REE x 1.73
Very hard exercise (twice per day, extra heavy workouts)	REE x 1.90

*REE: Resting energy expenditure (Harris & Benedict, 1918:370)

The Harris-Benedict prediction formula has been and is still the most used formula and proven to be valid and reliable. In a literature review, the closing reports and precision of resting metabolic rate measurements versus approximations of four studies were summarised by Frankenfield *et al.* (2005:786). Reported in their analysis was a correct estimate of 45 – 80%, with errors and an error scope of maximum underestimation to maximum overestimation of 23 – 42%. Kelly (2014:9) reviewed 25 validation studies and concluded that the Harris-Benedict equation demonstrated highly variable accuracy (45 – 80%), although the trend was to overestimate REE. In contrast, Hasson *et al.* (2011:348) found the Harris-Benedict equation to be the truest equation when compared to four other commonly used equations, with 57.5% of predicted REE values within ±10% of measured resting metabolic rate (RMR) derived from the MedGem indirect calorimetry measure. A greater percentage of predicted REE within 10% of measured REE was also demonstrated by Flack *et al.* (2016:4). Klein (1998:970) verified the validity and reliability of the Harris-Benedict energy equations when using the equation in non-obese men. In general, the prediction of group means is respectable with this equation (Kien & Ugrasbul, 2004:876). As the Harris-Benedict prediction formula only considers a subject's gender, age, height and weight, the margin for alternative errors to arise when predicting REE was relatively small. Unfortunately, this method can sometimes underestimate the daily caloric needs of very muscular athletes, as leaner bodies require more energy, and in this equation, lean body mass is not calculated (Harris & Benedict, 1918:370; Kelly, 2014:9). However, an underestimation of daily energy needs can also be calculated for other formulas, such as the Cunningham formula, that consider body composition. Firstly, an inaccurate anthropometric measure and, secondly, an error in the percentage body fat versus percentage lean body mass measurement, may occur with equations such as the Cunningham equation as mentioned, which will also lead to

incorrectly predicted REEs (Laquale, 2007:35). However, the Harris-Benedict formula is one of the oldest equations that is still used in clinical settings and therefore the preferred equation for this study.

The Harris-Benedict equation for males is as follows:

BMR = 66.47 + (13.75 x weight in kg) + (5 x height in cm) – (6.8 x age in years)

Where BMR represents basal metabolic rate.

3.6.1 Dietary manipulation

After a registered dietician determined each participant's daily energy consumption using the Harris-Benedict formula, individual iso-energetic high-CHO and high-FAT diets were prepared according to each participant's daily caloric needs. Evidence-based research (Guimaraes Couto *et al.,* 2014:318; Havemann *et al.,* 2006:195; Hulton *et al.,* 2013:166; Rowlands & Hopkins, 2002:679) that also used these diet approaches used different macronutrient distributions for both the high-CHO and the high-FAT diets. For this study, the researcher used the average macronutrient distribution of these four studies (Guimaraes Couto *et al.,* 2014:318; Havemann *et al.,* 2006:195; Hulton *et al.,* 2013:166; Rowlands & Hopkins, 2002:679) and ensured that the protein macronutrient needs were fairly equal for both high-FAT and high-CHO trials. An example of meal plans for the high-CHO and high-FAT diets are included in the Appendix list (Appendix A.16).

Table 3.3: Macronutrient distribution of the high-FAT and high-CHO diets of previous studies and the present study

Literature	Macronutrient distribution	
	High-FAT diet	**High-CHO diet**
Hulton *et al.* (2013:166)	Fat: 64% of energy CHO: 22% of energy Protein: 14% of energy	CHO: 61% of energy Fat: 22% of energy Protein: 17% of energy
Guimaraes Couto *et al.* (2014:381)	Fat: 60% of energy CHO: 25% of energy Protein: 15% of energy	CHO: 70% of energy Fat: 15% of energy Protein: 15% of energy
Rowlands & Hopkins (2002:679)	Fat: 70% of energy CHO: 15% of energy Protein: 15% of energy	CHO: 70% of energy Fat: 15% of energy Protein: 15% of energy
Havemann *et al.* (2006:195)	Fat: 68% of energy	CHO: 68% of energy
Calculations for this study	∑ ***(fat)*** = (64 + 60 + 70 + 68)/4 = 65.5 (**65% fat of energy**)	∑ ***(CHO)*** = (61 + 70 + 70 + 68) / 4 = 67.25 (**67% CHO* of energy**)
	∑ ***(CHO)*** = (22 + 25 + 15) / 3 = 20.6 (**21% CHO of energy**)	∑ ***(fat)*** = (22 + 15 + 15) / 3 = 17.3 (**17% fat of energy**)
	∑ ***(protein)*** = (14 + 15 + 15) / 3 = 14.6 (**14% protein of energy**)	∑ ***(protein)*** = (17 + 15 + 15) / 3 = 15.6 (**16% protein of energy**)
	∑ ***(total)*** = 64 + 21 + 15 = 100% macronutrient distribution	∑ ***(total)*** = 68 + 17 + 15 = 100% macronutrient distribution

*CHO = Carbohydrates

Participants alternated randomly between the high-FAT and the high-CHO diet for 48 hours before the testing day on the different trial occasions over the study period. The aim of a short-term dietary trial is to manipulate the energy stores of the body. For example, the aim of a short term high-FAT diet for one to three days is to lower resting liver and muscle glycogen stores and increase intramuscular triaglycerols and free fatty acids as an alternative fuel source, while a high-CHO diet provides supplementary CHO sources for the muscles rather than reducing existing glycogen stores that might exist (Burke & Hawley, 2002:1492). All meals and snacks were administered and prepared by a registered dietician according to the specific macronutrient distribution calculations indicated in Table 3.5. Each participant's energy needs were calculated by the dietitian after which the researcher personally delivered the meals to each participant to ensure maximal convenience. Testing was done after a twelve hour overnight fast to eliminate any probable changes in testing measurements due to uncontrolled macronutrient consumption.

3.7 Trial method

3.7.1 Procedure overview

The Metamax 3B system was switched on at least 45 minutes prior to calibration and was also calibrated before each test by using the calibration technique recommended by the manufacturer (Appendix A.3).

Each participant reported to the laboratory at specific, individual allocated timeslots on the morning of testing and after a 48 hour dietary trial period for experimental tests. Participants first had to complete the Pittsburgh Sleep Quality Index questionnaire and Short-form McGill Pain Questionnaire, as well as the urine colour chart after which they were taken to the testing room for body weight and height measurements.

Participants were familiarised with the Borg RPE scale on all occasions (Borg, 1982:378), as they had to indicate their perceived exertion during the last 15 seconds of each incremental stage of the graded exercise test (GXT). Thereafter, the participants moved to the Woodway treadmill (Pro; RS-232 Interface) to start the initial warm-up which consisted of jogging at eight kilometres per hour (8 km/h) for three minutes to increase the temperature of the muscles. The importance of increasing muscle temperature prior to stretching is generally accepted (Prentice, 2015:223). The

Metamax 3B chest and back carrying system was not worn during the warm-up. After the treadmill warm-up, participants completed a dynamic stretching protocol which the researcher compiled and supervised (Appendix A.5). The dynamic stretching protocol was executed due to the dynamic nature of running that requires repeated dynamic contractions of the agonist muscles. With this type of stretching, the muscle gradually adapts to the imposed demands of the upcoming activity as a result of the antagonist muscle that contracts eccentrically to decelerate the dynamic stretching of the agonist muscle (Prentice, 2015:224).

After completion of the stretching protocol, participants moved to the same treadmill where all Metamax 3B testing equipment, including the mask and head cap, were fitted snugly and comfortably to the face and head of each participant. After the mask and head cap were fitted, the volume transducer line was connected to the mask. The Metamax 3B was then fixed to the participants after which they were asked to breathe through their noses while closing their mouths to check for any leaks in the mask. Before the incremental test commenced, the maximum flow-volume-loop test was conducted (see Appendix A.4 for the detailed methodology of the maximum flow-volume-loop test). After all the above steps were completed, the $\dot{V}O_2$max treadmill test was started. The researcher was responsible for and present at all the $\dot{V}O_2$max tests. Figure 3.1 presents the workflow module for the cardio-pulmonary exercise test (CPET).

Figure 3.1: Workflow module CPET test (see Appendix A.4)

After completion of the test, the wellbeing of participants was the main focus of the researcher. When the exercise test was terminated, the recovery period was divided into two stages: (i) the first two minutes consisted of a cool-down that included a speed of 4 km/h and an incline of 0%, and (ii) for the third minute of cool-down, participants executed a passive recovery during which they stood stationary on the treadmill in a relaxed state. Heart rate recovery and blood lactate were measured at two, three and five minutes during the recovery period by the person who was responsible for

individual measurements during the treadmill test. After the exercise trial, a rehydration drink (Energade 500 ml) was offered to participants, but they were not forced to drink it. A person who has a Level 2 Emergency First Aid qualification was always present during testing in case of an emergency. Furthermore, the appropriate telephone numbers for emergencies and an automated external defibrillator (AED) medical system, were also available at all times during testing.

The reliability of the testing protocol was ensured by maintaining the exact same testing procedures (warm-up and running speed) during different testing sessions. Furthermore, testing was performed under similar environmental conditions (19 – 22°C temperature and 45 – 50% relative humidity) at the same time of the day (08h00 to 11h00) to minimise the effects of the circadian rhythm on physiological responses (Cerezuela-Espejo *et al.,* 2018:3). In addition, the same personnel conducted and were involved with all the tests. The researcher could only locate one study that assessed the validity and reliability of endurance performance protocols. In this study, the authors found a greater variation of 11.2% in time to exhaustion during an open-end GXT than a fixed time-trial test that obtained a 1.7% coefficient of variation (CV) (Hopkins *et al.,* 2001:212). They concluded that the greater variation in GXT results compared to the self-paced time-trial may be related to differences in motivation, and the monotonous nature of the GXT test.

a. Validity and reliability of measurement tools

i) The Pittsburgh Sleep Quality Index (PSQI) Questionnaire

Due to the fact that sleep is recognised as an important and critical aspect of both the post-exercise recovery process as well as for optimal performance in athletes, it was important to ensure that athletes obtained adequate sleep daily (Venter, 2012:167). The importance of sleep is further accentuated by research that established a link between the cognitive process, metabolic function, and sleep of high-performance athletes (Samuels, 2008:169). For the determination of the participants' sleep quality and quantity of the previous night, the Pittsburgh Sleep Quality Index (PSQI) questionnaire was completed on each test day. The PSQI is a reliable test with a reliability coefficient of 0.83 reported for the seven components that serve as indicators of sleep quality and have been used in numerous sleep studies of the general

population and high-performance athletes (Backhaus *et al.,* 2002:739; Juliff *et al.,* 2015:14; Knutson *et al.,* 2006:1505; Venter, 2012:172).

ii) Short-form McGill Pain Questionnaire

In view that physical recovery in sport improves performance and overall fitness (Martinent, 2014:326), participants also had to complete the Short-form McGill Pain Questionnaire (SF-MPQ-PRI) (Melzack, 1987:196) on each test day to ensure no musculoskeletal pain was experienced by any participant. The SF-MPQ-PRI includes the classifications of 15 adjectives that portray either affective or sensory measurements of pain, which are then categorised on a three point Likert scale from 0 to 3 with a test-retest reliability of $r > 0.7$ when testing for any musculoskeletal pain (Hawker *et al.,* 2011:S240) (Appendix A.9).

iii) Urine colour chart

A critical component to ensure optimal performance in athletes is the regular assessment of their hydration status to avoid hypohydration, of which the influence has been well established (Webb *et al.,* 2016:448). A urine colour scale is an effective and very practical means to determine an athlete's hydration status due to the high correlation ($r = 0.82$; $p < 0.001$) between urine gravity/osmolality and urine colour in adolescent athletes and university students (Lew *et al.,* 2010:334; Webb *et al.,* 2016:449). Armstrong (2005:s45) also indicates that the assessment of urine colour allows anyone to identify their rehydration needs. In this study, participants identified the colour of their urine using a five-point Likert scale, namely: "transparent", "shades of yellow", "light yellow", "dark yellow", or "very dark yellow".

iv) Borg RPE chart

Participants had to verbally give descriptors of total body effort after every three minute stage according to the Borg RPE scale (a 20-point intermediate interval tool), as displayed in Table 3.4 (Borg, 1982:378). The support behind the range of RPE from RPE6 ('no exertion at all', i.e. consistent with rest) to RPE20 ('maximum exertion', i.e. the rating equivalent to the theoretic maximal possible) is based on the perception of exercise intensity and the increased heart rate (HR) response with a rise in exercise intensity (Kenney *et al.,* 2015:520).

Table 3.4: Borg scale for ratings of perceived exertion during exercise

Rating	Description
6	No exertion at all
7	Extremely light
8	
9	Very light
10	
11	Light
12	
13	Somewhat hard
14	
15	Hard (heavy)
16	
17	Very hard
18	
19	Extremely hard
20	

(Borg, 1982:378)

v) Cortex Metamax 3B Portable Spiroergometry System

The Metamax 3B is an apparatus that uses indirect calorimetry via breath-by-breath measures for gas analysis. The system contains a turbine compilation and gas analysis data telemetry module. The system was created with a dimension and battery unit that are the same size (120 x 110 x 45 mm). The tube leading to the data telemetry and gas analysis system is clipped to the participant's chest using a yoke attached to the facemask and bidirectional digital turbine assembly (Vmask^{TM}, Hans Rudolph Inc., Shawnee, KS; dead space 40–49 mL). A Nafion/Permapure sampling tube of 60 cm in length is attached to the turbine that documents flow-transduced volume measurements over a range of 0.05–20.0 litres per second (L/sec). Simultaneously,

electrochemical oxygen ($O_{2)}$ and infrared carbon-dioxide (CO_2) analysers determine indirect calorimetry values ranging from 0 to 100% O_2 and 0 to 13% CO_2. The breath-by-breath data of gas concentrations and respiratory volume can either be stored via an on-board memory chip or immediately sent to a computer via telemetry.

MacFarlane and Wong (2012:2539) considered the performance of the Metamax 3B for (i) reliability, using a commercially accessible gas exchange emulator, (ii) drift or stability over three hours, and (iii) validity associated with the Douglas Bag method criterion and a formerly certified gas analysis system (Jaeger Oxycon Pro) during field tests in children and adolescents. They indicated that the practical changeability of the Metamax 3B measurements was agreeably low, with the comparative percentage error for $\dot{V}_E$, $\dot{V}O_2$ and $\dot{V}CO_2$ between tests being less than 2%, and the technical error of measurement (TEM) commonly less than 1.5% (MacFarlane & Wong, 2012:2545). Their reliability results were similar to that of Gore *et al.* (Gore *et al.,* 1997, cited by MacFarlane & Wong, 2012:2545), who found a 1% comparative error produced from a complex automated calibration system, which is less than the TEM reliability limit of 3%.

Vogler *et al.* (2010:733) also assessed the reliability and validity of the Metamax 3B. Their aims were (i) to evaluate validity of physiological measures for athletes that were endurance-trained by comparing outcomes with a first-principle metabolic calibrator and a Douglas Bag system, and (ii) to determine the reproducibility of the Metamax 3B measurements during identical trials. Vogler *et al.* (2010:740) found the Metamax 3B to be an exceptionally reliable apparatus for measuring $\dot{V}O_2$, $\dot{V}CO_2$, RER and $\dot{V}_E$. Although the representative error results were more than those achieved from the Douglas Bag method, the certainty limits for the Metamax 3B's typical error estimates were comprehensive given their small sample size, but still demonstrated a strong likelihood for trivial differences. They also reported that the results from the Metamax 3B could be compared with the results of the Cosmed K4b² (Vogler *et al.,* 2010:740). Volume quantities were within 4% of the goal value across the physiological range when comparing Metamax 3B measurements with the metabolic calibrator measurements (Vogler *et al.,* 2010:741). Values for $\dot{V}O_2$ and $\dot{V}CO_2$ were more changeable with differences between 3% to 8% for $\dot{V}O_2$, and 1% to 10% for $\dot{V}CO_2$. Overall, $\dot{V}O_2$ and $\dot{V}CO_2$ measurements were visibly outside the 4% range of the calibrator target, but for three out of the five assessed metabolic outputs, $\dot{V}O_2$ was

within the range. The RER value also differed from the one (1.00) target. Typical errors of 2% to 3% for $\dot{V}O_2$, $\dot{V}CO_2$ and $\dot{V}$ also indicated excellent reproducibility and validity for the Metamax 3B.

In addition, Macfarlane and Wong (2012:546) also found overestimation of $\dot{V}O_2$ and $\dot{V}CO_2$ during moderate to vigorous exercise of 10% to 17%, respectively, when compared to the Douglas Bag method system. They reported that previous studies on the validity of the Metamax 3B showed inconsistent findings, with some reporting an overestimation and others an underestimation of these mentioned $\dot{V}O_2$ kinetics. Overall, in their study, the error in $\dot{V}_E$ during moderate and intense exercise was smaller than 2.5% when compared to the Douglas Bag method, yet a relatively large technical error of measurement percentage (TEM%) score of greater than 7% was found. The high TEM% could be due to the sensitivity of the TEM scores to the degree of variability in the data pair (MacFarlane & Wong, 2012:2546).

MacFarlane and Wong (2012:2546) also argued that the errors could be partially attributed to the gradual upwards drift in $\dot{V}O_2$ and $\dot{V}CO_2$ by less than 4% at a low metabolic tempo, and less than 2% at two higher metabolic tempos. In addition, the fact that their study population included children and adolescents and not highly trained individuals might be the cause of the validation values not giving consequent and reliable results. A review by MacFarlane (2001:841) on automated metabolic gas analysis systems, assessed three studies that investigated the validity and reliability of the Cosmed K4b² Moveable Automated System.

In the first study (McLaughlin *et al.,* 2001:280), no significant differences in $\dot{V}O_2$ resting value and $\dot{V}O_2$ were found at 250 Watts (W) when the Cosmed K4b² portable system was evaluated against the Douglas Bag method. Only slightly higher values (less than 0.1 L/min) were observed at cycling intensities of 50 W, 100 W, 150 W, and 200 W for the Cosmed K4b² with an error of +3 to +9.6%. The authors concluded that the Cosmed K4b² was an acceptable, reliable, and valid $\dot{V}O_2$ measurement instrument across a wide range of exercise intensities. In the second study (Doyon *et al.,* 2001:1), no significant differences were found for the $\dot{V}O_2$ measurements at all working rates when the Cosmed K4b² system was compared to an ordinary laboratory mixing-box system. In the third study (Parr *et al.,* 2001:6), the Cosmed K4b² was again compared to the criterion Douglas Bag method at exercise intensities from rest to 250 W. In this

study, the fraction of end-tidal carbon dioxide ($FECO_2$) was underestimated, and a fraction of oxygen uptake (FEO_2) was overestimated by the Cosmed K4b² system, but accurate values were produced for $\dot{V}O_2$, $\dot{V}CO_2$, $\dot{V}_E$ and RER up to 200 W, with $\dot{V}O_2$ errors less than 60 mL/min. Although the Cosmed K4b² system showed a significant underestimation of 300 mL/min for $\dot{V}CO_2$ and RER at 250W, it was still considered to be an accurate spiroergometry system for measurements during exercise (MacFarlane, 2001:856).

Therefore, to conclude, the Metamax 3B can indeed be regarded as a valid and reliable system to acquire volume measurements during exercise. Although the Metamax 3B results did not meet the requirements of the Douglas Bag method, both studies of MacFarlane (2001:857) as well as MacFarlane and Wong (2012:2539) validated the Metamax 3B against the Cosmed K4b² systems.

vi) Lactate Scout + portable analyser

A GXT allows researchers to determine an athlete's anaerobic threshold by measuring and analysing blood lactate concentrations during the test in combination with heart rate measures (Beltz *et al.,* 2016:2). The anaerobic threshold usually indicates the anaerobic contribution to exercise through the accumulation of blood lactate. Therefore, the exercise intensity at which the blood lactate concentration exponentially increases reflects the breakdown of glycogen by the muscles (Attipoe & Deuster, 2015:268; Monahan, 2016:8).

The portable Lactate Scout+ lactate analyser used by the Department of Exercise and Sport Science, was used during all experimental trials. Lactate Scout+ is a small portable lactate analyser that needs just 0.2µl of capillary blood and yields results in just ten seconds. The device can store up to 250 results which also includes a stopwatch and countdown timer for performance measurement. Lactate Scout+ has been created for on field use as a training acquaintance for individuals or sports teams. Since lactate is a vital measurement for many different types of athlete competing in different climates, Lactate Scout+ operates in temperatures from 5 – 45°C and up to 85% humidity (SensLab GmbH, 2012; Artic Medical, 2011).

The manufacturers of the Scout+ report measurement errors of 3% (SensLab GmbH, 2012; Artic Medical, 2011). This measurement error (3.5%) was confirmed with the

similarity it showed to overall results from the study from Bonaventura *et al*. (2015:212) who studied the accuracy and reliability of six portable blood lactate analysers. The Scout+ showed a Pearson's correlation coefficient of r = 0.99 on the within lactate ranges from 0.5 – 21 mmol/L in a study done by the manufacturers. The point of their study was to validate the use of the Lactate Scout+ on equine whole blood in the sports medicine field environment by comparing it to a reference method (COBAS 6000) (SensLab GmbH, 2012; Artic Medical, 2011). Tanner *et al.* (2010:551) also found strong reliability values for the Scout+ when they compared it to two other portable handheld lactate analysers (Lactate Pro and Lactate Plus). For the lactate range of 4.1 – 8.0 mmol/L, all three analysers showed similar reliability with a TE of 0.4 mM, 0.3 mM and 0.3 mM and CV = 6.9, 3.8 and 5.2%, respectively, for the Lactate Pro, Lactate Scout+ and Lactate Plus. The typical error of measurement (TE) for the same blood samples was 1.0 mmol/L with a coefficient of variation (CV) value of 10.2% (Tanner *et al.,* 2010:556). Correlation analysis for repeated measurements of the same blood sample were 0.95 for the Lactate Scout+ (Tanner *et al.,* 2010:556). At higher lactate concentrations (>0.8 mmol/L) the Lactate Scout+ displayed weaker intra-analyser reliability when compared to the Lactate Pro and Lactate Plus. The Lactate Scout+ tended to produce lactate values that were 0.9, 0.6 and 0.5 mmol/L higher at lactate concentration zones of 0 - 4.0 mmol/L, 4.1 – 8.0 mmol/L and >0.8 mmol/L, respectively (Tanner *et al.,* 2010:557). Although Tanner *et al.* (2010:553) also confirmed the strong reliability of the Lactate Scout+ (0.95), they advised to rather use the Lactate Pro or Lactate Plus as the values of these two lactate meters correlated strongly (r = 0.99) with each other, as well as displayed good reliability and accuracy when compared at a wide range of lactate concentrations and a laboratory-based analyser, and was therefore a good choice to use as measurement tool.

vii) Body mass and height measurements

A portable weighing scale (Seca 813, SECA Precision for Health, Germany) was used with a maximum capacity of 200 kg. The scale was calibrated every morning according to manufacturer recommendations. A ten kilogram Olympic weighted plate was put on the scale to verify measurement accuracy. This was done before measuring the body mass of each participant to eliminate possible measurement errors. The participants,

dressed in minimum clothing, stood in the centre of the scale. Body mass was logged to the closest tenth of a kilogram (Carter, 2002:3).

Body height (stature) was measured with a stadiometer (Seca 217, SECA Precision for Health, Germany) according to ISAK guidelines (Norton & Eston, 2018:69). Participants' shoes and socks were removed before measurement. After their shoes and socks were removed, the participants were asked to stand with their shoulders, heels and buttocks all in contact with the vertical plane of the stadiometer. Their feet were to be flat on the floor with either ankles or knees remaining in contact. Measurement was taken to the nearest 0.1 centimetre and recorded.

3.8 Physiological measures

3.8.1 Heart rate

Participants were close-fitted with a short-range radio telemetry system (Polar, H10 Heart Rate Sensor) to monitor heart rate during all tests. The maximal heart rate of each subject was estimated using the formula of Roy and McCrory (2015:322) who concluded in their study that the formula, HRmax = 208 – (0.7 x age in years), was more accurate than the HRmax = 220 – age and HRmax = 226 – age formulas for predicting HRmax in subjects.

3.8.2 Assessment of respiratory gases

Expired O_2 and CO_2 were measured and analysed at frequent intervals (every 15 seconds) during all tests via the Cortex Metamax 3B portable metabolic system.

Indirect measures included:

- $\dot{V}_E$ (L/min)
- RER
- $\dot{V}O_2$ (L/min)
- $\dot{V}CO_2$(L/min)
- $\dot{V}O_2$ (ml/kg/min)

These values were used to determine certain thresholds or turning points that occurred during test execution. VT_1 and VT_2 were visually determined through analysing the

graphs plotted automatically by the Metamax 3B. Two unbiased researchers agreed on the detection of most VT_1 and VT_2. In cases where the researchers could not agree on the exact placement of the threshold points, the judgement of a third researcher was obtained. In all cases, the third researcher agreed on the placement of the threshold point with one of the two other researchers.

- *Ventilatory thresholds (VT)*

The ventilatory threshold 1 (VT_1) or aerobic threshold was verified using the principles of a rise in $\dot{V}_E/\dot{V}O_2$ with no simultaneous increase in $\dot{V}_E/\dot{V}CO_2$. VT_1 was easily detectable as it marks the first increase in both the ventilatory equivalent of oxygen ($\dot{V}_E/\dot{V}O_2$) and the end-tidal pressure of oxygen ($P_{ET}O_2$) with no parallel rise in $\dot{V}_E/\dot{V}CO_2$. In most cases, this point coincides with the point of "optimal ventilatory efficiency" (Lucia *et al.,* 2000:1788).

The ventilatory threshold 2 (VT_2) or respiratory compensation point (RCP) was determined using the principles of a rise in both $\dot{V}_E/\dot{V}O_2$ and $\dot{V}_E/\dot{V}CO_2$ with a decrease in $P_{ET}O_2$. This point refers to the point at which high-intensity exercise can no longer be continued due to blood lactate accumulation.

3.8.3 Assessment of substrate utilisation during exercise

Rates of whole-body carbohydrate oxidation (CHOox) (g/min) and fat oxidation (FATox) (g/min) were analysed from respiratory data measured during the GXT. The respiratory data were averaged over ten second intervals to analyse substrate metabolism. The raw numbers were then manually analysed for each athlete. The average values for $\dot{V}O_2$ and $\dot{V}CO_2$ were calculated over the last two minutes of every stage (Achten *et al.,* 2002:94). The calculation was made from $\dot{V}O_2$ and $\dot{V}CO_2$ measurements assuming non-protein RER values. Consequently, we presumed that the protein amount oxidised is unimportant and that other metabolic processes that involve O_2 and CO_2 production and/or utilisation are negligible compared to the oxidation of glucose and fatty acids (Stepto *et al.,* 2002:451). Calculations were done using the equation of Farinatti *et al.* (2016:80). This equation also assumes the negligible contribution of protein oxidation.

$$CHOox\ (g/min) = (4.585 \times \dot{V}CO_2) - (3.226 \times \dot{V}O_2)$$

FATox (g/min) = (1.695 x $\dot{V}O_2$) – (1.701 x $\dot{V}CO_2$)

Although the stoichiometric estimates used with indirect calorimetry are built on the fact that all the CO_2 stem from the oxidation of CHO, fat and protein, $\dot{V}CO_2$ will only be a reliable estimate of tissue CO_2 in the presence of a stable bicarbonate pool and therefore, it can be argued that a shift in the bicarbonate pool at higher exercise intensities could affect the results of the calculated oxidation rates (Achten & Jeukendrup, 2003c:605). However, Romijn *et al.* (1993) assessed the reliability of indirect calorimetry at high-intensity exercise by comparing the breath-by-breath method to the ^{13}C:^{12}C ratio method from which CHO and fat oxidation can also be measured independent of $\dot{V}CO_2$. They found that the oxidation of fat and CHO are the same between both these methods at rates up to 80–85% of the $\dot{V}O_2$max (Romijn *et al.,* 1993:E380). Therefore, for the current study, maximal fat oxidation was calculated using data up to 85% of the $\dot{V}O_2$max.

3.8.4 Assessment of maximal oxygen uptake ($\dot{V}O_2$max)

For calculation of $\dot{V}O_2$max, all subjects completed a GXT to exhaustion on a motorised treadmill. The GXT protocol that was validated by Peserico *et al.* (2015:732), was used to obtain the true $\dot{V}O_2$max or $\dot{V}O_{2peak}$ value of each subject. In their study, Peserico *et al.* (2015:730) started the treadmill protocol at 8km/h with a gradient set at 1%, which is regarded to be a reliable and accurate way to determine the endurance performance of recreational and amateur runners. The current study only included trained distance runners, which meant that the protocol of Peserico *et al.* (2015:733) was adapted by running the first incremental stage at ten km/h. The speed increments progressed by one km/h) every three minutes until exhaustion was reached. The treadmill was set at a 1% gradient throughout the test to simulate the energetic costs of outdoor running at the same speeds (Kuipers *et al.,* 2003:487). Subjects were verbally encouraged right through the GXT to deliver a maximum effort.

The $\dot{V}O_2$max value was described as the highest $\dot{V}O_2$ value attained during any 10-s period. However, the value was termed a $\dot{V}O_{2peak}$ value and not a $\dot{V}O_2$max value if two of the below listed criteria points were not met (Christie & Lock, 2001:19; Clark, 2012:1; St Clair Gibson *et al.,* 1999:1226).

(1) The $\dot{V}O_2$ values levelled off or reached a plateau with an increase in running intensity, which is expressed as an increase in $\dot{V}O_2$ of less than 2 ml/kg/min or 5% with an increase in exercise intensity.

(2) A RER value that exceeded 1.15.

(3) The onset of extreme exhaustion that led to a suspension of the test.

(4) The capillary blood lactate (CBL) value reached or exceeded 8 mmol/L.

(5) A RPE of 19 or 20 out of 20 was reached.

(6) The age-related maximum heart rate or a value that is within ten beats per minute of the age-related maximum was reached.

3.9 Perceptual measures

3.9.1 Rating of perceived exertion

All participants were requested to describe their RPE on a 20-point scale (Table 3.2, Borg, 1982:378) at the end of every three minute interval during the GXT.

3.9.2 Lactate threshold and capillary blood lactate (CBL) sampling and analysis

Lactate Threshold (LT) was determined by assessing the capillary blood lactate-speed relationship ($[Lact]_{BLOOD}$/km/h) during the GXT and set as the highest speed accompanying a rise in CBL of 0.8 mmol/L or more above the resting concentration (Cerezuela-Espejo *et al.,* 2018:4; Faude *et al.,* 2009:470). Blood sampling was done via finger prick method to obtain blood lactate samples at every three minute interval.

The following finger prick blood sample technique was repeated at every three minute interval during the GXT: The sensor strip was inserted into the device slot with the black connection contact surfaces facing up. The test mode spontaneously started upon the strip insertion. After a momentary code display (code 57), a flashing drop appeared on the display demonstrating that the device is ready to test. Next, a drop of sample blood was produced using a single use sterile lancet permitted for capillary blood sampling. The site where blood was drawn, was cleaned from sweat as lactate accumulates on the skin from perspiration and leads to falsely elevated results. The finger was disinfected and washed with fresh, hygienically safe water and the finger of each subject was dried thoroughly prior to blood sample collection. Extensive pressure

on the prick site was avoided to avoid a risk of sweat and/or tissue fluid merging with the blood droplet and falsify the test result. The fingertip with the droplet of blood was brought close to the tip of the sensor that had been inserted in the device so that the blood could be absorbed. Once the chamber of the sensor was filled, an audio approval signal sounded, and the test procedure was activated. “LAC” with a progress bar appeared on the display together with the allocated memory number. After ten seconds, a second audio signal sounded, and the test result was displayed in “mmol/L”. Each reading was stored automatically along with the date and time of the test.

3.9.3 Power output and active energy expenditure

Power output measured in absolute power output (W) and relative power output (W/kg) as well as work output measured in kilojoules (kJ) were automatically analysed by the Cortex Metabmax 3B at each 15 second interval. These individual values were then used to calculate the different running intensities at VT_1, VT_2 and $\dot{V}O_2max$, respectively.

3.10 Pilot study

After obtaining ethical approval for the study, a pilot study was performed on two male endurance athletes from a running club in Bloemfontein that did not form part of the primary subject group. All procedures, including dietary analysis, dietary manipulation and the entire exercise testing protocol was performed according to the described procedures. The researcher, along with the selected group of testing personnel, supervised execution of the pilot study tests. The tests were performed within a window period of 48 hours between testing sessions to verify test protocol reliability. Circadian variations in physiological reactions to testing were avoided by testing participants at exactly the same time of day during the two testing sessions, and the room temperature (19 – 21°C) as well as humidity (45 – 50% relative humidity) were maintained at the same values.

3.11 Statistical analysis

3.11.1 General

Data from the $\dot{V}O_2$max test (Section 3.3.4) were recorded by the researcher and captured from the Metamax 3B software programme during the test. All data were stored in a Microsoft Excel (Microsoft Office 2010) spreadsheet at the end of all two experimental trials for further analysis.

The statistical analyses were performed by Prof. Robert Schall, Statistical Consultation Unit, Department of Mathematical Statistics and Actuarial Science, UFS, using SAS (version 9.4 or higher). Statistical analyses were performed according to the guidelines of Fallowfield *et al.* (2005).

3.11.2 Descriptive statistics

The following descriptive statistics were calculated: percentages and frequencies for categorical data, and the means, standard deviations, minimum, median and maximum values for quantitative data. Descriptive statistics were calculated separately for the two study treatments (high-FAT and high-CHO).

3.11.3 Comparison of study treatments

The two study treatments were compared with respect to the various measurements using analysis of variance (ANOVA), applicable to a cross-over trial, namely with subject, period and treatment effects. From the ANOVA of each measurement, the mean values for each study treatment (high-FAT and high-CHO) were calculated, the point estimate and 95% confidence interval (CI) for the mean difference "high-FAT – high-CHO", the p-value associated with a test of the null-hypothesis of no difference between treatment means, and the effect size calculated as the ratio of the point estimate of the mean difference divided by the residual standard deviation from the ANOVA.

All ANOVA analyses were accompanied by residual plots to judge, among others, homoscedasticity, and symmetry of the distribution of the residuals. The residual plots did not reveal any serious deviations from homoscedasticity and symmetry.

3.12 Implementation of findings

Results of this study will be used to determine whether a more beneficial macronutrient "loading" approach is available for optimising the endurance performance of athletes.

Furthermore, results could indicate the response of each subject to a specific dietary trial. Subjects who wanted their individual results received detailed reports.

3.13 Ethical considerations

3.13.1 What is ethics?

The word "ethics" proposes a set of standards that enable a person to decide what components of the study are acceptable in the pursuit of study aims, and what acts can be categorised as right or wrong during the study execution (Olivier, 2007:30). Olivier (2007:30) based ethics on three fundamental principles, namely respect for persons, beneficence, and justice. He further suggested that the application of these principles to do research includes the sensitivity to autonomy (a subject's right to self-determination), responsibilities not to harm others (including social, psychological and physical harm), helpfulness (producing a net balance of benefits over harm), justice (distributing benefits and harms fairly), privacy and truthfulness.

3.13.2 Ethical aspects

All studies with humans as subjects include some extent of risk (Marczyk *et al.,* 2005). The researcher had to be aware of the ethical challenges that identified risks may present. Ethical approval (UFS-HSD2017/0033) for the study was given by the Health Sciences Research Ethics Committee (HSREC) of the Faculty of Health Sciences, University of the Free State (Appendix A.8). Furthermore, permission was obtained from the chairpersons of several Bloemfontein athletic clubs for athletes to participate in the study.

After ethical approval and the necessary permission were obtained, an information sheet (Appendix A.1) that contains a detailed explanation of the envisaged study and the potential risks associated with participation, was provided to each potential subject. Emphasis was placed on the fact that their participation was completely voluntary and refusal to take part would not lead to any penalty or loss of benefits. Subjects were given the opportunity to ask questions prior to signing a written informed consent form (Appendix A.2). The basic information provided in the informed consent form included:

- a reasonable description of the research processes that were to be followed.
- an explanation of the benefits and value of study participation.

- an agreement to answer any questions about the research processes.
- a statement to indicate to subjects that they could withdraw consent and cease study participation at any time.
- information related to the human rights of subjects.
- a statement that the right to privacy or non-participation of subjects will be respected.
- a statement to indicate to subjects that they have the right to stay anonymous.
- a statement to indicate to subjects that they have the right to discretion; and
- the right to demand responsibility from the researcher.

The information sheet and informed consent were available in English.

Participants did not get any financial reimbursement for their involvement in the study. Participant's gave consent for the publication of the results in the informed consent form. Every effort was made to keep personal information confidential. All information along with data collection results were captured and saved on a Lenovo laptop that only the researchers had access to. The laptop was locked up in a safe at the researchers' home every day up to the end of the study. Back-ups of data were also made and kept on two separate external hard drives and safely locked up. Data were stored on the hard drives until the final draft of the thesis were submitted, after which it would then be saved to a compact disc and kept in the Department of Human Movement Science safe for future article writing purposes.

CHAPTER 4

RESULTS

CHAPTER 4 - RESULTS

4.1 Introduction

In this chapter the results from the study will be presented. Tables provide descriptive statistics as well as the statistical comparison between the two trials via the Analysis of Variance (ANOVA). The figures provide illustrations of different plotted variables.

4.2 Diet

The descriptive statistics for the participants' basal metabolic rate (BMR) and total daily energy expenditure are presented in Table 4.1. The participants' BMR and daily activity rate was used to determine their individual energy expenditure in kilojoules (kJ), which was then used to calculate and design their individual diets for both experimental trials according to the macronutrient distribution laid out in Table 3.5.

Table 4.1: Basal Metabolic Rate and Energy expenditure (N=24)

	BMR (kJ/day)	Energy expenditure (kJ/day)
Mean	7957.80	12384.01
SD	683.79	1079.64
Min	6679.63	10873.88
Median	7864.04	12045.90
Max	9629.98	14926.42

Note: N: Number of participants; **BMR:** Basal Metabolic Rate; **SD:** Standard Deviation; **Min:** The smallest value in the data set; **Max:** The largest value in the data set; **kJ/day:** kilojoules per day

The performed diets in the 2 experimental conditions are presented in Table 4.2.

Table 4.2: Nutrient composition of the two experimental diets (N=24)

	High-FAT diet			High-CHO diet		
	Mean	**SD**	**Macronutrient %**	**Mean**	**SD**	**Macronutrient %**
Energy expenditure, kJ/day	12384.01	1079.64	100%	12384.01	1079.64	100%
CHO, kJ	2600.65*	226.73	21% CHO of energy	8297.29	723.29	67% CHO* of energy
FAT, kJ	8049.59*	701.78	65% fat of energy	2105.26	183.55	17% fat of energy
PROT, kJ	1733.77	151.13	14% protein of energy	1981.46	172.79	16% protein of energy

Note: N: Number of participants; **BMR:** Basal Metabolic Rate; **SD:** Standard Deviation; **Min:** The smallest value in the data set; **Max:** The largest value in the data set; **kJ:** kilojoules; **CHO:** carbohydrate; **PROT:** protein; *****Significantly different from high-CHO diet

4.3 Ventilatory and metabolic responses

4.3.1 $\dot{V}O_2$ kinetics

Descriptive statistics for the ventilatory and metabolic gas values measured during the two trials are presented in Table 4.3.

VT_1 and VT_2 were revealed in 100% of the cases. Two unbiased researchers agreed on most VT_1 and VT_2 detection points. In cases where disagreement existed, the opinion of a third researcher was obtained to verify the VT_1 and/or VT_2 points.

Table 4.3 shows that the $\dot{V}O_2$max of the subjects averaged 51.42 ± 4.58 ml/kg/min and 51.04 ± 5.24 ml/kg/min (mean ± SD) for the high-CHO- and high-FAT-trials, respectively. There was no significant difference between the two trials regarding $\dot{V}O_2$max ($p = 0.43$). All participants achieved at least two of the criteria for the attainment of a maximal $\dot{V}O_2$max value during the graded exercise test (GXT), therefore the $\dot{V}O_2$max values were verified.

Table 4.3: Descriptive statistics of ventilatory and metabolic responses (N=24)

Variables		VT_1		VT_2		VT_1 (% of max)		VT_2 (% of max)		Absolute Maximum Values	
		High-CHO	High-Fat	High-CHO	High-Fat	High-CHO	High-Fat	High-CHO	High-Fat	High-CHO	High-Fat
$\dot{V}O_2$/kg (ml/kg/min)	Mean	40.96	38.67	46.67	45.17	81.19	77.94	92.52	90.51	51.42	51.04
	Std	4.46	4.78	3.80	4.93	7.57	11.37	5.98	7.14	4.58	5.24
	Min	31.00	26.00	38.00	36.00	57.38	52.00	72.13	74.51	43.00	40.00
	Median	41.50	39.00	47.50	45.00	81.65	78.00	93.36	92.75	51.00	51.00
	Max	49.00	47.00	52.00	56.00	90.91	93.88	100.00	100.00	62.00	60.00
$\dot{V}O_2$ (L/min)	Mean	3.22	3.05	3.67	3.57	81.1.0	77.83	92.41	90.39	4.05	4.03
	Std	0.42	0.47	0.37	0.50	7.53	11.37	6.02	6.65	0.49	0.52
	Min	2.27	1.93	2.85	2.43	56.93	51.88	71.43	75.11	2.94	2.94
	Median	3.19	2.99	3.73	3.54	81.95	78.81	93.19	93.21	4.10	3.99
	Max	3.93	3.99	4.23	4.53	90.74	93.43	99.47	99.77	4.83	4.82
$\dot{V}CO_2$ (L/min)	Mean	2.88	2.81	3.55	3.48	73.38	72.61	90.51	89.01	4.05	4.01
	Std	0.50	0.55	0.41	0.50	10.22	14.28	8.22	7.29	0.49	0.51
	Min	1.78	1.81	2.61	2.47	47.3.0	44.10	67.82	74.25	2.65	2.90

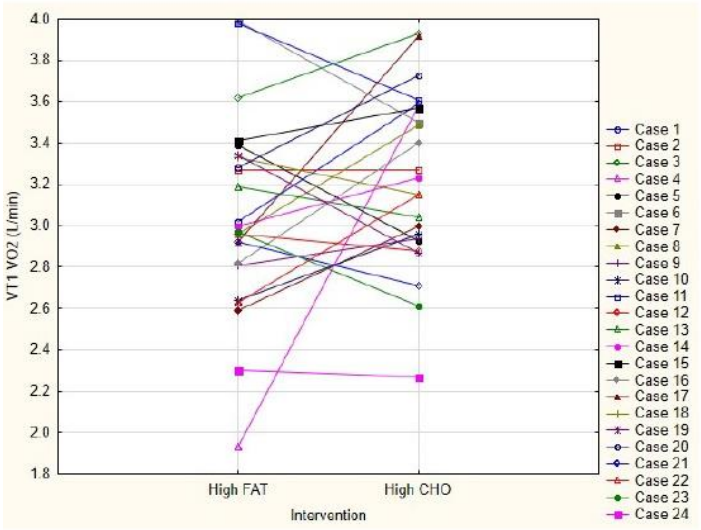
	Median	2.91	2.85	3.60	3.41	74.12	74.90	89.35	89.15	4.16	4.01
	Max	4.07	3.91	4.14	4.49	91.16	94.90	111.33	100.95	4.72	4.82
V̇E (L/min)	Mean	102.03	98.83	131.83	130.43	66.98	67.61	86.85	88.55	159.28	157.36
	Std	19.70	22.51	15.64	18.86	10.65	15.59	8.84	9.07	18.99	21.11
	Min	67.50	53.20	102.30	86.50	42.24	31.02	69.32	72.77	110.40	103.10
	Median	105.15	101.50	129.95	133.85	67.51	70.23	86.61	89.48	159.90	162.00
	Max	143.50	142.50	166.70	160.90	88.77	94.31	102.02	103.63	191.30	187.50
RER	Mean	0.89	0.90	0.97	0.98	89.92	90.79	97.96	98.42	1.05	1.05
	Std	0.05	0.06	0.05	0.04	4.45	6.74	4.58	4.13	0.05	0.05
	Min	0.78	0.78	0.91	0.91	82.57	78.79	92.08	90.48	0.98	0.95
	Median	0.90	0.91	0.95	0.97	89.00	91.99	97.36	97.99	1.05	1.05
	Max	0.97	0.99	1.11	1.06	98.98	102.06	114.74	107.22	1.18	1.15

Note: N: Number of participants; **SD:** Standard Deviation; **Min:** The smallest value in the data se;. **Max:** The largest value in the data set; **VT1 (% of max):** percentage of $\dot{V}O_2$max at VT1; **VT2 (% of max):** percentage of $\dot{V}O_2$max at VT2; **VT1:** first ventilatory threshold; **VT2:** second ventilatory threshold.

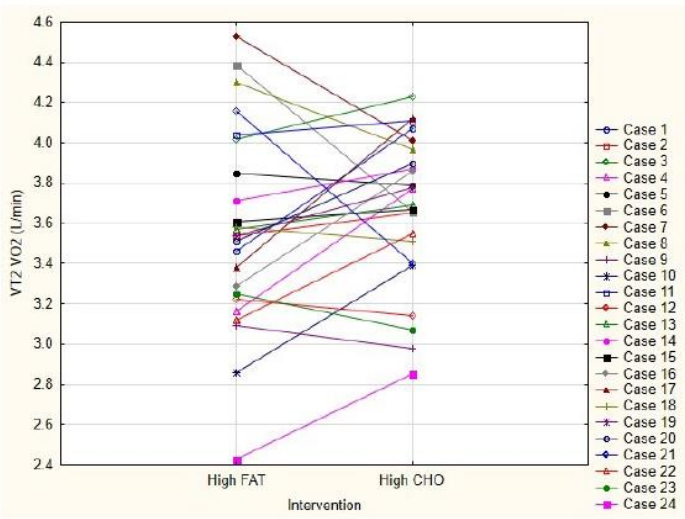
The $\dot{V}O_2$ values (L/min) at VT_1 (3.22 ± 0.42 vs. 3.05 ± 0.47 L/min), VT_2 (3.67 ± 0.37 vs. 3.57 ± 0.50 L/min) and $\dot{V}O_2$max (4.05 ± 0.49 vs. 4.03 ± 0.52 L/min) were slightly higher during the high-CHO trial. However, no statistically significant differences were detected between the two trials for $\dot{V}O_2$ (L/min) at VT_1 ($p = 0.06$), VT_2 ($p = 0.18$) and $\dot{V}O_2$max ($p = 0.47$) (Table 4.4). Nonetheless, Cohen's effect size value (d) showed that medium practical significant differences existed between the trials for $\dot{V}O_2$ (L/min) at VT_1 ($d = 0.58$) and VT_2 ($d = 0.41$) (Table 4.4).

Case plots (Figure 4.1a – c), illustrates the individual differences in responses for $\dot{V}O_2$ (L/min) at VT_1[(a)], VT_2[(b)], and $\dot{V}O_2$max[(c)] between the high-FAT and high-CHO trials. The plots show that nine participants obtained higher $\dot{V}O_2$ values during the high-FAT compared to the high-CHO trial at VT_1, whereas two participants showed no differences and 13 participants experienced lower values for the high-FAT compared to the high-CHO trial. At VT_2, nine participants obtained higher $\dot{V}O_2$ values during the high-FAT compared to the high-CHO trial, whereas 15 participants experienced lower values for the high-FAT compared to the high-CHO trial. At $\dot{V}O_2$max, $\dot{V}O_2$ values indicate that 16 participants obtained higher $\dot{V}O_2$ values during the high-FAT compared to the high-CHO trial, whereas eight participants experienced lower values for the high-FAT compared to the high-CHO trial.

(a)



(b)



(c)

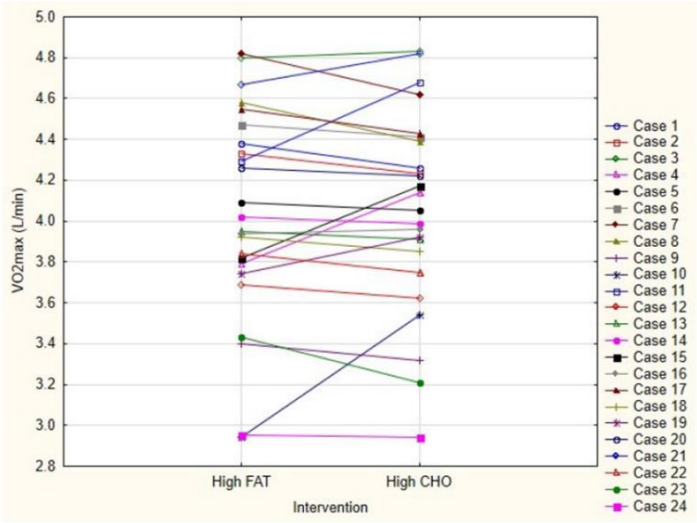
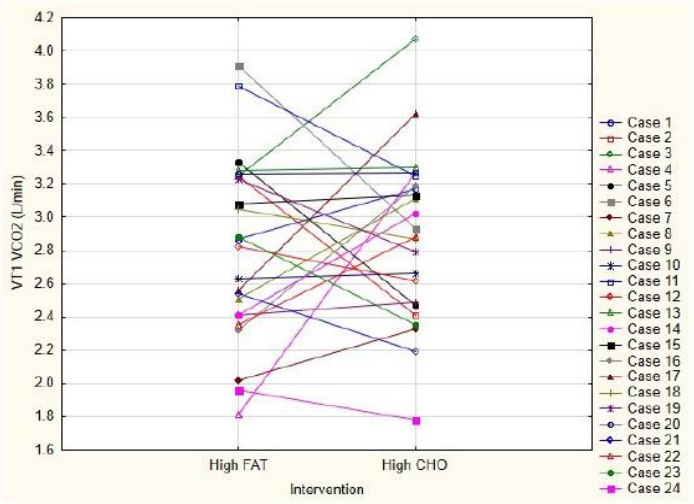


Figure 4.1a – c: Case plots to indicate the individual differences in $\dot{V}O_2$ at VT_1, VT_2 and $\dot{V}O_2$max between the high-FAT and high-CHO trials (N=24)

$\dot{V}CO_2$ (L/min) values at VT_1 (2.88 ± 0.50 vs. 2.81 ± 0.55 L/min), VT_2 (3.55 ± 0.41 vs. 3.48 ± 0.50 L/min) and $\dot{V}O_2$max (4.05 ± 0.49 vs. 4.01 ± 0.51 L/min) were slightly higher during the high-CHO trial. No statistically significant differences were observed between the two trials for $\dot{V}CO_2$ (L/min) at VT_1 ($p = 0.4791$), VT_2 ($p = 0.5149$) and $\dot{V}O_2$max ($p = 0.5426$) (Table 4.4). Cohen's effect size indicated small practically significant differences for $\dot{V}CO_2$ (L/min) at VT_1 ($d = 0.21$), VT_2 ($d = 0.20$), and $\dot{V}O_2$max ($d = 0.18$) and will therefore not be further discussed (Table 4.4).

Case plots (Figures 4.2a – c) illustrate the individual differences in responses for $\dot{V}CO_2$ (L/min) at VT_1[(a)], VT_2[(b)] and $\dot{V}O_2$max[(c)] between the high-FAT and high-CHO trials. Plots illustrate that ten participants obtained higher $\dot{V}CO_2$ values during the high-FAT compared to the high-CHO trial at VT_1, whereas 14 participants experienced lower values for the high-FAT compared to the high-CHO trial. At VT_2, eight participants obtained higher $\dot{V}CO_2$ values during the high-FAT compared to the high-CHO trial, whereas one participant showed no difference and 15 participants experienced lower values for the high-FAT compared to the high-CHO trial. At $\dot{V}O_2$max, $\dot{V}CO_2$ values indicate that 13 participants obtained higher $\dot{V}CO_2$ values during the high-FAT compared to the high-CHO trial, whereas eleven participants experienced lower values for the high-FAT compared to the high-CHO trial.

(a)



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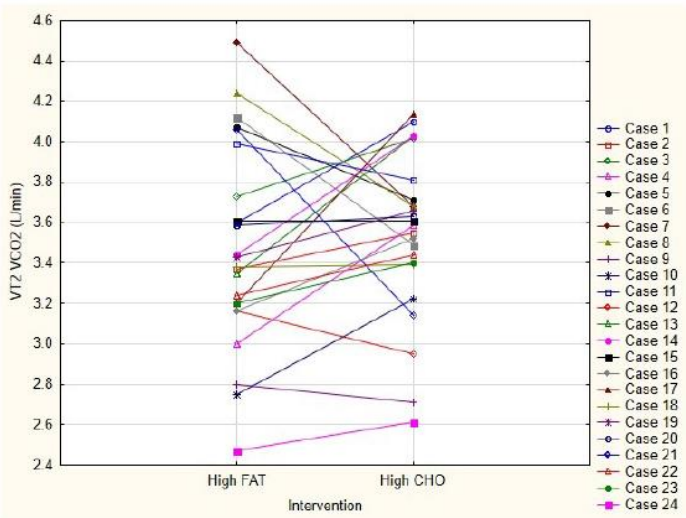


(c)

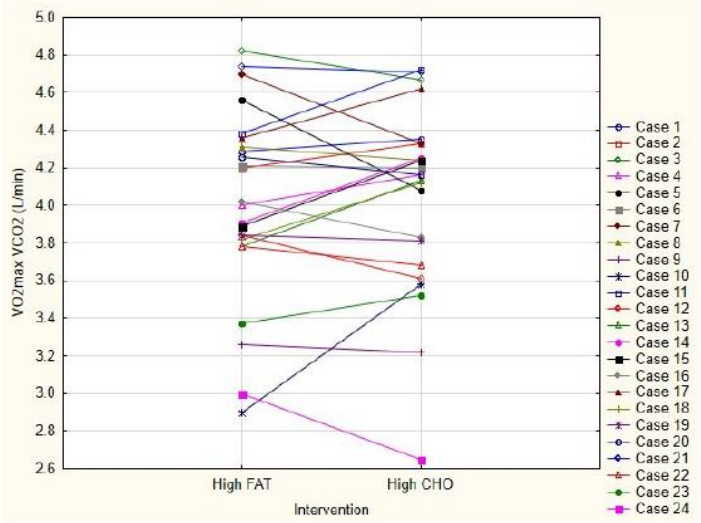
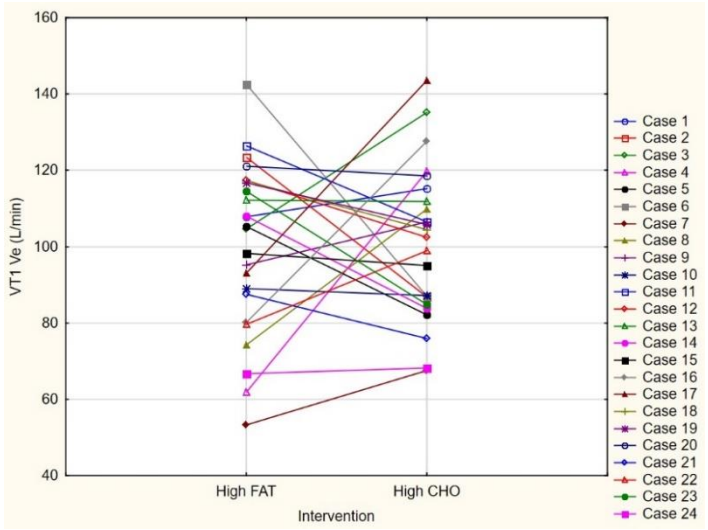


Figure 4.2a – c: Line graphs of the individual differences in $\dot{V}CO_2$ at VT_1, VT_2 and $\dot{V}O_2max$ between the high-FAT and high-CHO trials (N=24)

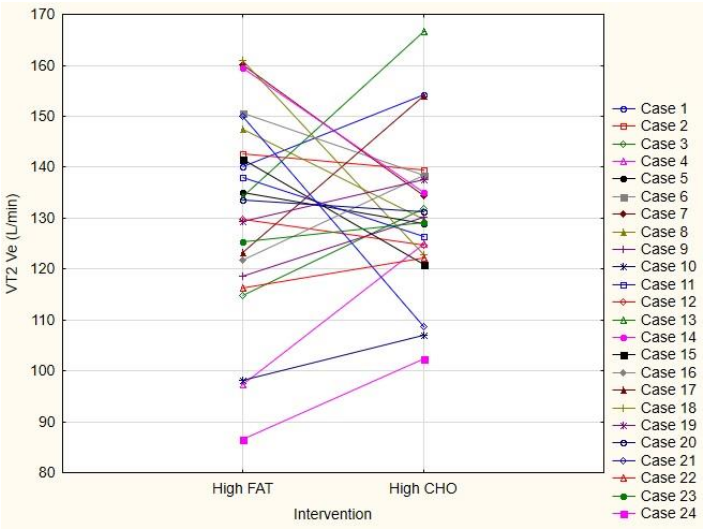
$\dot{V}_E$ (L/min) values at VT_1 (102.03 ± 19.70 vs. 98.83 ± 22.51 L/min), VT_2 (131.83 ± 15.64 vs. 130.43 ± 18.86 L/min) and $\dot{V}O_2max$ (159.28 ± 18.99 vs. 157.36 ± 21.11 L/min) were slightly higher during the high-CHO compared to the high-FAT-trial. No statistically significant differences were found between the two trials for $\dot{V}_E$ (L/min) at VT_1 ($p = 0.49$), VT_2 ($p = 0.78$) and $\dot{V}O_2max$ ($p = 0.62$). Cohen's effect size showed only small practically significant differences at VT_1 ($d = 0.01$), VT_2 ($d = 0.01$) and $\dot{V}O_2max$ ($d = 0.02$) and will therefore not be further discussed (Table 4.4).

Case plots (Figures 4.3a – c), illustrate the individual differences in responses for $\dot{V}_E$ (L/min) at VT_1[(a)], VT_2[(b)] and $\dot{V}O_2max$[(c)] between the high-FAT and high-CHO trials. The plots show that 14 participants obtained higher $\dot{V}_E$ values during the high-FAT compared to the high-CHO trial at VT_1, whereas ten participants experienced lower values for the high-FAT compared to the high-CHO trial. At VT_2, and twelve participants obtained higher $\dot{V}_E$ values during the high-FAT compared to the high-CHO trial, whereas twelve participants experienced lower values for the high-FAT compared to the high-CHO trial. At $\dot{V}O_2max$, $\dot{V}_E$ values indicate that nine participants obtained higher $\dot{V}_E$ values during the high-FAT compared to the high-CHO trial whereas 15 participants experienced lower values for the high-FAT compared to the high-CHO trial.

a)



b)



(c)

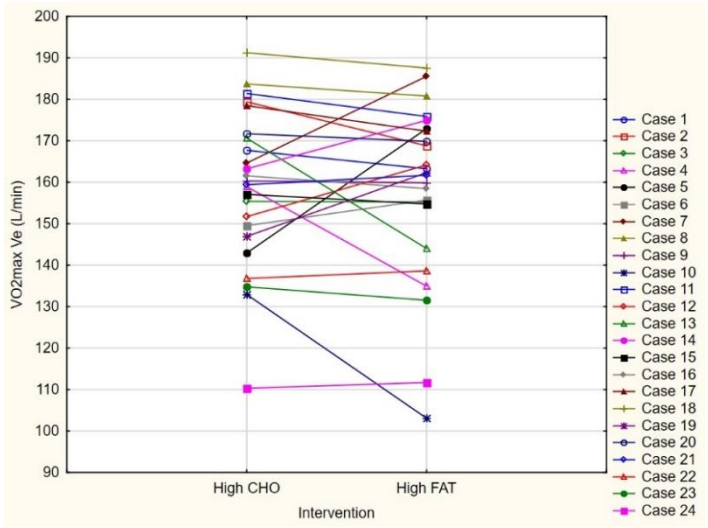


Figure 4.3a – c: Line graphs of the individual differences in $\dot{V}_E$ at VT_1, VT_2 and $\dot{V}O_2$max between the high-FAT and high-CHO trials (N=24)

Table 4.3 also suggests that the percentage of $\dot{V}O_2$max (%$\dot{V}O_2$max) corresponding to $\dot{V}O_2$ (L/min) and $\dot{V}CO_2$ (L/min) were higher during the high-CHO trial at both VT_1 (81.1% of HRmax vs. 77.83% $\dot{V}O_2$max; 73.38% $\dot{V}O_2$max vs. 72.61% $\dot{V}O_2$max) and VT_2 (92.41% $\dot{V}O_2$max vs. 90.39% $\dot{V}O_2$max; 90.51% $\dot{V}O_2$max vs. 89.01% $\dot{V}O_2$max), respectively. The %$\dot{V}O_2$max values corresponding with $\dot{V}_E$ (L/min) were higher during the high-FAT trial at both VT_1 (67.61% $\dot{V}O_2$max vs. 66.98% $\dot{V}O_2$max) and VT_2 (88.55% $\dot{V}O_2$max vs. 86.85% $\dot{V}O_2$max), respectively. No statistically significant differences were found between the two trials for %$\dot{V}O_2$max corresponding with $\dot{V}O_2$ (L/min) at VT_1 ($p = 0.13$), VT_2 ($p = 0.27$). However, Cohen's effect size showed a medium practically significant difference at VT_1 ($d = 0.47$), but a small practically significant difference at VT_2 ($d = 0.34$) (Table 4.5).

No statistically significant differences were found between the two trials for %$\dot{V}O_2$max corresponding with $\dot{V}CO_2$ (L/min) at VT_1 ($p = 0.65$) and VT_2 ($p = 0.55$). Also, Cohen's effect size showed small practically significant differences at VT_1 ($d = 0.14$) and VT_2 ($d = 0.18$) and will therefore not be discussed further (Table 4.5). No statistically significant differences were found between the two trials for %$\dot{V}O_2$max corresponding to $\dot{V}_E$ (L/min) at VT_1 ($p = 0.98$) and VT_2 ($p = 0.50$). Cohen's effect size also showed small practical significant differences at VT_1 ($d = 0.01$) and VT_2 ($d = -0.21$), which does not warrant further discussion (Table 4.5).

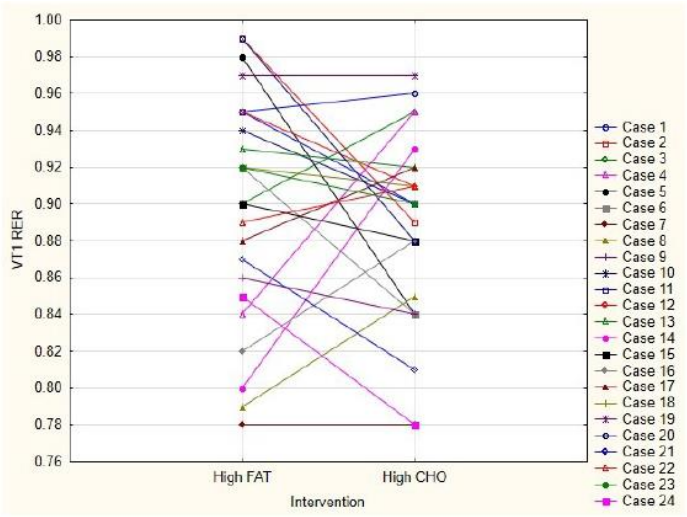
4.3.2 Substrate metabolism

No statistically significant differences ($p = 0.54$) were observed between the two trials for resting RER values (Table 4.6). Furthermore, no significant differences were found for the RER values at the ventilatory threshold points between the two trials (VT_1 - $p = 0.46$; VT_2 - $p = 0.32$; $\dot{V}O_2$max - $p = 0.92$) (Table 4.6). Cohen's effect size indicated a small practically significant difference at VT_1 ($d = -0.23$), VT_2 ($d = -0.30$), $\dot{V}O_2$max ($d = -0.24$) and Maximal Fat Oxidation (MFO) ($d = 0.34$) for the two trials (Table 4.6).

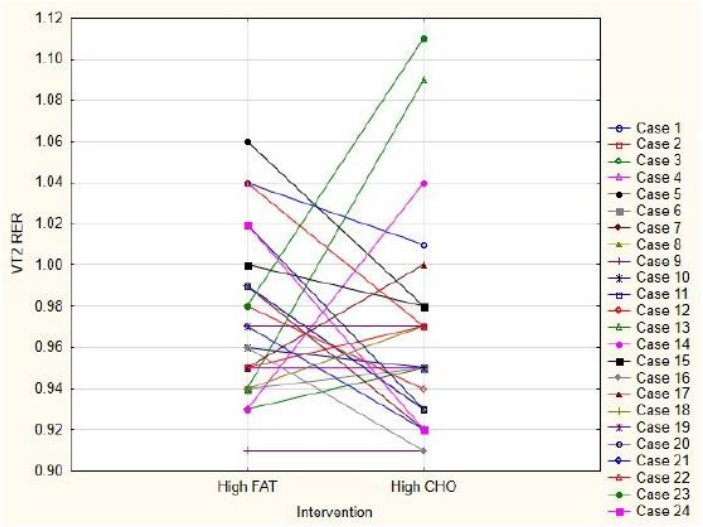
Figure 4.4a – c, and figure 4.5 illustrate the individual and overall differences in RER value responses at $VT_1^{(a)}$, $VT_2^{(b)}$ and $\dot{V}O_2max^{(c)}$ between the high-FAT and high-CHO trials. The RER case plots (Figure 4.4a – c) show that 13 participants responded positively to the high-FAT compared to the high-CHO trial at VT_1, whereas two participants showed no differences and nine participants experienced lower values for the high-FAT compared to the high-CHO trial. At VT_2, 13 participants responded positively to the high-FAT compared to the high-CHO trial, whereas three participants showed no differences and eight participants experienced lower values for the high-FAT compared to the high-CHO trial. At

$\dot{V}O_2$max, RER values increased for nine participants with the high-FAT compared to the high-CHO trial, whereas four participants showed no differences and eleven participants experienced lower values for the high-FAT compared to the high-CHO trial. Figure 4.5 illustrates the drifting of overall RER values for the two respective trials.

(a)



(b)



(c)

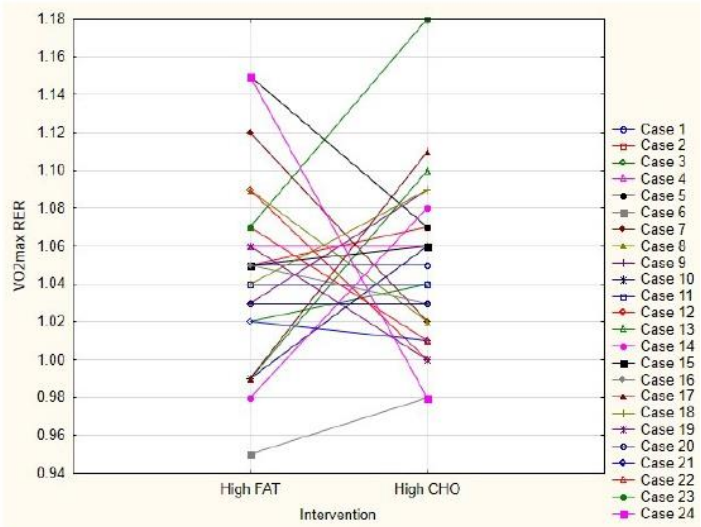


Figure 4.4a – c: Line graphs of the individual differences in RER at VT_1, VT_2 and $\dot{V}O_2max$ between the high-FAT and high-CHO trials (N=24)

Figure 4.5 shows the relationship between the rates of CHO oxidation (CHOox) and fat oxidation (FATox) during the high-CHO and high-FAT trials at the different exercise intensities, expressed as percentage of $\dot{V}O_2$max (%$\dot{V}O_2$max). In view of the fact that many subjects did not reach a running speed of higher than 15 km/h during the incremental running test, the only values that were considered for the calculation of CHOox and FATox rates were those collected during the first six exercise intensities – 10 to 15 km/h. Only 22 participants were able to complete these stages and, therefore, data of only 22 participants are displayed in Figure 4.5 as well as in Table 4.11. The same running intensity values were also applied for determination of MFO, since all running intensity stages obtained RER values of smaller than one, which is a prerequisite for the determination of MFO. The FATox rate of all subjects was the highest during the first exercise intensity (10 km/h) and decreased from this intensity level onwards. Consequently, the exercise intensities below FAT_{max} could not be determined. FAT_{max} represents the exercise intensity (%$\dot{V}O_2$max) where MFO was identified.

Table 4.9 indicates the statistically and practically significant differences of the FATox rates over increasing exercise intensities between the high-CHO and high-FAT trials. On the other hand, Table 4.10 presents the statistically and practically significant differences of the CHOox rates over increasing exercise intensity between the high-CHO and high-FAT trials. No statistically significant differences were observed for the CHOox and FATox rates over increasing exercise intensities between the respective trials.

Cohen's effect size value (d) showed small practically significant differences for FATox rates at all 6 stages (d = 0.36; d = 0.10; d =0.03; d = 0.5; d = 0.17, respectively) and also for CHOox rates at all stages (d = -0.45; d = -0.14; d = -0.00; d = -0.06; d = -0.04; d = -0.12, respectively) between the high-FAT and high-CHO trials (Table 4.9). During the high-CHO trial the mean FATox was 0.83 ± 0.22 g/min at 65% of $\dot{V}O_2$max with a maximal oxidation rate of 1.39 g/min. The high-FAT trial showed a similar mean FATox of 0.79 ± 0.22 g/min at 66% of $\dot{V}O_2$max with a maximal oxidation rate of 1.18 g/min.

Tables 4.7 and 4.8 show that resting CHOox rates over the two trials were 0.45 ± 0.26 and 0.51 ± 0.33 g/min for the high-CHO and high-FAT trials, respectively. Resting FATox rates were 0.33 ± 0.14 and 0.34 ± 0.15 g/min for the high-CHO and high-FAT trials, respectively. No statistically ($p = 0.72$ and $p = 0.38$) or practically significant differences (d = -0.27 and d = -0.11) were found for the resting FATox and CHOox rates, between the two trials, respectively.

Table 4.4: Ventilatory and metabolic response: Analysis of Variance (ANOVA)

Time point	Variable	Treatment Mean[1]		Mean difference[1]: CHO – FAT			
		High-CHO	High-FAT	Point Estimate	95% Confidence Interval	[2]P-value	[3]Effect size (d)
VT_1	RER	0.89	0.90	-0.01	-0.04 to 0.02	0.46	-0.23
	$\dot{V}O_2$/kg (ml/kg/min)	41.21	38.42	2.79	-0.02 to 5.60	0.05	0.61
	$\dot{V}O_2$ (L/min)	3.24	3.03	0.21	-0.01 to 0.42	0.06	0.58
	$\dot{V}CO_2$(L/min)	2.90	2.80	0.10	-0.18 to 0.37	0.48	0.21
	$\dot{V}E$ (L/min)	102.54	98.33	4.21	-8.28 to 16.69	0.49	0.21
VT_2	RER	0.96	0.98	-0.01	-0.04 to 0.01	0.32	-0.30
	$\dot{V}O_2$/kg (ml/kg/min)	46.78	45.05	1.73	-0.57 to 4.04	0.13	0.47
	$\dot{V}O_2$ (L/min)	3.68	3.56	0.12	-0.06 to 0.30	0.18	0.41
	$\dot{V}CO_2$(L/min)	3.55	3.48	0.07	-0.15 to 0.28	0.51	0.20
	$\dot{V}E$ (L/min)	131.75	130.50	1.25	-7.87 to 10.37	0.78	0.10
$\dot{V}O_2$max	RER	1.05	1.05	-0.002	-0.03 to 0.03	0.92	-0.03
	$\dot{V}O_2$/kg (ml/kg/min)	51.48	50.97	0.50	-0.78 to 1.78	0.43	0.24
	$\dot{V}O_2$ (L/min)	4.06	4.02	0.03	-0.06 to 0.12	0.47	0.22
	$\dot{V}CO_2$(L/min)	4.05	4.01	0.04	-0.09 to 0.16	0.54	0.18
	$\dot{V}E$ (L/min)	159.05	157.59	1.46	-4.55 to 7.48	0.62	0.15

1Treatment means, and point estimate and 95% confidence interval of for the mean difference “CHO – FAT” from an analysis of variance of the outcome variable, fitting treatment, period and subject as fixed effects (ANOVA of 2-treatment, 2-period cross-over data).

2P-value for t-test of the null-hypothesis that the mean difference is 0 (that is, null-hypothesis of no difference between CHO and FAT), from the analysis of variance.

3Effect size calculated as the ratio of the point estimate of the mean difference “CHO – FAT” divided by the residual standard deviation from the ANOVA.

Table 4.5: Percentage of $\dot{V}O_2$max: Analysis of Variance (ANOVA)

Time point	Variable	Treatment Mean[1]		Mean difference[1]: CHO – FAT			
		High-CHO	High-FAT	Point Estimate	95% Confidence Interval	[2]P-value	[3]Effect size (d)
VT_1	$\dot{V}O_2$ (L/min)	81.51	77.42	4.09	-1.31 to 9.48	0.13	0.47
	$\dot{V}CO_2$(L/min)	73.76	72.23	1.53	-5.43 to 8.49	0.65	0.14
	$\dot{V}E$ (L/min)	67.35	67.24	0.10	-7.69 to 7.90	0.98	0.01
VT_2	$\dot{V}O_2$ (L/min)	92.55	90.24	2.31	-1.95 to 6.57	0.27	0.34
	$\dot{V}CO_2$(L/min)	90.47	89.05	1.42	-3.49 to 6.29	0.55	0.18
	$\dot{V}E$ (L/min)	86.79	88.61	-1.83	-7.30 to 3.65	0.50	-0.21

1Treatment means, and point estimate and 95% confidence interval of for the mean difference “CHO – FAT” from an analysis of variance of the outcome variable, fitting treatment, period and subject as fixed effects (ANOVA of 2-treatment, 2-period cross-over data).2P-value for t-test of the null-hypothesis that the mean difference is 0 (that is, null-hypothesis of no difference between CHO and FAT), from the analysis of variance.

3Effect size calculated as the ratio of the point estimate of the mean difference “CHO – FAT” divided by the residual standard deviation from the ANOVA.

Table 4.6: Respiratory exchange ratio: Analysis of Variance (ANOVA).

Time point	Variable	Treatment Mean[1]		Mean difference[1]: CHO – FAT			
		High-CHO	High-FAT	Point Estimate	95% Confidence Interval	[2]P-value	[3]Effect size (d)
Resting	RER	0.80	0.81	-0.01	-0.05 to 0.02	0.54	-0.18
VT_1	RER	0.89	0.90	-0.01	-0.04 to 0.02	0.46	-0.23
VT_2	RER	0.96	0.98	-0.01	-0.04 to 0.01	0.32	-0.30
$\dot{V}O_2max$	RER	1.05	1.05	-0.002	-0.03 to 0.03	0.92	-0.24
MFO	RER	0.84	0.78	0.05	-0.04 to 0.15	0.26	0.34

1Treatment means, and point estimate and 95% confidence interval of for the mean difference "CHO – FAT" from an analysis of variance of the outcome variable, fitting treatment, period and subject as fixed effects (ANOVA of 2-treatment, 2-period cross-over data).

2P-value for t-test of the null-hypothesis that the mean difference is 0 (that is, null-hypothesis of no difference between CHO and FAT), from the analysis of variance.

3Effect size calculated as the ratio of the point estimate of the mean difference "CHO – FAT" divided by the residual standard deviation from the ANOVA.

Table 4.7: Resting CHO oxidation and FAT oxidation rates: Descriptive statistics (N=24)

		Test	
		High-CHO	High-Fat
FATox (g/min)	Mean	0.33	0.34
	Std	0.14	0.15
	Min	-0.03	0.01
	Median	0.34	0.33
	Max	0.54	0.57
CHOox (g/min)	Mean	0.45	0.51
	Std	0.26	0.33
	Min	-0.11	-0.02
	Median	0.39	0.51
	Max	1.09	1.44

Note: N: Number of participants; **SD:** Standard Deviation. **Min:** The smallest value in the data set. **Max:** The largest value in the data set.

Table 4.8: Resting CHO oxidation and FAT oxidation rates: Analysis of Variance (ANOVA)

Time point	Variable	Treatment Mean[1]		Mean difference[1]: CHO – FAT			
		High-CHO	High-FAT	Point Estimate	95% Confidence Interval	[2]P-value	[3]Effect size (d)
Resting	CHOox (g/min)	0.44	0.52	-0.07	-0.24 to 0.10	0.38	-0.27
	FATox (g/min)	0.33	0.34	-0.01	-0.09 to 0.07	0.72	-0.11

1Treatment means, and point estimate and 95% confidence interval of for the mean difference "CHO – FAT" from an analysis of variance of the outcome variable, fitting treatment, period and subject as fixed effects (ANOVA of 2-treatment, 2-period cross-over data).

2P-value for t-test of the null-hypothesis that the mean difference is 0 (that is, null-hypothesis of no difference between CHO and FAT), from the analysis of variance.

3Effect size calculated as the ratio of the point estimate of the mean difference "CHO – FAT" divided by the residual standard deviation from the ANOVA.

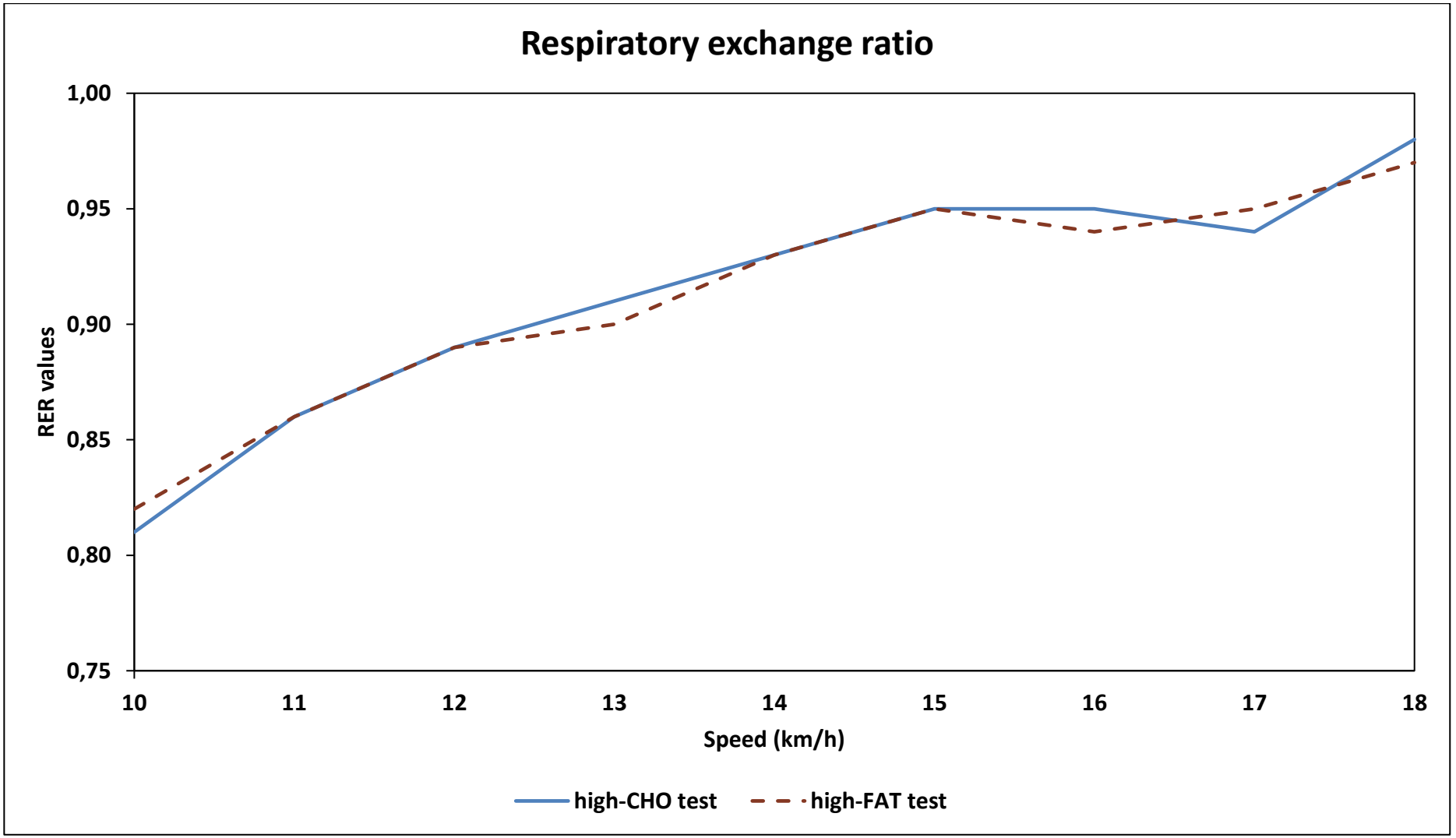


Figure 4.5: RER values plotted over speed for high-CHO and high-FAT tests (N=24)

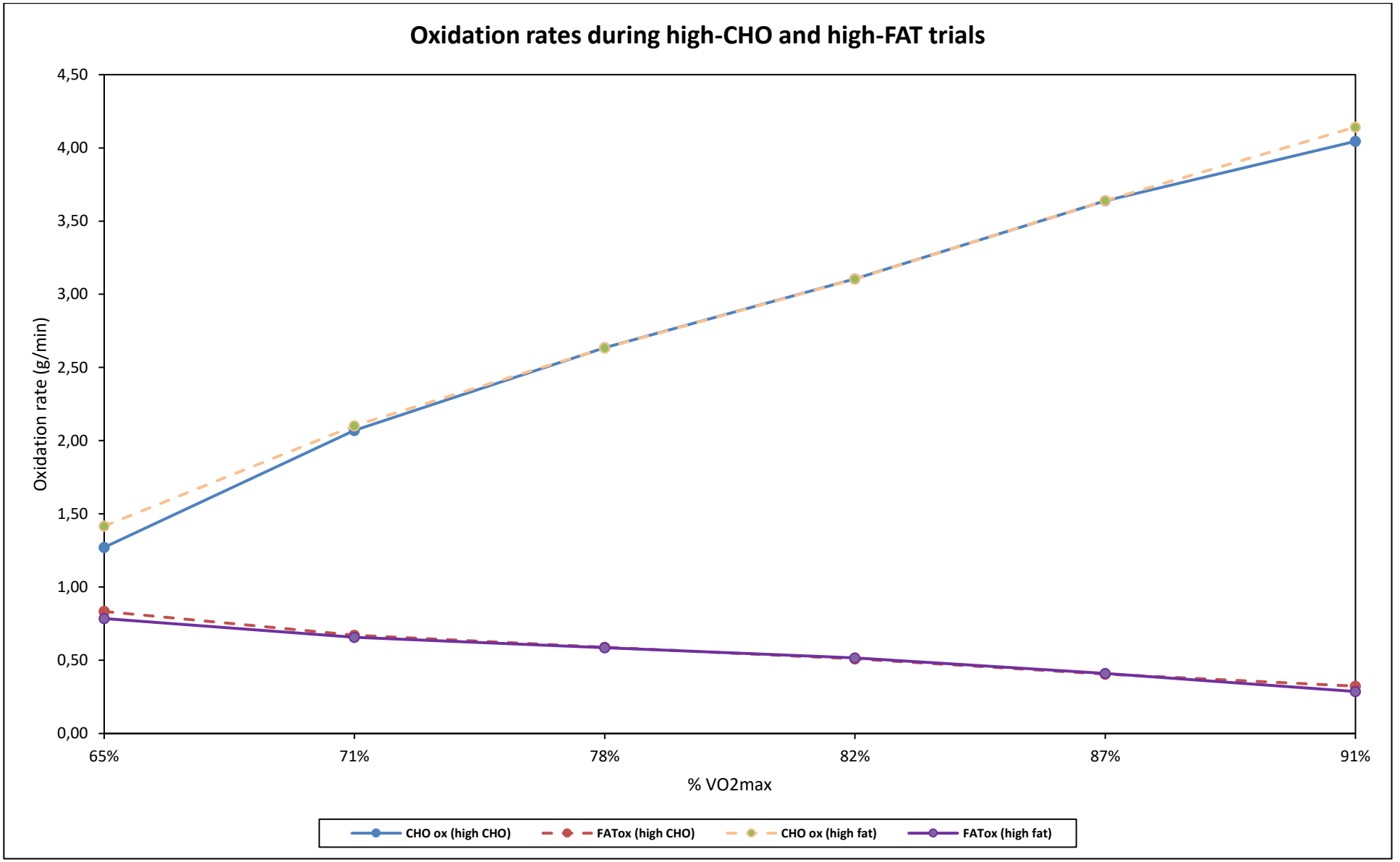


Figure 4.6: Oxidation rates during the high-CHO and high-FAT trials (N=22)

Table 4.9: FAT oxidation rate (g/min): Analysis of Variance (ANOVA)

Stage/Speed	Treatment Mean[1]		Mean difference[1]: CHO – FAT			
	High-CHO	High-FAT	Point Estimate	95% Confidence Interval	[2]P-value	[3]Effect size
Stage 1 / 10 km/h	0.84	0.78	0.06	-0.04 to 0.15	0.24	0.36
Stage 2 / 11 km/h	0.67	0.66	0.02	-0.08 to 0.11	0.74	0.10
Stage 3 / 12 km/h	0.59	0.59	-0.00	-0.10 to 0.11	0.93	0.03
Stage 4 / 13 km/h	0.51	0.51	-0.01	-0.11 to 0.12	0.92	0.03
Stage 5 / 14 km/h	0.41	0.40	-0.01	-0.11 to 0.13	0.89	0.05
Stage 6 / 15 km/h	0.28	0.25	0.03	-0.10 to 0.16	0.61	0.17

1Treatment means, and point estimate and 95% confidence interval of for the mean difference “CHO – FAT” from an analysis of variance of the outcome variable, fitting treatment, period and subject as fixed effects (ANOVA of 2-treatment, 2-period cross-over data).

2P-value for t-test of the null-hypothesis that the mean difference is 0 (that is, null-hypothesis of no difference between CHO and FAT), from the analysis of variance.

3Effect size calculated as the ratio of the point estimate of the mean difference “CHO – FAT” divided by the residual standard deviation from the ANOVA.

Table 4.10: Carbohydrate oxidation rate (g/min): Analysis of Variance (ANOVA)

Speed	Treatment Mean[1]		Mean difference[1]: CHO – FAT			
	High-CHO	High-FAT	Point Estimate	95% Confidence Interval	[2]P-value	[3]Effect size
Stage 1 / 10 km/h	1.26	1.43	-0.17	-0.42 to 0.07	0.15	-0.45
Stage 2 / 11 km/h	2.06	2.11	0.05	-0.26 to 0.17	0.65	-0.14
Stage 3 / 12 km/h	2.63	2.64	-0.00	-0.23 to 0.22	0.99	-0.00
Stage 4 / 13 km/h	3.09	3.12	0.03	-0.28 to 0.23	0.84	-0.06
Stage 5 / 14 km/h	3.63	3.65	0.02	-0.29 to 0.25	0.89	-0.04
Stage 6 / 15 km/h	4.18	4.23	-0.05	-0.36 to 0.25	0.72	-0.12

1Treatment means, and point estimate and 95% confidence interval of for the mean difference “CHO – FAT” from an analysis of variance of the outcome variable, fitting treatment, period and subject as fixed effects (ANOVA of 2-treatment, 2-period cross-over data).

2P-value for t-test of the null-hypothesis that the mean difference is 0 (that is, null-hypothesis of no difference between CHO and FAT), from the analysis of variance.

3Effect size calculated as the ratio of the point estimate of the mean difference “CHO – FAT” divided by the residual standard deviation from the ANOVA.

Table 4.11: Carbohydrate oxidation and FAT oxidation rates, respiratory exchange ratio and percentage $\dot{V}O_2$max during each stage of the graded exercise tests (N=22)

	High-CHO trial				High-FAT trial			
Stage/ Speed (km/h)	CHOox (g/min)	FATox (g/min)	RER	$\dot{V}O_2$/kg (ml/kg/min)	CHOox (g/min)	FATox (g/min)	RER	$\dot{V}O_2$/kg (ml/kg/min)
Stage 1 / 10 km/h	1.27	0.83	0.81	65%	1.42	0.79	0.82	66%
Stage 2 / 11 km/h	2.07	0.67	0.86	71%	2.10	0.66	0.86	72%
Stage 3 / 12 km/h	2.64	0.59	0.89	78%	2.63	0.59	0.89	78%
Stage 4 / 13 km/h	3.11	0.51	0.91	82%	3.11	0.51	0.90	83%
Stage 5 / 14 km/h	3.64	0.41	0.93	87%	3.64	0.41	0.93	88%
Stage 6 / 15 km/h	4.04	0.32	0.95	91%	4.14	0.29	0.95	91%

4.4 Physiological and Perceptual measures

This section provides the results on the respective individual physiological and perceptual measures as well as the comparison of relevant variables between the two trials.

4.4.1 Rate of perceived exertion

The rating of perceived exertion (RPE) (6 – 20) values were similar at VT_1 (10.29 ± 2.42 vs. 9.75 ± 2.94), VT_2 (15.25 ± 2.36 vs. 15.08 ± 2.39) and $\dot{V}O_2$max (20.00 ± 0.00 vs. 19.92 ± 0.28) during the high-CHO trial compared to the high-FAT trial (Table 4.12). However, no statistically significant differences were observed for RPE values at VT_1 ($p = 0.56$), VT_2 ($p = 0.90$) and $\dot{V}O_2$max ($p = 0.27$) between the high-CHO and high-FAT trials, respectively. Furthermore, only small practically significant differences were detected for REP values at VT_1 ($d = 0.18$), VT_2 ($d = 0.04$) and the $\dot{V}O_2$max ($d = 0.34$) between the different trials (Table 4.13).

4.4.2 Heart rate

Heart rate values recorded during both trials are presented in Table 4.12. No statistically significant differences were obtained for HR between trials at VT_1 ($p = 0.21$), VT_2 ($p = 0.23$) and $\dot{V}O_2$max ($p = 0.38$), respectively. Cohen's effect size showed a medium practically significant difference for HR at $\dot{V}O_2$max ($d = 41$) and small practically significant differences for HR at VT_1 ($d = 0.38$) and VT_2 ($d = 0.37$) (Table 4.14). VT_1 was reached at 86% and 85% of HRmax during the high-CHO and high-FAT trials, respectively. VT_2 was reached at 97% and 96% of HR max during the high-CHO and high-FAT trials, respectively (Table 4.18).

Table 4.12: Physiological and perceptual measures: Descriptive statistics (N=24)

		VT_1		VT_2		VT_1 (% of $\dot{V}O_2max$)		VT_2 (% of $\dot{V}O_2max$)		Absolute Maximum Values	
Variable		High-CHO	High-Fat	High-CHO	High-Fat	High-CHO	High-Fat	High-CHO	High-Fat	High-CHO	High-Fat
		24	24	24	24	24	24	24	24	24	24
Borg (6-20)	Mean	10.29	9.75	15.25	15.08	.	.	.	.	20.00	19.92
	Std	2.42	2.94	2.36	2.39	.	.	.	.	0.00	0.28
	Min	7.00	6.00	10.00	11.00	.	.	.	.	20.00	19.00
	Median	10.00	9.00	16.00	15.00	.	.	.	.	20.00	20.00
	Max	15.00	16.00	19.00	19.00	.	.	.	.	20.00	20.00
HR (bpm)	Mean	163.46	159.5	181.83	178.71	87.16	86.18	97.06	96.54	189.5	188.21
	Std	14.91	17.14	8.13	9.44	6.64	8.85	3.72	3.55	7.82	7.19
	Min	126.00	122.00	167.00	158.00	72.00	66.67	89.11	88.46	173.00	174.00
	Median	166.00	160.50	181.50	180.50	88.16	87.24	96.83	96.25	189.00	190.00
	Max	182.00	188.00	202.00	196.00	96.15	98.43	106.17	103.57	204.00	201.00
Speed (km/h)	Mean	12.88	12.21	15.04	15.21	.	.	.	.	16.04	16.25
	Std	1.60	1.50	1.57	1.82	.	.	.	.	1.33	1.67

	Min	10.00	10.00	12.00	12.00	.	.	.	.	14.00	13.00
	Median	13.50	12.00	15.00	15.00	.	.	.	.	16.00	16.00
	Max	16.00	15.00	18.00	19.00	.	.	.	.	19.00	20.00
Work (kJ)	Mean	672.58	649.57	1203.95	1258.88	47.72	49.56	84.45	92.10	1544.23	1547.58
	Std	359.49	358.53	375.77	465.22	24.12	26.82	15.35	18.09	324.68	401.33
	Min	112.67	46.02	627.60	585.76	10.15	3.74	47.67	53.05	949.77	648.52
	Median	719.65	692.45	1104.58	1184.07	53.13	53.01	84.17	95.71	2654.75	1458.12
	Max	1380.72	1355.62	2008.32	2267.73	92.53	91.73	116.49	121.50	2405.80	2422.54
Absolute power output (W)	Mean	270.58	268.08	320.75	327.25	78.42	78.83	92.69	95.80	358.92	359.04
	Std	43.55	50.81	42.39	51.79	10.44	12.40	5.44	6.35	44.53	48.45
	Min	165.00	166.00	224.00	226.00	56.31	47.54	78.78	82.68	243.00	259.00
	Median	271.50	278.00	315.00	325.50	79.03	81.94	92.60	97.11	371.00	357.00
	Max	349.00	345.00	390.00	423.00	95.74	96.21	105.31	105.02	431.00	435.00
Relative power output (W/kg)	Mean	3.45	3.38	4.07	4.14	78.91	78.96	92.80	95.89	4.53	4.54
	Std	0.49	0.51	0.47	0.56	10.49	12.50	5.80	6.70	0.40	0.52
	Min	2.60	2.40	3.30	3.40	56.60	47.17	77.27	82.93	3.90	3.60

	Median	3.45	3.45	4.05	4.00	80.20	81.39	92.68	97.33	4.40	4.40
	Max	4.40	4.20	5.00	5.40	97.56	95.24	106.38	105.13	5.50	5.60

Note: N: Number of participants; **SD:** Standard Deviation. **Min:** The smallest value in the data set. **Max:** The largest value in the data set; **VT1 (% of max):** percentage of $\dot{V}O_2$max at VT1; **VT2 (% of max):** percentage of $\dot{V}O_2$max at VT2; **VT1:** first ventilatory threshold; **VT2:** second ventilatory threshold.

Table 4.13: Rating of perceived exertion and speed: Analysis of Variance (ANOVA)

Time point	Variable	Treatment Mean[1]		Mean difference[1]: CHO – FAT			
		High-CHO	High-FAT	Point Estimate	95% Confidence Interval	[2]P-value	[3]Effect size (d)
VT_1	RPE (6-20)	10.29	9.75	0.54	-1.35 to 2.44	0.56	0.18
	Speed (km/h)	12.98	12.28	0.69	-0.21 to 1.60	0.12	0.48
VT_2	RPE (6-20)	15.20	15.13	0.07	-1.05 to 1.18	0.90	0.04
	Speed (km/h)	15.09	15.22	-0.13	-0.73 to 0.48	0.63	-0.13
Absolute maximum values	RPE (6-20)	19.99	19.93	0.07	-0.06 to 0.19	0.27	0.34
	Speed (km/h)	16.07	16.28	-0.20	-0.72 to 0.32	0.43	-0.24

1Treatment means, and point estimate and 95% confidence interval of for the mean difference "CHO – FAT" from an analysis of variance of the outcome variable, fitting treatment, period and subject as fixed effects (ANOVA of 2-treatment, 2-period cross-over data).

2P-value for t-test of the null-hypothesis that the mean difference is 0 (that is, null-hypothesis of no difference between CHO and FAT), from the analysis of variance.

3Effect size calculated as the ratio of the point estimate of the mean difference "CHO – FAT" divided by the residual standard deviation from the ANOVA.

Table 4.14: Heart rate (bpm): Analysis of Variance (ANOVA)

Time point	Treatment Mean[1]		Mean difference[1]: CHO – FAT			
	High-CHO	High-FAT	Point Estimate	95% Confidence Interval	[2]P-value	[3]Effect size (d)
VT_1	163.62	159.34	4.28	-2.63 to 11.18	0.21	0.38
VT_2	181.39	179.15	2.23	-1.48 to 5.94	0.23	0.37
$\dot{V}O_2$max (ml.kg/min)	189.27	188.44	0.83	-1.12 to 2.78	0.38	0.41

1Treatment means, and point estimate and 95% confidence interval of for the mean difference "CHO – FAT" from an analysis of variance of the outcome variable, fitting treatment, period and subject as fixed effects (ANOVA of 2-treatment, 2-period cross-over data).

2P-value for t-test of the null-hypothesis that the mean difference is 0 (that is, null-hypothesis of no difference between CHO and FAT), from the analysis of variance.

3Effect size calculated as the ratio of the point estimate of the mean difference "CHO – FAT" divided by the residual standard deviation from the ANOVA.

4.4.3 Time to exhaustion, absolute and relative power output

The total time to exhaustion for the high-FAT and high-CHO diets averaged 25.26 ± 4.80 and 25.37 ± 6.13 minutes, respectively (Table 4.15). No statistically ($p = 0.86$) or practically significant differences ($d = -0.05$) were observed for time to exhaustion values between the trials (Table 4.16).

Table 4.15: Time to exhaustion (N=24)

Time to exhaustion (minutes)		
	Test	
	CHO	Fat
Mean	25.26	25.37
Std	4.80	6.13
Min	18.15	13.45
Q1	22.23	21.3
Median	24.15	23.3
Q3	29.08	31.15
Max	37	38.15

Note: N: Number of athletes. **SD:** Standard Deviation. **Min:** The smallest value in the data set. **Q1:** The median of the lower half of the data. **Q3:** The median of the upper half of the data. **Max:** The largest value in the data set.

Table 4.17 presents the statistically and practically significant differences in absolute (W) and relative power output (W/kg) recorded at VT_1, VT_2 and $\dot{V}O_2max$. No statistically significant differences ($p > 0.05$) were observed between trials for absolute (W) and relative power (W/kg). Cohen's effect size values showed small significant differences for absolute and relative power output at VT_1 ($d = 0.17$ and $d = 0.25$), VT_2 ($d = -0.37$ and $d = -0.31$) and $\dot{V}O_2max$ ($d = 0.28$ and $d = 0.27$) (Table 4.17). The absolute and relative power outputs at VT_1 were 270.58 ± 43.55 and 268.08 ± 50.81 W, as well as 3.45 ± 0.49 and 3.38 ± 0.51 W/kg for the high-CHO and high-FAT trials, respectively. At VT_2, the absolute power outputs were 358.92 ± 44.53 and 359.04 ± 48.45 W, whereas the relative power outputs were 4.53 ± 0.4 and 4.54 ± 0.52 W/kg for the high-CHO and high-FAT trials, respectively (Table 4.12). The biggest difference in absolute power values between trials was observed for VT_2 (423 vs. 390 W) (Table 4.12). Table 4.18 provides a summary of the differences in absolute and relative power outputs at VT_1, VT_2 and LT.

Case plots (Figure 4.7a – c) for absolute power output show that twelve participants responded positively and twelve participants negatively to the high-FAT compared to the high-CHO trial at VT_1. At VT_2, 15 participants responded positively to the high-FAT compared to the high-CHO trial, whereas nine participants experienced lower values for the high-FAT compared to the high-CHO trial. Absolute power values at $\dot{V}O_2max$, indicate that 13 participants responded positively to the high-FAT compared to the high-CHO trial, whereas one participant showed no difference and ten participants experienced lower values for the high-FAT compared to the high-CHO trial.

Case plots (Figure 4.8a – c) for relative power output show that eleven participants responded positively to the high-FAT compared to the high-CHO trial at VT_1, whereas two participants showed no difference and eleven participants experienced lower values for the high-FAT compared to the high-CHO trial. At VT_2, 14 participants responded positively to the high-FAT compared to the high-CHO trial whereas one participant showed no difference and nine participants experienced lower values for the high-FAT compared to the high-CHO trial. Relative power outputs at $\dot{V}O_2max$, indicate that nine participants responded positively to the high-FAT compared to the high-CHO trial, whereas six participant showed no difference and nine participants experienced lower values for the high-FAT compared to the high-CHO trial.

Table 4.16: Time to exhaustion: Analysis of variance (ANOVA)

Variable	Treatment Mean[1]		Mean difference[1]: CHO – FAT			
	High-CHO	High-FAT	Point Estimate	95% Confidence Interval	[2]P-value	[3]Effect size (d)
Total time to exhaustion (min)	25.24	25.40	-0.15	-1.87 to 1.58	0.86	-0.05

1Treatment means, and point estimate and 95% confidence interval of for the mean difference "CHO – FAT" from an analysis of variance of the outcome variable, fitting treatment, period and subject as fixed effects (ANOVA of 2-treatment, 2-period cross-over data).

2P-value for t-test of the null-hypothesis that the mean difference is 0 (that is, null-hypothesis of no difference between CHO and FAT), from the analysis of variance.

3Effect size calculated as the ratio of the point estimate of the mean difference "CHO – FAT" divided by the residual standard deviation from the ANOVA.

Table 4.17: Absolute and relative power outputs: Analysis of Variance (ANOVA)

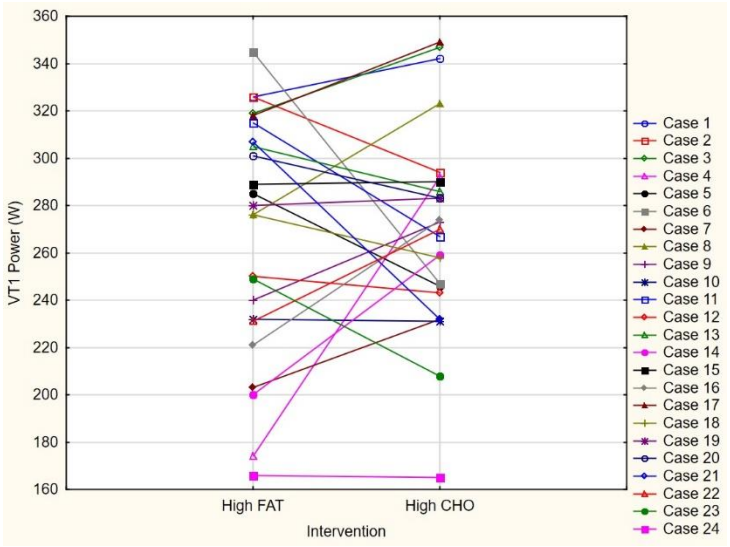
Time point	Variable	Treatment Mean[1]		Mean difference[1]: CHO – FAT			
		High-CHO	High-FAT	Point Estimate	95% Confidence Interval	[2]P-value	[3]Effect size (d)
VT_1	Relative power output (W/kg)	3.47	3.36	0.11	-0.15 to 0.37	0.41	0.25
	Absolute power output (W)	272.08	266.59	5.49	-14.80 to 25.77	0.58	0.17
VT_2	Relative power output (W/kg)	4.06	4.15	-0.08	-0.24 to 0.08	0.30	-0.31
	Absolute power output (W)	320.46	327.54	-7.09	-19.09 to 4.91	0.23	-0.37
$\dot{V}O_2max$	Relative power output (W/kg)	4.53	4.5	-0.01	-0.16 to 0.13	0.85	0.27
	Absolute power output (W)	358.74	359.22	-0.48	-11.61 to 10.66	0.93	0.28

1Treatment means, and point estimate and 95% confidence interval of for the mean difference "CHO – FAT" from an analysis of variance of the outcome variable, fitting treatment, period and subject as fixed effects (ANOVA of 2-treatment, 2-period cross-over data).

2P-value for t-test of the null-hypothesis that the mean difference is 0 (that is, null-hypothesis of no difference between CHO and FAT), from the analysis of variance.

3Effect size calculated as the ratio of the point estimate of the mean difference "CHO – FAT" divided by the residual standard deviation from the ANOVA.

(a)



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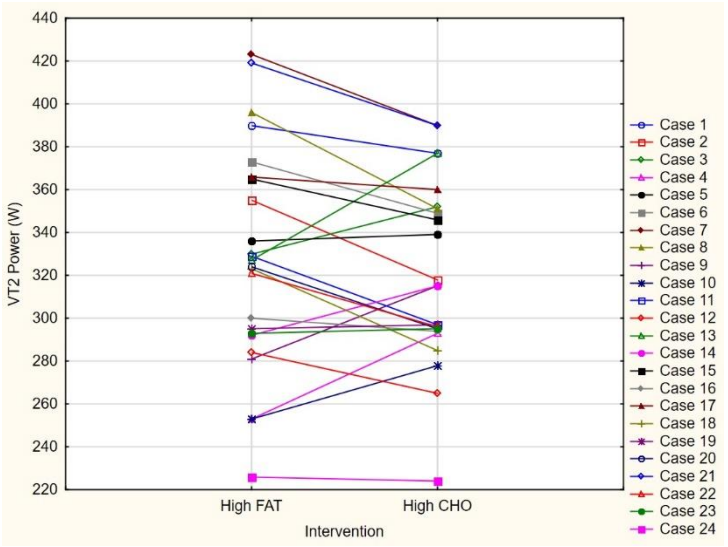

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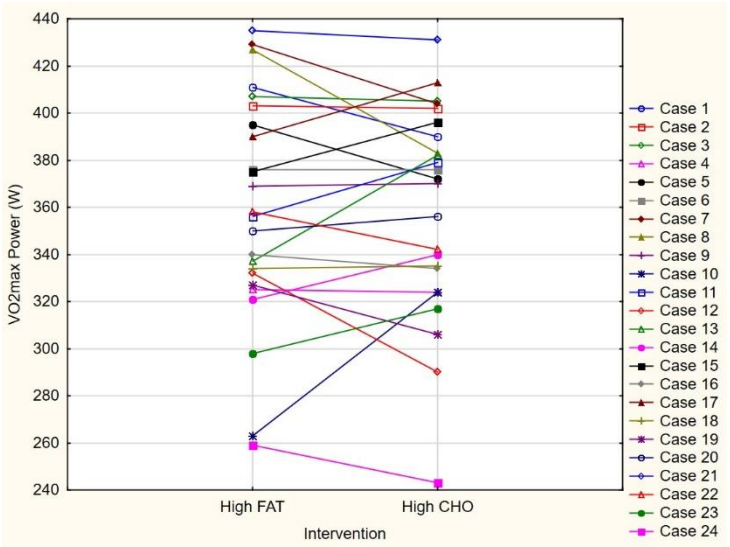
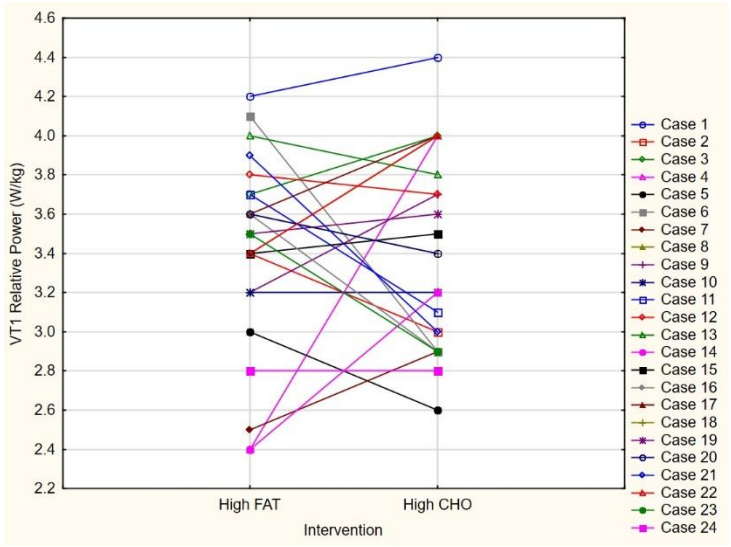
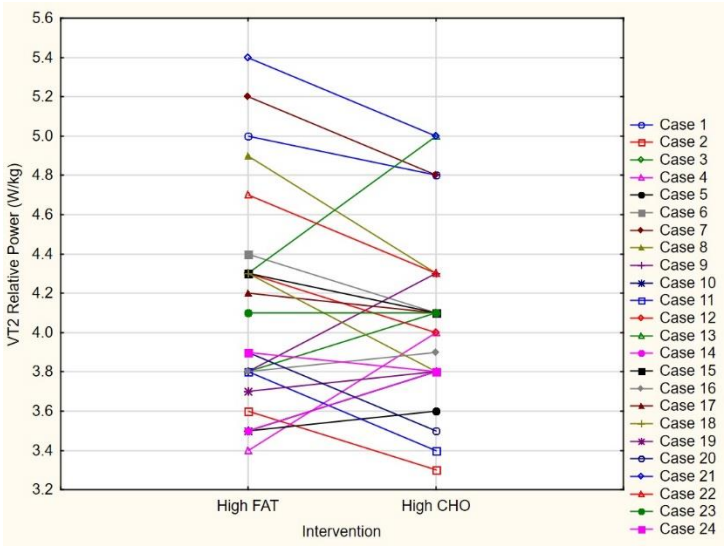


Figure 4.7a – c: Line graphs of the individual differences in absolute power output at VT_1, VT_2 and $\dot{V}O_2max$ between the high-FAT and high-CHO trials (N=24)

(a)



(b)



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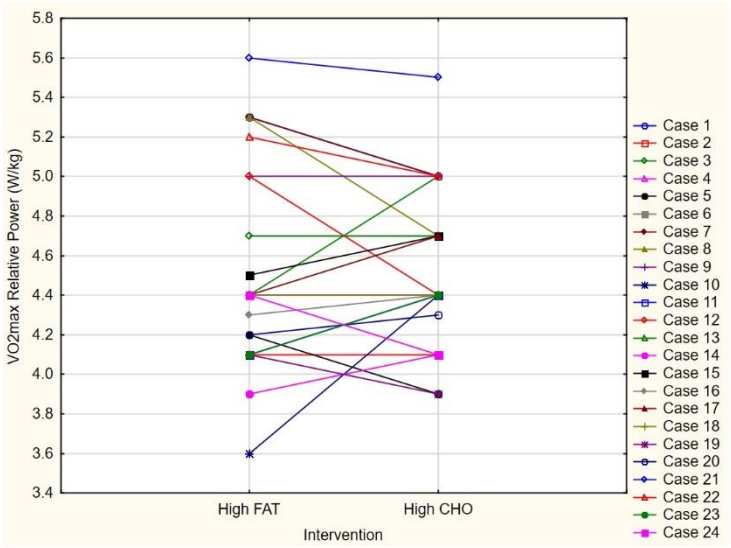


Figure 4.8a – c: Line graphs of the individual differences in relative power output at VT_1, VT_2 and $\dot{V}O_2max$ between the high-FAT and high-CHO trials (N=24)

4.4.4 Lactate Threshold

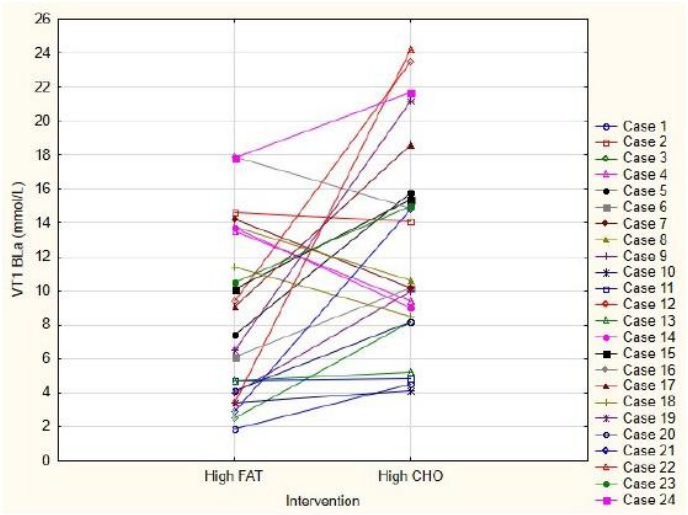
Lactate Threshold (LT) was identified for both trials as well as the HR (bpm), speed (km/h), absolute (W) and relative power outputs (W/kg) at the LT points (Table 4.18). Lactate threshold was identified at a speed of 14 km/h, and an absolute and relative power output of 303.51 ± 33.56 W and 3.84 ± 0.01 W/kg, respectively, for the high-CHO trial. The LT corresponded to 93% of the HRmax (HR = 177.33 ± 14.58 bpm) and 81% of the $\dot{V}O_2$max ($\dot{V}O_2$ = 51.41 ml/kg/min). During the high-FAT trial, LT was observed at a speed of 12 km/h, and an absolute and relative power output of 261.37 ± 28.89 W and 3.30 ± 0.01 W/kg, respectively. This LT point corresponded to 86% of the HRmax (HR = 163.54 ± 12.43 bpm) and 72% of the $\dot{V}O_2$max ($\dot{V}O_2$ = 51.04 ml/kg/min).

Case plots (Figures 4.9a – b) illustrate the individual differences in CBL responses at VT_1 and VT_2. The plots show that seven of the participants obtained higher values for the high-FAT compared to the high-CHO trial, whereas 17 participants experienced lower values for the high-FAT compared to the high-CHO trial at VT_1. However, twelve of the participants obtained higher values and twelve lower values for the high-FAT compared to the high-CHO trial, at VT_2. Figure 4.10 illustrates the CBL and HR responses of subjects for the different exercise intensities during the high-CHO and high-FAT trials, respectively.

Table 4.18 presents the speed, relative and absolute power outputs, $\dot{V}O_2$max, %HRmax and %VO_2max at VT_1, LT and VT_2 for the respective trials. The table shows that VT_1 was reached before LT during the high-CHO trials, with VT_2 that corresponded to 97% of the HRmax (HR = 181.83 ± 8.13 bpm) and 92% of the $\dot{V}O_2$max ($\dot{V}O_2$ = 46.67 ± 3.80 ml/kg/min). During the high-FAT trial, VT_1 was identified at the exact same running speeds and relative HR and $\dot{V}O_2$max values as LT, while VT_2 corresponded to 96% of the HRmax (HR = 178.71 ± 9.44 bpm), 90% of the $\dot{V}O_2$max ($\dot{V}O_2$ = 45.17 ± 4.93 ml/kg/min). Therefore, the tabulated results show relatively big differences between the percentage of the HR max where VT_1, VT_2 and LT were reached during the two trials.

Table 4.19 presents the comparison of HR and absolute power output values between VT_1 and LT for the high-CHO and high-FAT trials, respectively. Statistical and large practically significant differences were observed for absolute power output (d = 1.17) and HR (d = 1.12) during the high-CHO trial. During the high-FAT trial, significant differences were observed for both HR (p = 0.00) and absolute power output (p = 0.00), as well as a medium practically significant difference for HR (d = 0.46).

(a)



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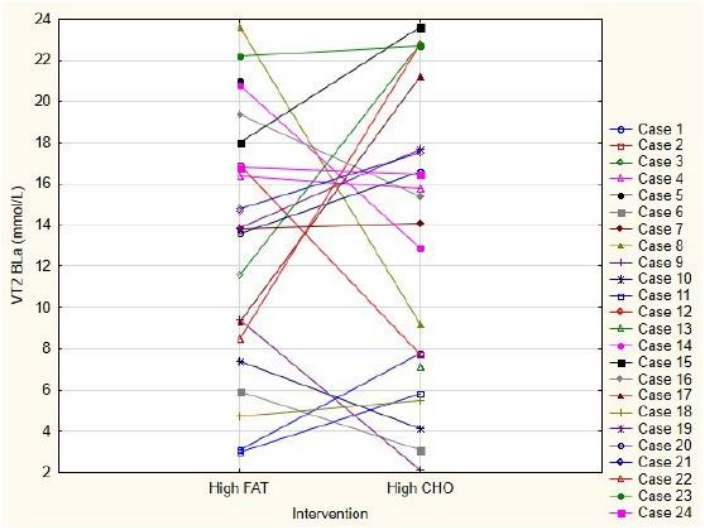

Figure 4.9a – b: Line graphs of the individual differences in CBL at VT_1 and VT_2 between the high-FAT and high-CHO trials (N=24)

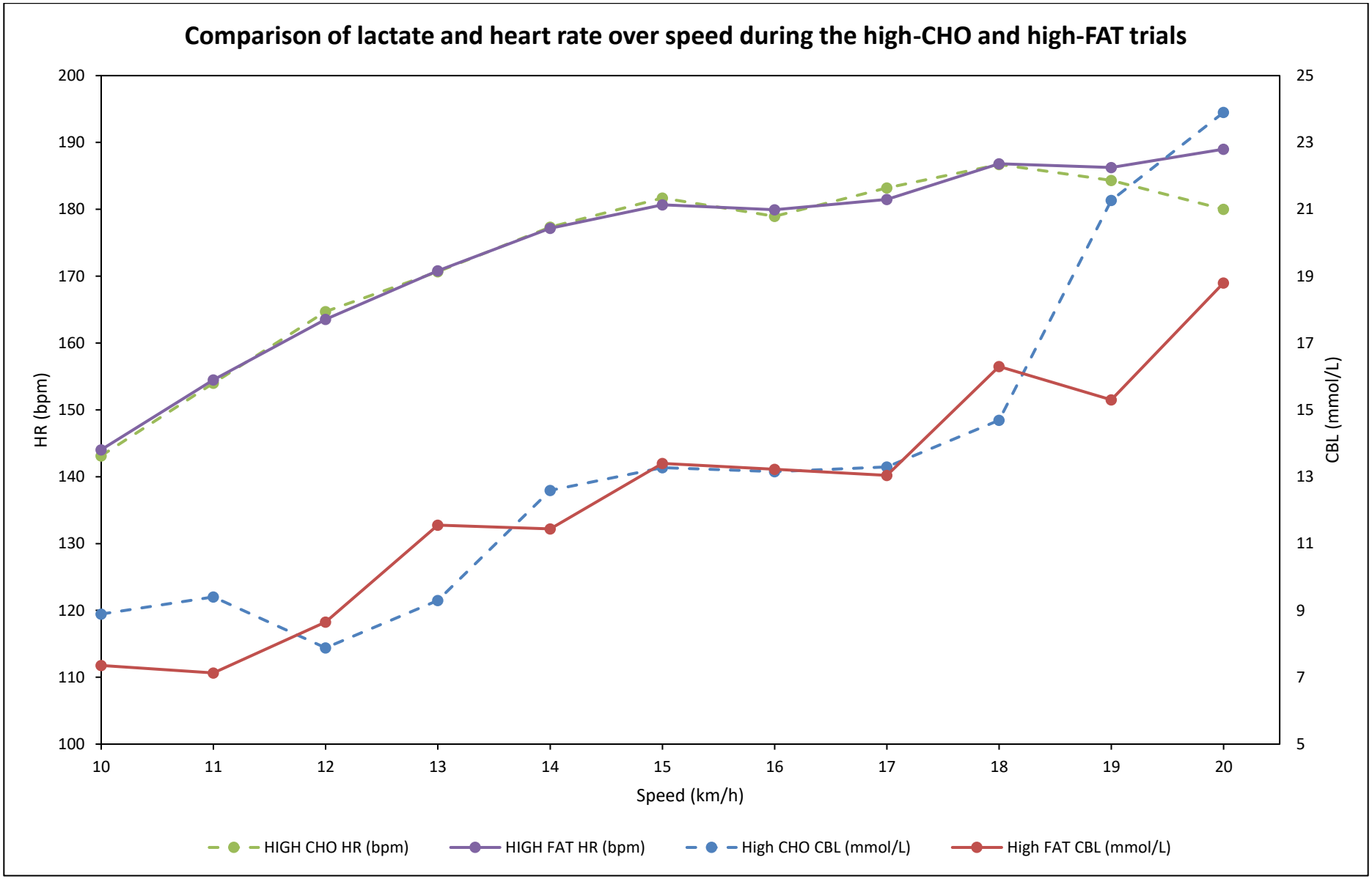

Figure 4.10: Lactate and heart rate over speed (N=24)

Table 4.18: Speed, absolute and relative power outputs, $\dot{V}O_2$max, %HR max and %$\dot{V}O_2$max at VT1, VT2 and LT (N=24)

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Variable	VT_1		LT		VT_2	
	High-CHO	High-Fat	High-CHO	High-Fat	High-CHO	High-Fat
Speed	12.00	12.00	14.00	12.00	15.00	15.00
Absolute power output (W)	270.58	268.08	303.51	261.37	320.75	327.25
Relative power output (W/kg)	3.45	3.38	3.84	3.30	3.30	3.00
$\dot{V}O_2$max (ml/kg/min)	40,96	38,67	41,82	36,85	92,52	90,51
%HR max	86%	85%	93%	86%	97%	96%
% $\dot{V}O_2$max	81%	77%	81%	72%	92%	90%

Note: N: Number of participants; **VT1:** first ventilatory threshold; **VT2:** second ventilatory threshold; **LT:** lactate threshold; **% HR max:** percentage of heart rate maximum; **%** $\dot{V}O_2$max **:** percentage of $\dot{V}O_2$max

Table 4.19: Heart rate and absolute power output between VT1 and LT

Treatment	Variable	Time point		Mean difference[1]: VT_1 - LT	
		VT_1	LT	[2]Effect size (d)	[3]P-value
High-CHO	HR (bpm)	163.46 ± 14.91	177.33 ± 14.58	1.12	0.17
	Absolute power output (W)	270.58 ± 43.55	303.51 ± 33.56	1.17	0.01*
High-FAT	HR (bpm)	159.50 ± 17.14	163.54 ± 12.43	0.46	0.00
	Absolute power output (W)	268.08 ± 50.81	261.37 ± 28.89	-0.24	0.00

1Treatment means, and point estimate and 95% confidence interval of for the mean difference "CHO – FAT" from an analysis of variance of the outcome variable, fitting treatment, period and subject as fixed effects (ANOVA of 2-treatment, 2-period cross-over data).

2R-value **is** a measure of the strength of the association between the two variables.

3P-value for t-test of the null-hypothesis that the mean difference is 0 (that is, null-hypothesis of no difference between CHO and FAT), from the analysis of variance.

*statistically significant: p = 0.01

4.4.5 Work output and energy contribution

Work output expressed in kilojoules (kJ) was not statistically significantly different between the high-CHO and high-FAT trials at VT_1 (684.25 ± 359.50 vs. 637.89 ± 364.18 kJ, p = 0.63), VT_2 (1199.30 ± 375.77 vs. 1263.48 ± 465.22 kJ, p = 0.33), and $\dot{V}O_2max$ (1543.90 ± 324.68 vs. 1547.91 ± 401.33 kJ, p = 0.95), respectively. Cohen's effect size showed a small practically significant difference for work at VT_1 (d = 0.15), VT_2 (d = -0.30) and $\dot{V}O_2max$ (d = -0.02) (Table 4.20).

Table 4.21 shows that CHO contributed less to the energy contribution at VT_1 (142.17 ± 51.7 vs. 145.92 ± 59.37 g/h) and VT_2 (216.38 ± 34.73 vs. 218.54 ± 36.8 g/h) during the high-CHO compared to the high-FAT trial, while the fat contributions at VT_1 (29.67 ± 15.39 vs. 24.63 ± 24.63 g/h) and VT_2 (10.92 ± 9.6 vs. 7.29 ± 7.98 g/h) were higher during the high-CHO compared to the high-FAT trial. At $\dot{V}O_2max$ slightly higher CHO (262.63 ± 34.39 vs. 261.38 ± 32.67 g/h) and fat contributions (56.25 ± 11.02 vs. 52.46 ± 10.8 g/h) were observed during the high-CHO compared to the high-FAT trials.

The results of the statistical comparison of the CHO and fat contribution values between the two trials are presented in Table 4.22. No statistically significant differences were observed between the two trials regarding both CHO and fat contribution at VT_1 (p = 0.88 and p = 0.19), VT_2 (p = 0.83 and p = 0.16) and $\dot{V}O_2max$ (p = 0.68 and p = 0.07). Cohen's effect size showed a medium practically significant difference for CHO contribution at VT_1 (d = 0.40) and medium practically significant differences for FAT contribution at VT_2 (d = 0.43) and $\dot{V}O_2max$ (d = 0.56) between the trials.

Case plots (Figure 4.11a – c) illustrate individual differences in CHO contribution at VT_1, VT_2 and $\dot{V}O_2max$. Nine participants showed higher values for the high-FAT compared to the high-CHO trial at VT_1, whereas 15 participants experienced lower values for the high-FAT compared to the high-CHO trial. At VT_2, twelve participants showed higher values for the high-FAT compared to the high-CHO trial at VT_2, with the other twelve participants experiencing lower values for the high-FAT compared to the high-CHO trial. At $\dot{V}O_2max$, eleven participants showed higher values for the high-FAT compared to the high-CHO trial, whereas thirteen participants experienced lower values for the high-FAT compared to the high-CHO trial.

Case plots (Figure 4.12a – c) of FAT contribution illustrate that 14 participants showed higher values for the high-FAT compared to the high-CHO trial at VT_1, whereas one

participant showed no difference and nine participants experienced lower values for the high-FAT compared to the high-CHO trial. At VT_2, 13 participants showed higher values for the high-FAT compared to the high-CHO trial at VT_2, with three participants showing no difference and eight participants experiencing lower values for the high-FAT compared to the high-CHO trial. At $\dot{V}O_2max$, ten participants showed higher values for the high-FAT compared to the high-CHO trials, whereas six participants showed no difference and eight participants experienced lower values for the high-FAT compared to the high-CHO trial.

Table 4.20: Work output (kJ): Analysis of Variance (ANOVA)

Time point	Treatment Mean[1]		Mean difference[1]: CHO – FAT			
	High-CHO	High-FAT	Point Estimate	95% Confidence Interval	[2]P-value	[3]Effect size (d)
VT_1	684.25	637.89	11.09	-35.62 to 57.80	0.63	0.15
VT_2	1199.30	1263.48	-15.34	-47.43 to 16.74	0.33	-0.30
$\dot{V}O_2max$	1543.90	1547.91	-0.97	-29.84 to 27.90	0.95	-0.02

1Treatment means, and point estimate and 95% confidence interval of for the mean difference "CHO – FAT" from an analysis of variance of the outcome variable, fitting treatment, period and subject as fixed effects (ANOVA of 2-treatment, 2-period cross-over data).

2P-value for t-test of the null-hypothesis that the mean difference is 0 (that is, null-hypothesis of no difference between CHO and FAT), from the analysis of variance.

3Effect size calculated as the ratio of the point estimate of the mean difference "CHO – FAT" divided by the residual standard deviation from the ANOVA.

Table 4.21: CHO and FAT contribution: Descriptive statistics (N=24)

		VT_1		VT_2		$\dot{V}O_2max$	
		Test		Test		Test	
Variable		High-CHO	High-Fat	High-CHO	High-Fat	High-CHO	High-Fat
CHO (g/h)	Mean	142.17	145.92	216.38	218.54	262.63	261.38
	Std	51.70	59.37	34.73	36.80	34.39	32.67
	Min	39.00	44.00	142.00	148.00	162.00	195.00
	Median	148.00	160.00	218.00	210.50	266.50	257.50
	Max	252.00	232.00	273.00	295.00	316.00	318.00
Fat (g/h)	Mean	29.67	24.63	10.92	7.29	56.25	52.46
	Std	15.39	17.19	9.60	7.98	11.02	10.80
	Min	3.00	0.00	0.00	0.00	37.00	30.00
	Median	25.00	26.00	11.00	4.50	56.00	55.00
	Max	63.00	63.00	28.00	23.00	79.00	70.00

Note: N: Number of participants; **SD:** Standard Deviation. **Min:** The smallest value in the data set. **Max:** The largest value in the data set; **VT1 (% of max):** percentage of $\dot{V}O_2max$ at VT1; **VT2 (% of max):** percentage of $\dot{V}O_2max$ at VT2; **VT1:** first ventilatory threshold; **VT2:** second ventilatory threshold.

Table 4.22: Carbohydrate and FAT contribution: Analysis of Variance (ANOVA)

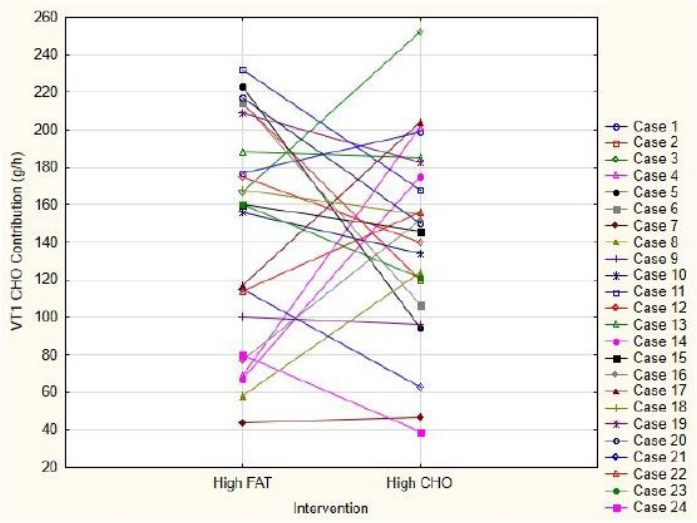
Time point	Variable	Treatment Mean[1]		Mean difference[1]: CHO – FAT			
		High-CHO	High-FAT	Point Estimate	95% Confidence Interval	[2]P-value	[3]Effect size (d)
VT_1	CHO (g)	142.92	145.16	-2.24	-33.11 to 28.62	0.88	-0.04
	Fat (g)	29.77	24.52	5.26	-2.83 to 13.34	0.19	0.40
VT_2	CHO (g)	216.40	218.51	-2.11	-22.17 to 17.95	0.83	-0.07
	Fat (g)	11.17	7.04	4.12	-1.75 to 9.99	0.16	0.43
$\dot{V}O_2max$	CHO (g)	262.77	261.23	1.53	-6.06 to 9.10	0.68	0.13
	Fat (g)	56.52	52.19	4.32	-0.45 to 9.10	0.07	0.56

1Treatment means, and point estimate and 95% confidence interval of for the mean difference “CHO – FAT” from an analysis of variance of the outcome variable, fitting treatment, period and subject as fixed effects (ANOVA of 2-treatment, 2-period cross-over data).

2P-value for t-test of the null-hypothesis that the mean difference is 0 (that is, null-hypothesis of no difference between CHO and FAT), from the analysis of variance.

3Effect size calculated as the ratio of the point estimate of the mean difference “CHO – FAT” divided by the residual standard deviation from the ANOVA.

(a)



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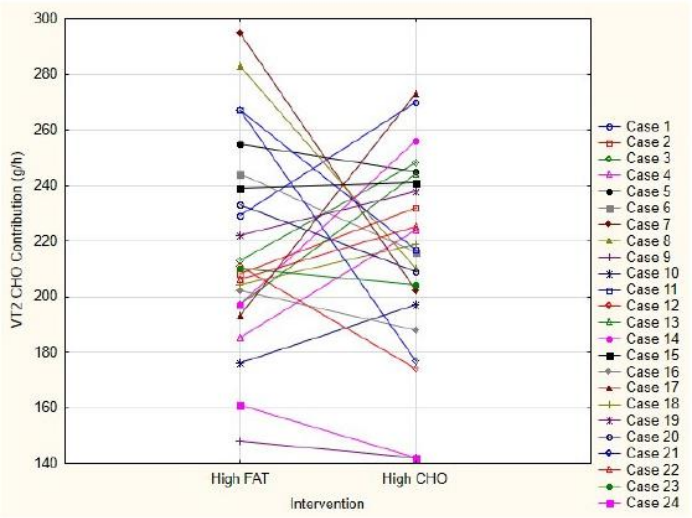

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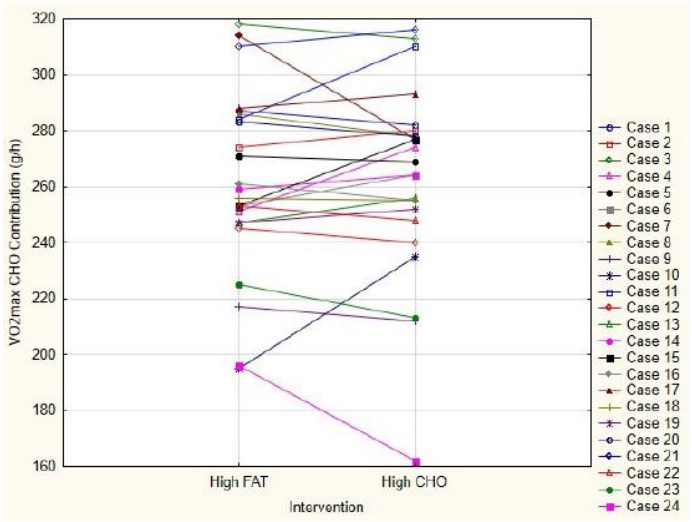
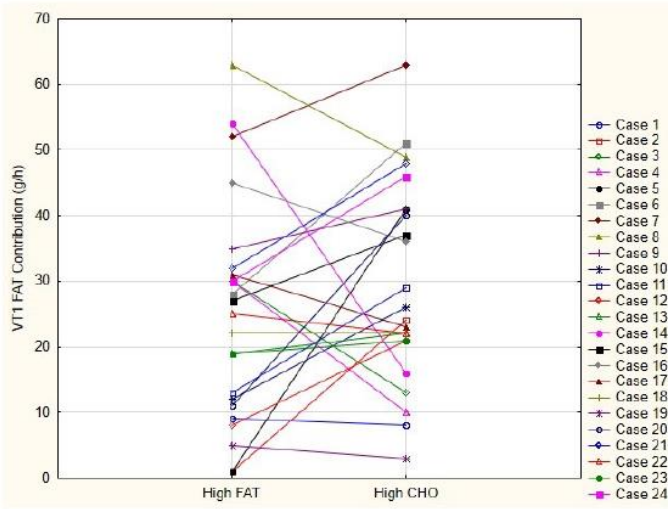


Figure 4.11a – c: Line graphs of the individual differences in CHO contribution at VT_1, VT_2 and $\dot{V}O_2max$ between the high-FAT and high-CHO trials (N=24)

(a)



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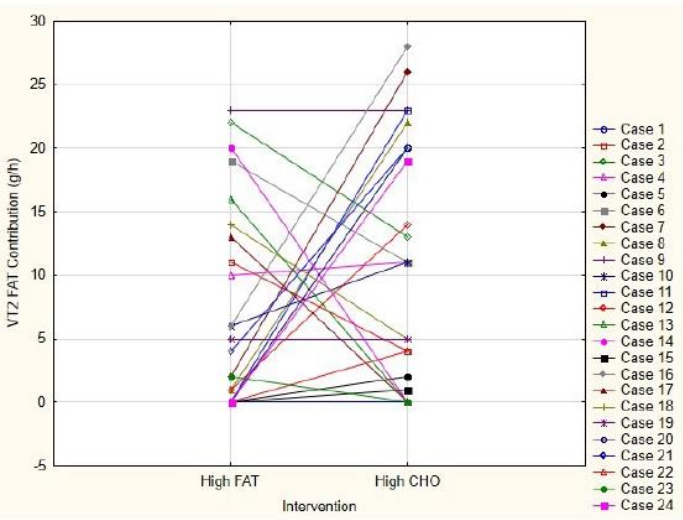

(c)

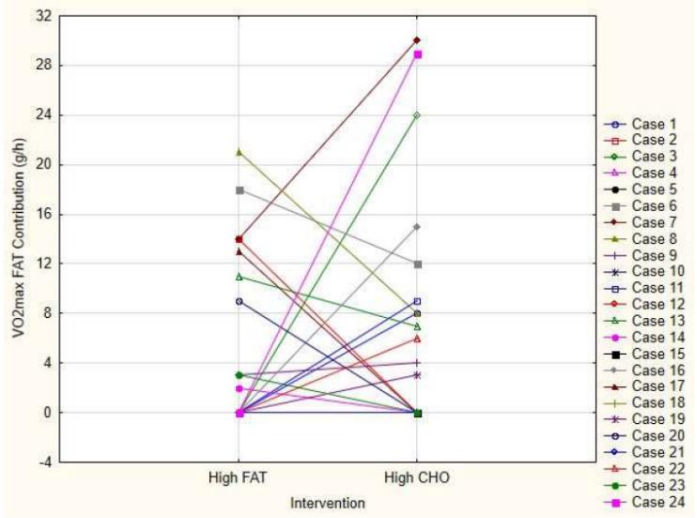

Figure 4.12a – c: Line graphs of the individual differences in FAT contribution at VT_1, VT_2 and $\dot{V}O_2max$ between the high-FAT and high-CHO trials (N=24)

CHAPTER 5

OVERALL DISCUSSION

CHAPTER 5 – OVERALL DISCUSSION

5.1 Introduction

The reviewed literature of this study (Chapter 2 & Table 2.2) indicates that research on the effects of short-term diets on the physiological responses of endurance runners is scarce. To the understanding of the research team, this is the first study to compare the effects of short-term (48-hour) high-carbohydrate (high-CHO) and high-FAT diets on the endurance capacity of runners by using a graded exercise test (GXT) to exhaustion on a treadmill. The uniqueness of the study also lies in the fact that a maximal exercise test was used to determine possible differences in performance outcomes and physiological thresholds [i.e. ventilatory threshold 1 (VT_1), ventilatory threshold 2 (VT_2) and $\dot{V}O_2max$] between two types of short-term diets. Although several previous studies also made use of high-FAT or high-CHO diets as interventions (see Table 2.2), the majority of these studies focussed on endurance cyclists, who performed trials at intensities between 60 – 70% of the participant's $\dot{V}O_2max$, followed by either a time-trial or a short sprint. These studies also made use of dietary interventions that were often implemented over a period of more than five days. However, none of these studies evaluated changes in the physiological thresholds (VT_1, VT_2 and $\dot{V}O_2max$) due to the dietary interventions.

The majority of published literature only focus on the effects of short-term fat adaptation followed by carbohydrate (CHO) restoration diets on endurance performance. Therefore, the current study focussed on endurance runners who had to follow either a strict high-CHO or a high-FAT diet for 48 hours before the execution of a GXT on a treadmill. The protocol for the GXT was compiled in such a manner that it simulated the demands of competitive endurance events which require that runners run at near maximal intensities. Finally, most of the listed studies (Table 2.2) had smaller sample sizes (4 to 20) compared to the current study (24 participants).

Furthermore, the statistical analysis of the data for the present study did not only determine the statistical significance of observed differences between the two diets, but also the corresponding effect sizes. Effect sizes were also calculated due to the fact that statistical significance alone is not sufficient for judging the noteworthiness or practical significance of observed treatment differences (Frölich *et al.,* 2009:175).

Effect size calculations are independent of sample size, which means that the size of the effect can be expressed irrespective of the sample. The independency of sample size helps researchers to avoid the difficult, and often subjective interpretation of inferential statistics because it is tied to the magnitude or practicality of the evaluated variables and is used to approximate a specific population parameter (Frölich *et al.,* 2009:175). Beside the calculation of effect sizes, case plots of individual differences in $\dot{V}O_2$ kinetics, absolute (W) and relative power output (W/kg), capillary blood lactate values (CBL) and CHO and FAT contribution between diets were compiled to illustrate individual differences in responses between the two interventions. Thus, the interpretation of the effect sizes as well as the case plots are designed to provide more in-depth information regarding individualised physiological response of the outcomes of the high-CHO and high-FAT diets, respectively.

5.2 Discussion

The main objective of this study was to investigate the effects of short-term (48-hours) high-CHO and high-FAT diets on oxygen consumption ($\dot{V}O_2$), carbon dioxide production ($\dot{V}CO_2$), respiratory exchange ratio (RER), minute ventilation ($\dot{V}_E$), substrate utilisation (FAT oxidation and CHO oxidation), time to exhaustion, absolute (W) and relative power output (W/kg), and work output (kJ) on the endurance capacity of long-distance runners. Among others, the objective was to establish whether there was a statistical and/or practical significance between-treatment difference in the variables, namely VT_1, lactate threshold (LT), VT_2 and $\dot{V}O_2$max, at certain thresholds that occur during execution of the GXT.

5.3 Diet

Two extreme diet compositions (Table 3.3) were analysed in the present study to determine the effects of these diets on the above-mentioned variables. The proportions of these diets were in agreement with the studies of Guimaraes Couto *et al.* (2014:318), Havemann *et al.* (2006:195), Hulton *et al.* (2013:166) and Rowlands and Hopkins (2002:679) (Table 3.3). The overall macronutrient distribution of FAT/CHO/PROTEIN was 65%/21%/14% for the high-FAT diet, and 17%/67%/16% for the high-CHO diets. The energy contribution from CHO during the 48-hour dietary trial was lower in the high-FAT diet (2600.65 ± 226.73 kJ) compared to the high-CHO diet

(8297.29 ± 723.29 kJ). In contrast, the energy contribution from dietary fat was considerably higher in the high-FAT diet (8049.60 ± 701.78 kJ) than in the high-CHO diet (2105.26 ± 183.55 kJ).

5.4 Ventilatory and metabolic response measures

5.4.1 $\dot{V}O_2$ kinetics

As discussed in Chapter 4, no statistically significant differences were observed for differences in any of the measured variables ($\dot{V}O_2$, $\dot{V}CO_2$, $\dot{V}_E$, and $\dot{V}O_2max$) at VT_1, VT_2 and $\dot{V}O_2max$, respectively, between the high-CHO and high-FAT trials (Table 4.4). However, Cohen's effect sizes showed that the observed differences were of medium practical significance for $\dot{V}O_2$ (L/min) at VT_1, and VT_2 as well as for $\dot{V}O_2$/kg (ml/kg/min) at VT_1 and VT_2. These results suggest that a high-CHO and high-FAT diet may cause notable differences in the responses of last-mentioned variables between trials.

Guimaraes Couto *et al.* (2014:383) did the only other study that used a running protocol to test the effects of a short-term high-CHO and high-FAT diet on running performance. The running protocol consisted of a submaximal exercise test at 65% of $\dot{V}O_2max$ during which ten-minute intervals were performed by adolescent boys between 14 and 17 years of age. Similar to the results of this study, they found no significant between-diet differences in $\dot{V}O_2$, $\dot{V}CO_2$, $\dot{V}_E$ and RER measured after a two-day high-FAT or high-CHO diet (2.43 ± 0.15 vs. 2.61 ± 0.15 L/min; 1.85 ± 0.12 vs. 2.07 ± 0.13 L/min; 55.5 ± 3.3 vs. 60.1 ± 2.6 L/min). Correspondingly, a three-day high-CHO or high-FAT diet also caused no significant difference in $\dot{V}O_2$ during a 20-minute cycle ride at 65% of $\dot{V}O_2peak$ (3.18 ± 0.27 vs. 3.27 ± 0.22 L/min), as well as during a high intensity interval session performed at 86% of $\dot{V}O_2peak$, immediately after the cycle ride (4.32 ± 0.32 vs. 4.53 ± 0.23 L/min) (Stepto *et al.*, 2002:451). However, none of the aforementioned researchers considered the physiological thresholds (VT_1, VT_2 and $\dot{V}O_2max$) as these turning points can only be observed through a GXT.

The results of all statistical analyses and the observed inter-individual differences from the case plots indicate that absolute and relative $\dot{V}O_2$ at the different threshold points during exercise (Figure 4.1a – c) may be influenced by dietary differences in the content of CHO. However, the variability in results also suggest that other factors than

dietary components may play a role in influencing $\dot{V}O_2$ during exercise. These factors will be discussed later in this section.

Although researchers assumed that higher fat intakes would result in higher fat oxidation (FATox) rates with corresponding increases in $\dot{V}CO_2$, this was neither confirmed by the results of the current study nor by the other mentioned short-term dietary manipulation studies (Guimaraes Couto *et al.* 2014:383; Stepto *et al.*, 2002:451). Even though results from the $\dot{V}CO_2$ case plots (Figure 4.2a – c) showed that 42% and 54% of the participants experienced a higher carbon dioxide output ($\dot{V}CO_2$) at VT_1 and $\dot{V}O_2$max, respectively, for the high-FAT compared to the high-CHO diet, the calculated effect sizes for differences were small.

The rapid increase in $\dot{V}_E$ at the onset of exercise is associated with nervous system stimulation due to joint receptor activation as a result of movements generated by the working muscle (Bruce, 2017:541). However, with a sudden increase in exercise intensity, as with the current study, changes in $\dot{V}_E$ are caused by a higher carbon dioxide (CO_2) blood concentration (Bruce, 2017:541). However, effect size value for differences for $\dot{V}_E$ between dietary interventions only delivered a small practically significant value. This result of no statistical or practically significant difference between interventions for $\dot{V}_E$, is similar to what previously mentioned short-term dietary manipulation studies found (Guimaraes Couto *et al.* 2014:383; Stepto *et al.*, 2002:451).

Factors other than dietary intake that may influence $\dot{V}O_2$ kinetics are highlighted in literature and are discussed next. Endurance exercise causes adaptations that are related to capacities and kinetics (Drescher *et al.,* 2018:1). In this context, capacities refer to the utmost limits of the physiological systems involved during endurance training, which include $\dot{V}O_2$max, $\dot{V}O_{2peak}$, maximal cardiac output (Q) and maximal heart rate (HRmax) (Basset & Howley, 2000:72; Drescher *et al.,* 2018:1). On the other hand, kinetics refers to the speed of adjustment to training stresses and to relative changes such as the dynamic response of $\dot{V}O_2$, HR and/or Q in reaction to changing metabolic requirements during provisional exercise (Drescher *et al.,* 2018:1). The $\dot{V}O_2$ kinetics are dependent on the oxygen supply and extraction which are influenced by enzyme activities, muscle fibre distribution and the size and number of mitochondria, while oxygen delivery relies on the amount of muscle perfusion as well as the capillary density (Drescher *et al.,* 2018:156). Therefore, responses from both the cardiovascular

and respiratory systems are pivotal in optimising endurance performance and should, therefore, not be divided into physiological independent variables (Drescher *et al.,* 2018:2). Consequently, individual adaptations in both capacities and kinetics due to endurance training may cause various responses during endurance running or exercise which are not related to dietary changes.

The $\dot{V}O_2$ kinetics can be categorised into three phases from the onset of exercise to the end of moderate intensity exercise. These phases include (1) the 'cardio-dynamic' phase that is characterised by an abrupt increase in $\dot{V}O_2$ at the beginning of exercise, facilitated by a raised ventricular output and an increase in venous return; (2) The 'fast' component which reflects the kinetics of muscle $\dot{V}O_2$ and, (3) where the steady state of $\dot{V}O_2$ is reproduced. During an intense bout of aerobic exercise, the oxygen demand of working muscles increases and is directly associated with the metabolic efficiency, weight of exercising muscle and exercise intensity, which means that aerobic exercise that involves larger muscles or requires a higher work rate will likely lead to a higher total $\dot{V}O_2$ (Haff & Triplett, 2016:117). Faster $\dot{V}O_2$ kinetics, which refers to the extent of oxygen delivery response, determines the oxygen deficit at the start of exercise, which leads to a couple of important adaptations, including (1) a quicker achievement of the required steady state $\dot{V}O_2$, which in turn leads to the reduced speed of fatigue development and a reduction in metabolic perturbations, and (2) an increased energy input from aerobic metabolism during high intensity running bouts which forms a very important part of any distance running event (Shaw, 2016:9).

However, more in-depth and invasive testing is necessary to examine the possible influences of all the above-mentioned factors on $\dot{V}O_2$ kinetics and the performance outcomes.

5.4.2 Substrate metabolism

Study results of comparisons showed that the resting RER values and RER values at all threshold points obtained no significant differences between the two trials (Table 4.6). The RER case plots (Figure 4.4a – c; Figure 4.5) indicated that large individual differences existed for the responses between the two trials. RER values calculated for each stage of the respective tests (Table 4.11), showed a shift in substrate metabolism (from aerobic to an increase in anaerobic metabolism) at stage 2/speed 11 km/h, with an approximate CHO energy contribution of 52.4% and a fat energy

contribution of 47.6% (Plowman & Smith, 2008:134). The percentage of $\dot{V}O_2$max (%$\dot{V}O_2$max) that corresponded to the above-mentioned RER values was 72% of $\dot{V}O_2$max and 73% of $\dot{V}O_2$max for the high-CHO and high-FAT trials, respectively. From stage three/speed 12 km/h, the RER values showed a progressive rise which signifies more reliance on CHO as primary fuel source. Furthermore, RER values showed a shift in substrate metabolism which started to reflect anaerobic metabolism to primarily CHO as fuel before VT_1 (0.89 vs. 0.90) and again progressively increased and showed almost complete reliance on CHO as fuel at VT_2 (0.97 vs. 0.98) during both high-CHO and high-FAT trials (Table 4.6).

The well-known table of non-respiratory quotient by Péronnet and Massicotte (1991:27) that is still used today, clearly states that RER values of above 0.85 indicates a shift in substrate metabolism and utilisation from primarily fat as fuel to CHO as fuel. As RER levels approach one, the execution of exercise at that exercise intensity relies progressively more on CHO as the primary fuel source, while any values over one indicates total dependence on CHO as fuel source (Farinatti *et al.,* 2016:77; Kenney *et al.,* 2015:124; Storm, 2017:13).

The CHOox and FATox were calculated by using the $\dot{V}O_2$ and $\dot{V}CO_2$ values obtained during the different stages of the incremental treadmill test. Resting CHOox and FATox showed no significant and only small practically significant differences between diets (Table 4.7). However, the only values that were taken into account for the calculation of CHOox and FATox were those collected during the first six running intensities (speeds of 10 to 15 km/h), as the sixth stage was the last stage that was completed by the majority of the participants. Therefore, the values after these stages were not considered. The CHOox and FATox that were observed for the high-CHO diet ranged between 1.27 – 4.04 g/min for CHO and between 0.32 – 0.83 g/min for fat, respectively. On the other hand, the CHOox and FATox for the high-FAT diet ranged between 1.42 – 4.14 g/min for CHO and 0.29 – 0.79 g/min for fat, respectively (Table 4.9 – Table 4.11).

For determination of maximal fat oxidation (MFO), the same running intensities as mentioned above were used to calculate FATox as a prerequisite as the determination of the MFO is that the RER must be smaller than one. Continuous GXT protocols consisting of three to five minute intervals of increasing exercise intensities are frequently being used to estimate MFO rates in trained and untrained participants.

Studies that used these protocol formats, typically observe RER values of one at 90% of $\dot{V}O_2$max, which also supports the assumption of negligible FATox beyond this intensity (Achten & Jeukendrup, 2003b:1021; Achten *et al.,* 2002:95; Romijn *et al.,* 1993:E389).

The maximal fat oxidation (MFO) was observed during the first interval (10 km/h) for both the high-CHO and high-FAT trials, after which a decrease in FATox was observed. The exercise intensity (%$\dot{V}O_2$max) less than where MFO was identified, also known as $FAT_{max,}$ could consequently not be determined. The FAT_{max} was identified to be 0.84 g/min at 65% of $\dot{V}O_2$max for the high-CHO trial and 0.78 g/min at 66% of $\dot{V}O_2$max for the high-FAT trial. No statistically or practically significant differences were found for MFO between the high-CHO and high-FAT diets. The researcher of this study could not identify any previous studies that investigated the influence of different short-term diets on the GXT protocol determined MFO of participants. Therefore, it is impractical to completely compare the results of this study to that of previous studies.

However, the following section provides results of studies that made use of a different type of testing protocol, when compared to the protocol of this study. In this regard, Stepto *et al.* (2002:449) reported that competitive endurance runners are capable of performing high-intensity intervals effectively after a high-FAT diet of three days. Their study included a randomised three-day diet of either high-FAT or high-CHO separated by an 18 day washout period. Tests on day one included a standardised training session which consisted of a 20 minute warm-up at 65% of $\dot{V}O_2$max followed by eight repetitions of five minute intervals at 86 ± 2% of $\dot{V}O_{2peak}$ (Stepto *et al.,* 2002:450). The last-mentioned study also observed similar RER values on day one for both their high-FAT and high-CHO interventions, respectively (0.91 ± 0.04 vs. 0.92 ± 0.03). However, RER values were found to be similar after the high-CHO diet (0.92 ± 0.03) on day four, but RER values fell to 0.85 ± 0.03 after the high-FAT diet.

The significant increase in the FATox after the high-FAT diet (31 ± 13 vs. 61 ± 25 µmol/kg/min) is expected with a decline in RER values as the RER provides an accurate reflection of substrate utilisation (Stepto *et al.,* 2002:450). In contrast to the current study, last-mentioned researchers evaluated the habitual diets of participants before the start of the experiments and found that the normal daily habitual diets contained a high proportion of CHO (Stepto *et al.,* 2002:450). However, due to the fact

that the current study did not evaluate habitual dietary intakes before the start of the experiments, no conclusion could be drawn regarding the possible effects of the habitual diet on the test results. The habitual high-CHO diet of participants in the study of Stepto *et al.* (2002:450) could serve as a reason for the significant difference in FATox after the high-FAT diet.

Furthermore, Stepto *et al.* (2002:450) made use of an intense aerobic interval training session as a testing protocol, compared to the GXT in our study. The last-mentioned study included highly-trained competitive ultra-endurance athletes as study participants, who were busy with a prolonged, strenuous training program at the time of the investigation. Despite the achievement of higher FATox after the high-FAT diet, researchers still concluded that competitive endurance runners are able to compete in intense aerobic interval sessions after either a three-day high-FAT or high-CHO diet (Stepto *et al.,* 2002:455). Similarly, we also found that endurance runners are able to perform a maximal GXT while either on a high-CHO or high-FAT short-term diet.

Guimaraes Couto *et al.* (2014:381) examined the short-term effect of a two-day, high-FAT (24.2% CHO, 15.5% protein, 60.4% fat), high-CHO diet (69.3% CHO,15.1% protein, 15.9% fat) or daily habitual diet (56.1% CHO, 16.5% protein, 27.5 % fat) on substrate utilisation and oxidation during submaximal exercise (65% $\dot{V}O_2$peak) after a 7 to 14-day washout period in mid- to late-pubertal trained adolescent boys. Even though they did not measure FATox intrinsically, the results showed a higher fat contribution after the high-FAT diet compared to the high-CHO diet with a significantly lower ($p = 0.01$) CHO contribution to energy expenditure (Guimaraes Couto *et al.,* 2014:382). Again, the larger amount of CHO in the habitual diets of their participants may explain the difference in results between their study and the present study (Guimaraes Couto *et al.,* 2014:384). Furthermore, they included mid- to late-pubertal boys in their study as participants. Research confirmed that the higher relative contribution from fat to energy expenditure during exercise reduces progressively as children mature (Riddell *et al.,* 2008:742; Tonson *et al.,* 2010:776). Consequently, the results of this study cannot be directly compared to the results of their study.

Støa *et al.* (2016:399) tested the day-to-day variability in FATox and the effect of a change in a one day long diet on moderately trained female athletes' exercise performance. They first tested the effects of the day-to-day variability in the habitual diet on FATox and used the results of this analysis to explain the acute effect of diet

manipulation on FATox. They found that the FATox was significantly lower during the high-CHO diet compared to the high-FAT diet (0.29 ± 0.13 vs. 0.42 ± 0.14 g/min) (Støa *et al.,* 2016:401). They also reported a 4 – 5% variation in work economy and test-retest variability for $\dot{V}O_2$ and $\dot{V}CO_2$, which indicated that the 2 – 3% variability in day-to-day FATox could be attributed to small non-significant differences in the composition of the habitual diet (Støa *et al.,* 2016:401). No significant differences were reported in $\dot{V}O_2$, $\dot{V}CO_2$, RER, HR, BLa and FATox (0.39 ± 0.08 vs. 0.42 ± 0.15 g/min). The RER values were significantly higher after the high-CHO, compared to the high-FAT diet (0.87 ± 0.0 vs. 0.83 ± 0.04). They also concluded that even when reporting 4 – 5% likely variations in work economy and $\dot{V}O_2$, 26 – 27% of the differences in FATox between the two diets was possibly caused by the altered contents of the fat and CHO in the diets (Støa *et al.,* 2016:397). No significant difference was found between the high-FAT and habitual diet's FATox, which could be reasoned by the already high relative proportion of fat (>40% of daily macronutrient intake) consumed in the participants' habitual diets (Støa *et al.,* 2016:402).

Other studies that made use of short-term diet manipulation interventions included cycling protocols with a 120-minute time-trial at 65% of $\dot{V}O_2$max (Starling *et al.,* 1997:1185) and a time-to-exhaustion protocol at 70% of $\dot{V}O_2$max (Pitsiladis & Maughan, 1999:992). The study of Starling *et al.* (1997:1185) incorporated a 120-minute cycling protocol at 65% $\dot{V}O_2$max to deplete glycogen stores. This was then followed by a twelve hour long high-CHO or high-FAT diet. After the 12-hour dietary period, a 1 600 kilojoule self-paced cycling bout was done. Important to note is that Starling *et al.* (1997:1185) did not test indirect indices or substrate utilisation *per se*, but made use of a needle biopsy sample from the vastus lateralis muscle to determine muscle triglyceride and muscle glycogen concentrations. They found muscle triglyceride significantly higher 24-hours after 120 min cycling for the high-FAT vs. the high-CHO trial. Muscle glycogen significantly lower 24-hours after 120 min cycling in high-FAT vs. high-CHO trials and time to complete the 1 600kJ time-trial significantly greater in the high-FAT trial (139.3±7.1 min) compared to high-CHO trial (117.1±3.2 min). In contrast, Pitsiladis and Maughan (1999:920) tested indirect indices in their study which involved an experiment where subjects first had to complete a 120-minute cycling time-trial at 70% $\dot{V}O_2$max to deplete muscle glycogen stores, followed by a

three-day high-CHO or high-FAT diet before a self-paced cycling time-trial until exhaustion.

Ferreira *et al.* (2018:4) studied the effects of a 48-hour high-CHO and high-FAT diet on post-exercise FATox and showed that the rate of FATox post-exercise was higher in the high-FAT than in the high-CHO diet groups (7.830 ± 1.864 mg vs. 6.264 ± 1.736 mg). Again, this is in direct contrast with the results of this study which found no difference between FATox after the last completed stage (Table 4.9), but this could again be attributed to differences between testing protocols. The testing method of Ferreira *et al.* (2018:4) included a protocol to manipulate pre-exercise CHO availability with a 90-minute cycling bout at 70% $\dot{V}O_2$max and six one-minute exercise bouts at 125% $\dot{V}O_2$max with one-minute rest intervals followed by a 48-hour high-CHO and high-FAT diet. The experimental trial after the dietary period consisted of a single high intensity effort until exhaustion, at a power output which corresponded to 95% $\dot{V}O_2$max (Ferreira *et al.,* 2018:4).

In the current study, the higher CHO intake did not lead to a reduction in FATox at the different running intensity stages. In addition, a restriction in the CHO content of the short-term diet (high-FAT) also did not lead to an elevation in FATox. This finding was unexpected as other short-term dietary-related studies found that marked isoenergetic increases in dietary CHO resulted in decreased FATox (Bergstrom *et al.,* 1967:140; Ferreira *et al.,* 2018:1; Helge *et al.,* 1996:293; Lambert *et al.,* 1994:287; Stepto *et al.,* 2002:449; Støa *et al.,* 2016:397).

Of all the above-mentioned studies, the study of Stepto *et al.* (2002:453) was the only study to include competitive endurance runners as participants while the other studies included either well-trained cycling or running endurance athletes. Some endurance athletes will deliberately exercise with low glycogen levels to improve their FATox capacity and, ultimately, to spare glycogen energy stores for usage during critical parts of the race which require high and maximal efforts, which generally depend on glycogen and glucose as energy sources (Burke, 2015:s36). Therefore, it is possible that some of the endurance athletes who served as participants for this study, showed higher FATox due to implementation of the low-glycogen training regimen during periods before commencement of the study. However, this is only an assumption as the training regimens of participants were not evaluated or analysed before

commencement of the study. Therefore, it can be recommended that future studies of this nature should also include and analyse information related to the training regimens as well as the habitual diets of especially endurance athletes, as it may influence the substrate metabolism of study participants.

Athletes that train continuously experience exponential increases in $\dot{V}O_2max$, running economy, skeletal muscle mitochondrial volume, as well as maximal CHOox and FATox (Barnes *et al.,* 2013:642; Galbraith *et al.,* 2014:1023; Hawley & Leckey, 2015:s5; Spriet, 2007:333; Stangier *et al.,* 2016:45). These training adaptations will improve the ability of skeletal muscles to take up free fatty acids (FFA) from the plasma and to store and oxidise intramuscular triacylglycerol (IMTG), which is inexhaustible, as stored energy source (Hammond, 2019:3; Spriet, 2007:333). Athletes will then have the capacity to oxidise fat at a high rate, despite the change in short-term diet content.

Periods of endurance training lead to a decrease in RER, a reduction in muscle glycogenolysis and greater FATox when compared to pre-training levels (Cox & Clarke, 2014:2; Hawley & Leckey, 2015:s5). All of the above-mentioned studies reported a significant decrease in RER values as an indicator of an increase in FATox after a high-FAT diet and an increase in RER after a high-CHO diet.

Along with the probability that the training status of participants may influence the results of a study, the experimental and habitual diets of studies that were either implemented or evaluated, all differed somewhat in macronutrient composition (Table 2.2 & Table 3.5). The macronutrient distribution of a diet may affect adaptations and responses because of the diet changes, which may explain the inconsistency and contrast in findings concerning changes in RER values and substrate metabolism between studies. The variability in wash-out periods between experimental trials, which varied from 2 – 18 days, also makes it difficult to determine if a change in fitness levels due to training during the wash-out period also influenced exercise responses (Støa *et al.,* 2016:399). Another factor that may explain differences between study results, is that participants may unintentionally change their daily eating habits a few days before the start of the diet manipulation. In addition, a 48-hour period may be too short to achieve significant metabolic adaptations due to diet manipulations. For these reasons, Støa *et al.* (2016:400) used a two-day wash-out period with the aim to show its relevance from a methodical perspective. However, they concluded that uncertainty

still remained concerning the sufficiency of a two-day wash-out period to normalise the metabolism after a diet manipulation (Støa *et al.,* 2016:400).

In view of the above-mentioned discussions, a possible reason for the finding that no statistically or practically significant differences were observed for substrate metabolism and RER values in the current study, is that the macronutrient distributions of participants' habitual diets were not evaluated and may have influenced the FATox. The variability in the macronutrient content of participants' daily diets before commencement of the study and the fact that no glycogen depletion test was done before the start of the dietary manipulation period, may also have influenced the different test responses.

Up until now, studies that have investigated the MFO on participants via a GXT, did not evaluate the possible influence of a short-term dietary manipulation on MFO (Achten & Jeukendrup, 2003c:604; Achten *et al.,* 2003:748; Achten *et al.,* 2002:93; Hetelid *et al.* 2015:1; Randell *et al.,* 2017:134). Nevertheless, Hetelid *et al.* (2015:1) concluded that MFO can occur anywhere between 47 – 75% of $\dot{V}O_2$max, after which a further increase in intensity will lead the primary use of CHO until approximately 85% of $\dot{V}O_2$max, after which the use of fat as a fuel source becomes negligible. Romijn *et al.* (1993:E380) were one of the first research groups to prove that FATox at low and high intensities (25% and 85% of $\dot{V}O_2$max) are lower compared to moderate intensities (65% of $\dot{V}O_2$max). The inter-individual variety in MFO rates between untrained and trained participants is influenced by exercise intensity, training status, gender differences, exercise duration and nutrition, which can all affect the cellular expression of FATox. Of these possible factors, exercise intensity controls substrate oxidation acutely, irrespective of nutritional influence and/or training status (Purdom *et al.,* 2018:4). Cellular and hormonal fluctuations which can lead to increased lipolytic rates and corresponding FATox during exercise, are causally associated to exercise intensity (Purdom *et al.,* 2018:5). However, Randell *et al.* (2017:139) concluded that although the variance in MFO rates can be explained by the above-mentioned factors, in their study, more than 50% of the variance in MFO could not be explained by these factors and remained unaccounted for. The MFO results of 1 121 athletes from different sporting codes and competitive levels that undertook a GXT in a fasted state showed that the average MFO was 0.59 ± 0.18 g/min, ranging between 0.17 and 1.27 g/min, with FATmax occurring at an average exercise intensity of 49.3% ± 14.8% of

$\dot{V}O_2$max with a range between 22.6% and 88.8% of $\dot{V}O_2$max (Randell *et al.,* 2017:133).

However, participants with a higher $\dot{V}O_2$max obtained a higher MFO (0.56 ± 0.14 g/min) compared to their counterparts with a lower $\dot{V}O_2$max (0.48 ± 0.15 g/min) (Achten & Jeukendrup, 2003c:606). Randell *et al.* (2017:137) also found a significant positive relationship between MFO and $\dot{V}O_2$max. These researchers also referred to the strong positive correlation between MFO and $\dot{V}O_2$max that Robinson *et al.* (2015) found (cited in Randell *et al.,* 2017:137). Another study by Achten *et al.* (2002:94) found FATmax to be equivalent to 64 ± 4% of $\dot{V}O_2$max and 74 ± 3% of HRmax with a MFO rate of 0.60 ± 0.07 g/min (range: 42 to 84 g/min). Trained males experienced FAT_{max} at 40% of $\dot{V}O_2$max versus 59% of $\dot{V}O_2$max in untrained males (Bergman & Brooks, 1999:479). In this study, the FAT_{max} was observed at 65% and 66% of $\dot{V}O_2$max, respectively which also falls between the exercise intensity scale for the FAT_{max} zone (47 – 75% of $\dot{V}O_2$max) set out by previous researchers. Researchers further revealed significantly higher FATox during running versus cycling (0.65 ± 0.05 vs. 0.47 ± 0.05 g/min), even though the exercise intensity which elicited MFO was not significantly different between the trials (62.1 ± 3.1 vs. 59.2 ± 2.8% of $\dot{V}O_2$max) (Achten *et al.,* 2003:750). The treadmill also led to significantly higher $\dot{V}O_2$max, HRmax and FATox rate values from 55% to 80% of $\dot{V}O_2$max during the treadmill test. The smaller muscle mass recruited during cycling, compared to running, may explain the higher CHOox and FATox as well as exercise responses that were observed for the running tests (Achten *et al.,* 2003:750). During exercise the release of catecholamines is related to the exercising muscle mass, which means that the release of catecholamines will be higher during running because of the potent activation of lipolysis (Achten *et al.,* 2003:751). Another hypothesis is that the work rate during cycling is divided over a smaller number of muscle fibres than during running, which means the metabolic stress level and energy requirement per fibre can only be met by an increase in oxidation rates (Achten *et al.,* 2003:751). The current study's high MFO results can be supported by the mentioned studies by Achten *et al.* (2003:748) and Randell *et al.* (2017:136), who both used a GXT protocol on a treadmill in a fasted state to determine MFO rates in either moderately trained or a variety of sporting codes.

The reasons for the wide range of FAT_{max} values that the literature provides, are that FAT_{max} does not only rely on the ability of a person to oxidise fatty acids but also on the study methods used to determine this value, along with the other factors already discussed in the above-mentioned sections. However, the constant increase in intensity until exhaustion GXT seems to be the best method to use when oxidation rates of different substrates are reviewed and especially when trying to verify the intensity where MFO occur.

Nevertheless, several concerns regarding the use of a standardised GXT to assess gas analysis-related variables and data (ventilatory, metabolic and substrate usage) of athletic populations have been highlighted. For instance, the duration of the test is unknown to athletes and athletes are not able to change the fixed progressive intensity of the test which makes the test non-specific to outside sport conditions (Truong *et al.,* 2018:305). In cases where athletes are able to apply a pacing strategy, it allows the brain to recruit the appropriate number of muscle motor units so that a certain activity can be performed without homeostatic failure and the onset of muscle fatigue (Truong *et al.,* 2018:305). In view of these shortcomings, several researchers discourage the use of the GXT as athletes are unable to replicate the exact sport-specific conditions that they are normally subjected to (Noakes, 2008:554; Truong *et al.,* 2018:305). Another general concern that researchers must consider when a GXT is used to determine substrate utilisation, is that substrate utilisation during the later stages of the test can be influenced by the duration and intensity of the formerly exercise stages, since the contribution of fat for energy production rises when exercise is continuous for long periods (Achten *et al.,* 2002:96).

Despite the mentioned shortcomings of the GXT, researchers prefer last-mentioned test for the comprehensive gas analysis of endurance or trained runners, especially when stage durations and intensity increments are not too large (Achten, *et al.,* 2002:96; Machado *et al.,* 2013:577; Peserico *et al.,* 2015:732). For example, Peserico *et al.* (2015:732) found that a GXT with one km/h speed increments and with a three-minute stage duration should be used as a standard for determining the highest value of $\dot{V}O_2$ attained upon an incremental test (V_{peak}) to predict endurance performance and to assess aerobic fitness, even though the study was done on recreational runners. Machado *et al.* (2013:577) also showed that a GXT comprising of three-minute test intervals is superior for accurately predicting the five and ten km running

performance of male endurance trained runners. The GXT from the current study with 3-minute stage durations with an intensity increment of one km/h was, therefore, verified as an accurate means of testing and interpreting gas analysis variables in endurance athletes.

5.5 Physiological and perceptual measures

5.5.1 Rating of perceived exertion

An athlete's behavioural, physical, and motivational state on any given moment or day during which the GXT is performed, contributes greatly to the overall validity of the RPE test. This is especially of concern when observing the relationships between RPE, HR and BLa (Beltz *et al.,* 2016:7). Furthermore, participants' understanding of the scale and associated verbal descriptors can influence the validity of the RPE outcome (Eston, 2012:175; Muotri *et al.,* 2017:6).

The data from the current study suggested that neither the high-CHO nor the high-FAT diets had an influence on the physical and sensorial exertion that the athletes experienced during the GXT. The RPE values did not show any statistically or practically significant differences between the different diet interventions at any of the threshold points (VT1, VT2, $\dot{V}O_2max$) (Table 4.13). Therefore, all athletes perceived the test exertion to be similar between trials. In contrast to the results of this study, Stepto *et al.* (2002:451) found significantly higher RPE values after a four day long high-FAT diet (16.0 ± 1.3) when compared to day 1 of the diet (14.8 ± 1.5). However, the high-CHO diet did not lead to any significant differences in RPE values between day 1 (14.1 ± 1.4) and day 4 (13.8 ± 1.8).

The RPE method of choice may serve as a likely clarification for the differences in RPE between this and previous studies. For example, athletes in the study of Stepto *et al.* (2002:450) reported the RPE according to the local exertion that was experienced by the legs. On the other hand, the current study requested athletes to indicate the RPE according to the perception of full body exertion after each 3-minute interval. Another possible reason for differences in RPE results between studies is that an incremental running test to exhaustion will lead to higher RPE values compared to a specific constant submaximal intensity test.

5.5.2 Heart rate

Data from the current study showed no significant differences between HR at any of the threshold points (VT1, VT2, $\dot{V}O_2$max) between diet interventions. However, Cohen's effect size showed a medium practically significant difference for HR at $\dot{V}O_2$max between the trials (Table 4.14). In agreement with the current study, Pitsilades and Maughan (1999:923), Stepto *et al.* (2002:451) and Støa *et al.* (2016:399) also found no significant differences in HR between short-term high-FAT and high-CHO diets, despite the result that higher FATox rates were achieved after subjection to a high-FAT diet. The identification of medium practically significant differences in HR between trials is therefore not a result that was verified by previous researchers.

As discussed, CHO is the preferred energy source at intensities above 65% of $\dot{V}O_2$max. Although other studies found a significant increase in HR after a high-CHO diet and attributed this result to the higher perceived effort and metabolic cost of CHO breakdown, these studies made use of longer-term adaptations diets (>28 days) (Burke *et al.,* 2017:2795; Burke, 2015:s45; Burke & Hawley, 2002:1496; Rosenkranz *et al.,* 2007:306). Therefore, results of previous studies cannot really be compared to the results of this study. However, the non-significant difference in HR between diet interventions, would suggest that HR during execution of the graded exercise test (GTX) is not influenced by short-term diet.

The problem with HR monitoring and use is that stimulation of the sympathetic nervous system at the onset of exercise causes an early increase in HR, which is also known as the cardiovascular drift (Haff & Triplett, 2016:116; Rivera, 2017:28). Only after this initial increase in HR, a relative linear increase in HR with a rise in exercise intensity is observed (Haff & Triplett, 2016:116; Rivera, 2017:28). At the end of the GXT, during which a maximal exercise intensity is reached, the HR begins to plateau (Kenney *et al.,* 2015:196). These trends in HR changes are related to autonomic nervous system activity and not diet. Furthermore, other factors that are not related to dietary intakes, may also influence the trends in HR changes during the execution of the GTX. These include: (1) age, (2) gender, (3) environmental factors, (4) hydration status, and (5) exercise intensity (Haff & Triplett, 2016:117; Rivera, 2017:28). For example, the rise in core temperature at the beginning of exercise and the increased demand for oxygen

by the working muscles with a rise in exercise intensity may all be factors that can be attributed to changes in HR during a running test (Rivera, 2017:28).

On the other hand, a blood volume decrease due to sweat loss and dehydration during exercise, may lead to an increase in HR and fatigue levels (Achten & Jeukendrup, 2003a:531; Rivera, 2017:29). The plasma and blood volume decrease will lead to a decrease in stroke volume, which can, in turn, cause an increase in HR by up to 7.5% (Achten & Jeukendrup, 2003a:531; Rivera, 2017:29). Therefore, the urine colour scale test before commencement of each GTX was an important tool to identify the possible occurrence of dehydration in the athletes. Fortunately, none of the athletes showed any signs of dehydration before test execution and were able to execute the test in a hydrated state.

All of the other above-mentioned factors that may influence HR during GXT execution were controlled to also ensure consistency in physiological responses during testing.

5.5.3 Time to exhaustion and Absolute and Relative power output

The main objectives of most short-term macronutrient manipulation studies were to examine the effects of dietary strategies on endurance performance (i.e. total time to complete a specific test). Only two studies tested the effects of different diet intervention strategies on the power output of athletes during high-intensity interval training (Burke *et al.,* 2000:1284; Stepto *et al.,* 2002:455).

The current study found that the total time to exhaustion was similar for the high-CHO and high-FAT trials – 25.26 ± 4.8 vs. 25.37 ± 6.13 minutes (Table 4.15). Even though the maximum time to exhaustion was slightly higher during the high-FAT trial (38.15 vs. 37.00 minutes), the overall time to exhaustion results along with the previously discussed results show no statistically or practically significant performance differences between dietary trials (Table 4.16). Similarly, absolute (W) and relative power output (W/kg) also obtained no significant differences between the high-CHO and high-FAT trials at VT_1, VT_2, and $\dot{V}O_2max$ (Table 4.12 and Table 4.17). However, the case plots of last-mentioned results revealed a lot of inter-individual differences for responses between the different trials (Figure 4.7a – c, Figure 4.8a – c).

In agreement with the current study, Burke *et al.* (2000:1284) explored the effects of a 3-day CHO-loading strategy on 100 km cycling performance. Seven well-trained

cyclists participated in the study and each cyclist received an individual food plan for 72 hours, which was created according to individual body mass and food preferences (Burke *et al.,* 2000:1285). Meal plans supplied each cyclist with six grams of CHO per kilogram body mass per day for breakfast and several sports bars for each day with a composition of 27 g CHO, 6.5 g fat and 2.7 g protein. No significant differences in time-trial performance or power output were found between treatments for the sprints. Results also showed an increase in muscle glycogen concentrations (572 ± 107 vs. 485 ± 128 mmol/kg dry weight) for the CHO-loading group when compared with the placebo group. However, total muscle glycogen utilisation did not differ between trials. Researchers concluded that CHO-loading prior to an event does not improve 100 km cycling time-trial performance (Burke *et al.,* 2000:1289).

Although participants in the study of Stepto *et al.* (2002:455) experienced no significant differences in endurance performance between the high-FAT and high-CHO trials, researchers recommended that different dietary strategies should be individually practiced before participation in an endurance event. They also concluded that FATox alone could not endure exercise intensity at power outputs requiring 60 – 65% of $\dot{V}O_2$peak, even in very well-trained athletes who are adapted to a high-FAT diet (Stepto *et al.,* 2002:455).

In contrast to the results of the current study, Guimaraes Couto *et al.* (2014:384) found a significantly faster 10 000 meter running time for the high-CHO compared to the high-FAT trial. Subsequently, they concluded that a short-term high-CHO diet is more effective to improve endurance running performance than a high-FAT diet (Guimaraes Couto *et al.,* 2014:384). Similarly, Pitsiladis and Maughan (1999:923) also observed significantly slower times during the cycle test to exhaustion at 70% $\dot{V}O_2$max after the high-FAT diet in both ambient temperature tests compared to high-CHO diet. In addition, various other research groups also reported significantly better endurance performance when a high-CHO diet was followed, compared to a low-CHO diet (Ferreira *et al.,* 2018:1; Rauch *et al.,* 1995:27; Williams *et al.,* 1992:24).

From the above-mentioned results, researchers would expect reduced exercise tolerance after a high-FAT diet due to a reduced glycogenolysis flux, caused by reduced muscle glycogen supply at high-intensity exercise and a very large ATP demand via CHO oxidation (Ferreira *et al.,* 2018:5). Researchers also argue that the lower exercise tolerance that is experienced during high intensity exercise due to low-

CHO availability can be attributed to impaired excitation-contraction coupling, increased exercise-induced strain, and increased effort perception (Ferreira *et al.,* 2018:6).

The lower rates of CHO contribution experienced by some of the participants in the current study during the high-FAT trial can be attributed to a reduced rate of glycogen oxidation or the capacity to utilise CHO. Nevertheless, the similar times to exhaustion between trials, despite a presumably greater availability of glycogen during the high-CHO trial, propose that the availability of glycogen was not a main factor of endurance performance in this study. However, one would think that the type of running protocol that was used in this study would cause enough exercise stress to reduce glycogen stores.

In view of the above-mentioned findings, the researchers of this study recognise that the factors determining endurance exercise performance are multi-factorial and complex. For example, the awareness of effort and pacing strategy can be just as important in determining performance outcomes as substrate provision (Fletcher, 2016:198).

The similarities in absolute and relative power outputs at the different threshold points (VT_1 and VT_2) between dietary interventions, could possibly be attributed to the study protocol that was used. A shift between FATox and CHOox as fuels during sub-maximal test protocols or high-intensity intervals interspersed with rest intervals, cause a slower depletion in glycogen and, as a result, a slower fatigue rate. The continuous incremental increase in exercise intensity during execution of the GXT does not allow for back and forth shifts between fat and CHOox as CHO becomes the dominant fuel source after an intensity of 85% of $\dot{V}O_2$max (Hetelid *et al.,* 2015:1). However, the similarity of results between diet interventions indicates that this notion needs to be further investigated.

5.5.4 Lactate

In the current study, LT was identified at a speed of 14 km/h for the high-CHO trial and at 12 km/h for the high-FAT trial. The blood lactate responses varied a lot between trials, with minimum and maximum CBL values at LT of between 4.10 and 24.20 mmol/L for the high-CHO trial and 1.90 and 17.90 mmol/L for the high-FAT trial (Figure 10). The average absolute and relative power outputs that were obtained at the LT

were higher for the high-CHO compared to the high-FAT trial (Table 4.12 and Table 4.18). The LT corresponded to 93% of the HRmax and 81% of the $\dot{V}O_2$max in the high-CHO trial and 86% of the HRmax and 72% of the $\dot{V}O_2$max in the high-FAT trial (Table 4.18).

Baseline CBL measures did not differ significantly between the high-CHO and high-FAT trials, although the high-CHO trial caused slightly higher values than the high-FAT trial (8.89 ± 5.86 vs. 7.35 ± 5.56 mmol/L). These results are in agreement with those of Pitsilades and Maughan (1999:924), as well as Stepto *et al.* (2002:452) which also found non-significantly lower CBL values after the intake of a high-FAT compared to a high-CHO diet. Furthermore, Pitsilades and Maughan (1999:924) only found significant differences in lactate values after 45 minutes of cycling, with the high-CHO trial indicating the highest values when compared to the high-FAT trial. Important to note is that LT results from this study could not be compared to other studies, as other studies did not include a GXT which is the accepted protocol to evaluate the LT and corresponding workloads or intensities (Faude *et al.,* 2009:469; Fernandes *et al.,* 2016:194; Jamnick *et al.,* 2018:1).

In theory, the LT represents the point where energy production comes predominantly from anaerobic glycolysis and represents the exercise intensity where the aerobic metabolism is insufficient to generate energy and lactate accumulates (Dunford & Doyle, 2015:79; Haff & Triplett, 2016:50; Pallarés *et al.,* 2016:2; Støren *et al.,* 2014:622). The differences in LT between diet interventions is an expected finding as literature clearly states that a high-FAT diet cannot sustain persistent increases in exercise intensity, especially after 65% of $\dot{V}O_2$max (Hetelid *et al.,* 2015:1).

A further analysis via case plot figures (Figure 4.9a – b) revealed that individual responses in CBL at VT_1 and VT_2 due to the high-FAT and high-CHO trials, varied a lot. Seventy-one percent of participants experienced lower CBL values during the high-FAT trial, which may suggest that the diet did have an influence on the substrate utilisation up to the VT_1 (lower) intensity. However, only half of the participants experienced lower values at VT_2 (higher) intensity during the high-FAT trial.

In view of the scarcity of studies that investigated LT changes due to different short-term diets, the researchers felt compelled to also discuss non-diet related studies that

also made use of the GXT to exhaustion to determine LT, VT_1 and VT_2 and related variables.

Støren *et al.* (2014:627) examined the relationships between LT expressed as a percentage of $\dot{V}O_2max$ and in watts (power output) (LT_w). They found that the best determinant of LT_w was the product of maximal aerobic power, which is calculated by dividing $\dot{V}O_2max$ by the cost of cycling (MAP), and the individual LT expressed as percentage of $\dot{V}O_2max$ (Støren *et al.,* 2014:627). They reported a significantly higher average LT value (78% of $\dot{V}O_2max$) for the elite compared to the regional cycling group (74% of $\dot{V}O_2max$). The LT in the current study corresponded to 76% of $\dot{V}O_2max$ during the high-CHO trial, which is very close to the last-mentioned values.

The literature shows that the blood lactate levels at the LT, can vary from 1.5 – 7.5 mmol/L in normal participants (Fernandes *et al.*, 2016:197). The OBLA method for determining LT does not take individual physiological responses into account and generally provides lower estimates of LT (Fernandes *et al.*, 2016:197; McGehee *et al.,* 2005:557). Therefore, the methodology that was used in this study to determine LT, is regarded to be the more accepted and accurate method.

On the other hand, the researcher also decided to determine the relationship between LT and VT_1 and VT_2 within trials. In the current study, both LT and VT_1 were identified at a speed of 12 km/h with only a 6.71 W difference in power output between last-mentioned thresholds during the high-FAT trial. With regard to the LT and VT_2 relationship during the high-FAT trial, VT_2 coincided with LT + 3 mmol/L (15 km/h). LT was identified at 14 km/h and VT_1 at 12 km/h for the high-CHO trial with VT_2 corresponding to LT + 1 mmol/L.

Studies concerning the relationship between VT_1 and LT found agreement between VT_1 and LT, obtained through incremental tests independent of whether tests were done on a treadmill or cycle ergometer (Amann *et al.,* 2006:27; Cerezuela-Espejo *et al.,* 2018:1; Lucia *et al.,* 2000:1777; Pallarés *et al.,* 2016:1; Solberg *et al.,* 2005:29; Wasserman *et al.,* 1973:236). The relationship between VT_1 and LT values in this study seems to suggest that the VT_1 and LT values are accurate as they agree with the relationships that other studies reported.

In contrast, some studies did not observe significant agreement between last-mentioned variables and indicated that the level of accession between variables

depended on the exercise test protocol and the training status of the study participants (Baumgart *et al.,* 2018:10; Leicht *et al.,* 2014:1642; Okano *et al.,* 2006:37e; Simon *et al.,* 1983:16).

The results of Pallarés *et al* (2016:11) show that there is indeed an accession between VT_1 and LT is in agreement with the works from Wasserman and co-workers (1973:242) and Lucia *et al.* (2000:1779), which also identified this positive relationship between these two thresholds (321±8 W vs. 319±10 W) in elite trained endurance cyclists.

In the current study, both LT and VT_1 was identified at a speed of 12 km/h with only a 6.71 W difference in power output between last-mentioned thresholds during the high-FAT trial. With regard to the LT and VT_2 relationship during the high-FAT trial, VT_2 coincided with LT + 3 mmol/L (15 km/h for the current study) which is in agreement with the results of a study by Cerezuela-Espejo *et al.* (2018:6). The LT was identified at 14 km/h and VT_1 at 12 km/h for the high-CHO trial. It is generally agreed that a rightward shift of the lactate curve can be explained in terms of an improved endurance performance, with a shift to the left typically reflecting a decline in endurance performance (Faude *et al.,* 2009:471).

As a GXT cannot and does not directly mimic endurance training or competition since athletes use different strategies throughout the set distance to be completed (e.g. slower pace and faster pace intertwined), the identification of the LT, for one, is not only challenging, but is also the result of a complex system of factors which makes it even more difficult to determine correctly (Heuberger *et al.,* 2018:2). The above-mentioned is also the reason why some athletic trainers rather use the Maximal Lactate Steady State (MLSS) method during which several sessions of different submaximal workloads are executed to approximate LT (Faude *et al.,* 2017:144).

The fact that participants in this study showed no differences in endurance performance between trials, despite a difference in LT occurrence, indicates that a downward shift in the lactate curve does not necessarily signify a decline in endurance performance (Faude *et al.,* 2009:469). Other factors that might influence CBL measurements should also be considered before interpreting LT curves. These factors include: O_2 delivery, mitochondrial capacity, the ability to clear and utilise lactate by other cells throughout the body, nutritional status, and prior exhaustive exercise

(Faude *et al.,* 2009:472). Furthermore, Hall *et al.* (2016:s12) concluded that although the LT may be a valuable tool for assessing a group of athletes, the low reliability of traditional lactate threshold measuring techniques may limit its applicability for individual athletes.

During the high-CHO trial, VT_2 was identified at LT + 1 mmol/L which is in agreement with the study of Pallarés *et al.* (2016:11). Notwithstanding, the absence of statistically significant difference between endurance performance times in the current study, results from the LT identification concerning the high-CHO trial can possibly indicate that a high-CHO diet may enable participants to sustain performance for a longer period than a high-FAT diet, execute the incremental treadmill test protocol successfully, and to obtain data that can be used to set performance parameters for training programme prescription. However, more research is needed before this can be validated. On the other hand, a high-FAT diet may possibly impair performance as CBL seems to accumulate faster with intensity increases than when a high-CHO diet is followed. However, despite the earlier onset of LT due to the high-FAT diet, running performance was not impaired and it is, therefore, suggested that researchers would need to further investigate outcomes that are related to short-term diet changes. Overall, researchers suggest that systematic differences in V_1, VT_2 and LT should be considered when making recommendations for training (Dickhuth *et al.,* 1999:125).

5.5.5 Work output and carbohydrate and fat contribution

Indirect calorimetry is seen as the current gold standard to assess work which is calculated by measuring the O_2 consumed and the CO_2 released and is based on the presumption that O_2 is consumed by the body when the muscles are working (Grieve, 2018:7; Klass *et al.,* 2019:2). A straight relationship occurs between the amount of $\dot{V}O_2$ and the amount of heat produced, therefore, the measurement of an athlete's $\dot{V}O_2$ during exercise allows a person to predict the athlete's metabolic rate (Grieve, 2018:7; Klass *et al.,* 2019:2). Therefore, indirect calorimetry, as used in this study, allows researchers to indirectly determine the energy release from substrate oxidation during exercise, measured in kilojoules (kJ). Also, as mentioned before, RER measurements also indicate to researchers the preferred fuel source (CHO or FAT) that was used at a given point in time during a GXT test. However, the work output (kJ) and substrate contribution did not differ significantly between trials in the current study (Table 4.20).

Nevertheless, one of the most important findings of this study were that, despite the non-significant differences for CHO and FAT contribution at VT_1, VT_2 and $\dot{V}O_2max$, medium practically significant differences were observed for FAT contribution at VT_1 and VT_2 between the high-FAT and high-CHO trials. In addition, a large practically significant difference was also found for FAT contribution at $\dot{V}O_2max$ between the two trials (Table 4.22). The case plot (Figure 4.11a – c and Figure 4.12a – c) results further revealed large differences for CHO and fat contribution at VT_1, VT_2 and $\dot{V}O_2max$ between individuals.

Guimaraes Couto *et al.* (2014:383) reported a lower CHO contribution and a significantly higher FAT contribution to work during the high-FAT trial, compared to the high-CHO trial. Similarly, Stepto *et al.* (2002:451) found an increase in FAT contribution to work during 20 minutes of cycling and during exercise at 85% of $\dot{V}O_2max$ after a high-FAT diet. The practically significant differences between CHO and FAT contribution at VT_1, VT_2 and $\dot{V}O_2max$ (Table 4.22), as well as the case plot revealed that a large proportion of the participants in this study experienced a lower CHO contribution (63%, 50% and 54%) or a higher FAT contribution (58%, 54% and 42%) to work at VT_1, VT_2 and $\dot{V}O_2max$, respectively, after a high-FAT trial when compared to the high-CHO trial. In contrast to the mentioned results, Ferreira *et al.* (2018:4) found work to be significantly lower during high-intensity exercise when comparing low- to high-CHO availability. Energy expenditure was also significantly lower in the high-FAT compared to the high-CHO group.

Therefore, above-mentioned studies do not provide any clarity on the influence of a specific dietary intake on contribution of different macronutrients to work output during a running test. Possible reasons for this are that influences such as the duration, intensity and tempo of the exercise, along with the amount of muscle mass and muscle fibres that are recruited during the specific exercise, also determine work output (Ferreira *et al.,* 2018:4; Grieve, 2018:10; Guimaraes Couto *et al.,* 2014:381; Stepto *et al.,* 2002:451). Generally, long continuous aerobic type of exercises, such as submaximal distance running, lead to greater work outputs than low-volume exercises while, during high intensity interval training (HIIT), the work output is greater due to the greater intensity at which the exercise takes place (Grieve, 2018:10). Nevertheless, a meta-analysis of 13 training studies showed that continuous aerobic exercise protocols were responsible for a 102% greater work output than the HIIT exercise

protocols due to a longer duration (Wewege *et al.,* 2017:7). Whether or not differences in protocols, methods, work performed, or the duration of the specific exercise had an influence on the amount of work remains uncertain.

CHAPTER 6

SUMMARY

CHAPTER 6 - SUMMARY

6.1 Synthesis of findings

The overarching theme of this thesis was to investigate the effects of short-term macronutrient manipulation on the endurance performance of long-distance runners.

More specifically, the aims of the thesis were:

- To investigate the effects of a short-term (48-hour) high-carbohydrate (high-CHO) diet on a long-distance runner's indirect respiratory indices, as well as physiological and perceptual measures during a maximal graded exercise test to exhaustion, using a treadmill protocol, including $\dot{V}O_2$ (ml/kg/min), $\dot{V}O_2$ (L/min), $\dot{V}CO_2$ (L/min), RER and $\dot{V}_E$ (L/min), substrate utilisation (CHO oxidation and fat oxidation), time to exhaustion, absolute power output (W), relative power output (W/kg) and work output (kJ) during a graded exercise test (GXT).
- To use these indirect respiratory indices' values to determine certain threshold points that occurred during the test, including Ventilatory Threshold 1 (VT_1), Ventilatory Threshold 2 (VT_2) or Respiratory Coefficient Point (RCP), Lactate Threshold (LT), $\dot{V}O_2$peak and $\dot{V}O_2$max after the high-CHO intervention period.
- To investigate the effects of a short-term (48-hour) high-FAT diet on a long-distance runner's indirect respiratory indices, as well as physiological and perceptual measures during a maximal graded exercise test to exhaustion, using a treadmill protocol, including $\dot{V}O_2$ (ml/kg/min), $\dot{V}O_2$ (L/min), $\dot{V}CO_2$ (L/min), RER and $\dot{V}_E$ (L/min), substrate utilisation (CHO oxidation and fat oxidation), time to exhaustion, absolute power output (W), relative power output (W/kg) and work output (kJ) during a graded exercise test (GXT).
- To use these indirect respiratory indices' values to determine certain threshold points that occurred during the test, including Ventilatory Threshold 1 (VT_1), Ventilatory Threshold 2 (VT_2) or Respiratory Coefficient Point (RCP), Lactate Threshold (LT), $\dot{V}O_2$peak and after the high-FAT intervention period.
- To explore whether there is a difference in indirect measures when comparing results between the two treatment interventions (high-CHO and high-FAT trials).

- To use the results of the study as a possible indication for individual preferential use of a particular fuel source (high-CHO or high-FAT) over a short-term period to enhance a distance runner's performance.

The intention of this chapter is to offer a summary of the findings in relation to the objectives and aims as set out in Chapter 1. A brief conclusion is then given whereby detailed courtesy is given to presented data and how it has advanced our understanding of the effects of short-term macronutrient manipulation on endurance performance in distance runners. A discussion of the strengths of the present study, the practical limitations as well as directions for future research are also presented.

6.2 Conclusion

Using a randomised controlled cross-over study of 24 trained endurance runners, chapter 4 was able to demonstrate that there is no significant difference in endurance performance when comparing a short-term high-FAT and high-CHO diet after 48-hours of randomising the two trial diets with a two-week wash-out period separating the experimental trials, but that the medium practical significance seen at VT_1 (d = 0.58), and VT_2 (d = 0.41) for $\dot{V}O_2$ (L/min), with a medium practical significance (d = 0.61) at VT_1 and VT_2 (d = 0.47) for $\dot{V}O_2$/kg (ml/kg/min) might suggest that there were notable differences between diets at these threshold points. Other noteworthy medium practical significance was observed at VT_1 for speed (d = 0.48), along with HR at $\dot{V}O_2$/kg (ml/kg/min) (d = 0.41) and fat contribution at VT_1 (d = 0.40), and VT_2 (d = 0.43) and $\dot{V}O_2$/kg (ml/kg/min) (d = 0.56) between trials.

In other words, the results from the current study suggested no statistically significant differences between the effects of the short-term high-CHO and high-FAT diets on any of the respiratory and other indices measured in endurance runners during a GTX to exhaustion. However, some moderate effects sizes observed for some of the indices suggest that further research is justified, possibly involving longer-term diets.

The only noteworthy difference was seen between the LT's identified within the high-CHO and high-FAT trials, respectively, with LT identified at stage five or speed 14 km/h during the high-CHO trial (12.59 ± 6.03 mmol/L) and at stage three or speed 12 km/h during the high-FAT trial (8.65 ± 5.03 mmol/L). Also, the mean maximum CBL values at LT, were 24.20 mmol/L and 17.90 mmol/L for the high-CHO trial and high-

FAT trials, respectively. This accounted for a work rate of 303.51 ± 33.56 vs. 261.37 ± 28.89 W and WR/kg of 3.84 ± 0.01 vs. 3.30 ± 0.01 W/kg, respectively. LT also corresponded to 93% of HRmax (177.33 ± 14.58 bpm) and 81% $\dot{V}O_2$max in the high-CHO trial and 86% of HRmax (163.54 ± 12.43 bpm) and 72% $\dot{V}O_2$max in the high-FAT trial.

Another attractive finding from the current study was the difference between LT and VT_1 identification during the high-CHO trial with no significant differences seen between these two variables during the high-FAT trial. During the high-CHO trial, LT was identified at 14 km/h with VT_1 identified at 12 km/h. Directing attention to this within dietary treatment trial's results, a large practical significance was observed between HR and LT at VT_1 (d = 1.12), as well as between W and LT at VT_1 (d = 1.17) during the high-CHO trial, whereas within dietary treatment trial results for the high-FAT diet suggested a medium practical significance between W and LT (d = 0.46). The within dietary treatment trial results observed, just as between diet results, may suggest that there were notable differences between the high-CHO and high-FAT trials at these threshold turning points.

Although LT was identified at different exercise intensity points during the two experimental trials, overall results still showed no significant difference in endurance performance. This therefore showcases the importance to acknowledge the comment of Faude *et al.* (2009) that a downward shift of the lactate curve does not essentially mean a decline in endurance performance and must not be falsely attributed (Faude *et al.,* 2009:469).

Although our study provided further information concerning the possible influence of short-term macronutrient manipulation on the physiological responses of athletes by examining the possible differences in GXT-derived threshold points (VT_1, VT_2, LT and $\dot{V}O_2$max) by mimicking the running demands of competitions, several questions still remain. These questions pertain to how to simplify (i) the minimal amount of time required to adhere to a specific dietary protocol, (ii) the ideal intensity and volume of exercise required to accomplish such metabolic changes after a dietary manipulation strategy, and (iii) the number of times such a protocol needs to be completed in the athlete's macro cycle to achieve the desired effect.

All the above will consequently require a series of independent studies to properly identify the optimal protocol benefit. From the novel findings of the current study, it can be concluded that 48 hours of macronutrient manipulation does not affect endurance performance outcomes when using our GXT protocol to potentially demonstrate performance differences, despite the obvious difference seen in LT which would have led a researcher, coach and sport scientist to think the high-FAT diet would have impaired the endurance performance. However, whether this would have had the same outcomes with the use of a submaximal or time-trial test protocol remains unresolved.

Given the significance of energy balance in endurance athletes, as discussed throughout the literature review of this study, a broader understanding of the effects that exercise duration, mode, and intensity have on these energy balances is needed.

The knowledge obtained from the current study along with future work will be beneficial when it comes to the designing and implementation of diet and conditioning programmes for endurance athletes and will help them to avoid the negative effects of energy deficit.

6.3 Recommendations and future research

Without a doubt, the necessity of further research on this topic cannot be overlooked. Future studies should attempt to, firstly, shift focus to a more modern-day dietary approach to improve endurance performance, regardless of whether it is runners, cyclist, or walkers. Despite the notion that high-FAT diets are superior to high-CHO diets for endurance performance, further well-planned studies are needed to provide more proof for this contention. However, we should acknowledge that new insights or research are needed to challenge current diet-related ideas and paradigms.

Secondly, future studies should focus on whether endurance athletes should work on objective processes of training intensity (i.e. power output) rather than subjective factors (i.e. RPE).

Lastly, future studies should focus on using different testing protocols and scenarios, including the comparison of submaximal and maximal exercise testing in a single study. Laboratory and on-field tests should both be done and can be compared, however it is important to keep the specific aims and objectives of a study in mind, to

ensure the most appropriate testing protocol is used for the goals outlined in that study. The different testing protocols may have an effect on ventilatory and subjective measures, as well as endurance performance. Different number of days of dietary manipulation exposure can be examined.

Such future results can help to identify a set of protocols that can be used when, for example, the use of indirect calorimetry is not possible due to unavailability of such equipment or the cost of such laboratory tests, the need for such testing results is necessary to optimise conditioning programmes. In view that universities are experiencing more and more pressure to cut expenses especially during the Covid epidemic, researchers all looking for alternative running protocols to test athletes' aerobic capacities and related parameters. The cost of specialized, sophisticated equipment, the unavailability of well-trained personnel, the amount of time needed to conduct indirect calorimetry tests and the inability to test a large number of participants when directly measuring maximal oxygen consumption by indirect calorimetry and computerized instrumentation in the laboratory, are compelling university-based researchers to develop and use less expensive indirect testing methods.

6.4 Strengths of the study

Strengths of our study include the randomised within-subjects design and each of the trials conducted in a controlled clinical setting. Also, the relatively large sample size (n = 24) when comparing our sample size to previous research studies on this topic. To our knowledge, this was also the first study to examine the effects of 48 hours of macronutrient manipulation on the endurance performance of long-distance runners that used a GXT to exhaustion protocol. The use of such a test is a design component that has been inconsistent in the literature. Additionally, our study was also the first to investigate the effects that such a dietary approach has on the different turning points that occur during a GXT test to exhaustion protocol as the detection of these turning points during a submaximal test or even a maximal specified time limit test is not possible.

6.5 Limitations

Protocol design – A limitation of the current study was the use of only a maximal GXT to exhaustion test, using indirect calorimetry (ventilatory, metabolic and substrate

usage). Previous research either used a submaximal test or a maximal test for a set time to examine the possible effects of dietary manipulation strategies. The duration being unknown to the athlete, as well as the athlete not having the ability to indicate or change the fixed progressive intensity set out by the test, which makes the effort in such protocols excessively different from the athletes outside sport specific conditions. When an athlete is able to apply a pacing strategy, it allows the brain to recruit the appropriate number of motor units in the muscles to be able to perform the activity without homeostatic failure and the onset of muscle fatigue, and for this reason the use of the GXT has also been discouraged as the athletes are unable to replicate the exact sport specific conditions. Also, the possibility that three recurrent instances of maximal GXT to exhaustion place a mental burden on participants should be considered, even with strong verbal encouragement during the respective tests.

Participants – The participants in this study were well-trained male endurance runners who were free from injury and currently practising multiple cross-training activities. Due to these factors, the training status of individual participants could have surely fluctuate due to the particular nature of their training history and that offers changed adaptive stimuli (i.e. frequency and intensity). As such, it is uncertain whether the findings from this study would be adaptable to recreational and elite endurance athletes, given the metabolic and physiological differences that would exist.

Dietary trial – Although all food was prepared and provided to the subjects for both 48-hour periods to try to maximise compliance, it is impossible to know whether adherence was indeed 100%, even though subjects might have claimed it was the case. Future studies should also attempt to make the dietary food plans more specific to each subject's food preference that they are used to eating on a day-to-day basis, although this can complicate the setup of the menus as certain macronutrient layouts should be followed. Also, due to the nature of the study, it was challenging to blind which experimental trial was which, and as such, subjects were aware of which was the high-FAT and high-CHO trial. While RPE did not significantly differ between the two trials, it is possible that subjects in some cases did arrive to the laboratory for the high-FAT trial with the perception of earlier fatigue. However, due to the fact that the current study did not evaluate habitual dietary intakes before the start of the experiments, no conclusion could be drawn regarding the possible effects of the

habitual diet on the test results and this is definitely a study limitation. Habitual diets could have served as potential confounders to the results of this study.

6.6 In summary

Future research on this topic is necessary to open the mind to the underlying question that still remains as to which dietary strategy will lead to the best possible endurance performance, which highlights the importance to rather look at this topic of discussion as an individual approach rather than an over generalised assumption acquired from published study results. In other words, it is our advice that the practicality of current and future recommendations and strategies needs to be respected on an individual basis and the consequence of try-out of an individualised dietary approach prior to endurance events cannot be overstated and should only be implemented after possible trial and error practices. Endurance athletes are urged to apply dietary strategies within the framework of their training setting, body composition, and physiological needs. All the revised literature directed that endurance athletes must pay attention when attending to their nutritional needs to ensure good health, encourage optimal performance, and decrease the likelihood of burn-out and, consequently, injuries. Suitable nutrition will result in better performance through reduced energy depletion and – if an injury is sustained – faster recovery.

With the emergent international attraction of endurance events, substantial research is required to encourage the wellbeing and health of athletes. Additional cross-sectional and more longitudinal studies are needed to discover the specific environmental and nutritional circumstances under which athletes perform optimally based on gender, age, body type, type of event, and other physiological influences. In this regard, more longitudinal–based studies will allow researchers to determine how long athletes need to adapt to certain diets and reap the possible physiological and performance benefits that can be derived from following a specific diet. This type of research method will also allow researchers to examine more in-depth the individual differences in physiological and performance responses that a group of middle- or long-distance athletes experience when following various diet interventions.

CHAPTER 7

REFLECTION ON THE RESEARCH PROCESS

CHAPTER 7 – REFLECTION ON THE RESEARCH PROJECT

"EDUCATION IS THE MOST POWERFUL WEAPON WHICH YOU CAN USE TO CHANGE THE WORLD."

- Nelson Mandela

7.1 Introduction

Albert Szent-Gyorgyi once said, "Research is to see what everybody else has seen, and to think what nobody else has thought".

Learning does not come from achievement alone, but from reflecting on what we do. More than often, we shift through our daily lifes without "processing" our encounters. This is not necessarily an unhealthy thing, since what we do throughout the day we usually do routinely, and it may therefore not hold a lot of momentous learning. When we contribute to new experiences – even those outside of our comfort zones or outside of our daily routines – there is often beyond-thinking learning that takes place. Research shows that reflection has some optimistic effect on the outlooks of the participants regarding service. However, the absence of reflection has a STRONG NEGATIVE influence on the volunteers' attitudes about service and the service activity.

Reflecting on your research journey encourages you to take charge of your own learning and provides you with the opportunity to share your accomplishments, struggles and even moments of confusion, and this encourage you to focus on your own personal strengths and weaknesses. Through the above, you become aware of the skills you have developed and the ones that require some more refining. Reflecting on the journey also allows you to take a step back and help you to foster analytical thinking skills and improve on future performance by analysing what you have learned and how far you have come. Lastly, reflecting on the entire process sparks social interaction. Sharing your experience with your peers, co-workers, friends, and family leads to engagement with one another, discussing and debating elements and what you have liked and disliked, found useful or thought could be improved. The in-depth opportunity you get to look at a questions and the answers

to that questions provides you with the knowledge and skills to become a better teacher and even a better researcher. Research will forever be an ongoing process and the more you equip yourself with knowledge, the better you will become in your field of practice. As Mervin Gordon once said, “No research is ever quite complete. It is the glory of a good bit of work that it opens the way for something still better, and this repeatedly leads to its own eclipse”. This was my drive for the completion of my PhD. The opportunity to better myself in the understanding of research and to be able to strengthen my ability to seek answers to question and be part of the world’s research population in the ongoing, never-ending pool of questions is something I have strived for my entire career.

7.2 Reflecting on the research process

I completed my Master’s degree in 2011 and, as a result, the entire research process was a bit vague in the beginning. Luckily, the steps I followed back then were helpful in rewiring my brain to start the process the correct way. Below are 6 steps I followed back then and used again now as a guideline through the entire process:

Step 1: Find the best suitable supervisor

Step 2: ASK! Do not be shy

Step 3: Choose the right topic

Step 4: Keep your plan sensible

Step 5: Plan a timeline

Step 6: Do not stop writing

Step one: Find the best suitable supervisor

I had quite an idea of what I was interested in doing and what I wanted to achieve through the topic, but the whole idea I had could simply be articulated into valuable research questions after I knew who my supervisor would be. I knew from the very start exactly who I wanted as my supervisor. Prof. F.F. Coetzee was also my supervisor during my Master’s degree study, and I knew he understood my way of doing things and accepted it that way, which I appreciated very much. Therefore,

he was the easy and obvious choice and there was no question. After we had a brief meeting on my idea for the study, we agreed that we had to identify co-supervisors that are experts in the field of study I am interested in. We then thought it well to ask Prof. Ben Coetzee from NWU if he would be willing to co-supervise. Prof. Ben is truly an expert in the $\dot{V}O_2$max and sport science testing field and he was an obvious choice, given my field of interest. We also thought it best to appoint another co-supervisor expert in the field nutrition, as we knew it would form a big part of the study. Dr Ronette Lategan-Potgieter was willing and truly eager to help with the study. Unfortunately for us but fortunately for her, she and her family got the amazing opportunity to emigrate to Canada to pursue her academic career further. However, she recommended we approach Dr Elmine du Toit to take over her duties as co-supervisor, and she was more than willing. This was indeed such a relief for me. Dr du Toit is one of the most caring and compassionate people I have ever met and her personality and the way she presented herself to me truly helped me keep my calm through everything, and for that I am truly grateful. All of my supervisors played a significant role in the outcome and their direction assisted me to present the thesis in such a good manner.

Step 2: Ask!! Do not be shy

The important thing is to never stop questioning. However, this also means to never stop asking questions. Sometimes we are afraid to ask questions, but the most important thing is to never be anxious to ask for help when it is needed. It is something we must do continuously. Asking for help does not mark weakness, but is actually a sign of your strength. It shows your courage to admit when you are uncertain about something and again opens the chance to learn. The professional and caring approach of my supervisors made it a lot easier to ask questions whenever something was unclear.

Step 3: Choose the right topic

Luckily, I had somewhat of a sketch of the what and the why I wanted to do the research. I knew that the topic I had in mind was one of major discussion in the last few years, so to try and pour some extra insight on the topic was really stimulating to me. Formulating the title and research question was relatively easy with the help

of my supervisors and the prospect of starting the journey was extremely exciting to me.

Step 4: Keep your plan sensible

It is important to know the questions you want to be resolved at the end of the project and stick to that. Do not over-complicate things for yourself. While I was busy reading similar articles, I started to overthink my plan and had to really go and sit down and tell myself to stick to the basics I wanted to test, and nothing more.

Step 5: Prepare a timeline

This was truly the part of the project I completely misjudged, or rather, life's plans got in the way. What was my plan to finish my project in 3 years, turned out to be 5 unexpected years. My PhD journey started in 2017. A lot of hardship in my personal life set back my starting timeline, but I finally started mid-2017 and really threw all my available time into it. By October 2017, we announced the expectance of our beautiful baby boy, and as expected, it juggled through my head as to how on earth I was going to finish this project being employed full-time, being a mom, and trying to have a life in between. However, my motivation and determination led me to finish my experimental trials early in 2018 and by the time I became a mother, my literature review was well underway to the finish line. However, after becoming a mom, things completely took halt. Four months of maternity leave, only to return to work full-time during an extremely busy time left me with extraordinarily little time to work on my studies. It was only in mid-2019 that I really started to focus on the end goal. I must give Prof. Robert Schall credit for his help during this time with all my statistics that had to be done, and constant changing of results, etc. He was never too busy, and I will forever be thankful to him for calming my nerves during this time. I also have to say that I would not change my journey for one moment, as all of it was a learning experience which showed me my determination and drive to complete it. The most important lesson I learned in this step was that life happens and you have to adapt to unexpected changes. However, do what you can with what you have and start from scratch every time if you have to, the end will come when the time is ready.

Step 6: Do not stop writing

For me, this part was incredibly difficult. I think my perfectionistic personality was mainly the factor that let me to experience this as difficult. I am someone that needs my words and writing to be extremely specific and precise as I formulate it in my head. To add, I do not think of this as a bad thing, this was only my drive to work harder. To communicate the suitable message in English can also be a challenge for an Afrikaans-speaking person like myself, but as the process goes on, you learn exactly how to state what you really want to say, which also shows you how much of a learning process research actually is. The greatest guidance I can give is to start writing from day one and, again, not to over-complicate things for yourself. Do not re-read your paragraphs over and over, because that can really lead you to re-writing a paragraph that was way better than you thought it was to start with. Yes, the literature review and overall write up was time-consuming – sometimes very frustrating and unpleasant to say the least. However, understanding what the literature had to say on my topic and to observe the shortcomings of previous research and to ultimately communicate what my research yielded made it worthwhile.

7.3 Personal remarks

"I BELIEVE THAT EDUCATION IS ALL ABOUT BEING EXCITED ABOUT SOMETHING."

Steve Irwin

To write this last paragraph of this project is such an honour and I can only thank my Heavenly Father – He that is my Strength and my Rock – for giving me the wisdom, knowledge, and perseverance to finish it. To be able to understand the meaning of a Living God and the work He does for and through you during such a time, is something I hope I can continue to share with the world.

I also believe and hope that this is only the start of my independent research journey and it also makes me hopeful for a future in academia. I have learned so much during the process and it would be a privilege, in the future, to assist and help other students on a similar journey.

As a bonus, professional relationships were developed during the project. I have the greatest admiration for my supervisors, Prof. Derik Coetzee, Prof. Ben Coetzee and Dr Elmine du Toit.

Lastly, I would like to thank my husband, Alf Deacon, for his constant support and being a super dad when I was busy with my work. Also, my dad, Basie Janse van Rensburg, who has always been my drive to pursue things I only dreamed of. I appreciate you more than words will ever be able to explain.

I leave you with this quote:

"You have to set goals that are almost out of reach. If you set a goal that is attainable without much work or thought, you are stuck with something below your true talent and potential"

\- Steve Garvey -

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APPENDICES

Appendix A.1

INFORMATION DOCUMENT

UNIVERSITY OF THE FREE STATE
UNIVERSITEIT VAN DIE VRYSTAAT
YUNIVESITHI YA FREISTATA
UFS UV

EFFECT OF SHORT-TERM MACRONUTRIENT MANIPULATION ON ENDURANCE CAPACITY OF LONG-DISTANCE RUNNERS

Dear Mr

I, Lizl Deacon, a Doctoral student in Biokinetics at the University of the Free State, am doing research on macronutrient manipulation and its effect on the endurance capacity of long-distance runners.

Research is the manner to seek and learn the answer to arising questions. In this study, we aspire to gain more knowledge of the effect that a specific macronutrient dietary manipulation strategy has on endurance of long-distance runners when taken 48 hours prior to and just before exercise testing commences. Macronutrients are energy-providing sources consumed by humans in large quantities and is important for providing energy, promoting growth and other bodily functions taking place during everyday living. The three main macronutrient substrates consumed by humans are Carbohydrates, fats and proteins.

We are requesting you to partake in a research study that involves 24 middle- and long-distance endurance runners.

The study will focus on the effect that specific macronutrient dietary intake has on an individual's endurance during a test to maximum exhaustion while running on a treadmill. In other words, we want to test the efficiency of your running performance on a treadmill on two separate occasions after you ingested a high amount of carbohydrates on one occasion and a high amount of fat on the other occasion for a 2-day period before the running test on the treadmill. You will therefore perform the same treadmill test on two separate occasions. These test will be scheduled two weeks apart. On each testing occasion, you will do a warm-up on the treadmill at a walking speed of 4 km/h followed by a dynamic stretching routine. After the warm-up and stretching routine you will start the test on the treadmill where your $\dot{V}O_2max$ or maximum oxygen consumption, blood lactate level via a finger prick test and degree of fatigue will be measured. The treadmill protocol will start at 10 km/h with speed increments of 1 km/h every 3-minute interval. After each interval, your blood lactate and heart rate will be captured. The test will be terminated when you are unable to maintain the requirements of the treadmill, or when the British Association of Sport Sciences (BASS) guidelines are reached, or when any two of the following criteria are met: (i) lactate peak ≥8 mmol/L; (ii) HR_{max} ≥95% of endurance-trained age-predicted

maximum heart rate; and (iii) RPE_{peak} ≥19 in the 6–20 Borg scale. $\dot{V}O_2max$ measurement will continue for 5 minutes after test completion.

The study will have two testing trials that will all include macronutrient dietary manipulation (which will be different on each occasion), followed by the same $\dot{V}O_2max$ test protocol at every trial. Results of each individual will be calculated and compared after the trial periods.

Should you agree to participate in the study, your total daily energy expenditure, this means the total amount of calories your body needs to survive on a day to day level with the inclusion of your daily activity level, will be calculated by a registered dietitian who will after determination of your daily energy consumption, prepare an individual isoenergetic carbohydrate-rich and fat-rich diet. Diets will be described as (i) high-fat and (ii) high-carbohydrate. Before the trial period commence, you will be assigned to either the high-carbohydrate diet or the high-fat diet that will be followed for 48 hours prior to the first exercise testing. On the next day just before exercise testing commences, you will receive a diet-specific beverage with the same macronutrient composition (either high-carbohydrate or high-fat) as the diet you followed for the previous 48 hours. The exact procedure will be followed for the second laboratory visit trial test except you will receive the opposite dietary meals than your previous trial period in the 48 hours prior to exercise testing, again followed by a dietary beverage similar to the dietary intake prior to exercise testing. On the day of testing, you will have to complete 2 questionnaires and provide a urine sample before testing can start. After you provided the completed questionnaires and urine sample you will sit in a relaxed state where your resting heart rate will be monitored along with an explanation of testing procedures, the rate of perceived exertion scale where you will have to supply the researcher with a certain number on a scale provided that reflects your total body exhaustion during the test and fitting of all relevant equipment to be used during the test.

All the results of your individual exercise trials will be analysed and compared, which might be of advantage to yourself for future meal preferences by providing knowledge about your own individual body's energy usage in the lead up to an endurance run.

As a lack of sleep, not sticking to the prescribed diet, excessive exercise and alcohol intake may affect performance, you will be kindly requested to strain from very heavy high-intensity exercise in the 24 hours prior to each exercise testing trial, get an adequate amount of sleep and restrain from high levels of alcohol intake for the period of time before testing (48 hours).

Participation is completely voluntary, and should you decide not to participate you are not eligible for any penalty or loss of benefits. You may withdraw your participation at any time.

Your personal information will not, in any circumstance, be shared with other individuals or organizations without your permission. Personal information may be released if compelled by law. The only parties that may review and/or copy your records for data analysis and quality guarantees include organisations such as the Health Sciences Research Ethics Committee.

Contact details of the principal researcher: cell phone number 071 354 4253; email address: lizljvr1985@gmail.com.

_____

Lizl Deacon

Appendix A.2

INFORMED CONSENT

UNIVERSITY OF THE FREE STATE
UNIVERSITEIT VAN DIE VRYSTAAT
YUNIVESITHI YA FREISTATA
UFS UV

EFFECT OF SHORT-TERM MACRONUTRIENT MANIPULATION ON ENDURANCE CAPACITY OF LONG-DISTANCE RUNNERS

You have been asked to partake in a research study as informed by Mrs. Lizl Deacon.

You may phone Mrs. Lizl Deacon at 071 354 4253 with any questions you might have regarding the study or if you experience any injury as a result of the study or if you are unable to continue with the study.

You may also contact the Health Sciences Research Ethics Committee, UFS at (051) 401 7795 if you have questions about your rights as a research participant.

Your personal information will not, in any circumstance, be shared with other individuals or organizations without your permission. Personal information may be released if compelled by law. The only parties that may review and/or copy your records for data analysis and quality guarantees include organisations such as the Health Sciences Research Ethics Committee.

Upon agreement to partake, you will receive an indorsed copy of this text as well as the information sheet, which is a written summary of the research.

The research experiment and study, with all the information provided, has been verbally explained to me. I appreciate what my participation in the study means and I willingly agree to participate.

_____ _____

Participant Date

_____ _____

Witness Date

Appendix A.3

METAMAX 3B CALIBRATION PROCEDURE

VI. Calibration

General

To maintain the highest possible accuracy, periodic calibration of the MetaMax® 3B analyzers with certified calibration equipment and in accordance with the instructions described in this manual is strongly recommended.

Please read the calibration instructions thoroughly prior to performing a calibration measurement. Calibration procedures should be performed by trained personnel only to ensure utmost operator safety and to maintain a proper function of your MetaMax® 3B system.

Each analyzer of the MetaMax® 3B is individually pre-calibrated on delivery of the system, enabling users to operate their MetaMax® 3B immediately upon installation. A calibration report is included in the extent of supply, specifying the calibration factors and the barometric pressure at the time of calibration.

Depending on the prevailing altitude level of your area or the operating conditions in which you intend to use your MetaMax® 3B upon installation, it may be necessary to recalibrate its gas analyzers prior to first use. It should be noted that the MetaMax® 3B is calibrated based on a barometric pressure level of approximately 1000 mbar when delivered. Should the mean level of barometric pressure in your area be lower than 900 mbar, a gas and volume measurement should be performed prior to first use of the MetaMax® 3B system.

Calibration Procedures

The following calibration procedures are available:

- Gas calibration of the O_2 and CO_2 analyzer (2-point calibration),
- Calibration of the volume transducer,
- Calibration of the pressure analyzer.

A proper calibration compensates for differences due to

- specimen differences between analyzers,
- ageing of analyzers,
- changing operating conditions.

Important

To ensure the highest possible accuracy, the MetaMax® 3B analyzers should be calibrated under conditions (temperature, pressure, humidity) similar to the environmental conditions in which the system is operated.

Example:
If the MetaMax® 3B is usally operated at normal barometric pressure, it is strongly recommended to recalibrate its analyzers under the new conditions if a test is to be performed at high altitude.

Calibration Practices

If the MetaMax® 3B is operated **in stable environmental conditions**, i.e. if it is used in the same environmental conditions or if environmental conditions, in which it is used, do not change significantly between the measurements, the following calibration practices are recommended to verify and maintain accuracy of its analyzers:

Gas analyzers

A two-point gas calibration should be executed **prior to a series of coherent tests, after oxygen sensor exchange or at least every 30 days**. Please note that an ambient air measurement must be performed prior to each measurement/test. For instructions on how to perform this please refer to the chapter „Perform ambient air measurement" of this user manual.

Volume transducer

The volume transducer should be calibrated at least **once a day before the first test.**

Pressure analyzer

It is not necessary to recalibrate the pressure analyzer unless barometric pressure changes significantly. It is recommended, however, to check the barometric pressure measured by MetaMax from time to time (e.g. every six months) against a reference barometer. If the values measured differ more than 10 mbar, the pressure analyzer should be recalibrated.

Important

Remember to execute a two-point gas calibration after the pressure analyzer has been recalibrated.

CORTEX Calibration Kit

1. Parts List

The calibration procedures and routines described in this manual are based on CORTEX calibration equipment which is optionally available and can be purchased from your CORTEX Biophysik sales partner or CORTEX Biophysik. Always use original parts offered or sold by CORTEX Biophysik or parts specified as equivalent by CORTEX Biophysik.

Equipment for Volume Calibration

1-3 Liter Calibration Syringe, adjustable

Equipment for Pressure Calibration

Digital Barometer

Common Equipment for Gas Calibration

Certified Calibration/Reference Gas,
with 15% O_2, 5% CO_2, bal. in N_2,
in a handy 1.2 liter bottle,

Reduction Valve for 1.2 liter gas bottle

CORTEX Calibration Kit: Parts List (continued)

Equipment for Gas Calibration with CORTEX Calibration Gas Saver

Calibration Gas Saver

Tube Set, including:
Tube to connect sample line to gas saver and
Tube to connect gas bottle to gas saver.

Power Supply, type Multinorm, with plug adapter(s) to operate calibration gas saver on mains supply and
9V Battery to operate calibration gas saver on battery.

Equipment for Gas Calibration with Automatic Gas Calibrator

CORTEX Automatic Gas Calibrator

Connection Set, including:
Data Cable for connecting PC and Automatic Gas Calibrator,
Tube to connect sample line to Gas Calibrator and
two tubes to connect gas bottle(s) to Gas Calibrator.

CORTEX Calibration Kits: Parts List (continued)

Equipment for Gas Calibration

Power Supply, type Multinorm, with country-specific power cord or plug adapter(s) to operate Automatic Gas Calibrator on mains supply

Optional Equipment for Calibration Kit

Transport Case, here shown with CORTEX calibration gas saver

Steps prior to Calibration

Run system for a warm-up period

To execute a calibration, your **MetaMax® 3B** base system must be powered and connected to a PC/notebook.

A warm-up time of 45 minutes is required for gas calibration. Volume and pressure calibration can be done already after 15 minutes.

Turn the MetaMax® 3B on by pressing the **on/off button** (first button on front panel of base system) for a second.

A short beep indicates that the system is now powered.

Check physical connections

Make sure the

- volume transducer
- sample line

are plugged in firmly prior to test start.

We recommend **not to disconnect** these parts unless they need to be replaced and/or cleaned.

Volume Calibration

Equipment required:

- Manual Calibration Syringe (included in CORTEX Calibration Kit or equivalent syringe) or certified automatic syringe

Calibration Steps:

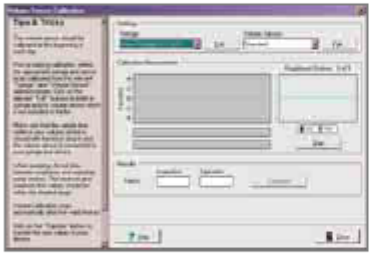
Fig. Equipment and volume calibration setup prior to calibrating the Triple® V volume transducer.

1. Follow the steps described in chapter **"Steps prior to calibration"**.
2. **Close the sample line outlet** of the volume transducer (DVT or Triple® V) with the black plug-in which is part of your volume transducer accessory.
3. Adapt transducer to calibration syringe.

1. Start MetaSoft®.
2. To access the volume calibration screen select **Volume**

from the **Calibration** Program Folder of MetaSoft®.

Note:
The volume (flow) sensor should be calibrated at the beginning of each day.



3. The *Volume Sensor Calibration* window will appear.

4.
   a. Select the appropriate calibration syringe from Syringe selection box.

   3 liter is the default syringe / syringe volume displayed. Last selection will be saved until changed.

   Note:
   The volume of the calibration syringe used has to be within the upper ventilation range expected to be measured during tests with MetaMax® 3B.

b. To enter a new syringe / syringe volume, first click in the

Edit Syringe button of the *Volume Sensor Calibration* dialog.

The *Syringe List* window will appear, displaying information on the selectable syringes.

c. To enter a new syringe / syringe volume, click in the

New Syringe button.

d. Type name, manufacturer of syringe and syringe volume in the appropriate boxes of the *Syringe Parameters* window. Click

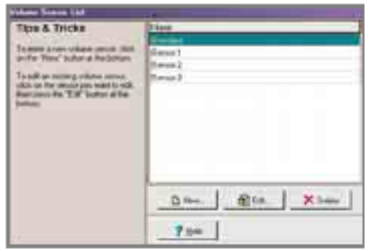
to save your new entry in the database. Click in the *Close* button to return to the *Syringe List*. Press the *Close* button again to return to the Volume Sensor Calibration screen.

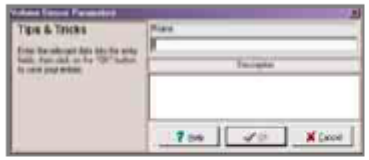
5.

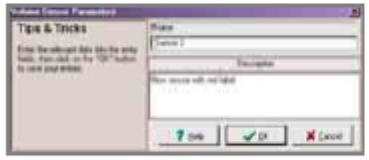
a. Select the appropriate volume (flow) sensor from Volume *Sensor* selection box.

Standard is the default volume (flow) sensor/ volume (flow) sensor displayed.

<u>Note:</u>
This setting allows you to handle several volume (flow) sensors. If you have only one volume (flow) sensor, select *Standard* from the *Volume Sensor* drop-down selection box.







b. To enter a new volume (flow) sensor, first click in the

Edit Volume Sensor button.

The *Volume Sensor List* window will appear, displaying information on the selectable volume (flow) sensors.

c. To enter a new volume (flow) sensor, click in the

New Volume Sensor button.

d. Type name and description of volume (flow) sensor in the appropriate boxes of the *Volume Sensor Parameters* window. Click

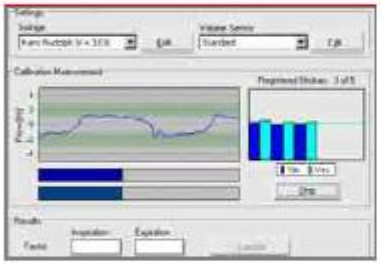
to save your new entry in the database. Click in the *Close* button to return to the *Volume Sensor List*. Press the *Close* button again to return to the Volume Sensor Calibration screen.

6. Press the

button to start calibration.

<u>Note:</u>
Make sure that the sample line outlet of your volume (flow) sensor is closed with the black plug-in and the volume (flow) sensor is connected both to your syringe and device.

Fig. Volume calibration

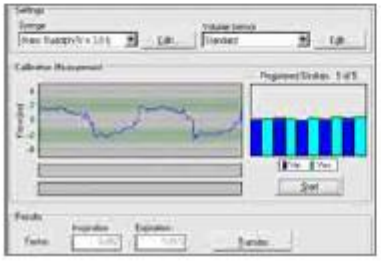


7. Start pumping by pulling the plunger of the calibration syringe **all the way** forward and backward.

Important!

Pump gently. Do not stop between inspiratory and expiratory pump strokes. The minimum and maximum flow values should be within the two shaded green ranges of the **"Calibration Measurement"** graph.

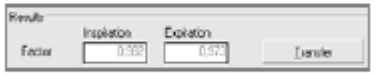
Use the **two rhythm bars at the bottom** as a guidance for performing suitable pump strokes. The upper bar is moving automatically, giving the rhythm. The lower bar moves along with your strokes. **When pumping, the two bars should move synchronously.**



8. Repeat this procedure until all pump cycles have been completed. During calibration measurement, the **Start** button turns into a Stop button.

   Volume calibration stops automatically after **5 valid strokes**.

   The actual calibration factors are displayed in the "Inspiration" and "Expiration" boxes under "Results".



9. If the calibration factors (values) calculated are lower than 0.8 or exceed 1.2, an error message will be displayed.

   Possible reasons may be:
   - syringe volume of the calibration: syringe volume selected from the syringe selection box may have been different to the volume of the calibration syringe used,
   - pumping procedure was not correctly performed,
   - volume (flow) sensor is damaged

   The volume (flow) sensor must be recalibrated.

10. Click in the **Transfer** button to transfer the values to your MetaMax® 3B measurement device.

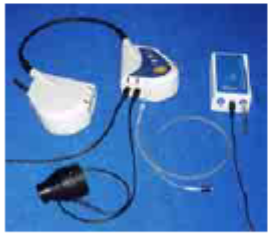
    The calibration values are stored in your CPX device until new calibration values are transferred.

Two-Point Gas Calibration using CORTEX Calibration Gas Saver

Equipment required:

- Gas 1 (clean ambient air or span gas with 0% CO_2, approx. 21% O_2, bal. in N_2)
- Calibration/Reference gas
  (Reference/calibration gas from CORTEX Calibration Kit or equivalent span gas with 4-6% CO_2, 14-16% O_2, bal. in N_2)
- Gas Saver with power supply or 9V battery (from CORTEX Calibration Kit)
- Set of various connection tubes to gas bottle and sample line
  (CORTEX Calibration Kit or equivalent tubes with connections)

Calibration Steps:



1. Follow the steps described in chapter "**Steps prior to calibration**".

2. Plug power cable into **DC IN** socket of gas saver and connect power supply to mains supply using the power adapter included in your CORTEX Calibration Kit.

   Note: The gas saver can also be operated on 9 V batteries. One battery is included in your CORTEX Calibration Kit.

   Connect appropriate tube from the Cortex Calibration Kit to **P OUT** socket of gas saver.

   Note:
   The sample line is not yet connected to gas saver.

Fig. Calibration equipment and test setup for a Gas 1 / ambient air calibration measurement.

3. Switch on gas saver.

4. Start MetaSoft® and select Manual Gas Sensor Calibration Method.

   To access this option please select first **General** in the **Configuration** Folder, then **General Settings**.

   The *General Settings* window will appear.

   Note: You can skip this step if the manual setting has not been changed since last calibration.

5. To access the gas analyzer calibration screen, select **Gas**

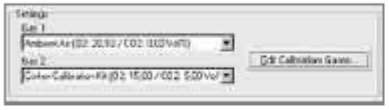
   from the **Calibration** Program Folder of MetaSoft®.

   Note:
   A two-point reference gas calibration should be executed prior to a test series or at least every 30 days. It must be executed if the oxygen analyzer has been exchanged.

   Prior to starting calibration, connect and check the equipment required and ensure that your device is sufficiently warmed up (45 minutes). Make sure that the room is sufficiently ventilated for an accurate Gas 1 measurement.

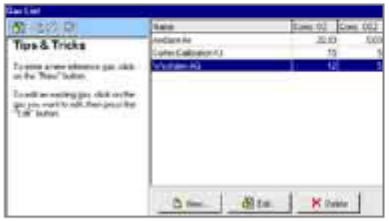
   If you use a span gas instead of ambient air for Gas 1 measurement, please proceed as described for Gas 2 measurement.

6. The *Gas Sensor Calibration* window will appear.



7.

a. Select the appropriate gas concentrations for **Gas 1** (ambient air or span gas) and **Gas 2** (calibration/reference gas from CORTEX Calibration Kit or see manufacturer label of calibration gas bottle) from the "Gas 1" and "Gas 2" selection boxes in the gas sensor calibration screen.



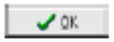
b. To enter a new reference / calibration gas, first click in the

Edit gas button. The *Gas List* window will appear, displaying information on the selectable calibration gases.

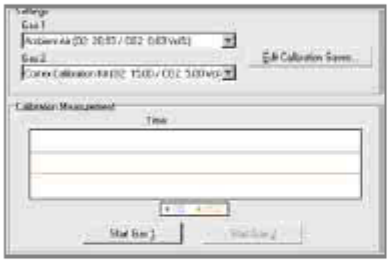
c. To enter a new gas, click in the

New gas button.

d. Type gas name, gas concentrations and description of the gas to be added in the appropriate boxes of the *Gas Parameters* window. Click



to save new gas entry in the database. Click in the *Close* button to return to the gas list. Press the Close button again to return to the Gas Sensor Calibration screen.



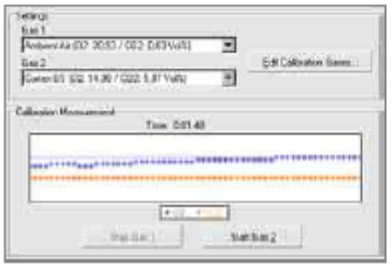
8. Press the

button to **start calibration with Gas 1** (ambient air or span gas).

If ambient air is used as Gas 1, the sample line must be exposed to fresh air.

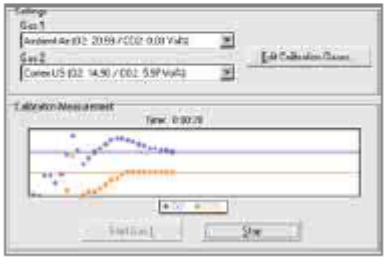
If a span gas is used as Gas 1, the sample line has to be connected to the span gas bottle for Gas 1 (refer to sections 10-14).

During Gas 1/ambient air calibration, the Gas 1 button turns into a **Stop** button.



9. Gas 1 calibration stops automatically once stable values have been reached.

The Gas 2 button is now enabled, the Gas 1 button is disabled.



10. Proceed with calibration of Gas 2 (reference/calibration gas).

    Check the bottle valve (**black** knob) on the pressure reduction valve and close it if still open. Counterclockwise turn out the pressure knob completely (on top of the reduction valve).

    Remove the protective cap from the gas bottle and screw the reduction valve in.

    Connect gas saver to calibration gas bottle using the appropriate connection tube. Plug connection tube into **P IN** socket of gas saver and connect it to gas bottle.

    Note:
    The sample line still remains unconnected to gas saver.

11. Now open the bottle valve and slowly screw in the pressure knob on top of the reduction valve.

    Visually check the correct pressure of gas flow via the LED displays of the gas saver.

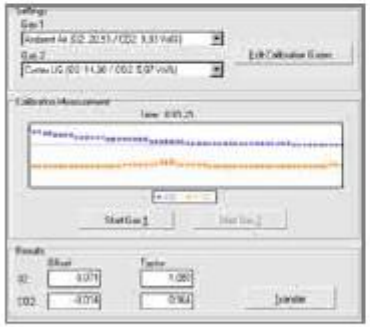
    Open the reduction valve of the calibration/reference gas bottle until the LED switches from green to yellow.

12. Press the

    button to **start calibration with Gas 2** (reference/calibration gas from CORTEX Calibration Kit or equivalent).

    During reference gas calibration, the Gas 2 button turns into a Stop button (see left illustration). The Gas 1 button is disabled.



13. Now connect sample line to the short tube connector on **P OUT** socket of gas saver

    Adjust gas pressure until green LED is flashing.

    P< = pressure too low, LED is red.
    P> = pressure too high, LED is yellow.
    OK = Pressure OK, LED is green.

14. Gas 2 (or reference gas) calibration stops automatically once stable values have been reached.

    The Gas 2 button

    is disabled, the Gas 1 button is now enabled.

    The calculated new offset / factors are displayed in the "O2" and "CO2" boxes under *Results*.

    Note:
    Please close the gas bottle valve and disconnect the sample line from gas saver.

15. Click in the **Transfer** button to transfer the O_2 and CO_2 values to your CPX device.

    The calibration values are stored in your CPX device until new calibration values are transferred.

Two-Point Gas Calibration using Automatic Gas Calibrator

Equipment required:

- Gas 1 (clean ambient air or span gas with 0% CO_2, approx. 21% O_2, bal. in N_2)
- Calibration/Reference gas
  (Reference/calibration gas from CORTEX Calibration Kit or equivalent span gas with 4-6% CO_2, 14-16% O_2, bal. in N_2)
- CORTEX Automatic Gas Calibrator with power supply (from CORTEX Calibration Kit)
- Set of various connection tubes to gas bottle(s) and sample line (CORTEX Calibration Kit)
- Connection Cable PC - Automatic Gas Calibrator (CORTEX Calibration Kit)

Calibration Steps:

1. Follow the steps described in chapter **"Steps prior to calibration"**.
2. Connect the power supply from your calibration kit with the calibrator's socket labeled **DC In** and a mains supply.

   Note: The gas calibrator can also be operated on either regular or rechargeable batteries. Use of alkaline batteries (AA / LR6 size) or rechargeable batteries (AA size) with a capacity of at least 1,500 mAh is recommended. The batteries compartment is located on the bottom side of the unit.

   Nearly exhausted batteries are indicated by a flashing **On / LowBat** LED. In this case you will be able to complete the calibration process before exchanging the batteries.

3. Plug the connection cable (from CORTEX Calibration Kit) into the PC COM port which is in use by MetaSoft® and into the **PC** socket on the calibrator.

   Connect the MetaMax® 3B **data** socket and the unit's **CPX** socket using the appropriate cable.

   Now connect the short tube, included in your Calibration Kit, to the **Sample Line** socket of the automatic calibrator.

Notes:

The Automatic Gas Calibrator allows you to perform the calibration procedure without needing to disconnect or unplug any connections even when using two gas bottles with different calibration gases.

If you are calibrating using ambient air and one calibration gas (e.g. from CORTEX calibration kit), the calibration gas bottle has to be connected to the **GAS 2** inlet. The **GAS 1** inlet is unused in that case.

If you are using a second calibration gas instead of ambient air, please connect this bottle to the **GAS 1** inlet.

The following steps refer to a calibration process using ambient air and one calibration gas (from CORTEX calibration kit).

4. Check if the bottle valve (black knob) on the pressure reduction valve is closed.
   To set the proper output pressure turn the pressure knob towards higher pressure (clockwise in '+' direction) until you reach the mechanical stop. Now screw it back out again ('-' direction) by 2.5 turns.
   Remove the protective cap from the gas bottle and screw the reduction valve in.

   Note: If your reduction valve is equipped with a manometer please adjust the pressure to a value of 14 - 29 PSI (1 - 2 Bar). Avoid pressure settings higher than 29 PSI (2 Bar).

5. Connect the **GAS 2** inlet at the automatic gas calibrator and the gas bottle using the appropriate tube from your calibration kit.
   Plug the end of the sample line into the short tube which is already connected to the calibrator (**Sample Line** socket).

6. Open the bottle valve by about one turn.

Fig.
Calibration equipment and test setup for performing an automatic gas calibration.

7. Switch on the automatic gas calibrator by pressing the **Off / On** switch. The green LED **On / LowBat** lights up.

8. Start MetaSoft® and select **General** from the **Configuration** Folder, then click on the **General Settings** icon.

   The *General Settings* window will appear. Click on the **Calibration** tab sheet and select the automatic gas sensor calibration method.

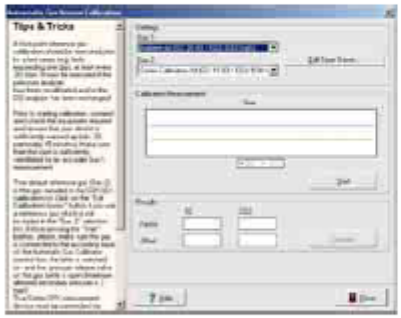
   Note: You can skip this step if the automatic setting has not been changed since last calibration.

9. To access the gas analyzer calibration screen, select Gas

from the **Calibration** Program Folder of MetaSoft®.

Note:
A two-point reference gas calibration should be executed prior to a test series or at least every 30 days. It must be executed if the oxygen analyzer has been exchanged.

Prior to starting calibration, connect and check the equipment required and ensure that your device is sufficiently warmed up (45 minutes). Make sure that the room is sufficiently ventilated for an accurate Gas 1 measurement.



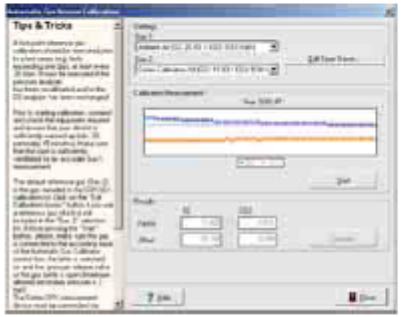
10. The *Automatic Gas Sensor Calibration* window will appear.

11. Select the appropriate gas concentrations for **Gas 1** (ambient air or span gas) and **Gas 2** (calibration/reference gas from CORTEX Calibration Kit or see manufacturer label of calibration gas bottle) from the "Gas 1" and "Gas 2" selection boxes in the gas sensor calibration screen.

    In case your calibration gas is not yet listed you can edit the MetaSoft® database. Please refer to section 'Two-point Gas Calibration using CORTEX Calibration Gas Saver'.

12. Press the

    button to start the automatic calibration. If you are using ambient air as GAS 1 the automatic calibrator must be exposed to fresh air. Please avoid breathing close to the unit and do not cover the little side hole of the unit's case.



13. The unit is now performing both calibration steps successively (ambient air > GAS 2 or GAS 1 > GAS 2) and will stop automatically after completion.

    The calculated new offset factors are displayed in the "O2" and "CO2" boxes under *Results*.

    In case of low gas pressure (bottle closed or empty) an error message window will pop up.

14. Click the

    button to transfer the O2 and CO2 values to your CPX device.

    The calibration values are stored in your CPX device until new calibration values are transferred.

Notes:

Please close the gas bottle(s) after finishing the calibration process since a complete pressure tightness of the calibrator's internal pneumatic system cannot be guaranteed.

If you won't be using the automatic gas calibrator for a long time, please remove the batteries.

Pressure Calibration

Equipment required:

- Reference barometer

Calibration Steps:

1. Follow the steps described in chapter **"Steps prior to calibration"**.

2. To access the pressure sensor calibration screen select **Pressure**

from the **Calibration Menu** in MetaSoft®.

3. The *Pressure Sensor Calibration* window will appear.

4. Type the actual barometric pressure in the *Actual pressure (mbar)* box of the pressure sensor calibration screen.

   To measure the actual barometric pressure you may use a reference barometer.

   Attention:
   The barometric pressure must be entered in mbar. Change the barometric pressure measured in mmHg to mbar using the following formula:

   P[mbar] = 1.33322 * P[mmHg]

5. Press the

button from the Pressure Sensor Calibration window to start the calibration of the pressure analyzer.

The calculated new offset is displayed in the Offset box under *Results*.

6. Click in the **Transfer** button to transfer the new offset value to your MetaMax® 3B.

Important

Always perform a two-point reference gas calibration after a pressure calibration.

Appendix A.4

MAXIMUM FLOW-VOLUME-LOOP TEST PROCEDURE

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UFS UV

1. Perform a "Maximum Flow-Volume-Loop" test

- Click on the start button ▶ ❻ (➡Fig. 51) to start recording the *Maximum Flow-Volume-Loop*. Ask the patient/subject to start with the Manoeuvres. Finish the text by clicking on the ■ button once a satisfactory Manoeuvre has been
- Then select the best from among
  nish the test by clicking on [CONF

⊡ **Note!** By clicking on [CONFIRM] an
to) ❻ and START ▶ you can start a

2. Perform blood pressure and

If you have confirmed the MAXIMU
function block for the BP MEASUREI

- Perform the blood pressure meas

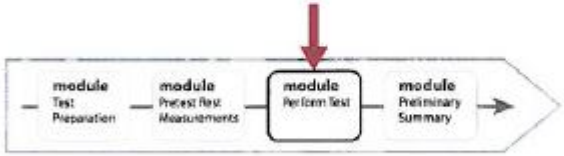
⊡ **Note!** If you have measured the bloc
values in the respective fields without performing the step as described above.

- Once the BP Measurement has been confirmed, the function block/ SPO_2 MEASUREMENT ❺ is activated. Proceed here as described above.

⊡ **Note!** By clicking on [CONFIRM] and then on [REPEAT] (Button changes to) and START ▶ you can start a new measurement.

7.2.2 Performing a CPET test

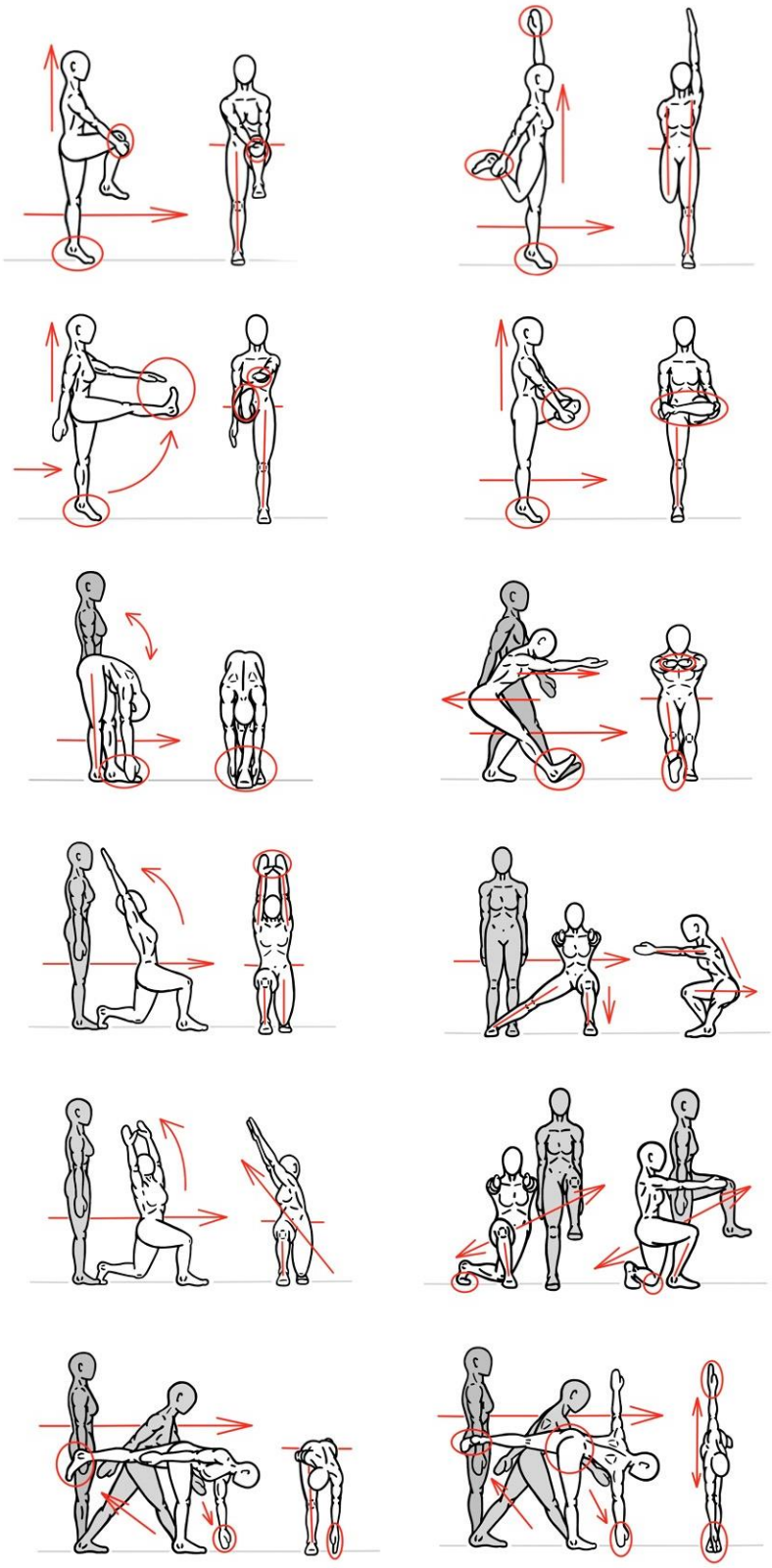
- Click on NEXT in the control bar. *You will proceed to the third workflow module* CPET PERFORM TEST:



The subject now performs the exercise testing.

Appendix A.5

DYNAMIC STRETCHING ROUTINE



Appendix A.6

DATA SHEET: $\dot{V}O_2$max protocol

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YUNIVESITHI YA FREISTATA
UFS UV

$\dot{V}O_2$max protocol

Name and surname	
Age	
ID NUMBER	

Stature (cm)	
Body weight (kg)	

Resting heart rate (beats/minute)	

$\dot{V}O_2$max

Interval (min)	Speed (km/h)		Heart rate (beats/minute)		RPE (6–20) (score)
0:00–0:59	0				
1:00-4:00	10				
4:00–4:30	**LACTATE:**				
4:30-7:30	11				
7:30-8:00	**LACTATE:**				
8:00-11:00	12				
11:00-11:30	**LACTATE:**				
11:30-14:30	13				
14:30-15:00	**LACTATE:**				
15:00-18:00	14				
18:00-18:30	**LACTATE:**				
18:30-21:30	15				

21:30-22:00	**LACTATE:**				
22:00-25:00	16				
25:00-25:30	**LACTATE:**				
25:30-28:30	17				
28:30-29:00	**LACTATE:**				
29:00-32:00	18				
32:00-32:30	**LACTATE:**				
32:30-35:30	19				
35:30-36:00	**LACTATE:**				
36:00-39:00	20				
39:00-39:30	**LACTATE:**				
39:30-42:30	21				
42:30-43:00	**LACTATE:**				
43:00-46:00	22				
46:00-46:30	**LACTATE:**				
46:30-49:30	23				

Recovery

2 minutes		LACTATE
3 minutes		
5 minutes		

Appendix A.7

ADULT PRE-EXERCISE SCREENING

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UFS UV

ADULT PRE-EXERCISE SCREENING

Name:_____
_____

Date of birth:_____ Date:_____

STAGE 1

1. Has your doctor ever point out that you have a heart condition?	Yes	No
2. Have you ever experienced a stroke?	Yes	No
3. Do you ever feel unexplained pains in your chest at rest?	Yes	No
4. Do you ever feel pains in your chest during physical activity/exercise?	Yes	No
5. Do you ever feel dizzy or have spells of faint during physical activity/exercise that bring about a loss of balance?	Yes	No
4. Have you ever experienced an asthma attack needing instant medical care at any time over the last 12 months?	Yes	No
5. Do you have diabetes (type I or type II)?	Yes	No
6. Do you have any identified bone, muscle or joint difficulties that could be made worse by physical activity/exercise?	Yes	No
If YES to item number 6 Please specify		
7. Do you have any additional medical condition(s) that may make it unsafe for you to participate in physical activity/exercise?	Yes	No
If YES to item number 6 Please specify		

IF YOU ANSWERED **'YES'** to any of the 7 questions, please seek guidance from your GP or appropriate health professional prior to undertaking physical activity/exercise

Signature_____**Date**_____

Appendix A.8

PITTSBURGH SLEEP QUALITY INDEX (PSQI) QUESTIONNAIRE

Name: _____ Date: _____

What is the PSQI, and what does it measure?
The Pittsburgh Sleep Quality Index (PSQI) is an effective measurement used to quantify the quality and patterns of adult sleep. It differentiates "poor" from "good" sleep quality by assessing seven areas (components): subjective sleep quality, the length of time it takes from lying down for the night until sleep onset, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleeping medications, and daytime dysfunction over the last month.

INSTRUCTIONS
The following questions relate to your usual sleep habits during the past month only. Your answers should indicate the most accurate reply for the majority of days and nights **in the past month**. Please answer all questions.

DURING THE PAST MONTH:

1. At what time have you usually gone to bed? _____
2. How long (in minutes) has it taken you to fall asleep each night? _____
3. What time have you usually gotten up in the morning? _____
4. How many hours of actual sleep did you get at night? _____

SELECT THE MOST APPROPRIATE OPTION

		Not during the past month (0)	Less than once a week (1)	Once or twice a week (2)	Three or more times a week (3)
5.	How often have you had trouble sleeping because you:				
	A Cannot get to sleep within 30 minutes				
	B Wake up in the middle of the night or early morning				
	C Have to get up to use the bathroom				
	D Cannot breathe comfortably				
	E Cough or snore loudly				
	F Feel too cold				
	G Feel too hot				

	Not during the past month (0)	Less than once a week (1)	Once or twice a week (2)	Three or more times a week (3)
(5. How often have you had trouble sleeping because you:)				
H Experience bad dreams				
I Are in pain				
J Other reason (s), please explain below				
6. How often have you taken medication (or over-the-counter or prescribed) to assist you sleep?				
7. Do have you had trouble staying alert or awake while driving, eating, or participating in social activity?				
8. Do you lack enthusiasm to do things day to day?				
	Very good (0)	**Fairly good (1)**	**Fairly bad (2)**	**Very bad (3)**
9. How would you rate your sleep quality overall?				

PSQI SCORING

Component 1: Subjective sleep quality

Refer to question number 9:

How would you value your overall sleep quality?

Response	Score
Very good	0
Fairly good	1
Fairly poor	2
Very poor	3

Component 1 score: _____

Component 2: The length of time it takes from lying down for the night until sleep onset

1. How long (in minutes) has it taken you to fall asleep each night?:

Response	Score
≤ 15 min	0
16–30 min	1
31–60 min	2
> 60 min	3

Question #2 score _____

2. Refer to question number 5a:

How often can you not sleep within 30 minutes:

Response	Score
Not during the past month	0
Less than once a week	1
Once or twice a week	2
Three or more times a week	3

Question number 5a score _____

3. Add number 2 score and number 5a score

Sum of number 2 and number 5a _____

4. Assign component 2 score as follows:

Sum of number 2 and number 5a	Score
0	0
1–2	1
3–4	2
5–6	3

Component 2 score: _____

Component 3: Sleep duration

Refer to question number 4:

How many hours of definite sleep did you get at night?:

Response	Score
> 7 hours	0
6–7 hours	1
5–6 hours	2
< 5 hours	3

Component 3 score: _____

Component 4: Habitual sleep efficiency

1. Write the number of hours slept (number 4) here: _____

2. Calculate the number of ours spent in bed (difference between number 3 and number 1):

Getting-up time (number 3): _____

Bedtime (number 1): _____

Number of hours spent in bed: _____

3. Calculate sleep efficiency as follows:

(Number of hours slept / Number of hours spent in bed) X 100 = habitual sleep efficiency (%)

(_____ / _____) X 100 = _____ %

4. Assign component 4 score as follows:

Habitual sleep efficiency %	Score
> 85%	0
75–84%	1
65–74%	2

< 65%	3

Component 4 score: _____

Component 5: Sleep disturbances

Refer to question number 5b – 5j:

How frequently have you had trouble sleeping because you:

B Wake up in the middle of the night or early morning
C Have to use the bathroom
D Have difficulty breathing comfortably
E Snore or cough noisily
F Feel overly cold
H Experience bad dreams
I Are in pain
J Other reason (s), please describe below, including how often you have had trouble sleeping because of these reasons

Response	**Score**
Not during the past month	0
Less than once a week	1
Once or twice a week	2
Three or more times a week	3

#5b score _____

#5c score _____

#5d score _____

#5e score _____

#5f score _____

#5g score _____

#5h score _____

#5i score _____

#5j score _____

2. Add the scores for questions number 5b–5j:

Sum of number 5b–5j: _____

3. Assign component scores as follows:

Sum of number 5b–5j	**Score**
0	0
1–9	1
10–18	2
19–27	3

Component 5 score: _____

Component 6: Use of sleep medication

Refer to question number 6:

How often do you use medication (over-the-counter or prescribed) to assist in sleep?

Response	Score
Not during the past month	0
Less than once a week	1
Once or twice a week	2
Three or more times a week	3

Component 6 score: _____

Component 7: Daytime dysfunction

1. Refer to question number 7:

   How frequently have you had difficulty staying alert or awake while eating, driving, or during social activities? Examine question number 7 and give scores as follows:

Response	Score
Not in the past month	0
Fewer than one occasion a week	1
Once or twice a week	2
More than three times a week	3

   Question number 7 score _____

2. Observe question number 8 and give scores as follows:

Response	Score
Not during the past month	0
Less than once a week	1
Once or twice a week	2
Three or more times a week	3

   Question number 8 score _____

3. Add the scores for questions number 7 and 8:

   Sum of number 5b–5j: _____

4. Assign component 7 scores as follows:

Sum of number 7 and 8	Score
0	0
1–2	1
3–4	2
5–6	3

Component 7 score: _____

GLOBAL PSQI SCORE

Add the seven component scores together:

Global PSQI score: _____

OUTCOME

A global PSQI score of 5 or more is indicative of poor sleep quality.

If you scored 5 or more, it is suggested that you discuss your sleep habits with a healthcare provider.

Appendix A.9

SHORT-FORM MCGILL PAIN QUESTIONNAIRE

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PARTICIPANT'S NAME: _____

DATE OF TEST: _____

	NONE	MILD	MODERATE	SEVERE
THROBBING	(0)	(1)	(2)	(3)
SHOOTING	(0)	(1)	(2)	(3)
STABBING	(0)	(1)	(2)	(3)
SHARP	(0)	(1)	(2)	(3)
CRAMPING	(0)	(1)	(2)	(3)
GNAWING	(0)	(1)	(2)	(3)
HOT-BURNING	(0)	(1)	(2)	(3)
ACHING	(0)	(1)	(2)	(3)
HEAVY	(0)	(1)	(2)	(3)
TENDER	(0)	(1)	(2)	(3)
SPLITTING	(0)	(1)	(2)	(3)
TIRING-EXHAUSTING	(0)	(1)	(2)	(3)
SICKENING	(0)	(1)	(2)	(3)
FEARFUL	(0)	(1)	(2)	(3)
PUNISHING-CRUEL	(0)	(1)	(2)	(3)

NO PAIN **I----------I** **WORST PAIN POSSIBLE**

PPI

0	NO PAIN	
1	MILD	
2	DISCOMFORTING	
3	DISTRESSING	
4	HORRIBLE	
5	EXCRUCIATING	

Appendix A.10

RPE Scale (Total body exertion)

6	No exertion at all
7	Extremely light
8	
9	Very light
10	
11	Light
12	
13	Somewhat heard
14	
15	Hard (heavy)
16	
17	Very hard
18	
19	Extremely hard
20	Maximal exertion

Appendix A.11

URINE COLOUR CHART

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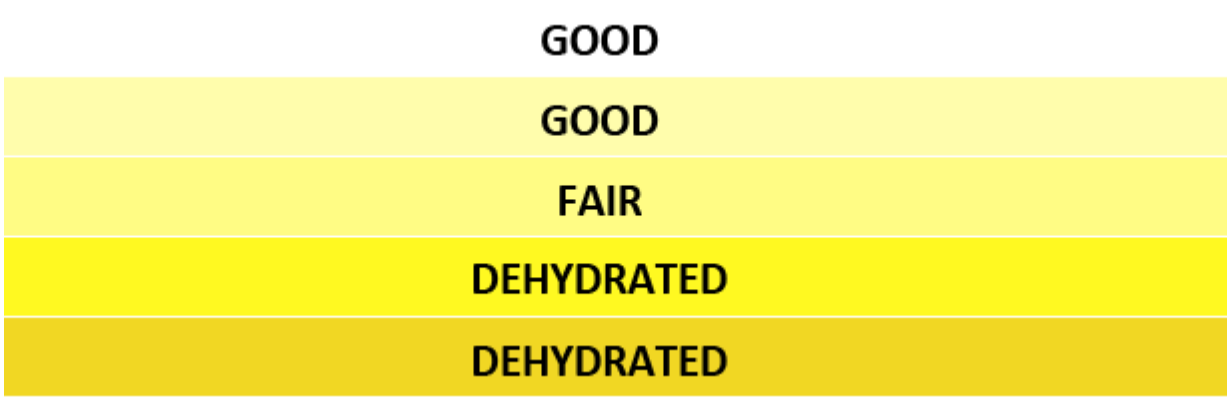
UFS UV

URINE COLOUR CHART



Transparent

Shades of yellow

Light yellow

Dark yellow

Very dark yellow

Appendix A.12

PERMISSION LETTER: DIRECTOR OF KOVSIE SPORT

UNIVERSITY OF THE FREE STATE
UNIVERSITEIT VAN DIE VRYSTAAT
YUNIVESITHI YA FREISTATA
UFS UV

PERMISSION LETTER

5 Kings Chase
Verster Street
Universitas
Bloemfontein
9301

09 January 2017

MR. D.B. PRINSLOO
DIRECTOR: KOVSIE SPORT
HEAD OF FREE STATE ATHLETICS

RESEARCH PROJECT: PhD in Exercise Sciences

EFFECT OF SHORT-TERM MACRONUTRIENT MANIPULATION ON ENDURANCE CAPACITY OF LONG-DISTANCE RUNNERS

Mrs. L. Deacon (PhD student) together with the University of the Free State's Department of Exercise and Sport Sciences, hereby request permission to conduct research on male long-distance runners from the Kovsie Athletics team and Bloemfontein athletic clubs. The research will be done under supervision of Prof. F.F. Coetzee (Adjunct Professor & Head of Department: Exercise and Sport Sciences), Prof. B. Coetzee (co-Professor Department: School for Biokinetics, Recreation and Sport Sciences, University of North-West) and Dr W. C. du Toit (Department: Nutrition and Dietetics).

Research is the development of answers to certain questions. In this study, we aspire to gain more knowledge about the effect that a specific macronutrient dietary manipulation strategy has on endurance of long-distance runners when taken 48 hours prior to and just before exercise testing commences. Macronutrients are energy-providing sources consumed by humans in large quantities and is important for providing energy, promoting growth and other bodily functions taking place during everyday living. The three main macronutrient substrates consumed by humans are Carbohydrates, fats and proteins.

We are requesting your permission for your athletes to participate in a research study that involves 24 long-distance endurance runners.

The study will focus on the effect that specific macronutrient dietary intake has on an individual's endurance during a test to maximum exhaustion while running on a treadmill. In other words, we want to test the efficiency of an athlete's running performance on a treadmill on two separate occasions after they have ingested a high amount of carbohydrates on one occasion and a high amount of fat on the other occasion for a 2-day period before the running test on the treadmill. Athletes will therefore perform the same treadmill test on two separate occasions. These tests will be scheduled two weeks apart. On each testing occasion, athletes will do a warm-up on the treadmill at a walking speed of 4 km/h followed by a dynamic stretching routine. After the warm-up and stretching routine athletes will start the test on the treadmill where their $\dot{V}O_2$max or maximum oxygen consumption, blood lactate level via a finger prick test and degree of fatigue will be measured. The study will have two testing trials that will all include macronutrient dietary manipulation (which will be different on each occasion), followed by the same $\dot{V}O_2$max test protocol at every trial. Results of each athlete will be calculated and compared after the trial periods.

Should the athletes agree to participate in the study, their total daily energy expenditure, this means the total amount of calories our bodies needs to survive on a day to day level with the inclusion of our daily activity level, will be calculated by a registered dietitian who will after determination of each athlete's daily energy consumption, prepare an individual isoenergetic carbohydrate-rich and fat-rich diet. Diets will be described as (i) high-fat and (ii) high-carbohydrate. Before the trial period commence, athletes will be assigned to either the high-carbohydrate diet or the high-fat diet that will be followed for 48 hours prior to the first exercise testing. On the next day just before exercise testing commences, each athlete will receive a diet-specific beverage with the same macronutrient composition (either high-carbohydrate or high-fat) as the diet followed for the previous 48 hours. The exact procedure will be followed for the second laboratory visit trial test except the opposite dietary meals than the previous period will be received for the 48 hours prior to exercise testing, again followed by a dietary beverage similar to the dietary intake prior to exercise testing. On the day of testing, athletes will have to complete 2 questionnaires and provide a urine sample before testing can start. After the above is provided the athletes will sit in a relaxed state where their resting heart rate will be monitored along with an explanation of testing procedures, the rate of perceived exertion scale where they will have to supply the researcher with a certain number on a scale provided that reflects their total body exhaustion during the test and fitting of all relevant equipment to be used during the test.

All the results of each individual exercise trials will be analysed and compared, which might be of advantage to each athlete individually for future meal preferences by

providing knowledge about their own individual body's energy usage in the lead up to an endurance run.

As a lack of sleep, not sticking to the prescribed diet, excessive exercise and alcohol intake may affect performance, athletes will be kindly requested to strain from very heavy high-intensity exercise in the 24 hours before to each exercise testing trial, get an adequate amount of sleep and restrain from high levels of alcohol intake for the period of time before testing (48 hours).

Participation in this study is completely voluntary, and rejection to partake will not involve any consequence or disadvantage to the athletes. They may withdraw involvement whenever they like.

Personal information will not, in any circumstance, be shared with other individuals or organizations without your permission. Personal information may be released if compelled by law. The only parties that may review and/or copy records for data analysis and quality guarantees include organisations such as the Health Sciences Research Ethics Committee.

Should results be published in the literature, it potentially may lead to individual identification.

Your permission for your athletes to participate in this study will be greatly appreciated.

Contact details of the principal researcher: cell phone number 071 354 4253; email address: lizljvr1985@gmail.com.

_____

Mrs. L. Deacon
Principle Investigator

Appendix A.13

PERMISSION LETTER HEAD OF FREE STATE ATHLETICS

UNIVERSITY OF THE FREE STATE
UNIVERSITEIT VAN DIE VRYSTAAT
YUNIVESITHI YA FREISTATA
UFS UV

PERMISSION LETTER

5 Kings Chase
Verster Street
Universitas
Bloemfontein
9301

09 January 2017

MR. D.B. PRINSLOO
DIRECTOR: KOVSIE SPORT
HEAD OF FREE STATE ATHLETICS

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EFFECT OF SHORT-TERM MACRONUTRIENT MANIPULATION ON ENDURANCE CAPACITY OF LONG-DISTANCE RUNNERS

Mrs. L. Deacon (PhD student) together with the University of the Free State's Department of Exercise and Sport Sciences, herewith request consent to conduct research on male long-distance runners from the Kovsie Athletics team and Bloemfontein athletic clubs. The research will be done under supervision of Prof. F.F. Coetzee (Adjunct Professor & Head of Department: Exercise and Sport Sciences), Prof. B. Coetzee (co-Professor Department: School for Biokinetics, Recreation and Sport Sciences, University of North-West) and Dr W. C. du Toit (Department: Nutrition and Dietetics).

Research is the process to learn the answer to a specific question. The goal of this study is to test the effect that a specific macronutrient manipulation strategy has on endurance of distance runners when taken 48 hours prior to and just before exercise testing commences.

We are requesting your permission for your athletes to participate in a research study that involves 24 long-distance endurance runners.

The study will focus on the effect that specific macronutrient dietary intake has on an individual's endurance during a test to maximum exhaustion while running on a treadmill. In other words, we want to test the efficiency of an athlete's running

performance on a treadmill on two separate occasions after they have ingested a high amount of carbohydrates on one occasion and a high amount of fat on the other occasion for a 2-day period before the running test on the treadmill. Athletes will therefore perform the same treadmill test on two separate occasions. These test will be scheduled two weeks apart. On each testing occasion, athletes will do a warm-up on the treadmill at a very slow speed of 4 km/h followed by a dynamic stretching routine. After the warm-up and stretching routine athletes will start the test on the treadmill where their $\dot{V}O_2$max or maximum oxygen consumption, blood lactate level via a finger prick test and degree of fatigue will be measured. The study will have two testing trials that will all include macronutrient dietary manipulation (which will be different on each occasion), followed by the same $\dot{V}O_2$max test protocol at every trial. Results of each athlete will be calculated and compared after the trial periods.

Should the athletes agree to participate in the study, their total daily energy expenditure, this means the total amount of calories our bodies needs to survive on a day to day level with the inclusion of our daily activity level, will be calculated by a registered dietitian who will after determination of each athlete's daily energy consumption, prepare an individual isoenergetic carbohydrate-rich and fat-rich diet. Diets will be described as (i) high-fat, low-carbohydrate and (ii) high-carbohydrate, low-fat. Before the trial period commence, athletes will be assigned to either the high-carbohydrate diet or the high-fat diet that will be followed for 48 hours prior to the first exercise testing. On the next day just before exercise testing commences, each athlete will receive a diet-specific beverage with the same macronutrient composition (either high-carbohydrate or high-fat) as the diet followed for the previous 48 hours. The exact procedure will be followed for the second laboratory visit trial test except the opposite dietary meals than the previous period will be received for the 48 hours before exercise testing, again followed by a dietary beverage similar to the dietary intake prior to exercise testing. On the day of testing, athletes will have to complete 2 questionnaires and provide a urine sample before testing can start. After the above is provided the athletes will sit in a relaxed state where their resting heart rate will be monitored along with an explanation of testing procedures, the rate of perceived exertion scale where they will have to supply the researcher with a certain number on a scale provided that reflects their total body exhaustion during the test and fitting of all relevant equipment to be used during the test.

All the results of each individual exercise trials will be analysed and compared, which might be of advantage to each athlete individually for future meal preferences by providing knowledge about their own individual body's energy usage in the lead up to an endurance run.

As a lack of sleep, not sticking to the prescribed diet, excessive exercise and alcohol intake may affect performance, athletes will be kindly requested to strain from very heavy high-intensity exercise in the 24 hours before each exercise testing trial, get an

adequate amount of sleep and restrain from high levels of alcohol intake for the period of time before testing (48 hours).

Personal information will not, in any circumstance, be shared with other individuals or organizations without your permission. Personal information may be released if compelled by law. The only parties that may review and/or copy records for data analysis and quality guarantees include organisations such as the Health Sciences Research Ethics Committee.

Should results be published in the literature, it potentially may lead to individual identification.

Your permission for your athletes to participate in this study will be greatly appreciated.

Contact details of the principal researcher: cell phone number 071 354 4253; email address: lizljvr1985@gmail.com.

I, _____, ID nr., _____ hereby give permission to Mrs. L. Deacon to collect data for this study from athletes of the Kovsie Athletics team.

_____
Signature

_____
Mrs. L. Deacon
Principle Investigator

Appendix A.14

HSREC – APPROVAL LETTER

UNIVERSITY OF THE FREE STATE
UNIVERSITEIT VAN DIE VRYSTAAT
YUNIVESITHI YA FREISTATA

UFS·UV
HEALTH SCIENCES
GESONDHEIDSWETENSKAPPE

IRB nr 00006240
REC Reference nr 230408-011
IORG0005187
FWA00012784

07 February 2017

L DEACON
DEPT OF EXERCISE AND SPORT SCIENCES
FACULTY OF HEALTH SCENCES
UFS

Dear L Deacon

HSREC 04/2017 (UFS-HSD2017/0033)
PROJECT TITLE: EFFECT OF SHORT-TERM MACRONUTRIENT MANIPULATION ON ENDURANCE CAPACITY OF LONG DISTANCE RUNNERS

1. You are hereby kindly informed that the Health Sciences Research Ethics Committee (HSREC) approved this protocol after all conditions were met. This decision will be ratified at the next meeting to be held on 28 February 2017.
2. The Committee must be informed of any serious adverse event and/or termination of the study.
3. Any amendment, extension or other modifications to the protocol must be submitted to the HSREC for approval.
4. A progress report should be submitted within one year of approval and annually for long term studies.
5. A final report should be submitted at the completion of the study.
6. Kindly use the HSREC NR as reference in correspondence to the HSREC Secretariat.
7. The HSREC functions in compliance with, but not limited to, the following documents and guidelines: The SA National Health Act. No. 61 of 2003; Ethics in Health Research: Principles, Structures and Processes (2015); SA GCP(2006); Declaration of Helsinki; The Belmont Report; The US Office of Human Research Protections 45 CFR 461 (for non-exempt research with human participants conducted or supported by the US Department of Health and Human Services-(HHS), 21 CFR 50, 21 CFR 56; CIOMS; ICH-GCP-E6 Sections 1-4; The International Conference on Harmonization and Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH Tripartite), Guidelines of the SA Medicines Control Council as well as Laws and Regulations with regard to the Control of Medicines, Constitution of the HSREC of the Faculty of Health Sciences.

Yours faithfully

.....
DR SM LE GRANGE
CHAIR: HEALTH SCIENCES RESEARCH ETHICS COMMITTEE
cc: FF Coetzee

Health Sciences Research Ethics Committee
Office of the Dean: Health Sciences
T: +27 (0)51 401 7795/7794 | F: +27 (0)51 444 4359 | E: ethicsfhs@ufs.ac.za
Block D, Dean's Division, Room D104 | P.O. Box/Posbus 339 (Internal Post Box G40) | Bloemfontein 9300 | South Africa
www.ufs.ac.za

Appendix A.15

LETTER FROM THE LANGUAGE EDITOR

Confirmation of Language Editing

24 November 2020

To whom it may concern,

CONFIRMATION OF LANGUAGE EDITING

In relation to the PhD Thesis of Lizl Deacon, entitled:

EFFECT OF SHORT-TERM MACRONUTRIENT MANIPULATION ON ENDURANCE CAPACITY OF LONG-DISTANCE RUNNERS

To be submitted in fulfilment of the qualification PhD in Human Movement Sciences at the University of the Free State, I, in my capacity as Language Practitioner, confirm that the abovementioned document has been edited with specific focus on the following:

- Language use and spelling (UK English)
- Coherence and linguistic flow
- Consistency of terminology and formatting

In relation to the above, Track Changes were used in MS Word to indicate changes, and comments were provided where necessary. Please note that changes are made solely at the client's discretion and remain their own responsibility. Any comments provided are purely suggestions and reflect the best efforts and opinions of the Editor and not necessarily subject-specific expertise. It remains the responsibility of the client to confirm the content of their final submission.

For any questions, please feel free to contact me at guillaume.annam@gmail.com during normal business hours.

Kind regards,

A.M. Guillaume-Combrink
LANGUAGE PRACTITIONER

Appendix A.16

EXEMPLAR OF MEAL PLANS

Participant XXX **High fat diet**

Food group	Day 1	Day 2
Breakfast		
1 milk portion	250 ml full cream milk	250 ml full cream milk
1 starch portions	1 slice low GI bread	1 slice low GI bread
1 fruit portion	80 g banana without peel	100 g pear unpeeled
4 fat portions	½ avocado (80g peeled, without pip) or 60 ml mashed avocado	½ avocado (80g peeled, without pip) or 60 ml mashed avocado
3 high fat meat portions	90 g cooked boerewors	60 g bacon
Snack		
1 fruit portion	120 g apple unpeeled	80 g banana without peel
5 fat portions	50 ml peanut butter	43 g peanuts
Lunch		
3 high fat meat portions	30 g cheddar + 60 g Russian	90 g regular mince (cooked) + 10 ml oil to fry meat and onions
2 starch portions	200 g boiled potato (allow to cool down before eating, can be reheated, but not too hot)	250 ml cooked pasta made from durum wheat
1 vegetable B portion	85g boiled peas	60 g McCains Ruby roast vegetables baked with 10 ml oil
Vegetable A	125 ml lettuce + 50 g cucumber	25 g raw carrot strips + 25 g cucumber
4 fat portions	40 ml mayonnaise (on potato)	-
Snack		
1 fruit portion	100 g pear unpeeled	120 g apple unpeeled
5 fat portions	43 g peanuts	50 ml peanut butter
Supper		
3 high fat meat portions	90 g fatty meat stew + 40 g boiled mushrooms in stew	200 g spareribs
1 starch portion	80 ml cooked Basmati rice	80 ml cooked frozen corn / mealies
1 vegetable B portion	125 ml cooked mixed vegetables with corn, peas, carrots	125 ml (100g) green beans with potato + 10 ml margarine
vegetable A	-	125 ml lettuce + 50 g tomato
5 fat portions	25 ml canola oil (use for preparation of stew)	20 ml canola oil for spareribs or 60 ml regular salad dressing on salad
1 milk portion	250 ml full cream milk	250 ml full cream milk
Snack		
4 fat portions	53 ml cream cheese (not cottage cheese)	53 ml cream cheese (not cottage cheese)
1 starch portion	3 Provitas	3 Provitas

Macronutrient breakdown: 12 164.74 kJ Total Daily energy Expenditure

CHO/FAT/PROT: 2 554.60kJ/7 907.08kJ/1 703.06kJ

Participant XXX **High CHO diet**

Food group	Day 1	Day 2
Breakfast		
1 milk portion	250 ml fat free/skimmed milk	250 ml fat free/skimmed milk
4 starch portions	4 slices low GI bread	4 slices low GI bread
1 fruit portion	80 g banana without peel	100 g pear unpeeled
1 fat portion	10 ml 40% fat spread (Stork margarine)	10 ml 40% fat spread (Stork margarine)
2 sugar portions	20 ml mixed berry jam	20 ml mixed berry jam
1 low fat meat portion	1 extra large egg	30 g sardines / Pilchards
Snack		
1 fruit portion	120 g apple unpeeled	80 g banana without peel
4 starch portions	12 Provitas	4 slices low GI bread
1 fat portion	10 ml peanut butter	8 g peanuts or 10 ml 40% fat spread (Stork margarine)
1 sugar portion	10 ml mixed berry jam	10 ml mixed berry jam
Lunch		
3 low fat meat portions	90 g tuna in brine	90 g extra lean mince + 5 ml oil to fry onions
4 starch portions	400 g boiled potato (allow to cool down before eating, can be reheated, but not too hot)	500 ml cooked pasta made from durum wheat
1 vegetable B portion	85g boiled peas	60 g McCains Ruby roast vegetables baked with 5 ml oil
Vegetable A	125 ml lettuce + 50 g cucumber	25 g raw carrot strips + 25 g cucumber
1 fat portion	10 ml mayonnaise	
Snack		
1 fruit portion	100 g pear unpeeled	120 g apple unpeeled
4 starch portions	4 slices low GI bread	12 Provitas
1 fat portion	10 ml 40% fat spread (Stork margarine)	10 ml peanut butter
Supper		
3 low fat meat portions + 2 fat portions	90 g stew made with meat without fat + 10 ml canola oil for preparation + 40 g boiled mushrooms in stew	90 g cooked chicken (cooked without skin) + 10 ml canola oil to fry onion and chicken
4 starch portions	240 g (500 ml) cooked pearled wheat (stampkoring)	360 ml cooked Basmati rice
1 vegetable B portion	125 ml cooked mixed vegetables with corn, peas, carrots	125 ml (100g) green beans with potato
Vegetable A	-	125 ml lettuce + 50 g tomato
1 milk portion	250 ml fat free/skimmed milk	250 ml fat free/skimmed cream milk
4 sugar portions	220 ml jelly	220 ml jelly
Snack		
2 fat portions	10 ml oil to prepare popcorn	10 ml oil to prepare popcorn
2 starch portions	3 Cups popcorn (popped)	3 Cups popcorn (popped)
	(15 ml unpopped gives 3 Cups)	

Macronutrient breakdown: 12 164.74 kJ Total Daily energy Expenditure

CHO/FAT/PROT: 8150.38 kJ/2 068.01kJ/1946.36kJ