THE EFFECT OF PRE-EXERCISE NUTRIENT INTAKE ON METABOLISM

by

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DECLARATION

I, Celesti Juanine Jansen van Rensburg, identity number 8105270083081 and student number 2000006524, hereby declare that the work submitted here is the result of my own independent investigation. Where help was sought, it was acknowledged. I further declare that this work is submitted for the first time at this university/faculty towards a Magister Artium degree in Sport Science and that it has never been submitted to any other university/faculty for the purpose of obtaining a degree.

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C.J. Jansen van Rensburg

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"We give thanks to you, O God, we give thanks, for your Name is near; men tell of your wonderful deeds." Psalm 75:1

This project is dedicated to my mother.
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LIST OF DEFINITIONS AND ABBREVIATIONS	
Acetyl-CoA	The major fuel for the oxidative processes in the body, being derived from the breakdown of glycogen, glucose and fatty acids.
Adipocyte	An adipose tissue cell whose main function is to store triacylglycerol (fat).
ADP (adenosine diphosphate)	Breakdown product of ATP.
Aerobic	Occurring in the presence of free oxygen.
Anaerobic	Occurring in the absence of free oxygen.
ATP (adenosine triphosphate)	A high energy compound that is the immediate source for muscular contraction and other energy requiring processes in the cell.
ATPase	An enzyme that splits the last phosphate group off ATP, releasing a large amount of energy and reducing the ATP to ADP and inorganic phosphate (Pi)
β-oxidation	Oxygen-requiring process in the mitochondria whereby 2-carbon units are sequentially removed from the hydrocarbon chain of a fatty acid in the form of Acetyl-CoA, which can then enter the Krebs cycle.
Breath-by-breath system	An automated system to analyze gas exchange to estimate energy expenditure and substrate utilization. These systems are able to measure CO_2 production and oxygen consumption from every breath.
Buffer	A substance that, in solution, prevents rapid changes in hydrogen ion concentration (pH).
C	Caffeine trial
Caffeine	A stimulant drug found in many food products such as coffee, tea, and cola drinks. Stimulates the central nervous system and used as an ergogenic aid.
Calorimeter	An insulated chamber to estimate energy expenditure

	by measuring heat dissipation from the body. This method is called direct calorimetry.
cAMP	Cyclic AMP
Carbohydrate (CHO)	A compound composed of carbon, hydrogen, and oxygen in a ration of 1:2:1.
CAT 1 and 2	Carnitine Acyl Transferase 1 and 2
Catabolism	Destructive metabolism whereby complex chemical compounds in the body are degraded to simpler ones.
Carbon dioxide (CO_2)	Gas produced during oxidation of carbohydrates and fats.
Ch	Carbohydrate trial
CoA (coenzyme A)	A molecule that acts as a carrier for acyl or acetyl groups.
CK	Creatine Kinase-the enzyme that facilitates the breakdown of PCr to creatine and inorganic phosphate (Pi)
CPT 1	Carnitine Palmitoyl Transferase 1
Ergogenic aids	Substances that improve exercise performance and are used in attempts to increase athletic or physical performance capacity.
F	Fat trial
Fa	Fasting trial
Fatty acid	A type of fat having a carboxylic acid group (COOH) at one end of the molecule and a methyl group (CH_3) at the other end, separated by a hydrocarbon chain that can vary in length.
FABP	A protein found in liver and muscle that binds fatty acids in order to maintain a low intracellular free fatty acid concentration.
FAD	Flavin Adenine Dinucleotide, oxidized form-an enzyme

	important in energy metabolism
$FADH_2$	Flavin Adenine Dinucleotide, reduced form of coenzyme FAD
Fasting	Starvation; abstinence from eating that may be partial or complete.
Fat	Fat molecules that contain the same structural elements as carbohydrates but with little oxygen relative to carbon and hydrogen and are poorly soluble in water.
FC	Fat and caffeine trial
	Fat and Carbohydrate Crossover Point
FFA	A fatty acid that is not esterified to glycerol or any other molecule.
GLUT	Glucose transporter found in cell membranes, including those of the muscle and liver.
Glycemic index (GI)	Increase in blood glucose and insulin in response to a meal. The GI of a food is expressed against a reference food, usually glucose.
Glycogenolysis	The breakdown of glycogen into glucose-1-phosphate by the action of phosphorylase.
Glycolysis	The sequence of reactions that converts glucose (or glycogen) to pyruvate.
H^+	Hydrogen ion or proton.
H_2O	Water
Hormone Sensitive Lipase (HSL)	Enzyme that splits triacylglycerols into fatty acids and glycerol. It is regular by hormones (mainly by epinephrine and insulin).
IDH	Isocitrate dehydrogenase
IMTG	Storage form of fat found in muscle fibers.
Indirect calorimetry	A method to measure energy expenditure and

	substrate utilization on the basis of gas exchange measurements. The term *indirect* refers to the measurement of O_2 uptake and CO_2 production rather than the direct measurement of heat transfer.
Lactate dehydrogenase (LDH)	Enzyme that catalyzes the reversible reduction of pyruvate to lactate
Lactic acid [La^-]	Metabolic end product of anaerobic glycolysis
LC	Lactate clamp
LCT	Long Chain Triglyceride-part of triacylglycerols. Hydrocarbon chains with 12 or more carbon atoms and the most abundant type of fatty acids
LDH	Lactate Dehydrogenase-a key glycolytic enzyme involved in the conversion of pyruvate to lactate
Lipolysis	The breakdown of triacylglycerols into fatty acids and glycerol
LPL	Lipoprotein Lipase
MCT	Medium Chain Triglyceride-a fatty acid with 8-10 carbon atoms
MCT	Monocarboxylate Transport Protein
Metabolic acidosis	A metabolic derangement of acid-base balance where the blood pH is abnormally low.
NAD^+	Nicotinamide Adenine Dinucleotide, oxidized from-a coenzyme important in energy metabolism
NADH	Nicotinamide Adenine Dinucleotide-reduced form of the coenzyme NAD
Oxygen (O_2)	Oxygen molecule.
PCr	Phosphocreatine-an energy rich compound that plays a critical role in providing energy for muscle action by maintaining ATP concentration.
PDH complex	Pyruvate Dehydrogenase complex-a complex

	multienzyme system that catalyzes the conversion of pyruvate to acetyl CoA + CO_2
pH	A measure of acidity/alkalinity.
Phosphagen	The term given to both high-energy phosphate compounds, adenosine triphosphate and phosphocreatine.
Pi	Inorganic phosphate
PKA	Protein Kinase A
RBC	Red Blood Cells
RER	The ratio of carbon dioxide produced divided by oxygen consumption, representing a measure of substrate utilization at the whole-body level.
S-FABP	Sarcolemmal Fatty Acid Binding Protein
TCA cycle	A series of reactions that are important in energy metabolism and take place in the mitochondrion. Also known as the Krebs cycle.
Triacylglycerol	The storage from of fat composed of three fatty acid molecules linked to a 3-carbon glycerol molecule. Also known as triglyceride.
$V'CO_2$	Rate of carbon dioxide production.
$V'O_2$	Rate of oxygen uptake.
$V'O_{2max}$	Maximal oxygen uptake. The highest rate of oxygen consumption by the body that can be determined in an incremental exercise test to exhaustion.
$V'O_{2peak}$	This term indicates that no plateau was reached during the test and the RER was not more than 1.1.

CHAPTER 1

RESEARCH DESIGN

1.1. INTRODUCTION

When comparing the limited capacity of humans to store carbohydrates to the almost limitless stores of endogenous fat depots, it is clear that the oxidation of these fat stores is limited during intense exercise and ultimately carbohydrates remain the fuel for oxidative metabolism. In the exploration for tactics to advance athletic performance, current interest has focused on numerous nutritional actions which may hypothetically promote fatty acid oxidation, ease the rate of muscle glycogen depletion and ultimately improve exercise capacity (Hawley et al., 1998:241).

Interventions aimed at improving the metabolism of fat could potentially reduce the symptoms of metabolic diseases such as obesity and type 2 diabetes and may have incredible clinical significance. In order to reach this objective an understanding of the factors that enhance or reduce fat oxidation is vital. Exercise duration and intensity are very important regulators of fat metabolism. Fat oxidation is maximal at low to moderate intensity exercise but as the intensity of exercise increases too much, less reliance on fat metabolism is evident and more reliance on carbohydrate metabolism becomes clear. Maximal rates of fat oxidation have been noted in trained individuals at around 59–64% $V'O_{2max}$, whilst in untrained individuals, maximal fat oxidation occurs around 47–52% $V'O_{2max}$ (Achten and Jeukendrup, 2004a:716).

Numerous factors are known to influence the selection of fuel for exercise, and there can be noteworthy interactions between several of them. These factors include: substrate availability; nutritional status; diet; mode; intensity; duration of exercise; muscle fiber type composition; physical fitness; the effect of training, drugs, hormones, prior exercise; environmental factors for example temperature and altitude (Maughan et al., 1997:29).

It has been found that a direct relationship exists between the rates of carbohydrate oxidation and improved marathon running speed. According to researchers the goal of a marathon athlete should be to 'train, eat and run at a pace' such that muscle glycogen stores are depleted as the athlete crosses the finish line (Lambert et al., 1997:315).

The two principal substrates used by muscles are carbohydrates and fats. Research has suggested that endurance training leads to adaptations on a metabolic and cellular level that actually allows trained muscles to use more fats for energy and hence spare carbohydrates. In addition to this, investigators have suggested that this might be the key to delaying muscular fatigue and in effect spare muscle glycogen and blood glucose (Costill et al., 1977; Hickson et al., 1977; Holloszy and Coyle, 1984; Hoppeler et al., 1985; Saltin and Astrand, 1993). It has been calculated by Oberholzer et al. (1976) that intracellular stores account for roughly 50% of the energy needed in an ultra-long distance event (25% lipids and 25% glycogen). The rest of the energy needed must be supplied by the blood and may depend on blood glucose and fat concentrations. Therefore increasing fat availability immediately before exercise will enhance the capacity of trained subjects to perform prolonged exercise. Due to this, it has been suggested that medium chain triglycerides (MCTs) and free fatty acids (FFAs) may be a readily obtainable energy source for muscles (Knoepfli et al., 2004:402).

1.2. PROBLEM STATEMENT:

The question that arises from this research project is the following: Does pre–exercise nutrient intake have an effect on macronutrient metabolism of man during subsequent exercise?

1.3. RATIONALE OF THE RESEARCH PROJECT

Should pre-exercise nutrient intake have an effect on macronutrient metabolism of man during subsequent exercise, the following notions within the sport and well-being industries ensue:

1.3.1. Athletic industry

Athletes are subjected to evaluation protocols to identify areas of strength and weaknesses. Should the postulates (see section 1.7) bear truth, the implications would render “fitness testing results” invalid in the sense that the findings of one athlete cannot be compared to the same athlete at a later stage (re-testing) or any other athletes at any stage of testing.

1.3.2. Well-being industry

The health risks associated with being obese or overweight include coronary heart disease, hypertension, diabetes mellitus, abnormal lipid and lipoprotein concentrations, impaired heat tolerance, osteoarthritis, gout, renal and pulmonary disease (Kerr et al., 2002:407). In addition, many scientists and athletes are aware of the facts that a negative correlation between percentage of body fat and performance exists.

New research, which still has to be done, on how to improve fat oxidation by the synchronization of training and nutritional manipulating strategies to alter body

composition could provide groundbreaking results on unresolved research questions:

- “Why combine diet and physical activity in the same international research society?” (Baranowski, 2004:2-19).
- “Diet and exercise for weight loss?” (Volek et al., 2005:1-9).

1.4. PURPOSE/OBJECTIVES OF THE RESEARCH

- The primary purpose of the present investigation is to evaluate the effect of various “nutrient intake interventions” prior to exercise (training/competition) on metabolism during exercise in man.

- The secondary purposes of this investigation is to investigate whether nutrient intake within the hours prior to training influence peak treadmill running velocity (indicative of athletic performance capacity) and the fatty acid threshold (indicative of fat utilization).

1.5. NECESSITY OF THE RESEARCH

Not only will the results from this investigation provide constructive information to athletes on fuel utilization, but such information could also serve those individuals who would like to promote well-being (correct body composition by reducing fat mass). Newly founded perspectives on the research objectives may also provide information for other researchers wanting to explore this field of study. Since the problem statements require a new field of study [information relating to pre-exercise nutritional manipulations on exercise metabolism during graded exercise tests appears to be absent (Achten and Jeukendrup, 2003:1022)], it could also explain contrasting research results presented in the peer reviewed scientific literature on weight loss up to date.

1.6. FOCUS AND HYPOTHESIS

The focus and the hypothesis of this investigation will be discussed.

1.6.1. Focus

The area of discipline relates to physiology, exercise physiology, bioenergetics, indirect calorimetry, nutritional principles, nutritional manipulations and the synchronization of exercise and nutrition sciences.

1.6.2. Hypothesis:

Pre-exercise nutrient intake and the timing thereof prior to a graded exercise test until voluntary fatigue sets in, manipulates metabolism (fat and/or carbohydrate oxidation) during exercise.

1.7. POSTULATES

- Fasting, fat intake, caffeine intake, fat in combination with caffeine intake and carbohydrate intake prior to a graded exercise test influence macronutrient metabolism in different ways.
- Due to genetic predisposition and/or the current physiological profile images of an individual, not all individuals will respond to the aforementioned interventions to the same extent.

CHAPTER 2

LITERATURE SURVEY

2.1. INTRODUCTION

Athletic activities can be classified into three categories according to their duration and energy expenditure characteristics: power, speed and endurance. Power can be seen in events such as shot put and 100m sprint, whereas speed can be seen in a 400m sprint and endurance is manifested in marathons. For all three of these components of athletics to thrive, they have to depend on energy yielding metabolic processes (energy systems).

Skeletal muscle has three energy systems which supply energy to each of these three kinds of activities. The first energy system is known as the phosphagen systems and provides energy in an anaerobic fashion. These systems supply energy for a few seconds. The first process involves the splitting of the high-energy phosphagen, phosphocreatine (PCr), which together with the stored ATP in the cell provides an immediate energy source. The immediate energy is utilized in the initial stages of intense or explosive exercises. For speedy, forceful activities lasting more than a few seconds (>±8s) up to 1 minute, muscles will be fueled by glycolytic energy sources in combination with immediate energy sources. For activities lasting more than 2 minutes, there is a requirement to depend on oxidative metabolism (Brooks et al., 2000:28-29).

The second process involves the anaerobic breakdown of blood glucose and/or muscle glycogen to pyruvic acid. The end products of glycolysis can be envisaged to implicate lactic acid and Acetyl CoA. The third process, aerobic or

oxidative metabolism, involves the oxidation of Acetyl CoA [originating from carbohydrates (mainly glucose), fats (fatty acids) and under some circumstances proteins (amino acids)]. These processes require oxygen, hence oxidative phosphorylation (Gastin, 2001:725).

2.1.1. Phosphagen systems

The most important characteristic of the phosphagens is that the energy store they represent is available to the muscle almost immediately. The PCr in muscle can be used to resynthesize ATP at a very high rate. This relatively high rate of energy transfer corresponds to the ability to produce rapid forceful actions during the initial stages of high intensity exercise. The major disadvantage of this system is its limited capacity- the total amount of energy available is diminutive (Maughan et al., 1997:17). If no other energy source is available to the muscle, fatigue will occur quickly (within 2 seconds). During short sprints over a distance of 30-50 m, where no slowing down takes place over the last few meters, full power can be maintained all the way and the energy requirements are met by breakdown of the phosphagen stores. Over longer distances, running speed decreases as these stores become exhausted and power output declines. However, the rate of recovery from a short sprint is quite speedy, and a second burst can be completed at the same speed after only 2-3 min recovery. For longer sprints (100m and more), much longer recovery periods are needed before the capability to produce a maximum performance is restored (Maughan et al., 1997:18).

Some of the energy for ATP resynthesis is supplied quickly and without the need for oxygen. Within the muscle fiber, the concentration of PCr is 3 to 4 times larger than that of ATP. When PCr is broken down to creatine and inorganic phosphate (Pi) by the action of the enzyme creatine kinase (CK), a large quantity of free energy is released (Jeukendrup and Gleeson, 2004:35). Because PCr has a higher free energy of hydrolysis than ATP, its phosphate is contributed directly to the ADP molecule to re-form ATP. When the ATP content starts to fall during

exercise, PCr is broken down, releasing energy for restoration of ATP. During very intense exercise PCr can become almost entirely depleted. However the reactions of ATP and PCr hydrolysis are reversible, and whilst energy is readily available from other sources (oxidative phosphorylation), creatine and phosphate can be rejoined to form PCr (Jeukendrup and Gleeson, 2004:36).

Take note that the resynthesis of ATP via breakdown of PCr buffers some of the hydrogen ions formed as a result of ATP hydrolysis. This facilitates the prevention of rapid acidification of the muscle sarcoplasm, which could induce premature failure of the contractile mechanism (Jeukendrup and Gleeson, 2004:36).

Maughan et al. (1997:17) states that under usual conditions muscle clearly does not fatigue after only a few seconds of effort, so a source of energy other than the phosphagens must be available. This is derived from glycolysis, the name given to the pathways involving the breakdown of blood glucose and muscle glycogen via glucose-1-phosphate (G1P) and a consequent series of chemical reactions to pyruvate.

2.1.2. Glycolysis:

According to Brooks et al. (2000:55), the major product of dietary sugar and starch digestion is glucose which is released into the blood of the systemic circulation. The glucose enters various cells, including myocytes and hepatocytes and is either catabolized immediately or accumulated as glycogen for later use.

The overall capacity of the glycolytic system to re-phosphorylate ADP to ATP is large in comparison with the phosphagen systems. However, the rate and power output at which the glycolytic system can produce ATP is lower than the phosphagen system. It is for this reason that maximum speeds cannot be sustained for more than a few seconds (Maughan et al., 1997:18).

Glycolysis (a process which is not oxygen-dependent) contributes towards the re–phosphorylation of ADP to ATP by means of some reactions involving substrate level phosphorylation. For the reactions to continue, the pyruvate must be removed. In low intensity exercise, when the rate at which energy is required can be met aerobically, pyruvate is converted to carbon dioxide and water by oxidative metabolism in the mitochondria. Although lactate is always present in the blood, pyruvate is shunted and converted to lactic acid formation anaerobically especially during high intensity exercise (see 2.1.3). The rate of lactate formation is dependent mainly on the intensity of the exercise, but depends more on the relative exercise intensity (% $V'O_{2max}$) than the actual absolute intensity. Activation of the glycolytic system occurs almost immediately at the onset of exercise and is triggered by calcium (Ca^{2+})-release from the sarcoplasmic reticulum in response to the end-plate potential. In high intensity exercise, the muscle glycogen stores are broken down rapidly with a correspondingly high rate of lactate formation. Some of the lactate diffuses out of the muscle fibers where it is produced and appears in the blood (Maughan et al., 1997:17–18).

According to Billat et al. (2003:409), the decrease in lactate production coincides with an improvement in the maintenance of cell phosphorylation and with an improved removal of lactate. This modification was associated with a superior potential for phosphorylation. It has been mentioned that sarcolemmal carrier–mediated lactate transport, which has a significant role in lactate release during and after heavy exercise, is more elevated in athletes than in less fit or untrained participants. It lately been reported that lactate transport was mediated by a monocarboxylate transport protein (MCT) which has numerous isoforms. These carriers are also responsive to endurance and intensive training and are modified after exhaustive exercise. Some data propose that among the family of MCTs, MCT1 and MCT4 are mostly responsible for lactate uptake from the circulation and lactate extrusion out of muscle, respectively.

Maughan et al., (1997:17-18), states that a great part, but not all, of the muscle glycogen stores can be used for anaerobic energy production during high intensity exercise, and will supply the major part of the energy requirement for maximum intensity efforts lasting from 20 seconds to 5 minutes. For shorter durations, the phosphagen systems are the main energy source, whereas oxidative metabolism becomes progressively more important as exercise duration increases.

2.1.2.1. The routes of glycolysis

In glycolysis two equivalents of ATP is required to activate the process, with the subsequent production of four equivalents of ATP and two equivalents of nicotinamide adenine dinucleotide (NADH). Thus, conversion of one mole of blood borne glucose to two moles of pyruvate is accompanied by the net production of two moles each of ATP and NADH. The conversion of one mole of glucose originating from glycogen to two moles of pyruvate is accompanied by the net production of three moles of ATP and two moles of NADH.

$$\text{Glucose} + 2\ \text{ADP} + 2\ \text{NAD}^+ + 2\ \text{P}_i \longrightarrow 2\ \text{Pyruvate} + 2\ \text{ATP} + 2\text{NADH} + 2\text{H}^+$$

According to Jeukendrup and Gleeson (2004:37) the NADH generated during glycolysis is used to:

- Fuel mitochondrial ATP synthesis via oxidative phosphorylation producing either two or three equivalents of ATP depending upon whether the glycerol phosphate shuttle or the malate-aspartate shuttle is used to transport the electrons from cytoplasmic NADH into the mitochondria. The net yield from the oxidation of 1 mole of glucose to 2 moles of pyruvate is, therefore, either 6 or 8 moles of ATP. Complete oxidation of the 2 moles of pyruvate, through the TCA cycle (Krebs cycle), yields an additional 30 moles of ATP; the total yield, therefore being either 36 or 38 moles of ATP from the complete oxidation of 1 mole of blood borne glucose to CO_2 and H_2O.

- Assist in the formation of lactic acid and eventually lactate. Pyruvate is transformed into lactic acid by the action of NADH to NAD^+. The NAD^+ is then used in two other places in glycolysis, i.e. when glyceraldehyde-3-phosphate is converted to 1,3-diphosphoglycerate and when pyruvate is converted to acetyl–CoA which enters the Krebs cycle.

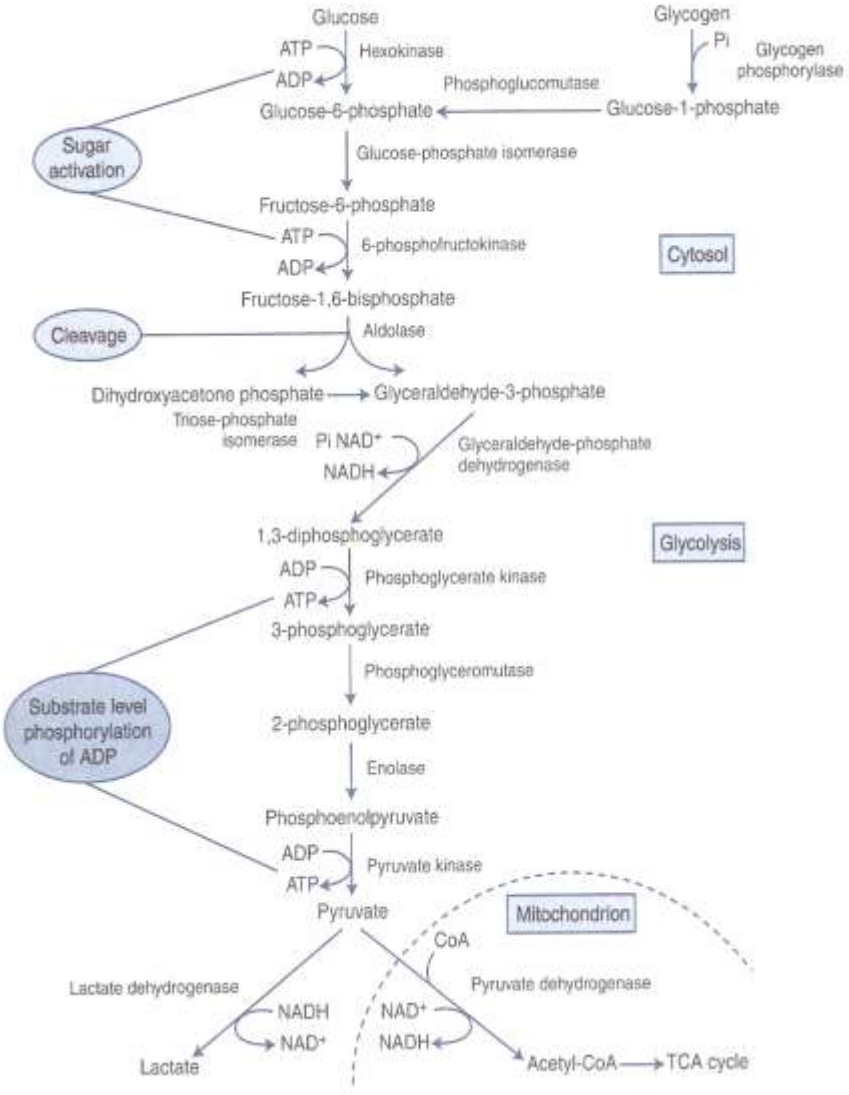


Figure 1–The reactions of glycolysis-After Jeukendrup and Gleeson (2004).

2.1.2.2. Glycolysis (blood borne glucose)

According to Hargreaves and Thompson (1999:169) glucose is transported across the cell membrane via assisted diffusion. A family of glucose transporter proteins named glucose transporters (GLUT) has been acknowledged. Although the different isoforms (GLUT1 to 7) are all able of transporting glucose they have different characteristics and tissue distribution. Since GLUT 1 is resident in the sarcolemma independently of stimulation by insulin and/or muscle contractions, its foremost function is thought to be to supply basal glucose transport. GLUT 4 is the most plentiful and most significant glucose transporter in skeletal muscle and is dependant upon insulin actions. It is distinctive in the sense that it is able to translocate from an intracellular storage site to the sarcolemma upon stimulation with contractions and/or in the presence of insulin (Hargreaves and Thompson,1999:169). Jeukendrup and Gleeson (2004:37) suggest that once the glucose molecule is within the muscle cell, an irreversible phosphorylation, catalyzed by the enzyme hexokinase, occurs to prevent the loss of glucose from the cell. The glucose is transformed to glucose-6-phosphate. The hexokinase reaction is an energy-consuming reaction, necessitating the investment of one molecule of ATP for each molecule of glucose. This reaction also guarantees a concentration gradient for glucose across the cell membrane, down which transport can occur. Hexokinase is inhibited by an accumulation of glucose-6-phosphate, and during high intensity exercise, the increasing concentration of glucose-6-phosphate limits the contribution that blood borne glucose can make to carbohydrate metabolism in the active muscles.

2.1.2.3. Glycolysis (glycogenolysis)

According to Jeukendrup and Gleeson (2004:38), if glycogen, rather than blood borne glucose, is the substrate for glycolysis, a solitary glucose molecule is split off by the enzyme glycogen phosphorylase, and the products are glucose-1-phosphate and a glycogen molecule that is one glucose residue shorter than the original. The substrates are glycogen and inorganic phosphate, thus unlike the hexokinase reaction, no breakdown of ATP occurs. Phosphorylase acts on the α-

1,4 carbon bonds at the free ends of the glycogen molecule but cannot break the α-1,6 bonds outlining the branch points. These bonds are hydrolyzed by the collective actions of a debranching enzyme and amylo-1,6-glucosidase, releasing free glucose, which is quickly phosphorylated to glucose 6-phosphate by hexokinase. Free glucose accrues within the muscle cell only in very high intensity exercise, where glycogenolysis is proceeding rapidly. Because relatively few α-1,6 bonds exist, no more than 10% of the glucose residues emerge as free glucose.

2.1.2.4. The combined glycolytic pathway

Jeukendrup and Gleeson (2004:38) suggest that the enzyme phosphoglucomutase quickly converts the glucose-1-phosphate formed by the action of glycogen phosphorylase to glucose-6-phosphate, which then proceeds down the glycolytic pathway. Refer to figure 1. After an additional phosphorylation, the glucose molecule is cleaved to form 2 molecules of the 3–carbon sugar glyceraldehydes–3–phosphate. The second stage of glycolysis is the conversion of glyceraldehyde–3–phosphate into pyruvate, in conjunction with the formation of ATP and reduction of NAD^+ to NADH. The net result of glycolysis is the conversion of 1 molecule of glucose to 3 molecules of pyruvate with the formation of 2 molecules of ATP and the conversion of 2 molecules of NAD^+ to NADH. If glycogen rather than glucose is the starting substrate, 3 molecules of ATP are formed because no initial investment of ATP is made when the first phosphorylation step occurs. Although this net energy yield appears diminutive, the relatively large carbohydrate store available and the rapid rate at which glycolysis proceeds makes energy supplied in this way crucial for the performance of intense exercise. The 800 meter runner, for example, acquires about 60% of the total energy requirement from anaerobic metabolism and may convert about 100g of carbohydrate to lactate in less than 2 minutes. The amount of ATP released in this way far exceeds the ATP available from PCr hydrolysis. This high rate of anaerobic metabolism not only allows a quicker “steady state” speed than is possible with aerobic metabolism alone but also allows a faster

pace in the early stages, before the cardiovascular system has adjusted to the demands and the delivery and utilization of oxygen have increased in response to the exercise stimulus.

The reactions of glycolysis occur in the sarcoplasm and some pyruvate will escape from active muscle tissues when the rate of glycolysis is high. The destiny of the pyruvate produced depends not only on factors such as exercise intensity but also on the metabolic capacity of the tissue. When glycolysis proceeds speedily, the availability of NAD^+, which is necessary as a cofactor in the glyceraldehyde-3-phosphate dehydrogenase reactions, could become limiting (Jeukendrup and Gleeson, 2004:38).

Reduction of pyruvate to lactate will regenerate NAD^+ and also bind two hydrogen atoms in the process (indicative of buffer capacity). Lactate can collect within the muscle fibers, reaching much higher concentrations than those reached by any of the glycolytic intermediates. When lactate collects in high concentrations however, the associated hydrogen ions cause a decrease in pH (acidosis), inhibiting some enzymes such as phosphorylase and phosphofructokinase, and the contractile mechanisms begins to fail. A low pH arouses free nerve endings in the muscle, causing the perception of pain. Although the unconstructive effects of the acidosis resulting from lactate accumulation are often stressed, the energy made available by anaerobic glycolysis allows the performance of high intensity exercise that would otherwise not be possible (Jeukendrup and Gleeson, 2004:38).

2.1.3. Lactate kinetics

The metabolic conditions that cause an increase in lactate production are themes of research in exercise physiology and biochemistry. Despite evidence for lactate production during conditions of low or no oxygen, lactate production can also take place in the presence of adequate oxygen. As a result, lactate production

should not be viewed as evidence of hypoxia (anaerobic conditions) (Robergs and Roberts, 2000:38).

2.1.3.1. Production of lactate

During anaerobic glycolysis, that period of time when glycolysis is proceeding at a high rate (or in anaerobic organisms), the oxidation of NADH occurs through the reduction of an organic substrate. Erythrocytes and skeletal muscle (under conditions of exertion) derive all of their ATP needs through anaerobic glycolysis. The large quantity of NADH produced is oxidized by reducing pyruvate to lactate. This reaction is catalyzed by lactate dehydrogenase (LDH). The lactate produced during anaerobic glycolysis diffuses from the tissues and is transported to highly aerobic tissues such as cardiac muscle, erythrocytes and liver. The lactate is then oxidized to pyruvate in these cells by LDH and the pyruvate is further oxidized in the TCA cycle. If the energy level in these cells is high, the carbons of pyruvate will be diverted back to glucose via the gluconeogenesis pathway (Robergs and Roberts 2000:36).

Pyruvate can be reduced to lactate by the enzyme lactate dehydrogenase (LDH), as specified in the equation below:

$$\text{Pyruvate} + \text{NADH} + \text{H}^+ \longleftrightarrow \text{Lactate} + \text{NAD}^+$$

Robergs and Roberts (2000:36) claims that it is classically explained that this reaction first produces lactic acid, which then immediately releases a proton when produced at physiological pH, leaving lactate. Mounting evidence exists however, to indicate that the acidosis accompanying lactate production may be more complex than this, and is perhaps due to the accumulation of NADH + H^+ and/or increased net ATP dephosphorylation. These authors state that it is more correct to declare that acidosis accompanies increased lactate production.

A basal level of lactate production exists in skeletal muscle, ensuing in a resting muscle lactate concentration of 1 mMol/kg wet wt. This resting concentration results from a balance between lactate production, metabolism within the identical muscle fiber, and its removal from the cell for metabolism in other tissues (i.e. other skeletal muscle fibers, heart and the liver tissues) (Robergs and Roberts, 2000:36).

Except if the free protons released during conditions of increased lactate production are buffered, increases in lactate production coincide with decreases in cellular pH. For example as exercise intensity increases, the rate of proton liberation eventually exceeds the buffering capacity of the cell, and pH decreases and acidosis ensues. Despite this occurrence, lactate production is not necessarily disadvantageous to muscle metabolism during exercise. The creation of lactate involves the reduction of pyruvate, and the electrons and protons required for this are provided by NADH + H^+. Lactate production therefore engages the oxidation of NADH, which regenerates NAD^+ for glycolysis. Lactate production therefore maintains the ratio between NAD^+ and NADH (termed the cytosolic redox potential), and supports sustained glycolysis and a high rate of ATP regeneration during repeated intense muscle contractions. Consequently, during sustained high intensity exercise bouts, the production of lactate is essential to enable glycolysis, and therefore a high rate of ATP production, to continue even when muscle creatine phosphate concentrations become low (Robergs and Roberts, 2000:37).

When the rate of pyruvate production exceeds the rate of pyruvate entry into the mitochondria, pyruvate will be transformed to lactate (refer to figure 1). This condition has been termed the mass action effect. Therefore the production of lactate is not a damaging occurrence. Because pyruvate and lactate can be removed from the muscle for metabolism in other tissues, lactate should be seen as a substrate of metabolism (Robergs and Roberts, 2000:38).

2.1.3.2. Lactate and performance

Lactic acid [La^-] in excess of 99% dissociates into lactate anions and protons (H^+) at physiological pH. During exercise and muscle contractions, muscle and blood [La^-] and [H^+] (=HLa) can rise to very high levels. Most researchers have argued that any harmful effects of HLa on exercise performance are due to H^+ rather than La^-. According to Gladden et al. (2004:6-7) numerous researchers indicate that a decline in maximal muscle force generation is correlated with a decrease in muscle pH. Evidence from abundant experimental approaches suggests that an elevated muscle [H^+] could depress muscle function by the following:

- Reducing the transition of the cross-bridge from the low- to the high-force state;
- Inhibiting maximal shortening velocity;
- Inhibiting myofibrillar ATPase;
- Inhibiting glycolytic rate;
- Reducing cross-bridge formation by competitively inhibiting Ca^{2+} binding to troponin C, and
- Reducing Ca^{2+} re-uptake by inhibiting the sarcoplasmic ATPase activity (leading to subsequent reduction of Ca^{2+} release).

Particularly over the previous 10 years, the role of acidosis as an important cause of fatigue has been challenged. These studies have reported that the effect of increased [H^+] to reduce Ca^{2+} sensitivity, maximal tension, and shortening velocity in isolated muscle fibers *in vitro,* is absent when the experiments are performed at temperatures that are closer to those met physiologically. There is also a report that muscle acidity does not decrease muscle glycogenolysis/glycolysis during intense exercise in man. One study in isolated rat soleus muscles *in vitro* observed that, rather than diminishing force generation, lactic acidosis actually protected the muscle fiber against the detrimental effects of elevated external potassium concentration [K^+] on muscle excitability and force (Gladden et al., 2004:7).

2.1.3.3. Lactate and acidosis

Gladden et al. (2004:7-8) says in place of acidosis, studies on isolated muscle fibers are pointing towards P_i as a main cause of muscle fatigue. P_i increases during forceful muscle contractions or exercise due to breakdown of PCr. However, these studies have not evaluated the effects of high $[H^+]$ on peak power or the mutual effects of a reduced Ca^{2+} release, a low pH and an elevated P_i. Accordingly, it is noted that it is untimely to dismiss H^+ as an important factor in muscle fatigue. Further, at least two questions arise relating to the role of P_i as a primary fatigue agent during short-term intense exercise in intact humans. Firstly, since most of the PCr breakdown occurs within the first 10 s of such intense exercise, would the main role of P_i be restricted to that time frame? Secondly, can changes in P_i explain the decline in performance observed in humans following prior intense exercise with different muscle groups? Regardless of well over 150 years of active research, the exact causes of muscle fatigue remain elusive. (Gladden et al., 2004:8).

Over the years, La^- has been considered insignificant in the development of fatigue. However, in the 1990s, several studies raised the likelihood that La^- *per se* might play some role in the fatigue process. In isolated dog gastrocnemii *in situ*, perfusion with L-(+)-lactate reduced twitch contraction force by 15% even though muscle pH was not changed from control conditions. These results were subsequently sustained by studies on muscles *in vitro*, skinned muscle fibers and sarcoplasmic reticulum vesicles. In Langendorff perfused rat hearts, La^- appeared to irrevocably depress developed pressure. More recently, studies of skinned mammalian muscle fibers have reported negligible effects (5% or less) of La^- on muscle contractility. While these recent studies on isolated fibers suggest a negligible role for La^- in the fatigue process, further studies on more intact systems are needed (Gladden et al., 2004:8).

Gladden et al. (2004:9) also reports that what is now known as the cell-to-cell lactate shuttle was merely known as the lactate shuttle. Since its introduction, this

hypothesis has been repetitively supported by studies using a wide variety of experimental approaches. It poses that La^- formation and its consequent distribution throughout the body is a major mechanism whereby the coordination of intermediary metabolism in different tissues, and cells within those tissues, can be accomplished. The importance of La^- as a "carbohydrate fuel" source is emphasized by the fact that during moderate intensity exercise, blood La^- flux may exceed glucose flux. Because of its great mass and metabolic capacity, skeletal muscle is probably the major component of the lactate shuttle, not only in terms of La^- production but also in terms of net La^- uptake and utilization as well.

2.1.3.4. Lactate removal

From interstitial fluid of active muscles, La^- penetrates the plasma. During intense exercise, a system intended to co- transport La^- and H^+ from the plasma into the red blood cells (RBC) could aid in establishing a gradient between the plasma and interstitial fluid, and enhancing the available space for efflux of La^- and H^+ ions from the exercising muscles. Indeed, the transport of La^- across the RBC membrane proceeds by three distinctive pathways: (1) non-ionic diffusion of undissociated HLa, (2) an inorganic anion exchange system, often referred to as the Band 3 system, and (3) a monocarboxylate-specific carrier mechanism (MCT). MCT1 is the monocarboxylate transporter in RBC membranes and it is the main pathway of La^- transport. As blood circulates through the body to liver, heart, inactive and active skeletal muscles, and all tissues, the pathway is classically reversed with La^- exiting the plasma into the interstitial fluid and on into the various tissues down the $[La^-]$ gradient. As plasma $[La^-]$ reduces, La^- will leave the RBC. Several investigations have satisfactorily illustrated the role of plasma and RBC in picking up La^- from active muscles and delivering it to inactive muscles (Gladden et al., 2004:9).

Gladden et al. (2004:8) declares that at rest, muscles gradually release lactic acid into the blood on a net basis, although at times they may show a small net uptake. During exercise, mainly short-term, high-intensity exercise, muscles

produce La^- rapidly while La^- clearance is slowed. This results in an enlarged intramuscular [La^-] and an increased net output of La^- from muscles into the blood. Later, during recovery from short-term exercise, or even during continuous, prolonged exercise, there is net La^- uptake from the blood by resting muscles or by other muscles that are exercising at a low to moderate intensity. During prolonged exercise of low to moderate intensity, the muscles that initially released La^- on a net basis at the onset of the exercise may actually reverse to net La^- uptake. Particularly during moderate to high intensity exercise, glycolytic muscle fibers are expected to be producing and releasing La^-.

Gladden et al. (2004:9) also states that while some of the La^- escapes into the circulation, some of it may disperse to neighbouring oxidative muscle fibers which can take up the La^- and oxidize it. Clearly, La^- exchange is a dynamic process with concurrent muscle uptake and release at rest and during exercise. Most of the La^- taken up by muscles is removed through oxidation with the absolute rate depending on the metabolic rate of both exercising and resting muscles. Oxidative skeletal muscles that are contracting in a submaximal steady state condition are perfectly suited for La^- consumption. Since cardiac muscle is more highly oxidative than even the most oxidative skeletal muscle, it is not astonishing to find that that the heart is an active La^- consumer. Evidence from several dissimilar experimental approaches suggests that as blood [La^-], myocardial blood flow and myocardial $V'O_2$ increase, La^- becomes the preferred fuel for the heart, accounting for as much as 60% of the substrate utilized. Tracer studies indicate that fundamentally all of the La^- taken up by the heart is oxidized. Even the brain can take up La^- from the blood. Lately it has been shown that net La^- uptake by the brain during high intensity is even continued during a 30-min recovery period.

2.1.3.5. The use of lactate by muscle tissues

Numerous studies by Gladden and colleagues (Gladden 1991; Gladden *et al.* 1994; Gladden, 2000; Hammann et al., 2001; Kelley et al., 2002) have

demonstrated that isolated, blood-perfused oxidative skeletal muscle readily consumes exogenously infused La^- as a fuel. Recently, these findings have been established and extended in lactate clamp (LC) studies in humans. Researchers have examined subjects exercising at moderate exercise intensity (~55% $V'O_{2max}$) with La^- infusion to maintain [La^-] at ~4 mM. Overall, researchers found a noteworthy increase in La^- oxidation accompanied by a decrease in glucose oxidation; the interpretation is that La^- competes successfully with glucose as a carbohydrate fuel source, thus sparing blood glucose for use by other tissues. In addition, they found that although La^- served as a gluconeogenic substrate, the absolute rate of gluconeogenesis was unchanged by LC. In contrast, LC enlarged the absolute gluconeogenic rate during low intensity exercise, ~34% $V'O_{2max}$. At both low and moderate intensities, La^- was an imperative gluconeogenic precursor. These LC studies along with many other investigations of dissimilar types emphasize the role of La^- as arguably the most important substrate for gluconeogenesis. The obvious conclusion from numerous studies is that La^- is a useful metabolic intermediate that can be exchanged quickly among tissue compartments. The cell-to-cell lactate shuttle provides the fundamental framework for interpretation of La^- metabolism (Gladden et al., 2004:9).

2.1.4. Krebs cycle

Jeukendrup and Gleeson (2004b:38) states that the bulk of ATP used by many cells to maintain homeostasis is produced by the oxidation of pyruvate in the TCA cycle. During this oxidation process, reduced nicotinamide adenine dinucleotide (NADH) and reduced flavin adenine dinucleotide ($FADH_2$) are generated. The NADH and $FADH_2$ are principally used to drive the processes of oxidative phosphorylation, which are responsible for converting the reducing potential of NADH and $FADH_2$ to the high energy phosphate in ATP.

Pyruvate is also a substrate which undergoes oxidative metabolism so as to produce carbon dioxide and water. This process occurs in the mitochondrion of a muscle cell and the pyruvate which is formed in the sarcoplasm of the muscle

cell during glycolysis is transported to the inside of the mitochondrion by a monocarboxylic acid transporter situated in the inner membrane of the mitochondria. Pyruvate is then transformed by oxidative decarboxylation into a 2-carbon acetate group, which is linked to coenzyme A and finally forms Acetyl CoA (see figure 2). This reaction is catalyzed by pyruvate dehydrogenase. Acetyl CoA is also produced from the oxidation of specific amino acids as well as fats (Jeukendrup and Gleeson, 2004:38).

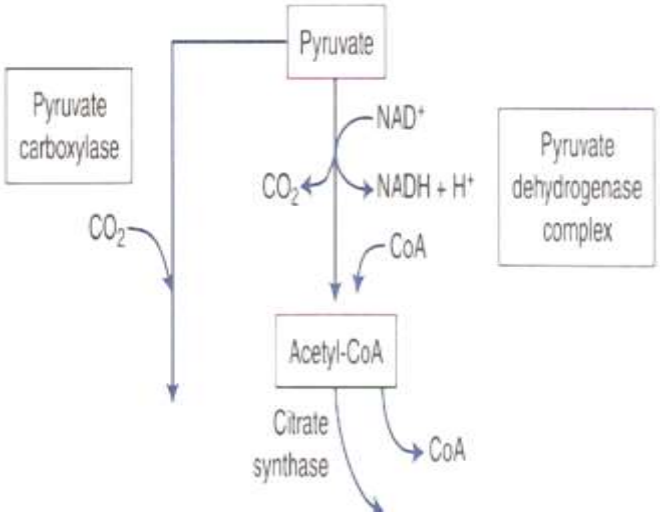


Figure 2–The transformation of pyruvate to Acetyl–CoA–After Jeukendrup and Gleeson (2004)

The fate of pyruvate depends on the cell energy charge. In cells or tissues with a high energy charge, pyruvate is directed toward gluconeogenesis, but when the energy charge is low pyruvate is preferentially oxidized to CO_2 and H_2O in the TCA cycle, with generation of 15 equivalents of ATP per pyruvate (Jeukendrup and Gleeson, 2004:38).

The enzymatic activities of the TCA cycle (and of oxidative phosphorylation) are located in the mitochondrion. When transported into the mitochondrion, pyruvate encounters two principal metabolizing enzymes: pyruvate carboxylase (a gluconeogenic enzyme) and pyruvate dehydrogenase (PDH), the first enzyme of

the PDH complex. With a high cell-energy charge, coenzyme A (CoA) is highly acylated, principally as Acetyl-CoA, and allosterically enables the activation of pyruvate carboxylase, directing pyruvate toward gluconeogenesis. When the energy charge is low, CoA is not acylated, pyruvate carboxylase is inactive, and pyruvate is preferentially metabolized via the PDH complex and the enzymes of the TCA cycle to CO_2 and H_2O. Reduced NADH and $FADH_2$ generated during the oxidative reactions can then be used to drive ATP synthesis via oxidative phosphorylation. (Jeukendrup and Gleeson, 2004:38).

Although the Krebs cycle is commonly regarded as a cycle, it is important to notice that it is imperfect. Many substances can leave and penetrate the Krebs cycle at various levels.

Brooks et al. (2000:98) says that the function of pyruvate dehydrogenase (PDH) in the Krebs cycle is the formation of carbon dioxide, ATP production and NADH production. In the Krebs cycle there are 4 places where NAD^+ is reduced to NADH and one place where FAD^+ is reduced to FADH and where ATP is produced. Each NADH is equal to 3 ATP molecules and each FADH is equal to 2 ATP molecules. Table 1 indicates the ATP tally from the catabolism of 1 molecule of glucose.

Table 1–ATP tally from the catabolism of 1 molecule of glucose

Metabolic Process	High Energy Products	ATP from Oxidative Phosphorylation	ATP Subtotal
Glycolysis	2 ATP		2 (anaerobic)
	2 NADH	6	8 (aerobic)
Pyruvate to Acetyl-CoA	2 NADH	6	14
Krebs Cycle	2 GTP	2	16
	6 NADH	18	34
	2 FADH	4	38
Grand Total			**38**

The purpose of the Krebs cycle is thus to enable metabolism of pyruvate from glucose to continue, as well as the intermediate products of fat and protein metabolism, and to trap part of the energy released in the forms of ATP and high energy reduced compounds NADH and FADH (refer to figure 3). Furthermore, high ATP/ADP, Acetyl CoA/CoA and $NADH/NAD^+$ act to decrease glycolytic flux to the Krebs cycle by the inactivation of PDH. On the contrary, dephosphorylation by a certain phosphatase activates the enzyme. Dephosphorylation is encouraged by high levels of pyruvate and calcium as well as by reductions which are present in ATP/ADP, Acetyl CoA/CoA, and $NADH/NAD^+$ (Brooks et al., 2000:98).

Acetyl CoA is the substrate which enters the Krebs cycle. Acetyl CoA can be produced from pyruvate/amino acids/fatty acids. Acetyl CoA joins with oxaloacetic acid by means of the enzyme citrate synthase to form citric acid. The existence of oxaloacetic acid may be an adaptable factor which controls the rate of the Krebs cycle (Brooks et al., 2000:101).

The rate limiting enzymes of the Krebs cycle is known as isocitrate dehydrogenase (IDH) and citrate synthetase to a lesser extent. Like PDH, IDH is an allosteric enzyme which is stimulated by ADP. IDH and any other dehydrogenases in the Krebs cycle are responsive to the redox potential of the cell. The redox potential refers to the $NADH/NAD^+$ ratio. The dehydrogenases are inhibited by a high redox potential and stimulated by a reduction in the redox potential (Brooks et al., 2000:101–102).

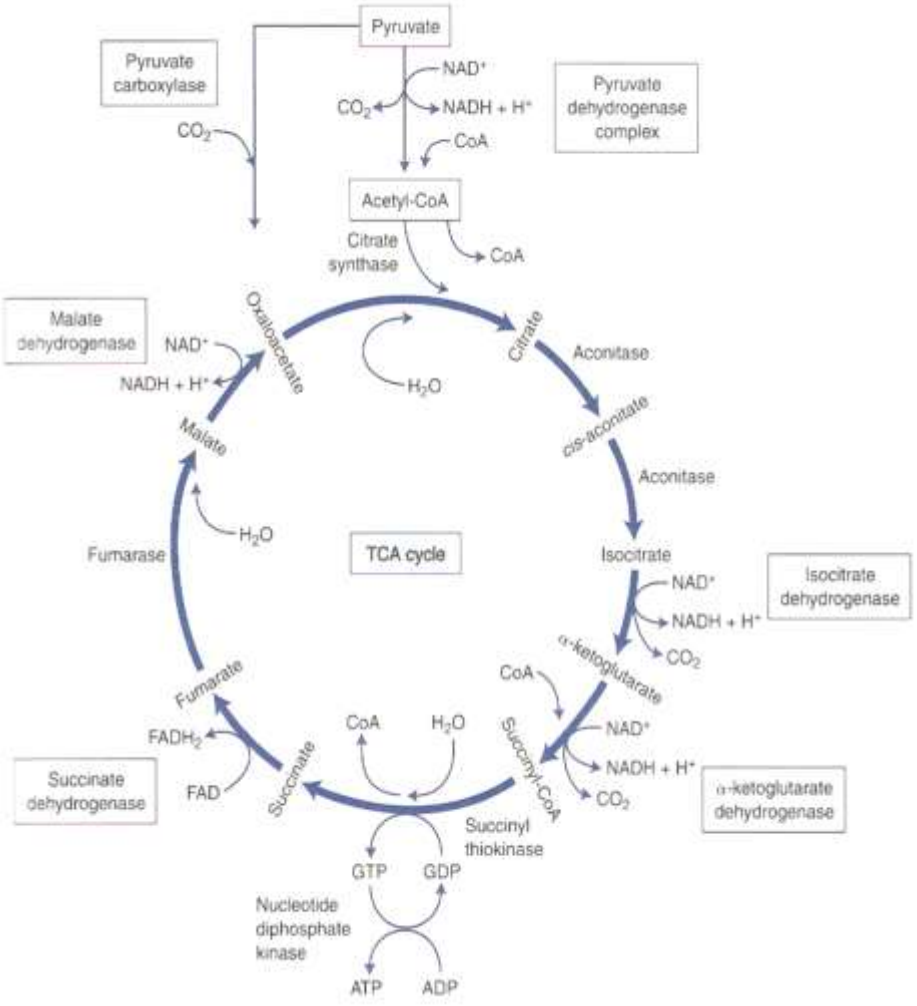


Figure 3–The reactions of the Krebs cycle showing sites of substrate level phosphorylation and NAD and FAD reduction–After Jeukendrup and Gleeson (2004).

2.1.4.1. Electron Transport Chain and oxidative phosphorylation

The key function of the ETC is the regeneration of ATP by means of ADP phosphorylation. If oxygen supply is satisfactory and substrate is available, NAD^+ and FAD will be continuously regenerated and thus the Krebs cycle can continue to function. This process is an aerobic process and is termed oxidative phosphorylation. For each molecule of NADH that enters the ETC, 3 molecules of ATP are produced (P:O=3) and for each molecule of $FADH_2$ that enters the

ETC, 2 molecules of ATP are generated (P:O=2) (Jeukendrup and Gleeson, 2004:40).

2.1.5. Fat catabolism

Fats and carbohydrates are the major substrates for energy supply during muscular contraction. Since Acetyl-CoA is also a product of fat oxidation, the sequence of reactions beyond the Acetyl–CoA level, involving the Krebs cycle and oxidative phosphorylation is alike for carbohydrates and for fats (Jeukendrup and Gleeson, 2004:41).

2.1.5.1. The utilization of lipids during exercise:

In order to achieve the end goal i.e. to gain access to and activate a 2-carbon skeleton acetyl group from fat, a series of reactions must first take place. The process of lipid metabolism during exercise can be summarized as follows:

a) Mobilization–the breakdown of adipose and intramuscular triglyceride by a process called lipolysis.
b) Circulation–the transport of free fatty acids from adipose to muscle.
c) Uptake–the entry of free fatty acids into muscles from blood.
d) Activation–raising the energy level of fatty acids preparatory to catabolism.
e) Translocation–the entry of activated fatty acids into mitochondria.
f) Beta Oxidation–the production of acetyl–CoA from activated fatty acids and the production of reducing equivalents (NADH and FADH).

a. Mobilization from Adipose:

The first step initiates the breakdown of stored fat in the form of triacylglycerol into fatty acids and glycerol. This process is labeled lipolysis and essentially begins with the hydrolytic removal of a fatty acid molecule from the glycerol backbone of the triacylglycerol molecule. This step is catalyzed by hormone–

sensitive lipase (Jeukendrup and Gleeson, 2004:41–42) and is activated or suppressed by hormonal activity as indicated in figure 4.

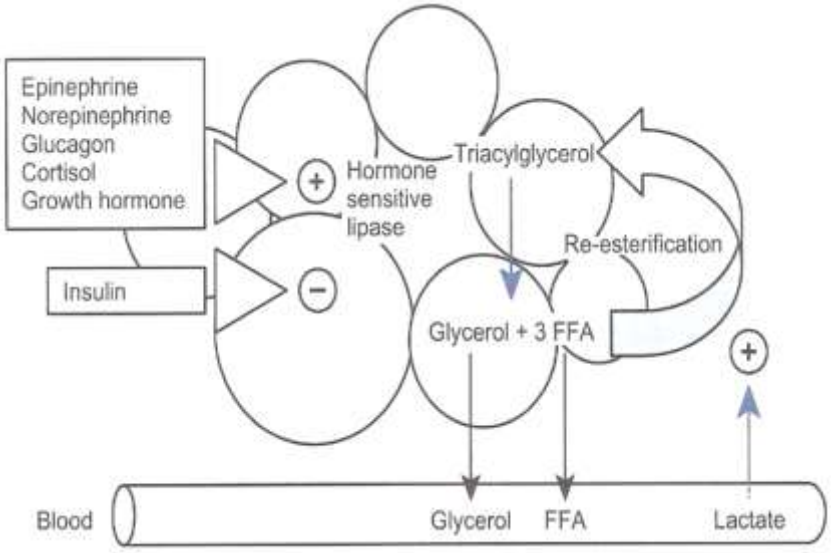


Figure 4–Lipolysis in adipose tissue mobilizes FFAs – After Jeukendrup and Gleeson (2004b).

In addition to the hormone sensitive lipase (HSL) in adipose tissue, adipocytes enclose a second lipase enzyme called lipoprotein lipase (LPL). The control and action of these two lipases are fundamentally reversed. While the adipose capillary wall LPL is stimulated by insulin and glucose and promotes fat storage, the HSL stimulates fat breakdown, is inhibited by insulin and is stimulated by other hormones, including the catecholamines (epinephrine and norepinephrine) and growth hormone (Jeukendrup and Gleeson, 2004:42).

A specific lipase removes another fatty acid from diacylglycerol and an additional lipase is accountable for the removal of the last fatty acid from the monoacylglycerol carbon skeleton. Thus, from each molecule of triacylglycerol, three fatty acids and one molecule of glycerol is created. Glycerol disperses into the circulation and fatty acids are transported via albumin in the circulation. Entry of fatty acids and glycerol into the circulation is narrowed down by two factors:

the rate of lipolysis as well as adipose tissue blood flow. During exercise at an intensity of 50% $V'O_{2max}$, blood flow to adipose tissue escalates, but during high intensity exercise, sympathetic vasoconstriction causes a fall in blood flow through adipose tissue. This results in the accumulation of fatty acids inside adipose tissue and thus less fatty acids and glycerol enter the circulation to act as an energy source (Jeukendrup and Gleeson, 2004:42).

Another factor that limits the mobilization of fatty acids during high intensity exercise is an increase in the blood lactate levels [La^-]. Lactate promotes the re-esterification of fatty acids back to triacylglycerol and thus limits the entry of fatty acids into the bloodstream. The glycerol in the blood can be converted by the liver to yield glycerol-3-phosphate which can be used to form triacylglycerol ultimately. In addition to this route, glycerol can be oxidized to dihydroxyacetone phosphate which enters the glycolytic pathway or can be transformed to glucose in liver tissue (Jeukendrup and Gleeson, 2004:42)–see figure 5.

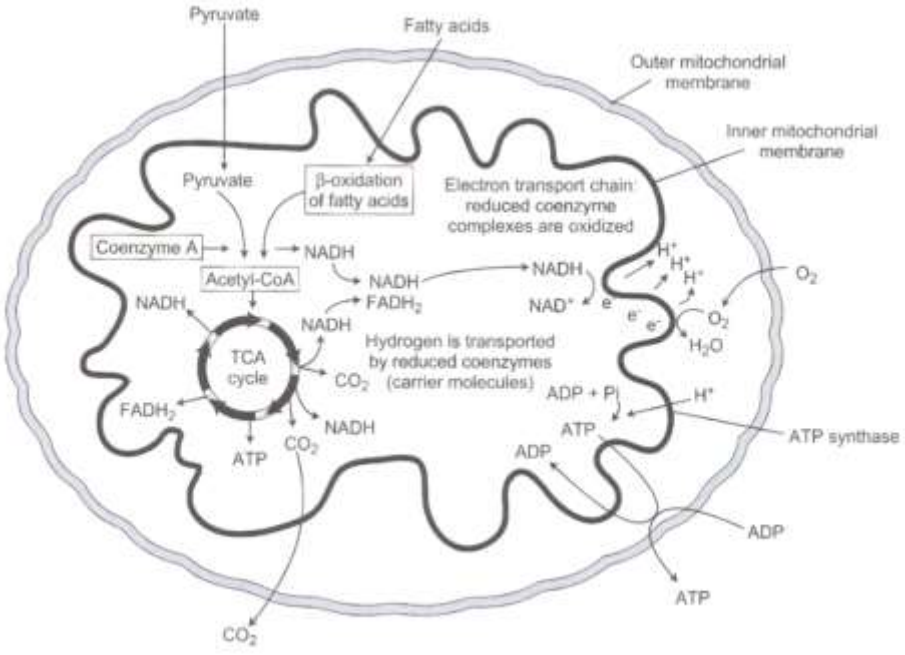


Figure 5–The Krebs cycle generates the reduced coenzymes NADH and $FADH_2$–After Jeukendrup and Gleeson (2004).

The activity of HSL is completely controlled by the presence of cyclic AMP (cAMP), which in turn is regulated by the adenylate cyclase system. Thus the onset of fat breakdown is much like the initiation of glucose breakdown. Two activators of the HSL system (epinephrine and growth hormone) access adipose tissue via circulation, whereas norepinephrine is released locally by sympathetic nerve endings within the adipose tissue. Compared to the release of growth hormone, which is slow, the release of the catecholamines is rather rapid. The catecholamines therefore trigger lipolysis at the onset of exercise. In contrast to the actions of the catecholamines, it takes 10–15 minutes for blood levels of growth hormone to increase during intense exercise in order to sustain lipolysis during prolonged exercise (Brooks et al., 2000:124).

b. Circulation and Uptake:

Due to the fact that fatty acids are insoluble in aqueous media, they require active transportation by means of a carrier molecule (albumin) in the blood. (Brooks et al., 2000:125).

Because the uptake of FFAs in lipid delivery to the active musculature depends to a large degree on the arterial fatty acid content, the rate of adipose tissue lipolysis directly affects FFA uptake by the muscle. Thus a key to lipid oxidation during exercise is the arterial concentration of FFAs. Any factor (e.g. caffeine) that stimulates adipose lipolysis and raises blood FFA levels could enhance exercise endurance (Brooks et al., 2000:125).

The uptake of fatty acids into muscle released from blood albumin is made possible via a specific receptor site on the sarcolemma. This receptor is known as sarcolemmal fatty acid binding protein (S-FABP). The S-FABP is one of a family of fatty acid binding proteins (FABP) that exist throughout the muscle cell matrix. Together these muscle cell FABPs function to move fatty acids throughout the cell. As a result of there being an inconsistent number of receptor binding sites in sarcolemmal membranes, the entry of fatty acids from blood into

muscle cells is more rapid in the heart than in red or to an even lesser extent white skeletal muscle fibers. Nevertheless, red muscle has more FABPs than white and endurance training increases the number of S-FABPs (Brooks et al., 2000:125).

c. Activation and Translocation:

The activation lifts the fatty acids to a higher energy level and requires one molecule of ATP. The process however, diverges from the activation in glycolysis in that the fatty acid is attached to coenzyme A, with the formation of a CoA derivative, termed fatty acyl CoA-see figure 6.

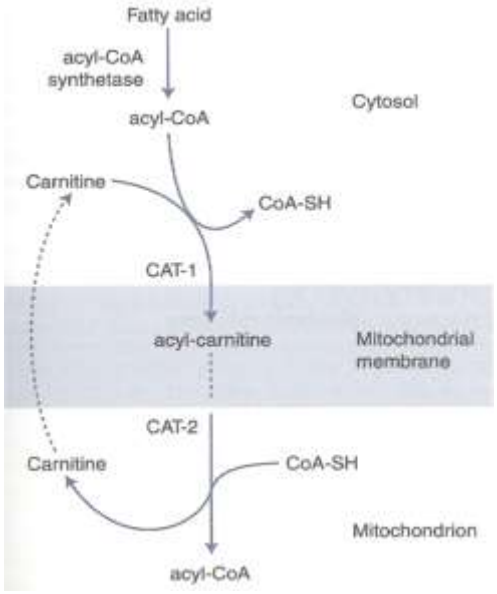


Figure 6–Activation and translocation of fatty acids–After Jeukendrup and Gleeson, 2004).

Fatty acyl-CoA is found in the inner mitochondrial membrane. Nevertheless, the site of fatty acid oxidation is the mitochondrial matrix. Like a wide diversity of substances, activated fatty acids gain entry into or exit from mitochondria by a transport mechanism. For fatty acids, the mechanism engages a carrier, carnitine, and the carnitine acyl - transferase enzymes. This mechanism involves the stripping off of CoA and its return to the cytosol as well as the reception of the

fatty acid by carnitine, with the formation of fatty-acyl carnitine. The procedure is catalyzed by a family of enzymes collectively called CAT1. The fatty – acyl complex is liberated to move across the mitochondrial membrane, where on the inner side, CAT 2 catalyzes the reverse reaction, leaving carnitine within the membrane and releasing fatty acyl-CoA into the mitochondrial matrix (Brooks et al., 2000:125-127).

d. Beta Oxidation (ß-oxidation)

The ß-oxidation cycle, located in the mitochondrial matrix, serves numerous purposes. First it degrades the fatty acyl-CoA to Acetyl-CoA by slivering the carbon atoms two at a time. Beginning from the carboxyl end of a fatty acid, the first carbon is the α-carbon and the second carbon is the β-carbon. In the β-oxidation pathway, cleavage occurs after the β carbons. The Acetyl-CoA that is formed as a result of ß-oxidation can then enter the Krebs cycle, each Acetyl-CoA residue resulting in the formation of 12 ATP molecules. Note that the subsequent breakdown of the remaining fatty acid skeleton does not require an additional molecule of ATP for the process to proceed. These views are illustrated graphically in figure 7.

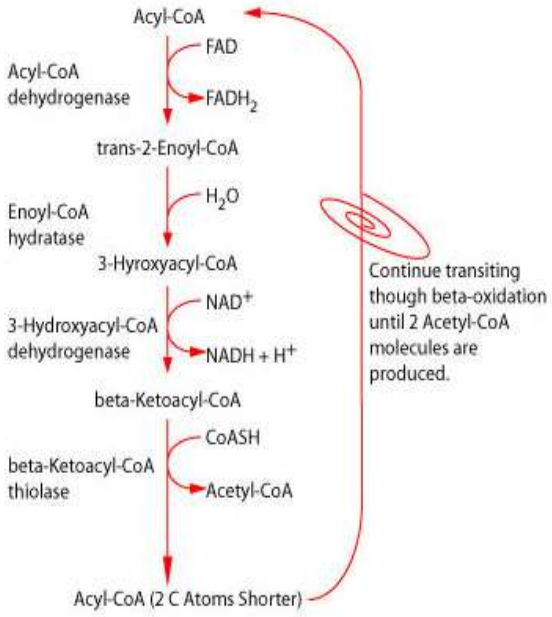


Figure 7–The process of ß-oxidation-After Gillespie (www.reactome.org) (2003)

The β-oxidation pathway is also regulated by mitochondrial redox. Reduction (i.e. high NADH / NAD^+) inhibits the dehydrogenases, while oxidation activates them. The second function of the β-oxidation pathway is to generate high energy reducing equivalents NADH and FADH. For each cycle of the β-oxidation pathway, one each of NADH and FADH is produced-the net tally in ATP production being 5 molecules of ATP (Brooks et al., 2000:129).

e. The regulation of lipid metabolism during exercise

At rest and during sub-maximal exercise skeletal muscle is the major site of oxidation of fatty acids. After an overnight fast most of the energy requirement at rest is covered by the oxidation of fatty acids resultant from adipose tissue with carbohydrate only making a relatively small contribution (glucose is utilized mainly by the brain). During low intensity exercise, the energy requirement is increased several fold above resting conditions and both carbohydrate and fat oxidation increases but not to the same extent: Fat is utilized to a greater extent compared to carbohydrate reaching the fatty acid threshold at an exercise intensity of ±65% $V'O_{2max}$, after which a decline in the rate of fat oxidation is observed and is accompanied by an exponential increase in carbohydrate oxidation with increasing exercise intensities (Jeukendrup, 2003:1270)-see figure 8 and 9.

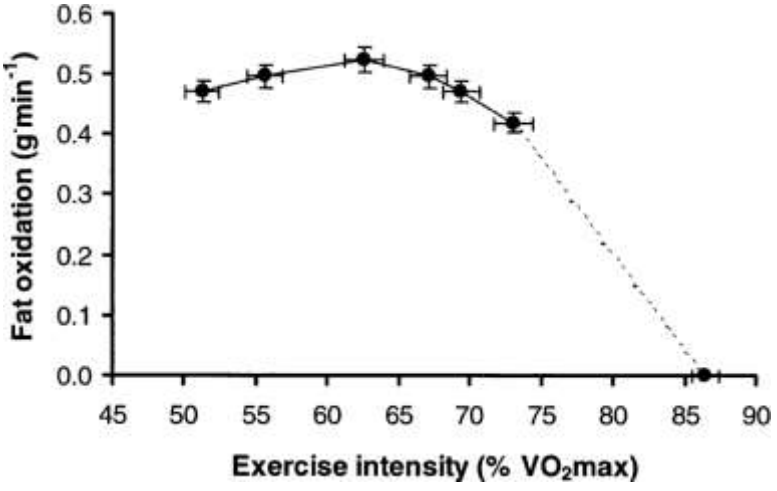


Figure 8–Regulation of lipid metabolism–After Jeukendrup and Gleeson (2004).

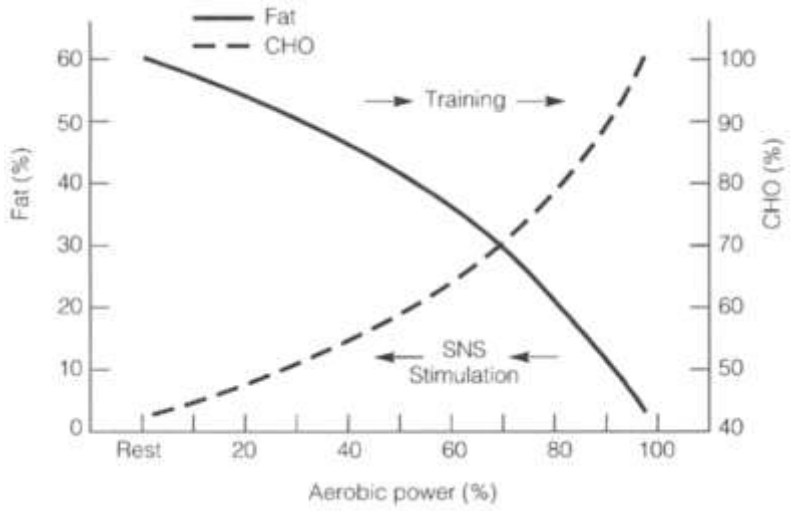


Figure 9–The crossover concept–After Brooks et al. (2000).

The factors that up-regulate fat metabolism in the conversion to moderate intensity exercise and the factors that result in a down regulation of fat metabolism at higher intensities are incompletely understood. Lipolysis in adipose tissue is generally dependent on β-adrenergic stimulation and the endocrine environment. When exercise is started, adrenaline concentrations increase and insulin concentrations decrease. As an outcome the rate of lipolysis and adipose tissue blood flow increase, and more fatty acids are released from the adipose tissue. During moderate intensity exercise, lipolysis increases roughly 3 fold, mainly because of an increased β-adrenergic stimulation. Additionally, during moderate intensity exercise the blood flow to adipose tissue is doubled and the rate of re-esterification is halved. Also blood flow to skeletal muscle is increased radically and therefore the delivery of fatty acids to the muscle is increased several fold. During the first 15 minutes of exercise, plasma fatty acid concentrations generally decrease because the rate of fatty acid uptake by the muscle exceeds the rate of fatty acid delivery by means of lipolysis. Thereafter, the rate of appearance is in surplus of the utilization by muscle, and plasma fatty acid concentrations increase. Fatty acids are also mobilized from intramuscular triglycerides (IMTG) and there is accrued evidence that well trained

individuals have larger IMTG stores and oxidize more fatty acids from IMTG than untrained controls (Jeukendrup, 2003:1270-1271).

The duration of exercise also influences substrate oxidation. Fat oxidation increases and carbohydrate oxidation decreases as the exercise duration increases. This increased fat oxidation is prone to be caused by a reduction in muscle glycogen stores towards the latter stages of prolonged exercise (Jeukendrup, 2003:1270-1271).

2.2. THE INFLUENCE OF NUTRIENT INTAKE ON METABOLISM

It is the goal of this study to explore the effect that nutrient intake prior to incremental treadmill running has on metabolism. Five interventions where current scientific information will compel sport nutritionists and physiologists to embrace new information about the pre–competition meal are identified. These are: (1) fasting; (2) carbohydrate; (3) fat as medium chain triglycerides (MCT); (4) caffeine; and (5) the combination of fat and caffeine.

It is also the function of this literature survey to provide trained and untrained individuals with information pertaining to the effect that nutrient intake prior to exercise can have on metabolism, performance and well-being.

Aragon-Vargas (1993) claims that scientists have been interested for many years in the combined effect of fasting and exercise performance. This intervention has served to provide information on the utilization of fuels for exercise, the use of intramuscular triglycerides and the changes that occur during exercise in terms of muscle and liver glycogen stores (Aragon-Vargas, 1993:255). The fasting results will provide us with “baseline” values for each trained/untrained individual. From these results one can see which nutritional intervention manipulated the metabolism. In addition to this it would be interesting to see whether athletes with

white muscle fibers versus athletes with red muscle fibers react the same to exercising in a fasted condition followed by the comparison between athletes and untrained individuals.

Over the past 25 years, research has accentuated the role of carbohydrates in endurance exercise and the advice that the ingestion of a high carbohydrate diet prior to exercise and the continual carbohydrate supplementation during exercise has been accepted by various athletes (Hawley and Hopkins, 1995:245). The goal is to exploit this generalized recommendation where all athletes are urged to consume carbohydrates prior to exercise. The question is if these recommendations are true for all athletes.

A nutritional strategy that might improve the training adaptation, presumably by allowing athletes to train for longer, would be to utilize an alternative source to carbohydrate and/or to slow its normal rate of utilization during exercise. Such a fuel is fat (Hawley et al., 2006:713). The type of fat which would be used in this study is medium chain triglycerides in the form of olive oil. MCTs empty quickly from the stomach and directly enter the systemic circulation through the portal vein. Unlike LCTs they are less dependent on CATI to enter the inner mitochondrial membrane. These physical properties have lead to the suggestion that MCTs could be an important source of energy for contracting skeletal muscle during submaximal exercise (Hawley, 2002:1486). The question posed which asks if all athletes respond accordingly when ingesting fat and if fat ingestion augments fat oxidation could in the long run supply information to fight against lifestyle diseases such as obesity and diabetes.

Caffeine has been reported to enhance exercise performance by enhancing fat oxidation and therefore sparing glycogen (Hadjicharalambous et al., 2006:876). Many studies which have been done in the past suggested that the primary mechanism related to performance improvements associated with caffeine ingestion relates to the influence it has on lipid substrate availability and

utilization. One should take into account that this is not the only factor that contributes towards the actions of caffeine in the human body during exercise (Bridge and Jones, 2006:437). Additional findings indicate that caffeine increases high intensity exercise performance by additional means other than caffeine's effect on fat availability and utilization. This might be due to the fact that caffeine directly affects skeletal muscle and/or influences the transmission of neural signals in regions between the brain and neuromuscular junction. The contradictions reported in the literature may be due to the numerous sites of action of caffeine, within both the central nervous system and peripheral tissues. Therefore an alternative approach, which attempts to distinguish between primary and secondary effects of caffeine, is necessary (Hadjicharalambous et al., 2006:876).

The ingestion of a combination of fat and caffeine is a new research area. Only one study, done by Hadjicharalambous et al. (2006), explored the influence of caffeine on perception of effort, metabolism and exercise performance following a high fat meal. No other articles supporting this area of research have been found. The reasoning behind this intervention is: since both fat and caffeine have the capability to enhance fat oxidation, it stands to reason that the combination must have an enhanced effect on fat oxidation and the subsequent sparing of muscle glycogen stores. It also stands to reason that caffeine increases high intensity exercise, which might indicate that the combination of fat and caffeine can also favour increased carbohydrate oxidation.

2.2.1. The influence of fat intake on metabolism:

The following information pertains to the effect that fat intake prior to exercise will have on metabolism during subsequent exercise.

2.2.1.1. Functions of fat

Fatty acids are engaged in a variety of different physiological functions, such as energy production, lipid biosynthesis, protein modification, regulation of

transcription, and intracellular signaling. In addition to this, fatty acids have been concerned with pathological conditions such as insulin resistance, atherosclerosis and obesity (Vassilis et al., 2003:476). Even though they are normally treated as one entity, in recent years it has become clear that different types of fatty acids exhibit distinct functions. They have opposing effects on liver lipoprotein metabolism and on glucose transport into skeletal muscles. Due to this fact, one can assume that any changes in the plasma fatty acid profile will affect the metabolism of various tissues by modifying the composition of the mixture of fatty acids delivered to these tissues (Vassilis et al., 2003:476).

2.2.1.2. Fat as a nutritional intervention:

A number of interventions have been used to increase fatty acid availability before/during exercise including fasting, caffeine ingestion, L-carnitine supplementation, ingestion of medium chain triglyceride solutions, ingestion of long chain triglyceride (LCT) solutions and infusion of intralipid emulsions. Although fasting increases the availability of plasma free fatty acids and rates of fatty acid oxidation during low to moderate intensity exercise, such an intervention does not have a positive effect of exercise capacity, largely due to a reduction in endogenous glycogen stores (Hawley, 2002:1485-1486). Studies have demonstrated that increased FFA availability, classically associated with fasting or a high fat diet resulted in weakened muscle glucose uptake and a decreased glycolytic flux are possibly due to the inhibition of phosphofructokinase (Lambert et al., 1997:318). It has been proposed that the capability to uphold exercise may be extended if the provision of fats is immediately increased before exercise, as the FFA oxidation rate is directly correlated to their serum concentration level (Ferreira et al., 2003:422).

A nutritional strategy that might improve the training adaptation, presumably by allowing athletes to train for longer, would be to utilize an alternative source to carbohydrate and/or to slow its normal rate of utilization during exercise. Such a fuel is fat, and there has been current interest in the effects of both acute and

chronic fat supplementation on metabolism and exercise performance. Of interest here is whether such dietary modification can augment the adaptive response to training. Certainly when well trained individuals use a high fat/low carbohydrate diet for 5–7 days, there is a rapid and marked capacity for these changes in macronutrient availability to modulate the expression of mRNA–encoding proteins that are necessary for fatty acid transport and oxidative metabolism. Accompanying these changes are hefty shifts in substrate metabolism in favour of fat, and a sparing of muscle glycogen. Even when carbohydrate availability is increased following “fat adaptation”, by the restoration of muscle glycogen stores and provision of exogenous carbohydrate during exercise, the enhanced capacity for muscle fat oxidation continues (Hawley et al., 2006:713–714).

2.2.1.3. Fat storage

Fats and carbohydrates are regarded as the main substrates that fuel aerobic ATP production in skeletal muscle. The majority of endogenous fat is stored as triacylglycerol in subcutaneous and deep visceral adipose tissue. Lesser quantities of triacylglycerol are stored as lipid droplets inside muscle fibers. The possible role of intramyocellular triacylglycerol (IMTG) as a source of energy during exercise in humans has recently recaptured much of its interest (Van Loon, 2004:1170). Some fats are also present in the circulation as free fatty acids which are bound to albumin (Van Loon, 2004:1171).

An electron microscope analysis of skeletal muscle tissue has revealed that intramyocellular lipid droplets are bordering muscle mitochondria, which means that IMTG can be regarded as an available source of energy during oxidative energy release. Several studies have discovered that there is threefold greater lipid content in type I muscle fibers than in type II muscle fibers. This means that the greater fat oxidative capacity in type I fibers is also connected to greater IMTG storage. In addition to this, the presence of hormone sensitive lipase in skeletal muscle tissue and its accounted activation by means of catecholamines

and muscle contraction strongly suggest that the IMTG pool will be able to function as a dynamic fuel during exercise. This argument is further sustained by the observation that hormone sensitive lipase content compares with IMTG content and oxidative capacity of various muscle fiber types (Van Loon, 2004:1172).

2.2.1.4. Fat utilization:

Fat does have some characteristics that will benefit an athlete. Fat is a very energy–dense fuel and yields much more energy per molecule than carbohydrates. They are also stored in much greater quantities than carbohydrates in the body. Due to these reasons fat can provide an extensive amount of fuel for oxidative phosphorylation during extended exercise at a low to moderate intensity (Spriet, 2002:1477). The total amount of energy which is available as triacylglycerol is more than 60 times the amount which are stored as glycogen. (Horowitz and Klein, 2000:559).

2.2.1.5. Factors affecting fat oxidation

The incapacity to oxidize lipids appears to be an important factor in the etiology of obesity. A study exploring Pima Indians has shown that an elevated 24 hour respiratory quotient, indicative of reduced levels of fat oxidation, is associated with a high rate of weight gain. Obesity is a condition connected with enhanced intramuscular triglycerides and insulin resistance, in which resting levels of fat oxidation is disturbed. This defect endures after weight loss and may predispose to weight regain. A better understanding of factors influencing fat oxidation is important for the development of interventions allowing efficient treatment of conditions in which fat oxidation is disturbed (Venables et al., 2004:161). Interventions aimed at improving the metabolism of fat could potentially reduce the symptoms of metabolic diseases such as obesity and type 2 diabetes and may have incredible clinical significance. In order to reach this objective an understanding of the factors that enhance or reduce fat oxidation is vital.

Exercise duration and intensity are very important regulators of fat metabolism (Achten and Jeukendrup, 2003:1021).

1. Exercise intensity

Fat oxidation is maximal at low to moderate intensity exercise but as the intensity of exercises increases too much, less reliance on fat metabolism is evident and more reliance on carbohydrate metabolism becomes clear. Maximal rates of fat oxidation have been noted in trained individuals at around 59-64% V'O_{2max}, whilst in untrained individuals, maximal fat oxidation occurs around 47-52% V'O_{2max}. The ingestion of carbohydrates prior to exercise training significantly reduces the rate of fat oxidation compared to fasting conditions, whereas fasting for longer than 6 hours maximizes fat oxidation (Achten and Jeukendrup, 2004a:716). The ingestion of carbohydrates prior to exercise in a graded exercise test has decreased maximal fat oxidation by 28% (Achten and Jeukendrup, 2003:1021). During low intensity exercise, lipids supply somewhat more than half of the energy, whereas, as exercise intensity increases, the relative contribution from lipids diminishes and that from carbohydrate increases This crossover point occurs between 48 and 53% of the V'O_{2max}, consistent with the 50% V'O_{2max} recommended by Brooks and Mercier (1994:2253). Furthermore, there is a rising body of evidence to recommend that it is indeed an increase in carbohydrate metabolism that regulates fat metabolism because studies have confirmed that an increase in glycolytic flux through increases in the exercise intensity or by inducing hyperglycemia and hyperinsulinemia will reduce long chain fatty acid oxidation (Venables et al., 2004:167).

Factors such as exercise intensity, exercise duration, diet, training status, gender, age, obesity and type 2 diabetes all affect IMTG usage during exercise. Generally, plasma free fatty acid oxidation offers the greater part of the energy needs during exercise of low intensity (<30% V'O_{2max}), with very little usage of intramuscular and lipoprotein derived triglycerides. During exercise of moderate

intensity (40-65% V'O_{2max}), fat oxidation reaches maximal rates and then normally provides between 40 and 60% of total energy expended. About 50–70% of the fat oxidized is normally derived from plasma free fatty acids, leaving muscle and or lipoprotein derived triglyceride to supply the other part of total fat oxidation. When the intensity of exercise is further increased to about 70–90% V'O_{2max}, total fat oxidation decreases (Van Loon, 2004:1183)-see figure 10.

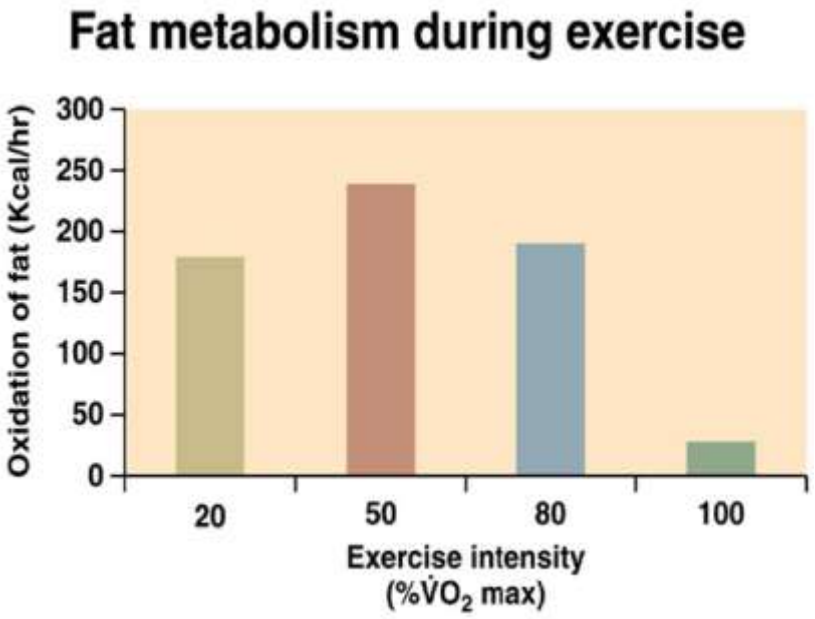


Figure 10–The exercise intensity (% V'O_{2max}) at which maximal fat oxidation occurs–After Young (2004)

One of the most significant regulators of substrate oxidation is exercise intensity, because it has been established that increases in glycolytic flux will inhibit long chain fatty acid transport into the mitochondria and therefore diminish long chain fatty acid oxidation. It has been recommended that the reduction in long chain fatty acid transport into the mitochondria could be an end result of the reduced pH brought about through an accumulation of H^+ during high intensity exercise, because a diminution in pH has been demonstrated to restrain the activity of carnitine palmitoyl transferase I (CPTI), a key enzyme in fatty acid transport. Such increases in glycolytic flux will also have the effect of escalating the

production of pyruvate and ultimately an increase in lactate accumulation. It has been shown in dogs and humans that lactate can openly inhibit adipose tissue free fatty acid release. More recently it has been demonstrated that in healthy trained men, the onset of plasma lactate accumulation arises at the same exercise intensity as for maximal fat oxidation (Venables et al., 2004:160; Achten and Jeukendrup, 2004b: 32).

2. Gender differences

When fat oxidation was scaled for free fat mass (FFM), researchers established the results of prior gender studies in which it was confirmed that the contribution of lipids to oxidative metabolism is superior in women than in men. In addition, they have exposed that women utilize both a higher absolute rate of lipids and have an enhanced relative contribution to total energy expenditure from lipids than men over a wide range of exercise intensities. Men also display a crossover point at lower exercise intensities compared to comparable data than the women, implicating earlier dependency on carbohydrate utilization during exercise. It has been demonstrated in both longitudinal and cross-sectional training studies that trained individuals use more fat at the same relative (higher absolute) exercise intensity than untrained individuals. Exercise training has also been exposed to be an efficient means of increasing fat oxidation during exercise in obese men and women and that the intensity found to induce these changes was low (40% $V'O_{2max}$). In their study it has been found that maximal fat oxidation rates are found at relative low intensities (44.9±0.9% $V'O_{2max}$) in men compared to women (51.9±1.0% $V'O_{2max}$) (Venables et al., 2004:167).

3. Effect of carbohydrate ingestion on fat metabolism

It has been reported that lipolysis was suppressed by 60% at rest after an increase in insulin levels occurred following carbohydrate ingestion. This suppression in conjunction with the high rate of triglyceride reesterification at rest emerges to account for the observation that small elevations in the concentration of plasma insulin of resting individuals reduced the rate of free fatty acid

appearance in blood, free fatty acid plasma concentration as well as for the rate of free fatty acid disappearance from plasma. This means that at rest, a reduction in the mobilization of free fatty acids from adipose tissue, after a fairly small increase in the concentration of plasma insulin, may in fact limit fat oxidation. By reducing lipolysis and fat oxidation on a daily basis, by ingesting carbohydrates prior to exercise, will increase the probability of being in a positive lipid balance and this might in fact increase fat deposition. Due to the sensitivity of lipolysis to even minute increases in insulin, it appears that in order to maintain high rates of fat oxidation at rest as well as during exercise, people should refrain from eating even small amounts of carbohydrates prior to exercise (Horowitz et al., 1997:773).

4. Interactions of IMTG and FFA originating from adipocytes

IMTG is regarded as a very important source of energy during moderate intensity exercise. IMTG is hydrolyzed by a rate limiting enzyme called hormone sensitive lipase which is controlled by both local and hormonal regulators. Hormone sensitive lipase is phosphorylated and activated by protein kinase A (PKA) in adipose tissue. PKA activity is increased by its response to epinephrine via the c-AMP dependent pathway and its activity is also reduced by insulin via increased phosphodiesterase activity. Thus in adipose tissue, plasma epinephrine amplifies HSL activity and subsequent adipose tissue lipolysis, whereas insulin diminishes HSL activity and subsequent lipolysis (Watt et al., 2004:145; Johnson et al., 2004:155).

Very few studies have investigated the regulation of HSL activity in human skeletal muscle. Watt et al. (2004) found that HSL activity is increased at the onset of exercise and is mostly dependent on exercise intensity. The data from their study indicated that the large elevation in plasma insulin and relatively small increase in plasma epinephrine were sufficient to inhibit the normal exercise–induced increase in HSL activity (Watt et al., 2004:145).

5. MCTs versus LCTs and their effect on fat metabolism

Medium chain triglycerides include fatty acids with a chain length of C6–10 and because of their relatively small molecular size they are thus more soluble than long chain triglycerides. MCTs empty quickly from the stomach and directly enter the systemic circulation through the portal vein. Unlike LCTs they are less dependent on CATI to intersect the outer mitochondrial membrane. These physical properties have lead to the suggestion that MCTs could be an important source of energy for contracting skeletal muscle during submaximal exercise (Hawley, 2002:1486).

The theoretical potential of MCT as an energy source during exercise is based on the quick rate of hydrolysis and absorption compared with LCTs. The hydrophilic nature of medium chain fatty acids enables absorption in the enterocytes without incorporation into micelles of bile salts and phospholipids, and the majority of medium chain fatty acids passes the enterocytes and enters the portal vein, which flows to the liver. The rapid metabolism of MCT was evident when 70% of the MCT ingested was oxidized during 3 hours of exercise (Lambert et al., 1997:318-319; Jeukendrup et al., 1998:372; Vistisen et al., 2003:2435). Research has proposed that the ingestion of MCTs may have a glycogen sparing effect due to its absorption and distribution properties. By thus by increasing the concentration of medium chain fatty acids in the blood it may lower carbohydrate oxidation and enable the sparing of glycogen, which has previously been connected with fatigue (Vistisen et al., 2003:2435).

Eating a high fat meal, which consists mainly of long chain triacylglycerols, prior to exercise, is not sensible as an instant resource of fat during exercise, because of the belated and inadequate availability of ingested fat for skeletal muscle oxidation. Since LCTs empty slowly from the stomach and have to follow a whole series of reactions before ending up in the blood, only a small part of LCTs are oxidized within 6 hours of ingestion. Another factor is that MCTs are not reesterified and thus are more easily transported into the mitochondria for

oxidation. The prospective value of MCTs as a readily accessible source of energy has lead to its addition as an ingredient in some sports bars (Horowitz and Klein, 2000:564). Unlike LCTs, they are less dependent on carnitine palmitoyltransferase I to cross the inner mitochondrial membrane. According to Bucci (1993:21) MCTs have been shown to possess at least four properties of vital interest to exercise performance: (1) they are a readily available energy source; (2) they mobilize body fat stores; (3) they increase metabolic rate; and (4) they spare lean muscle mass.

2.2.2. The influence of caffeine intake on metabolism

The following information regards the intake of caffeine prior to exercise on metabolism during subsequent exercise.

2.2.2.1. Caffeine as an ergogenic aid

Caffeine is related to uric acid since it is one of three methylated xanthine alkaloid derivatives that are present in many plant species in the world and is not originally produced by the human body, though it can be considered as a nutritional ergogenic aid, since it is commonly found in beverages like coffee (http://www.medicdirectsport.com). When caffeine is ingested, 100% of all the caffeine is absorbed and peak levels in blood is seen between 15–120 minutes after an oral dose of about 250mg. Since the concentration of caffeine is highest in skeletal muscle tissue, it may be reason for its ergogenic effects in athletes (http://www.afpafitness.com).

Caffeine is absorbed by the stomach and intestine and in contrast to other authors (http://www.afpafitness.com), caffeine levels peak in the blood after about 45–60 minutes after ingestion. Once caffeine is in the blood, caffeine causes various responses in the body. The first response is the stimulant effect that caffeine has on the brain. In addition to this, caffeine increases blood pressure, pulse rate, stomach acid production, fat stores are broken down and fatty acids are released into the blood. These effects of caffeine can last from a

few up to 12 hours. The body can build up resistance towards caffeine ingestion within 4 days of regular usage. This means that if a regular caffeine user consumes caffeine prior to exercise, the athlete will not experience factors such as high blood pressure and a high pulse rate (http://www.rice.edu). The nutritional status of an athlete will also influence the athletes' responsiveness to caffeine. Those who normally consume a high carbohydrate diet may demonstrate a dulled fat mobilization effect. Individual differences in the sensitivity of caffeine sensitivity, hormonal responses as well as tolerance may impact on the ergogenic effect of caffeine (http://www.pponline.co.uk).

2.2.2.2. Dosage

Current studies used minute amounts of caffeine (1-2 mg/kg). In numerous studies, coffee was used whilst others have used caffeine. However, they all show that small amounts of caffeine are efficient in improving exercise performance and these smaller amounts, as little as 90mg caffeine, are not associated with any unnecessary side effects (Doherty and Smith 2004:626).

2.2.2.3. The effects of caffeine:

The following effects are seen with the ingestion of caffeine.

1. The workings of caffeine on the human body

Caffeine has been reported to enhance exercise performance by enhancing fat oxidation and therefore sparing glycogen, particularly during the early stages of prolonged high intensity exercise. Numerous mechanisms have been proposed to explain this caffeine-induced sparing of muscle glycogen: (1) caffeine may reduce muscle glycogenolytic rate by inhibiting glycogen phosphorylase activity; the flux generating step for muscle glycogenolysis; (2) caffeine can enhance free fatty acid mobilization by stimulating the release of epinephrine, and hence increase the potential for fat oxidation; and (3) caffeine may indirectly promote fat oxidation and carbohydrate sparing by inhibiting adenosine receptors in adipose tissue, which otherwise inhibit free fatty acid mobilization from adipocytes.

On the other hand it is also likely that caffeine may augment endurance performance, not by sparing muscle glycogen but through other effect(s). For example it has been found that caffeine: (a) reduce perception of effort (RPE); (b) attenuate “central fatigue” by reducing brain serotonin turnover, through an inhibition of the enzyme tryptophan hydroxylase; and (c) inhibit central adenosine receptor activation, thereby attenuating “central fatigue” by increasing the dopamine to serotonin ratio in the brain. Otherwise caffeine, or one of its byproducts, could directly affect skeletal muscle and/or influence the propagation of neural signals in regions between the brain and neuromuscular junction. The contradictions reported in the literature may be due to the numerous sites of action of caffeine, within both the central nervous system and peripheral tissues. Therefore an alternative approach, which attempts to distinguish between primary and secondary effects of caffeine, is necessary (Hadjicharalambous et al., 2006:876).

The proposed effects of caffeine appear to be:

- increased work time to exhaustion;
- decrease lactate levels;
- reduced rate of glycogen oxidation;
- improved mobilization of free fatty acids and in effect glycolysis and glucose uptake is limited;
- increased muscle tension and contractile state, assist impulse transmissions;
- increase membrane extractability which may help in the recruitment of motor units spreading the tension demand over a larger muscle mass;
- increase the tidal volume of lungs and
- enhanced inspiratory muscle endurance and lower sense of effort.

All of these purported effects have not been confirmed but with further research a true understanding of caffeine’s actions will be observed (http://www.afpafitness.com).

2. Effects of caffeine on hormonal regulators

Caffeine which has been accepted as a well-known stimulant of the central nervous system, increases both motor neuron recruitment as well as the frequency of potentials of the motor end plate through the release of acetylcholine.

Caffeine enhances the production of catecholamines in the blood during and at the end of exercise. The action of catecholamines is regarded as vital since it allows the body to adapt to stress which is created by exercise. Catecholamines are involved in various reactions in the body: glycogenolysis, glucose uptake, gluconeogenesis, lipolysis of muscle and adipose tissue, contraction of muscles, inotropic and chronotropic responses of the heart and circulatory adjustments. The sensitivity towards caffeine is not experienced to the same extent by all muscle fiber types. Muscle bundles which contain a majority of type I (slow twitch) muscle fibers are more sensitive to the effects of caffeine than muscles which contain a majority of type II muscle fibers (Nehlig and Debry, 1994:216-217).

3. Effects of caffeine on metabolism

Caffeine also influences metabolism of man during exercise. Fatty acids are used dynamically by skeletal muscles during exercise, which normally results in a “glycogen–sparing” effect. The improvement in performance after the ingestion of caffeine during endurance exercise which involves aerobic metabolism is due to the stimulation of lipolysis. The hydrolysis of triglycerides of adipose tissue results in an increase in plasma free fatty acids levels. Increased usage of intramuscular triacylglycerols after the ingestion of caffeine can inhibit glycogen breakdown, especially by modifying muscle concentrations of Acetyl-CoA and citrate (Nehlig and Debry, 1994:216-217).

Caffeine has shown to indirectly stimulate the mobilization of fatty acids by increasing the circulating epinephrine levels, even directly, by antagonizing

adenosine receptors that generally inhibit hormone sensitive lipase and fatty acid oxidation. The improvement in performance can be explained by the increased availability of fatty acids, which would ultimately lead to the suppression of carbohydrate oxidation and the subsequent decreased glycogen usage (Jeukendrup et al., 1998:372–373).

2.2.2.4 The effect of caffeine ingestion on different training modalities

Used in fairly small quantities before exercise, caffeine can enhance performance in both short, high intensity effort as well as in endurance exercise. Well-controlled research studies have shown that caffeine is an efficient ergogenic aid for a wide selection of exercise modes and intensities. For runners it appears that middle distance and endurance event athletes benefit the most. Caffeine may also be an efficient ergogenic aid for shorter events such as the 100, 200 and 400m sprints, but less scientific data that supports this claim is available (http://www.medicdirectsport.com). Furthermore, the use of only two cups of coffee can speed up the 1500m time by four seconds and improve kicking speeds at the ends of 1500m races by three percent. Other research has indicated that caffeine can boost 100m swimming velocity and improve sprinting capacity on a bicycle. Another investigation has shown that the ingestion of caffeine during exercise does not increase the athlete's threat of dehydration, as had been previously believed (http://www.pponline.co.uk).

In a current study done by Bridge and Jones (2006) it was found that the ingestion of a small dose of caffeine (3 mg/kg body mass) one hour prior to an 8km race actually resulted in obvious performance gains. Caffeine ingestion resulted in a 23.8 seconds average improvement, which is equal to a 1.2% improvement in performance. Improvement varied from 10–61 seconds, indicating that athletes differ in their responsiveness towards caffeine ingestion. Differences in these ranges of responsiveness could be a suggestion that there may be responders and non-responders towards this ergogenic aid. Thus the

ergogenic advantage of caffeine should be considered on an individual basis for competitive athletes (Bridge and Jones, 2006:436).

Many studies which have been done in the past suggested that the primary mechanism related to performance improvements associated with caffeine ingestion relates to the influence on lipid substrate availability and utilization (Ivy et al., 1979; Essig et al., 1980; Erickson et al., 1987; Graham and Spriet, 1991; Spriet et al., 1992; Ryu et al., 2001) One should take into account that this is not the only factor that contributes towards the actions of caffeine in the human body during exercise. Researchers have questioned the hypothesis that metabolic factors were the key mechanism of improvement in endurance capacity as a result of caffeine intake, since there is a wealth of research indicating no significant increase in lipolysis and a reduction in either glycogenolysis or muscle glycogen stores. Another factor is that caffeine also has the capacity to increase performance when glycogen sparing is not a limiting factor, such as high intensity, short term exercise. In actual fact, this means that other mechanisms or a mixture of various mechanisms may contribute to these increases in performances, including metabolic, central nervous system, cardiovascular and skeletal muscle effects (Bridge and Jones, 2006:437).

Caffeine ingestion and succeeding exercise, results in an increase in mean heart rate, which means that athletes are able to work at a higher percentage of their maximal heart rate. Researchers have found an average increase of four beats per minute. The higher mean heart rates could actually be attributed to the fact that caffeine is a stimulant by means of caffeine's effect on central perceptions of effort (Bridge and Jones, 2006:437).

2.2.2.5 Caffeine ingestion, blood lactate levels and performance

One hour after caffeine ingestion showed no alterations in blood lactate levels. However, blood lactate levels were considerably higher three minutes after exercise. There are a variety of reasons for the increase in blood lactate levels,

the first of which may be associated with substrate metabolism. It has been suggested that free fatty acid availability reduces lactate production. In order to increase the rate of lactate utilization the rate of pyruvate metabolism needs to speed up (mass action effect). Theory has suggested that caffeine may inhibit pyruvate oxidation, consequently inhibiting lactate utilization and increasing lactate production (Bridge and Jones, 2006:437-438).

In addition to these perspectives, lactate accumulation in muscles decreases the pH levels inside the muscle cells, which in reality interferes with the contractile properties of muscles as well as the activity of glycogenolytic enzymes. In light of this evidence, it actually appears that increases in blood lactate concentrations may appear paradoxical considering that performance is improved. It should also be taken into account that increases in blood lactate concentrations are not always signs of fatigue or even reduced performance. An alternative theory might be that caffeine actually promotes increased ATP resynthesis through anaerobic glycolysis, which actually enables more rapid energy provision facilitated by the increased activity of phosphofructokinase or an increased efflux of H^+ from the muscle cell. Besides this, caffeine has been shown to facilitate calcium release, which actually activates both enzymatic transformation of glycogen phosphorylase b and glycogen phosphorylase a, and in the process, accelerates glycogenolysis and glycogenolytic adrenaline secretion. Therefore it is possible that, although increased lactate proposes a metabolic response to caffeine, the supporting cause of this is through caffeine's action on skeletal muscle (Bridge and Jones, 2006:437-438).

2.2.3. The influence of carbohydrate intake on metabolism

The following information regards the intake of carbohydrate prior to exercise on metabolism during subsequent exercise.

2.2.3.1. Carbohydrates recommended to all athletes

It has long been accepted that there is a close association between dietary carbohydrate intake, muscle glycogen concentration, and endurance capacity. For this reason, it is recommended that individuals training for sports in which carbohydrate is the most profoundly metabolized fuel should consume a diet rich in carbohydrate. It should however, be noted that only a few researchers have chronically manipulated dietary carbohydrate intake in well-trained individuals and studied the effect on subsequent training responses/adaptations and performance (Hawley et al., 2006:713).

Over the past 25 years, research has accentuated the role of carbohydrates in endurance exercise, and the advice that the ingestion of a high carbohydrate diet prior to exercise and the continual carbohydrate supplementation during exercise, has been accepted by various athletes. Only in the last decade have sport scientists explored the idea that lipids can contribute towards performance (Hawley and Hopkins, 1995:245).

During sub-maximal exercise, fat and carbohydrate in the body are both considered main fuel sources, with the oxidation ratio of each being determined by training, the duration and intensity of exercise, and dietary intake before and during the session. For example, during high intensity training, carbohydrate is oxidized more than fat, and an athlete oxidizes more fat than an unfit person. For many years, sports dieticians have supported extra dietary carbohydrate as glycogen stores are limited and the extra carbohydrate consumed before and during a training session will continue to make this valuable fuel available and in the process delaying the onset of fatigue (http://www.sportsdieticians.com).

2.2.3.2. Past and present usage of carbohydrates by athletes

The intake of carbohydrates prior to exercise will help guarantee that liver glycogen stores are ‘optimal’. Work however, in the 1970s, suggests that carbohydrates ingested prior to exercise was damaging to exercise performance,

which resulted in pre-exercise carbohydrate intake being avoided by athletes for many years. This effect was attributed to a momentary increase in blood glucose levels following carbohydrate ingestion, thus bringing about a rapid release of insulin, and resulting in a decline in blood glucose concentration, the inhibition of free fatty acid release, and the premature development of fatigue. In retrospection, it is clearer today that this response may have been intervened by the large amounts of carbohydrates ingested prior to exercise. More recent studies have shown that the intake of smaller amounts of carbohydrates within the immediate vicinity of the onset of exercise results in: (a) no increase of plasma insulin levels during exercise; (b) no rebound hypoglycemia; and (c) an enhancement of performance. As a result of this, the intake of carbohydrate solutions before exercise has become more widespread (Maughan et al., 1997:168).

2.2.3.3. Factors influencing carbohydrate oxidation

During submaximal steady state exercise, the magnitude of carbohydrate and fat based fuels at any known time depend on a number of aspects, including exercise intensity, substrate availability, training status, and gender.

1. Exercise intensity

Of these, the relative intensity has been proposed to be the most important determinant of skeletal muscle flux and ultimately, substrate metabolism. Indeed, an augmentation in exercise intensity results in greater contraction-induced muscle glycogenolysis and glycolysis and stimulates sympathetic nervous system activity, which leads to a superior use of carbohydrate-derived fuels (Jeukendrup et al., 1997:836; Wagenmakers et al., 2001:296; Arkinstall et al., 2004:2275; Jeukendrup et al., 2004:1551).

2. Pre–exercise substrate availability

Pre-exercise substrate availability has emerged as a remarkable regulator of the patterns of fuel oxidation during exercise. Numerous studies have shown that the

rate of glycogen utilization during exercise is directly related to starting muscle glycogen concentration. As a result, high pre-exercise muscle glycogen content after a carbohydrate rich diet would support a larger rate of glycogenolysis during subsequent exercise, whereas low pre-exercise muscle glycogen content, after a diet low in carbohydrate, would decrease rates of glycogenolysis (Arkinstall et al., 2004:2275).

2.2.3.4. The use of carbohydrates for exercise intensities above 65% V'O$_{2max}$

The term 'prolonged exercise' is usually used to define exercise intensities which can be continued for durations ranging between 30 and 180 minutes and corresponding values of 60 and 85% of the V'O$_{2max}$. Continuous exercise of any periods of longer duration (i.e. an intensity of less than 60% V'O$_{2max}$) is possibly not limited by muscle substrate availability and, providing adequate hydration is maintained, can most likely be sustained for several hours or even days. Carbohydrate is without a question, the most significant fuel for maximal intensity exercise. The main benefit of carbohydrate oxidation, which is often overlooked, is that, per unit oxygen utilized, it will supply the greatest amount of ATP compared with the oxidation of any other substrate (Maughan et al., 1997:158).

Since glycogen is stored close to the site of contraction and oxidation, and due to the fact that it is able to sustain the phosphorylation of ADP to produce ATP, glycogen is viewed as the key fuel for the maintenance of moderate to intense exercise (i.e. ≥65% V'O$_{2max}$). Due to this reason, endurance athletes are encouraged to maximize the availability of muscle glycogen through the ingestion of moderate amounts of carbohydrates prior to competing (Johnson et al., 2004:151). According to Lambert et al. (1997:315) the goal of a marathon athlete should be to 'train, eat and run at a pace such that muscle glycogen stores are depleted as the athlete crosses the finish line'. An important objective of an athlete's daily diet is to supply the muscles with substrates to fuel exercise in order to attain optimal adaptation and performance improvements. Fat sources

are regarded limitless, but carbohydrate stores are limited in terms of energy supply. In fact the energy which is supplied for muscular contraction and maintenance of the central nervous system is quite quickly depleted during exercise of submaximal intensity lasting longer than 90 minutes (Burke et al., 2004:15).

It has previously been shown that the ingestion of carbohydrates prior to exercise may maintain blood glucose availability as well as high rates of carbohydrate oxidation late during exercise when muscle glycogen stores start to deplete (Jeukendrup et al., 1997:836; Jeukendrup et al., 2004:1555–1556). This improvement in glucose uptake and carbohydrate oxidation might be responsible for the improvements which have been noticed in exercise time to exhaustion when carbohydrates are ingested (Jeukendrup et al., 1997:836).

2.2.3.5. The inhibition of fat oxidation by means of carbohydrate ingestion

Pre-exercise carbohydrate ingestion has a very strong inhibiting effect on fat oxidation. The ingestion of 50-100g of carbohydrate in the hours before exercise will inhibit lipolysis and will also reduce fat oxidation by about 30–40 %. It has been demonstrated that the reduction is due partly to less fatty acids being available for oxidation (Jeukendrup, 2003:1272; Watt et al., 2004:145). One reason for the decrease in fat oxidation during carbohydrate supplementation is the fact that ingestion of carbohydrates increases insulin levels in the blood, and insulin is an inhibitor of adipose tissue lipolysis. It has only been recently discovered that this suppression of fat oxidation persists for 4 hours after a meal. This means that fat oxidation in normal active people is always under the influence of insulin from normal dietary carbohydrates (Horowitz et al., 1997:768). The exact mechanism by which glucose and/or insulin reduce fat oxidation at an intramuscular level is still subject to debate (Jeukendrup, 2003:1272).

2.2.4. The influence of fasting on metabolism

Scientists have been interested for many years in the combined effect of fasting and exercise performance. This intervention has served to provide information on the utilization of fuels for exercise, the use of intramuscular triglycerides and changes that occur during exercise in terms of muscle and liver glycogen stores (Aragon-Vargas, L.F. 1993:255).

2.2.4.1. Increased fat oxidation: the goal of fasting

Since the established review by Hermansen et al. (1967), muscle glycogen has been considered as an influential aspect for submaximal exercise performance. Since then many researchers (Conlee, 1987) have investigated nutritional manipulations which can improve sub-maximal exercise performance and most of them concluded that whenever the plasma FFA concentration was elevated, there was a drop in glycogen utilization during exercise and as a result of this drop, the endurance ability of the athletes was improved (Koubi et al., 1991:1337).

Through research it has been suggested that fasting can be a way to increase the utilization of fat and in the process sparing glycogen and improving performance. The effects of short term fasting (less than 12 hours) on rats indicate that fasting increases the concentrations of epinephrine and norepinephrine as well as the concentration of circulating fatty acids and in effect an increase in lipolysis is noted. In effect this leads to an increase in fat oxidation and the subsequent sparing of muscle glycogen which will lead to a similar or even increased running time to exhaustion in rats (Dohm et al., 1983:830). In humans there is an increase in catecholamines, an increase in lipolysis and a decreased glucose turnover. Since glycogen stores, however, cannot be maintained during fasts which last longer than 12 hours, it is evident that there will be a decrease in exercise performance (Jeukendrup et al., 1998:373). After an overnight fast the fatty acids which are oxidized for energy are derived mainly from adipose triacylglycerols. (Horowitz and Klein, 2000:559).

2.2.4.2. Factors influencing fat oxidation

The following factors influence fat oxidation.

1. Carbohydrate ingestion

Exercise and fasting are effective means of increasing fat oxidation. Thus, exercise is often prescribed to individuals attempting to reduce body fat. The findings of Horowitz et al. (1997:774) indicate that when exercise is preceded by a carbohydrate meal, lipolysis and fat oxidation are reduced. How this affects body fat and weight regulation over periods of days to months is not clear. It has been reported that total fat oxidation was reduced by about 30% over an 8 hour period when carbohydrate was ingested before exercise, compared with ingestion after exercise. Reducing lipolysis and impairing daily fat oxidation by ingesting carbohydrate before exercise increases the likelihood of being in a positive lipid balance and thus may increase fat deposition. Horowitz also demonstrated that ingestion of either a low GI-carbohydrate or a high GI-carbohydrate suppresses lipolysis and fat oxidation during the 2 hour period of his study. Due to sensitivity of lipolysis to even small elevations in insulin, it appears that to maintain high rates of fat oxidation at rest and during subsequent exercise, people should not eat even small amounts of carbohydrates before exercise. The reduction in lipolysis and fat oxidation after carbohydrate ingestion necessitates a compensatory increase in carbohydrate oxidation to maintain energy production during exercise (Horowitz et al., 1997:774).

The combination of ingesting glucose and lipids prior to exercise increased fat oxidation, but did not restore fat oxidation to levels observed during fasting. This is an indication that carbohydrate ingestion prior to exercise inhibits lipolysis and fat oxidation. Exercise and fasting are regarded as an effective means to increase fat oxidation (Horowitz et al., 1997:773). Due to the sensitivity of lipolysis to even diminutive increases in insulin, it appears that in order to maintain high rates of fat oxidation at rest as well as during exercise individuals should refrain from eating even small amounts of carbohydrates prior to exercise

(Horowitz et al., 1997:774). A lower respiratory exchange ratio (RER) value in exercising athletes indicates a relative increase in fat oxidation. Carbohydrate oxidation increases during exercise as a result of eating a pre-exercise meal four hours prior to training. Thus the fed nutritional condition manipulates subjects to carbohydrate oxidation regardless of the training state or the exercise intensity (Bergman and Brooks, 1999:480).

2. Catecholamines

The rate of lipolysis is highly dependable on the actions of several hormones which include the catecholamines adrenalin and nor-adrenaline (nor-epinephrine) and insulin (Robergs and Roberts, 2000:192). During exercise of increasing intensities and duration, plasma catecholamine levels and sympathetic neural activity rise exponentially to a certain degree, especially at work rates above 70% $V'O_{2max}$. The plasma adrenaline level increases during sustained exercise, especially during very long bouts of exercise. The level of hormonal stimulation also depends on the exercise mode. Plasma catecholamine levels are highest during exercise involving muscles with a large mass, such as cycling ergometry using both legs, and are lower during exercises involving a small muscle mass, such as single-arm flexions or bicep curls. Although the length of an exercise bout seems to have a direct effect on blood FFA levels, the exact mechanism by which catecholamine concentration induces this increase is not well understood (Ranallo and Rhodes, 1998:34).

3. Growth hormone

The rate of lipolysis at rest is directly related to the level of catecholamine stimulation. Other hormones may also promote lipolysis, directly or in combination with other catecholamines. Plasma growth hormone levels are significantly elevated above resting values after 40 minutes of exercise. The degree of elevation is not affected by physical training, but is directly related to exercise intensity and duration. The rise in the plasma growth hormone level is too slow to account for the rapid stimulation of lipolysis at the onset of exercise.

Furthermore, the growth hormone levels decrease during prolonged exercise to exhaustion, which is when FFAs are most in demand. It is therefore unlikely that growth hormone has a role in mediating the increase in FFA turnover (Ranallo and Rhodes, 1998:34).

2.2.4.3 The effect of fasting on metabolism

During fasting a reduced provision of exogenous substrates will lead to progressive depletion of the glycogen stores. In addition to this, there is an increase in lipolysis and proteolysis to supply fuel for liver and muscle metabolism. In rodents, fasting of prolonged duration is attached to progressive depletion of fat mass. There is a progressive decrease in circulating insulin concentrations and hence an increased dependence on endogenous energy sources. The liver plays a very significant role in maintaining glucose levels during fasting. After the hepatic glycogen stores are depleted, the liver relies on gluconeogenic substrates like lactate, alanine, glycerol and pyruvate in order to maintain glucose output (Barzilai et al., 1995:819).

During the fasted state, dietary fat is trafficked to the muscle, presumably for oxidation rather than to adipose tissue for storage (Voltruba et al., 2002:1757). Although fasting increases the availability of FFA and the rates of fat oxidation during exercise (Astrand and Rodahl, 1986:550), such perturbations do not have a positive effect on exercise performance, which is largely due to the presence of endogenous glycogen reserves (Hawley et al., 1998:241).

2.2.5. The influence of fat and caffeine intake on metabolism

Very little information regarding the simultaneous intake of fat and caffeine exist. One study done by Hadjicharalambous et al., (2006) is the first article that can shed some light on this topic of discussion:

This study was designed to distinguish between the putative metabolic and CNS effects of pre-exercise caffeine ingestion. This was achieved by having the

participants exercise after elevating their circulating plasma [FFA] with a high fat meal and subsequently co-ingesting caffeine or placebo. Assuming that perception of effort reflects, in part at least, CNS responses, the present results indicate a differentiation between the putative metabolic effects and the CNS actions of caffeine during constant load exercise, as perception of effort was reduced after caffeine ingestion (despite an elevation in cardiopulmonary and metabolic responses), but there were no differences in substrate utilization and no improvement in exercise performance. Twelve of the eighteen participants from both experiments ranked the fat plus caffeine trial as the easiest trial, which was in line with the perception of effort results obtained during exercise. This ranking of the order of difficulty of the trials was irrespective of performance time, since some participants who ranked the fat plus caffeine trial as the easiest, did not perform better on this trail. These results demonstrate a clear dissociation between perception of effort, metabolic responses and exercise performances.

It is difficult, however, to explain why the participants in the present experiments perceived it easier to exercise with than without caffeine, particularly when one considers the accompanying elevation in blood [lactate], $V'O_2$, V_E and heart rate that typically would be expected to augment, rather than attenuate, perception of effort. It is proposed that these effects on perception of effort are the result of caffeine directly stimulating the CNS; the exact mechanism, however, remains unclear. Caffeine may reduce perception of effort by inhibiting brain adenosine receptors (A_1 and A_2), which otherwise suppress synaptic transmission within the motor cortex, and/or by reducing the excitation threshold of motor neurons facilitating motor unit recruitment. Alternatively caffeine may attenuate perception of effort by enhancing the secretion of endorphins, which is widely known to reduce pain perception and promote euphoria. Caffeine has previously been shown to reduce fatigue and effort sensation associated with inspiratory muscle contraction. Caffeine may also enhance respiration by blocking central adenosine receptors, which act to depress ventilation by inhibiting respiratory motor centres (Hadjicharalambous et al., 2006:884–885).

Several studies (McNaughton, 1986; Flinn et al., 1990; Jackman et al., 1996) utilizing non–glycogen depletion exercise have reported an enhancement in high intensity/incremental exercise performance after caffeine ingestion and suggest CNS involvement in the fatigue process. In the first experiment in this study,(incremental exercise performance) a non–glycogen depletion protocol was employed in an attempt to examine a possible metabolic effect of caffeine during the early stages of exercise and to further differentiate between a putative metabolic and CNS effect of caffeine during high intensity exercise. The failure of caffeine to improve incremental exercise performance in experiment 1 is not consistent with several previous reports (McNaughton, 1986; Flinn et al., 1990). This may be due to the effect of caffeine in elevating $V'O_2$ and energy expenditure and, therefore, metabolic rate. Thus, the increased metabolic rate and therefore higher ATP demand during the 30 min constant load exercise phase after caffeine ingestion may have negated any ergogenic effect of caffeine on incremental exercise performance.

Alternatively it is possible that the pre–exercise high fat meal employed in the experiments conducted by Hadjicharalambous et al., (2006) showed no synergistic effects on the increased levels of fat oxidation previously attributed to the sole effect of caffeine intake. Typically, the limitation in fat oxidation during the early stages of exercise is the inadequate delivery of free fatty acids to the active skeletal muscles, rather than the inability of the muscle to oxidize free fatty acids. In their experiments, however, the plasma [FFA] was elevated before exercise by acute fat ingestion. Although increased lipolysis was evident after caffeine ingestion, the saturation threshold for free fatty acid uptake and, possibly, oxidation by skeletal muscle was probably achieved on both fat trials due to the high fat meal. Consequently, any caffeine–induced lipolysis would not further enhance free fatty acid utilization. The performance results also confirm this. For example, if there was a significant contribution of the plasma [FFA] to fat oxidation, endurance performance would be increased following caffeine ingestion due to a greater sparing of muscle glycogen.

Based on the present substrate oxidation findings, it is unlikely that the significant increase in $V'O_2$ observed during exercise after caffeine ingestion reflects a marked shift towards fat oxidation. It is possible that caffeine increased $V'O_2$ due to its concomitant effect of increasing the whole–body metabolic rate, without predominantly elevating the relative rates of carbohydrate or fat utilization. In agreement with this is the higher energy expenditure observed at rest and during exercise in the fat plus caffeine trial relative to the fat only trial. This elevation in energy expenditure observed in previous studies was ascribed to the thermogenic effect of caffeine via increased epinephrine secretion. Increased epinephrine secretion is known to enhance lipolysis and muscle and liver glycogenolysis. The higher blood [glucose] and [lactate] observed following caffeine ingestion is consistent with many previous studies (Gaesser and Rich, 1985; Spriet et al., 1992; Jackman et al., 1996; Graham et al., 2000; Laurent et al., 2000). The higher blood [glucose] has been suggested to be due to an increased liver glycogenolysis, although a reduction in blood glucose uptake by skeletal muscle and/or adipose tissue cannot be excluded. Similarly, an increase in blood [lactate] may result from the inability of the mitochondria to handle the high pyruvate load, consequent to an increase in skeletal muscle glycogenolysis, therefore providing more substrate for lactate production. The higher [pyruvate] and [lactate:pyruvate] ratio observed in experiment 2 (endurance exercise performance) after caffeine ingestion supports the notion of an increase in skeletal muscle glycogenolysis providing more substrate for lactate production. The elevation, however, in blood [lactate] cannot solely be attributed to an increase in muscle lactate production through anaerobic metabolism. For example, it was found that an increase in blood [lactate] after caffeine ingestion, without a concomitant elevation in muscle lactate production at rest or during exercise, therefore imply inhibition of lactate uptake by non–exercising muscles and other tissues e.g. liver (Hadjicharalambous et al., 2006:885-886).

2.3. INDIRECT CALORIMETRY

The following information will pertain to the use of indirect calorimetry for testing purposes.

2.3.1. Introduction to indirect calorimetry

The use of indirect calorimetry for the evaluation of net rates of carbohydrate and fat oxidation and conversion of glucose to fat or glycogen in man and other animals is appealing because it provides kinetic information about the general pattern of metabolism and because the technique is noninvasive. The principles of indirect calorimetry were recognized by Zuntz and Shumberg (1901) and later by Lusk (1928). Indirect calorimetry and the respiratory quotient *per se* have been the subjects of several key reviews, scientific papers and letters (Livesey and Elia, 1988:608).

The science that quantifies the heat release from metabolism is termed calorimetry. Calorimetric methods that involve the direct measurement of heat dissipation from the body are termed direct calorimetry. When heat dissipation is calculated from other measurements, these methods are termed indirect calorimetry. Indirect calorimetry can also be subdivided into open and closed circuit systems. Closed circuit indirect calorimetry involves the recirculation of inhaled and exhaled air, thus necessitating the removal of carbon dioxide and the replenishment of oxygen. Open circuit indirect calorimetry can involve the inhalation of atmospheric air and the sampling and measurement of exhaled air for respiratory gas analysis. Other forms of indirect open circuit calorimetry exist, such as measuring total carbon and nitrogen exchange and measuring the exchange of labeled water within the body (Robergs and Roberts, 2000:61).

2.3.2. Open circuit indirect calorimetry

Although the mathematical calculations of open circuit indirect calorimetry have remained unchanged since the respiration calorimetry work of Atwater (1904),

the equipment that was originally used differs considerably from the computerized systems that exist today.

When concerned with exercise, the predominant application of indirect calorimetry is for the measurement of oxygen consumption, and therefore an assessment of the metabolic intensity of exercise. Also the ratio between carbon dioxide production and oxygen consumption is used to indicate the contribution of fat and carbohydrate substrates to energy production (Robergs and Roberts, 2000:65).

2.3.3. Respiratory Quotient and the Respiratory Exchange Ratio:

The ratio of $V'CO_2/V'O_2$ is called the gas exchange ratio or respiratory exchange ratio (RER). Under steady state conditions, the RER equals the respiratory quotient (RQ), whose value is determined by the fuels used for metabolic processes. An RQ of 1.0 indicates metabolism of primarily carbohydrates, whereas an RQ of less than 1.0 indicates a mixture carbohydrates with fat (RQ, about 0.7) or protein (RQ, about 0.8). The term RQ is often reserved for expressing events at the tissue level, which is difficult to measure and is not determined during clinical exercise testing. The term RER is usually measured by gas exchange at the mouth. In true steady state, the blood and gas transport systems are keeping pace with tissue metabolism, thus the RER can be used as an index of metabolic events (RQ) (Johnson et al., 2003:230).

However an RER greater than 1.0 could also be caused by CO_2 derived from lactic acid or by hyperventilation because of the 20–fold or higher tissue solubility of CO_2 compared with O_2. This difference in solubility is due both to the 20–fold higher direct solubility of CO_2 in water compared with O_2 and to the fact that [HCO_3^-] and proteins are significant forms of transport for CO_2 in body tissues, whereas the only significant form of transport for O_2 is by combination with haemoglobin. Thus, in practical testing situations, both lactic acid and

hyperventilation must be considered when the RER is greater than 1.0 (Johnson et al., 2003:230).

$$RER = V'CO_2 / V'O_2$$

The percentage energy release from catabolism for every liter of oxygen consumed at different nonprotein RER values is listed in table 2

Table 2–The percentage energy release from the catabolism of carbohydrate and fat for every liter of oxygen consumed at different nonprotein RER fractions-After Robergs and Roberts (2000).

RER	% CHO	% Fat
1	100%	0
0.99	96.80%	3.18%
0.98	93.60%	6.37%
0.97	90.40%	9.58%
0.96	87.20%	12.80%
0.95	84%	16.00%
0.94	80.70%	19.30%
0.93	77.40%	22.60%
0.92	74.10%	25.90%
0.91	70.80%	29.20%
0.9	67.50%	32.5
0.89	64.20%	35.80%
0.88	60.80%	39.20%
0.87	57.20%	42.50%
0.86	54.10%	45.90%
0.85	50.70%	49.30%
0.84	47.20%	52.80%
0.83	43.80%	56.20%
0.82	40.30%	59.70%
0.81	36.90%	63.10%
0.8	33.40%	66.60%
0.79	29.90%	70.10%
0.78	26.30%	73.70%
0.77	22.30%	77.20%
0.76	19.20%	80.80%
0.75	15.60%	84.40%
0.74	12.00%	88.00%
0.73	8.40%	91.60%
0.72	4.76%	95.20%
0.71	1.10%	98.90%
0.707	0	100%

The RQ is important because when carbon dioxide production is occurring only from cellular metabolism, and assuming that no change in protein catabolism occurs during exercise, the RQ value can be used to accurately reflect the proportion of fat and carbohydrate catabolized for energy during exercise.

For many metabolic and exercise conditions, the RER is often assumed to be equal to the RQ, and is then used to calculate contributions of either fat or carbohydrate to catabolism and caloric expenditure. The assumption of equality between RER and RQ cannot however be made under certain conditions (Robergs and Roberts, 2000:65–67).

2.3.4. Energy Expenditure: (Robergs and Roberts 2000:68)

The calculation of energy expenditure using the nonprotein data of Lusk is a simple process, involving the multiplication of oxygen consumption (L/min), time (min), and the caloric equivalent for the respective RER of the exercise (kcal/L O_2).

Kcal = $V'O_2$ (L/min) x RER caloric equivalent (kcal) x time (min)

For example when performing exercise for 30 minutes requiring a $V'O_2$ of 1.5 L/min, with an average RER of 0.9, caloric expenditure can be calculated as:

Kcal = 1.5 x 4.924 x 30 = 221.6

Based on the table above, the contribution of fat and carbohydrate to energy expenditure can be calculated. For example, from the previous calculation of $V'O_2$, RER and kcal,

% kcal from fat = [(1–RQ)/(1–0.7)] x 100
% kcal from carbohydrate = 100–(% kcal from fat)

With a reported RQ = 0.9, 33.3% of the kcal is derived from fat and 66.6% are derived from the carbohydrates:

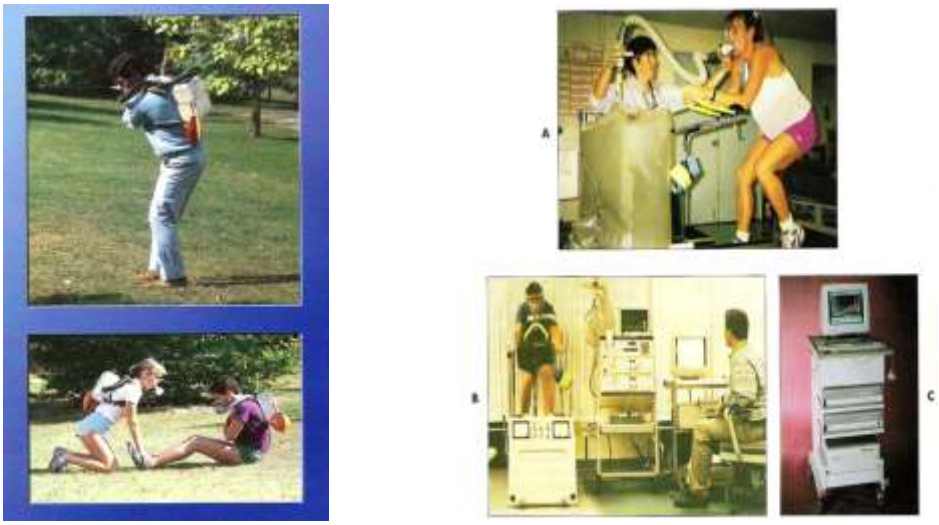
Kcal from fat = 0.33 x (221.6 kcal/30 min) = 2.46 kcal/min
Kcal from carbohydrate: = 0.667 x (221.6kcal/30 min)= 4.93 kcal/min

Assume caloric densities of 4 kcal/g for carbohydrates and 9 kcal/g for fat:

Fat usage	= (2.46 kcal/min)/(9 kcal/g)= 0.27g fat/min
Carbohydrate usage	=(4.93 kcal/min)/(4 kcal/g)
	=1.23g carbohydrates / min

2.3.5. Systems used in indirect calorimetry

Within the last 20 years the sophistication of the equipment used in indirect calorimetry has increased remarkably. Today, data is obtained, processed and calculated within seconds, enabling the monitoring of changes during very small time intervals. Ventilation measurements are now performed by advanced electronics less than one–tenth the size of the original volume meters, and the response time of the electronic analyzers for oxygen and carbon dioxide are now as short as 100ms (Robergs and Roberts, 2000:69).



After McArdle et al. (2001) After Robergs & Roberts (2000)

Figure 11–Systems used in indirect calorimetry

2.3.6. Breath–by–breath calculations of oxygen consumption

The circuitry of a breath–by–breath system is a little different from that of time averaged systems. Expired air is sampled close to the mouth, avoiding the need for a mixing chamber, and ventilation is measured by sophisticated devices such as a pneumotach or a low–mass impeller, rather than the traditional volume meter (Robergs and Roberts, 2000:69).

With the ready availability of online digital computer analysis of physiologic measurements, it has become practical to compute V'CO_2 and V'O_2 on a breath–by–breath basis. Utilizing algorithms first reported in 1973, a signal proportional to expired airflow and signals proportional to fractional concentrations of CO_2 and O_2 measured near the mouth are typically sampled 50 or 100 times per second. In this way, each breath is broken down into a large number of parts and the O_2 uptake and CO_2 output are calculated for each interval. These measurements are summed over the entire expiration to compute the total volume of O_2 uptake and CO_2 output per breath, V'O_2 and V'CO_2, respectively. The values of each breath are extrapolated to the minute (Casaburi et al., 2003:220).

Although breath–by–breath data collection/analysis is presently the most popular, it is important to recognize that the confidence with which these "metabolic" indicators can be calculated depends on the combined measurement errors for each of the determined values. Unless great care is taken with the calibration of the sensors, these errors can be additive and large (Casaburi et al., 2003:220).

2.3.7. Incremental graded treadmill running

Incremental graded treadmill running refers to the increase of exercise intensity over time, when the participant is running on a treadmill (refer to figure 12)

2.3.7.1. Workload and V'O_{2max}

Incremental exercise involves the increase in exercise intensity over time. Other names for this type of exercise are described as being "progressive" or "graded". Incremental exercise-protocols can be either continuous, where successive increases in intensity occur without rest periods, or intermittent, where a rest period is provided between increments. In addition, incremental protocols can be maximal, requiring the subject to exercise against increasing exercise intensities until volitional fatigue, or sub-maximal, when exercise is terminated at a predetermined intensity prior to volitional fatigue. Incremental exercise protocols can vary in the duration and specific intensity (stage) or any combination of these

two variables, designating the magnitude of the increment. In addition, protocols exist that continuously increase intensity over time (speed and/or grade on a treadmill), and these are termed ramp protocols.

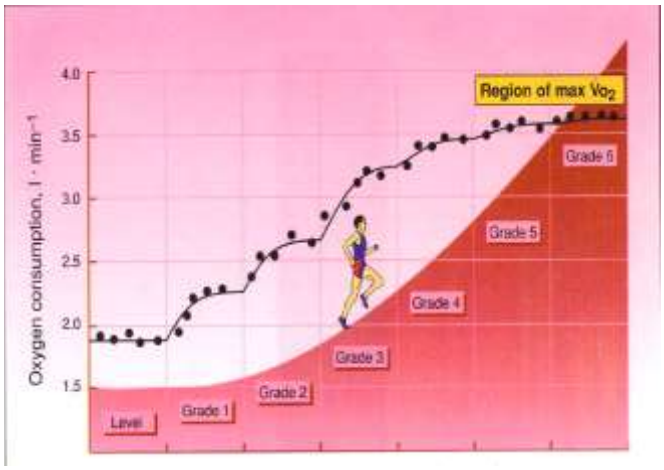


Figure 12-Incremental treadmill running–After Young (2004).

Maximal oxygen consumption is the maximal rate at which the body can consume oxygen during exercise. Traditionally $V'O_{2max}$ is verified when it coincides with a plateau in $V'O_2$ despite further increases in intensity, an RER that exceeds 1.1 and a maximal heart rate within ±10 beats/minute from predicted values (220–age). A true $V'O_{2max}$ is most likely to be attained in healthy, moderately to highly endurance–trained individuals, and when using a protocol duration between 8 and 12 minutes (Robergs and Roberts, 2000:112).

Oxygen uptake quickly increases when dynamic exercise is begun or increased. During staged exercise testing, oxygen uptake usually remains relatively stable (steady state) after the second minute of each intensity of exercise below the ventilatory threshold. $V'O_{2max}$ is the greatest amount of oxygen a person can take in from inspired air while performing dynamic exercise involving a large part of total muscle mass. It is considered the best measure of cardiovascular fitness and exercise capacity. $V'O_{2max}$ represents the amount of oxygen transported and used in cellular metabolism. $V'O_{2max}$ is influenced by age, gender, individuality,

exercise intensity, exercise habits, heredity and cardiovascular clinical status. For comprehensive review on these variables kindly refer to the work of Fletcher et al. (2001:1695).

2.3.8. Individual variation in $V'O_{2max}$:

$V'O_{2max}$ values are dispersed between extremely low capacities, like those of chronically ill individuals (<20 ml / kg / min), and the capacities of untrained, well trained and elite endurance athletes (>80 ml / kg / min). The factors that combine to influence $V'O_{2max}$ are a high proportion of slow twitch motor units, high central and peripheral cardiovascular capacities, and the quality and duration of training. Having more slow twitch muscle fibers increases the oxidative capacity of the muscle (Fletcher et al., 2001:1695).

2.3.9. Reliability

There has been current interest in whether the conventional interpretation of a plateau in $V'O_2$ during incremental exercise is valid. Since the original work on the $V'O_{2max}$ concept by Hill and Lupton (1923), it has been commonly accepted that the plateau in $V'O_2$ during incremental exercise to volitional exhaustion represents the incapability of the body's cardiovascular system to provide adequate oxygen to the working muscles. This supposition has resulted in the interpretation of $V'O_{2max}$ to signify the body's maximal cardiorespiratory capacity or "maximal aerobic power" (Robergs and Roberts, 2000:114).

The main condemnation of the $V'O_{2max}$ concept is that a true plateau in $V'O_2$ during incremental exercise has only been shown in 30–50% of tests performed on healthy subjects. This occurrence decreases radically when testing individuals who are sedentary, diseased, or disabled. Noakes (1988, 1997, and 1998) has disputed that $V'O_{2max}$ must not actually be a true maximal value, but the end result of not central limitations to oxygen delivery, but limitations in oxygen use by the contracting muscles. Basset and Howley (1997) have argued against this perception. Robergs (1999) responded to the discussion on the validity of $V'O_{2max}$

by providing a different review of the topic, and revealed that the $V'O_{2max}$ concept is valid, but that it is difficult to truthfully measure in all individuals. Just because a cardiovascular limitation to $V'O_2$ may not arise in all individuals does not mean that it does not exist. Evidently, in individuals that will have difficulty in exercising to true volitional fatigue caused by cardiovascular limitations to oxygen consumption, the expression $V'O_{2max}$ should be substituted with $V'O_{2peak}$. If the two main criteria for the accomplishment of a $V'O_{2max}$ are not met (plateau and RER > 1.1), then the term $V'O_{2peak}$ should be used. Once again, the individuals who are more likely to achieve a $V'O_{2peak}$ rather than $V'O_{2max}$ according to Robergs and Roberts (2000:114–115) are:

- Prepubescent children
- Untrained individuals
- Individuals with acute illness
- Individuals with disease

All breaths collected should be used in the processing of the data. However, erroneous breaths caused by swallowing, coughing and so one, should not be included. For the final tabular and graphic report, 30-60 second intervals for averaging data are recommended although 20 second intervals may be acceptable (Casaburi et al., 2003:221).

2.3.10. Sources of error in the reproducibility of measured values (Casaburi et al., 2003:223-224)

It is important to take into consideration the reproducibility of the variables measured during clinical exercise testing for appropriate interpretation of the results. Factors that may contribute to variability in these measurements include the following:

- Patient motivation
- Patient instructions/inducement

- The time of day
- Testing procedures
- Equipment/calibration errors
- Potential learning effect and therefore the need for preliminary/familiarization testing

Care must be taken to ensure that these factors, which may contribute to alteration of measured exercise responses, are meticulously controlled.

CHAPTER 3

MATERIALS AND METHODS

3.1. INTRODUCTION

The following information pertains to the materials and methods of this study. It includes the study design, study site, study population, exercise mode and apparatus, interventions and measurement techniques.

3.2. STUDY DESIGN

The study is a quantitative analytical investigation that examines the influence of various dietary prescriptions on specific metabolic parameters. This study was presented to the Ethics Committee of the University of the Free State prior to commencement.

3.3. STUDY SITE

The research was conducted at the Free State Sport Science Institute in Bloemfontein, Free State Province.

3.4. STUDY POPULATION

The next paragraphs describe the study population.

3.4.1. Number of subjects

Six trained individuals participating at a national level (training background of at least 8-10 years) and six untrained healthy individuals were recruited between June 2005 and December 2005. Individuals who complied with the inclusion criteria and gave their written informed consent of the experimental procedures and the possible risks and benefits involved (see appendix A) were enrolled.

3.4.2. Inclusion criteria

Participants were included in the investigation when classified as being apparently healthy and:

- Belonged to any population group
- Were aged between ages 15 and 35 years
- Were of either gender
- Were prepared to give consent

3.4.3. Exclusion criteria

- Refused to participate
- Refused to comply with the inconvenience due to the subscribed interventions
- Did not feel familiarized with treadmill running
- Refused to participate due to the repeated nature of the protocol design (wash-out period of 24 hours between interventions).
- Refused to participate due to the strenuous nature of the prescribed exercise protocol design (adhering to 5 consecutive maximal physical efforts until voluntary fatigue performed in 5 days).

3.4.4. Justification for the inclusion and exclusion criteria

- The participants had to give consent before they could be enrolled in this investigation for ethical reasons.
- To be representative the inclusion criteria included participants from any population group.
- The participants had to be familiar with the treadmill running to avoid hormonal fluctuations being introduced which could affect metabolism by means of stress/fear.
- The participants had to be prepared to be subjected to strenuous and repeated bouts of exercise within the time limits of a 5 day period to avoid muscular and/or systemic adaptations becoming additional interventions.

3.4.5. Subject identification

Individuals were randomly selected by means of the inclusion of any of the first 6 participants to accept the invitation in the two specified groups (trained and untrained individuals) to participate in the study.

3.4.6. Withdrawal

Although participation was voluntary, participants could withdraw from the project at any stage without any consequences and would be replaced with suitable candidates.

3.4.7. Financial implications for the participants

No costs were involved.

3.5. EXERCISE MODE AND APPARATUS

3.5.1. Exercise mode

All participants will be introduced to and familiarized with treadmill running. Participants will perform a graded exercise test to the point of volitional fatigue on a treadmill on five occasions spread over a period of 5 days (wash-out period of

24 hours between interventions). Individuals will be exposed to a graded progressive incremental (2 km/h every two minutes) treadmill running test commencing at a speed of 4km/h until the subject could no longer keep pace with the treadmill speed (demarcated as the onset of recovery).

3.5.2. Apparatus

The treadmill running test will be performed on a Technogym RUNRACE 1200HC. By means of indirect calorimetry the following quantitative variables will be obtained by means of a calibrated and automated computerized breath-by-breath analysis system (Jaeger: Oxycon Pro; Masterscreen CPX Ergospirometry-Germany):

- Treadmill speed (km/h)
- $V'O_2$ (ml/min)
- $V'CO_2$ (ml/min)
- $V'O_{2peak}$
- Heart rate [beats/min; [Technogym short range radio telemetry (Polar Electro)]
- Respiratory exchange ratio (RER)
- Fat utilization (g/day)
- Carbohydrate utilization (g/day)
- Ventilatory effort [(VE (L/min)]

The mechanisms involved during the trial that could explain alterations in metabolism during the trial fall beyond the ambit/scope of the present protocol design and require alternative methods of investigation (including tracer methods).

3.6. INTERVENTIONS

At 08h00 on five (5) consecutive days the following interventions adhering to a wash-out period of 24 hours between the interventions will be implemented in a double blind crossover protocol fashion prior to participation in a scheduled treadmill run:

1. Post absorptive state (Fasting state of 8 hours)
2. Olive oil (5 ml) and peanut butter (30 ml) ingestion 120 minutes prior to testing in the post absorptive state
3. Caffeine ingestion (150 mg) 90 minutes prior to testing in the post absorptive state
4. Olive oil (5 ml) and peanut butter (30 ml) ingestion 120 minutes prior to testing followed by caffeine ingestion (150 mg) 90 minutes prior to testing in the post absorptive state
5. Carbohydrate ingestion (4 slices white bread with apricot jam and no butter + 340ml coke) 240 minutes prior to testing.

3.7. MEASUREMENT TECHNIQUES

The following paragraphs will describe the measurement techniques used in this investigation.

3.7.1. Procedures

- All $V'O_{2peak}$-tests were performed on the Jaeger: Oxycon Pro; Masterscreen CPX, provided by the Free State Sport Science Institute in Bloemfontein–see figure 13.
- Before the participants are tested, the system is calibrated in terms of temperature and humidity, flow volume and gas analysis sensors.
- The participant's height and weight is measured and fed into the computerized system.

- The necessary equipment, i.e. head strap, mask, vest and heart rate monitoring device are then fitted.
- The test procedure is once again thoroughly explained to each participant prior to each and every treadmill run:
    - The first minute of the test, commences at 4 km/h after which the participant is informed that the speed of the treadmill will increase to 8 km/h that would last for a period of 2 minutes.
    - Thereafter, the speed of the treadmill will increase with 2 km/h every two minutes.
    - A few seconds before every augmentation of speed, the participant is informed of this.
- The participant runs until volitional fatigue sets in and by raising the hand the test is terminated.
- The estimated time of the test depends on the level of fitness of the participant.
- Thereafter the equipment is removed from the participant and the complete report sheet is printed and analyzed.

Figure 13-Jaeger: Oxycon Pro; Masterscreen CPX.

3.7.2. Quality control

Quality control of the apparatus is performed on a daily basis according to the manufacturer's specifications. This is to verify that the Jaeger Oxycon Pro, Masterscreen CPX is performing correctly and also to ensure accuracy and reliability.

3.7.3. Analysis of data

The following paragraphs pertain to the analysis of the data.

3.7.3.1. Absolute values

Table 3 (see Appendix B) is a typical example of the tabular print-out format indicating the absolute breath-by breath analysis values for the entire treadmill run for a any participant.

3.7.3.2. Relative values (calculated values) for a specific variable

For the purpose of the present investigation and for reasons to be discussed (see Chapter 5-section 5.1), the breath-by-breath values for any variable (see 3.5.2) are pooled for 30 second intervals and the average value calculated for all interventions (see 3.6).

SUBJECT X		CARBOHYDRATE							
Time [min]	Speed [km/h]	V'O2 [ml/min]	V'CO2 [ml/min]	V'O2peak	RER	HR [1/min]	CHO [g/day]	FAT [g/day]	V'E [L/min]
00:01:00	4.0	776	705	12.3	0.75	130	890	163	25
00:01:30	8.0	1367	1235	21.7	0.76	142	1559	366	40
00:02:00		1769	1596	28.1	0.86	153	2016	390	55
00:02:30		1934	1723	30.7	0.89	154	2078	483	56
00:03:00		2055	1887	32.6	0.92	158	2540	423	63
00:03:30	10.0	1970	1929	31.3	0.93	161	3146	188	66
00:04:00		2088	1971	33.1	0.94	165	2902	271	67
00:04:30		2229	2085	35.4	0.93	165	2983	322	70
00:05:00		2257	2154	35.8	0.95	167	3280	236	73
00:05:30	12.0	2374	2239	37.7	0.94	170	3290	313	73
00:06:00		2539	2358	40.3	0.93	170	3310	409	78
00:06:30		2570	2402	40.8	0.93	170	3440	378	79
00:07:00		2648	2536	42.0	0.96	172	3909	255	86
00:07:30	14.0	2693	2587	42.7	0.96	176	4019	236	86
00:08:00		2793	2597	44.3	0.96	175	3660	447	86
00:08:30		3018	2905	47.9	0.96	177	4539	299	100
00:09:00		2910	2874	46.2	0.99	178	4810	106	102
00:09:30	16.0	2898	2838	46.0	0.98	179	4647	129	101
00:10:00		3143	2973	49.9	0.98	180	4422	242	102
00:10:30		3174	3139	50.4	0.99	181	5277	36	111
00:11:00		3225	3249	51.2	1.01	183	5712	-83	114
00:11:30	18.0	3254	3309	51.6	1.02	184	5951	-163	119
00:12:00		3388	3449	53.8	1.02	187	6217	-177	123
00:12:30		3536	3597	56.1	1.02	187	6472	-200	124
00:13:00		3497	3574	55.5	1.02	187	6500	-224	124
00:13:30	20.0	3524	3621	55.9	1.03	189	6670	-265	130
00:14:00		3652	3762	58.0	1.03	190	6969	-308	136
00:14:30		3736	3895	59.3	1.04	191	7408	-417	138
00:15:00		3763	3925	59.7	1.04	191	7467	-421	141
00:15:25	22.0	3828	4033	60.8	1.05	191	7837	-526	146

Table 3-A typical example of the calculated average pooled (30 second intervals) data for the carbohydrate trial (Ch) for the entire treadmill run.

The variables that are measured in this intervention are: time, speed, $V'O_2$, $V'CO_2$, $V'O_{2peak}$, RER, heart rate, carbohydrate oxidation, fat oxidation and V'E.

	SUBJECT X			
FAST	CARBS	FAT	CAF	FAT + CAF
V'O2				
857	776	868	772	785
1148	1367	1266	1139	1161
1618	1769	1682	1657	1663
1930	1934	2024	2013	1911
1933	2055	1814	2016	1924
2055	1970	2031	1930	1940
2154	2088	2088	2150	2037
2233	2229	2157	2297	2244
2275	2257	2133	2252	2318
2327	2374	2256	2289	2320
2423	2539	2456	2432	2480
2640	2570	2603	2546	2548
2631	2648	2532	2579	2599
2764	2693	2621	2654	2722
2813	2793	2827	2832	2760
2971	3018	2824	2844	2894
2948	2910	2908	2945	2937
3131	2898	2968	3034	2932
3182	3143	2921	3110	3106
3188	3174	3237	3237	3185
3229	3225	3229	3257	3200
3339	3254	3200	3322	3276
3371	3388	3320	3359	3300
3353	3536	3388	3509	3452
3444	3497	3404	3540	3480
3533	3524	3486	3637	3582
3684	3652	3617	3649	3612
	3736		3702	3740
	3763		3724	3768
	3828		3760	3903

Figure 14-A typical example of one of the variables ($V'O_2$-Oxygen consumption) calculated as average pooled (30 second intervals) data for all interventions for a specific participant (x).

The interventions used: fasting, carbohydrates, fat, caffeine and the combination of fat and caffeine

3.7.3.3. Illustrations

For explanatory purposes specific variables are graphed from the relative calculated values-see Chapter 4; section 4.4.1 and 4.4.2.

3.7.4. Statistical analysis

To verify the effect of the interventions on specific variables for a specific subject, descriptive statistics were mainly used in this investigation. The following statistical procedures are also used to validate findings and are indicated in the text where appropriate:

- Percentages are determined to express the degree of change between test variables.
- Means and standard deviations are calculated for specified test variables-(M±SD).
- The population mean (μ) and the Confidence Interval for the μ (CI for μ) at a 95% confidence level indicated as [M(CI 95%) population mean]-example [66(24.9 ; 107.28) population mean]-is implemented to evaluate the degree of change for specified test variables or examine the effect of the interactions between test variables for a specific subject.
- Unpaired Student t-test on means and averages and the Confidence Intervals based on these means and averages at a 95% confidence level are mainly used when findings for a specific test variable are pooled to compare the outcome between trained and untrained subjects.

CHAPTER 4

THE RESULTS OF THE INVESTIGATION

4.1. INTRODUCTION

The findings on the trained individuals will be followed by the untrained individuals.

4.2. ABSOLUTE RESULTS

A typical example of a print-out report indicating the absolute breath-by breath raw data values for the entire treadmill regime for a specific intervention and participant can be found in Appendix B. Due to volumetric proportions, the remainder of the reports on the absolute breath-by breath data values for the entire treadmill run for a specific intervention and participant are not included.

4.3. SPECIFIC RESULTS

Unless otherwise indicated the average calculated breath-by-breath pooled value for 30 second intervals for all variables (see 3.5.2), the entire exercise regime, interventions (see 3.6) and participants are presented in the format of tables–see Appendix C.

4.4. RELATIVE RESULTS

The results of each participant will be viewed independently from the other participants and presented in the format of a synchronized, general informative graphical illustration of all the variables involved for the entire exercise regime. Accordingly, two illustrations represent a participant and are comprised of 10 graphs in total- each graph illustrating the findings for a single variable throughout the entire exercise regime.

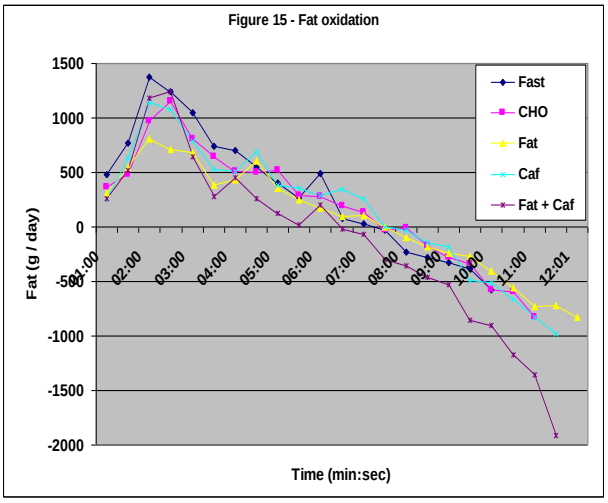
The core focus of the synchronized and general informative combined report back format of the relative results relates to indicating the largest difference(s) between any two (or more) of the various interventions for a specific participant and the accompanying variables for the specific intervention respectively.

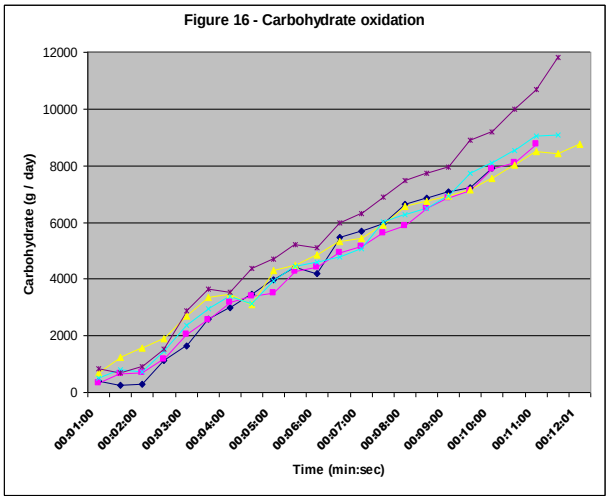
The following graphs present data collected from trained and untrained subjects.

4.4.1. Trained subjects

The following graphs show data from the group of trained subjects.

Trained individual 1





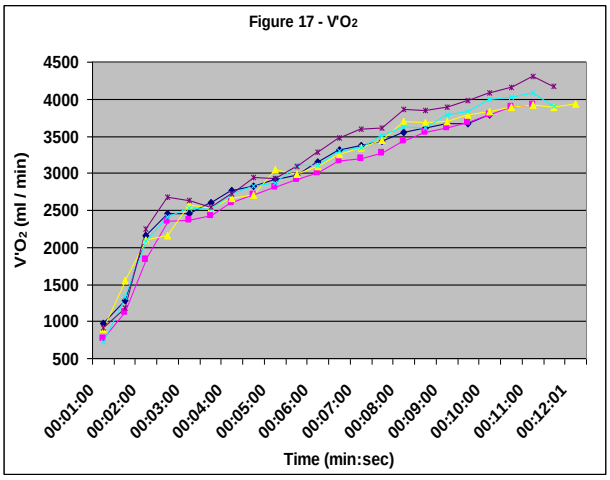


Figure 17 - V'O2
V'O2 (ml / min)
4500
4000
3500
3000
2500
2000
1500
1000
500
00:01:00
00:02:00
00:03:00
00:04:00
00:05:00
00:06:00
00:07:00
00:08:00
00:09:00
00:10:00
00:11:00
00:12:01
Time (min:sec)

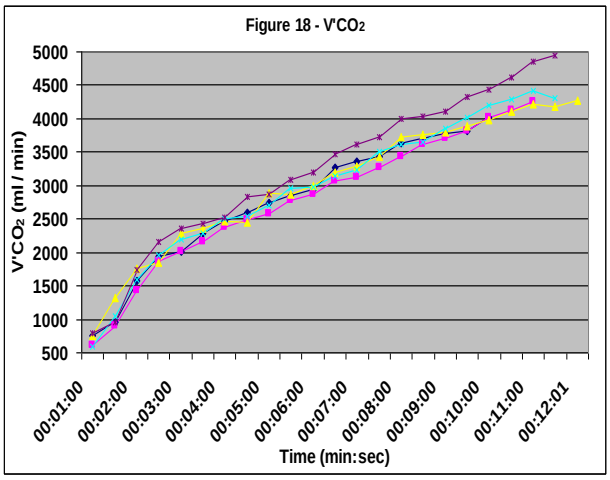


Figure 18 - V'CO2
V'CO2 (ml / min)
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
00:01:00
00:02:00
00:03:00
00:04:00
00:05:00
00:06:00
00:07:00
00:08:00
00:09:00
00:10:00
00:11:00
00:12:01
Time (min:sec)

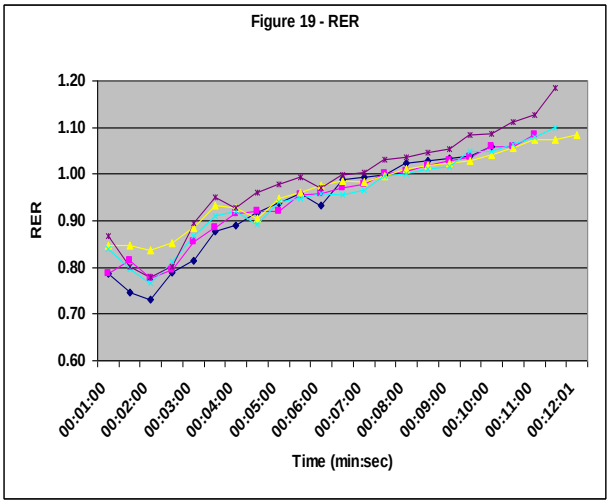


Figure 19 - RER
RER
1.20
1.10
1.00
0.90
0.80
0.70
0.60
00:01:00
00:02:00
00:03:00
00:04:00
00:05:00
00:06:00
00:07:00
00:08:00
00:09:00
00:10:00
00:11:00
00:12:01
Time (min:sec)

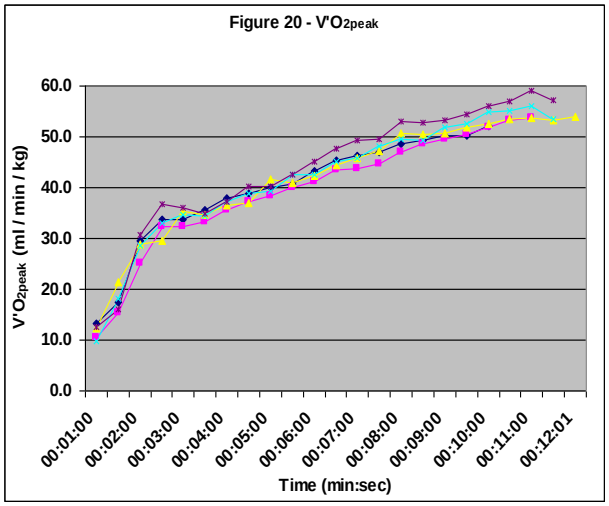


Figure 20 - V'O2peak
V'O2peak (ml / min / kg)
60.0
50.0
40.0
30.0
20.0
10.0
0.0
00:01:00
00:02:00
00:03:00
00:04:00
00:05:00
00:06:00
00:07:00
00:08:00
00:09:00
00:10:00
00:11:00
00:12:01
Time (min:sec)

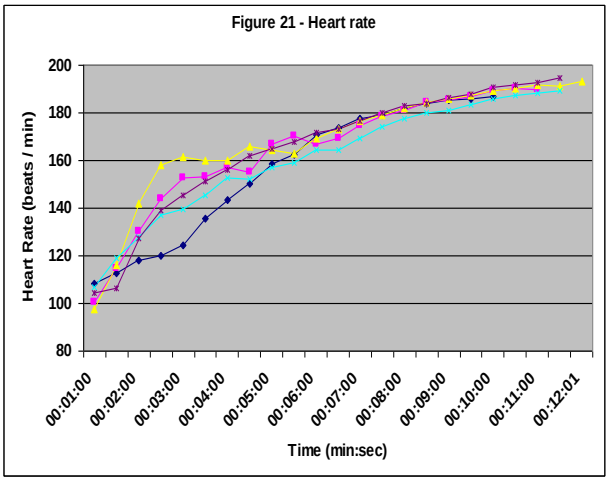


Figure 21 - Heart rate
Heart Rate (beats / min)
200
180
160
140
120
100
80
00:01:00
00:02:00
00:03:00
00:04:00
00:05:00
00:06:00
00:07:00
00:08:00
00:09:00
00:10:00
00:11:00
00:12:01
Time (min:sec)

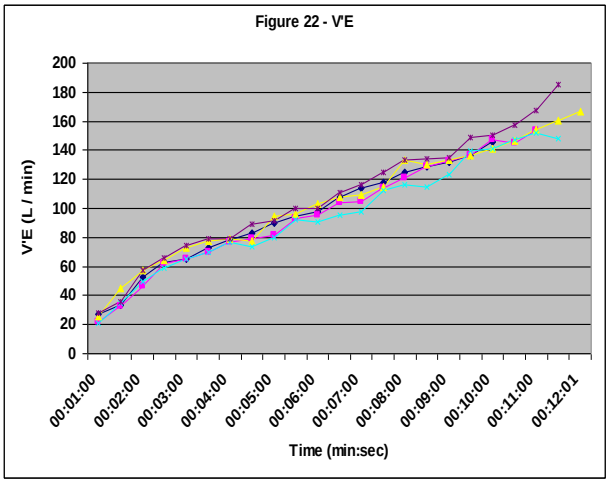
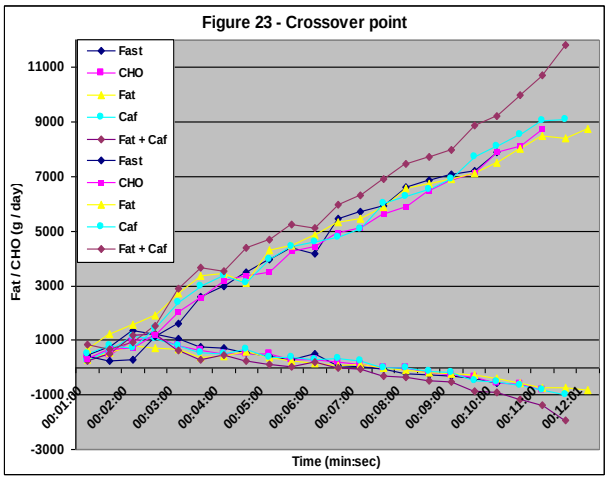
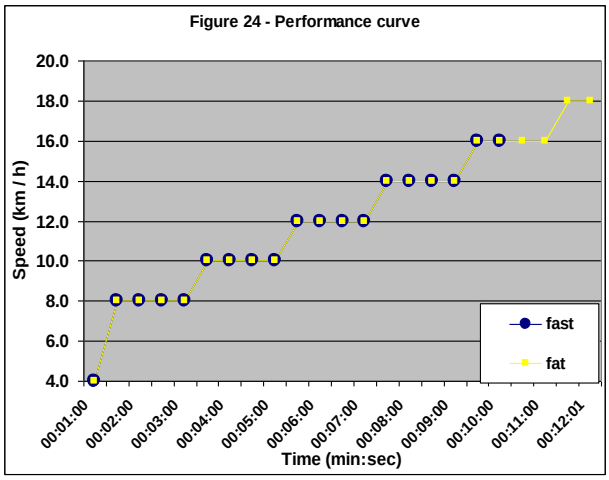


Figure 22 - V'E
V'E (L / min)
200
180
160
140
120
100
80
60
40
20
0
00:01:00
00:02:00
00:03:00
00:04:00
00:05:00
00:06:00
00:07:00
00:08:00
00:09:00
00:10:00
00:11:00
00:12:01
Time (min:sec)





Figures 15–24: The compiled results in graphic format for the various interventions and their corresponding variables.

- Figures 15 and 16 respectively show that the Fasting trial (F) produced the highest fat oxidation and lowest carbohydrate oxidation capacities at a low exercise intensity (8 km/h) level, which coincides with a decrease in the RER (Fig. 19) which are rather caused by an increase in the amount of oxygen consumed ($V'O_2$) (Fig. 17) relative to the increased amount of carbon dioxide produced ($V'CO_2$) (Fig. 18).

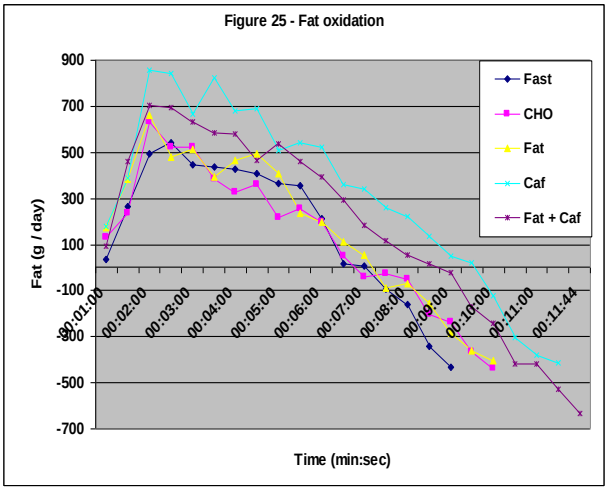
- As from exercise intensities ranging between 10km/h until voluntary fatigue sets in, the Fat and Caffeine trial (FC) show the lowest values for fat and the highest values for carbohydrate oxidation compared to any of the other interventions. This finding is accompanied by an increase in the $V'O_2$, $V'CO_2$, RER, VE and $V'O_{2peak}$ when compared to the corresponding values for any of the other interventions – see figures 17, 18, 19 and 22 respectively.

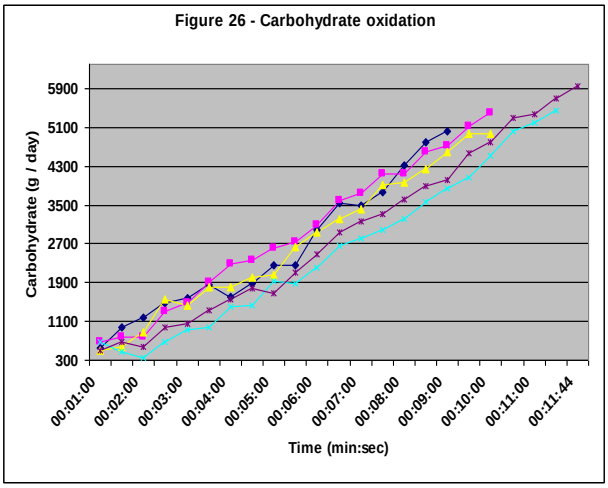
- The heart rate (HR) (Fig. 21) for the Fat trial (F) show higher values compared to the Fasting trial (Fa). No differences in the heart rate values were recorded between these interventions at the point of voluntary fatigue.

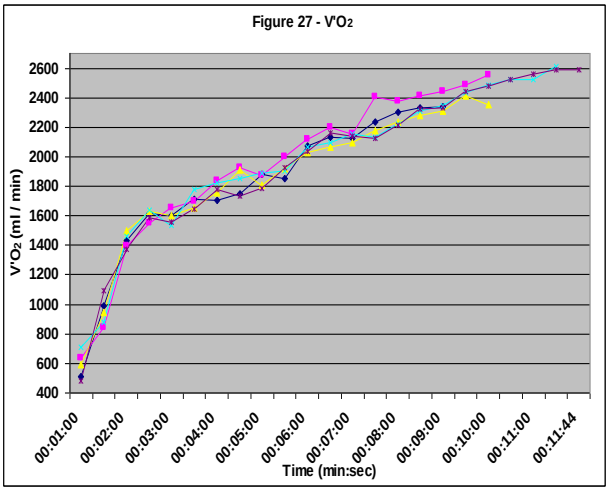
- For the Fat trial, no FCCP (Fig. 23) exist, whereas the FCCP for all other interventions occurred at a treadmill speed of 8 km/h–see Appendix D.

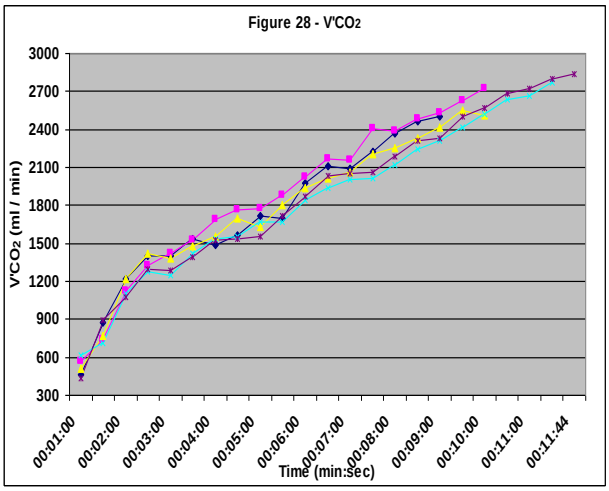
- When comparing the best and worst performance results (Fig. 24), the Fat trial (F) shows that the participant ran 1 minute longer at an intensity of 16 km/h and could continue an additional minute at 18 km/h compared to the Fasting trial (Fa)-see Appendix C.

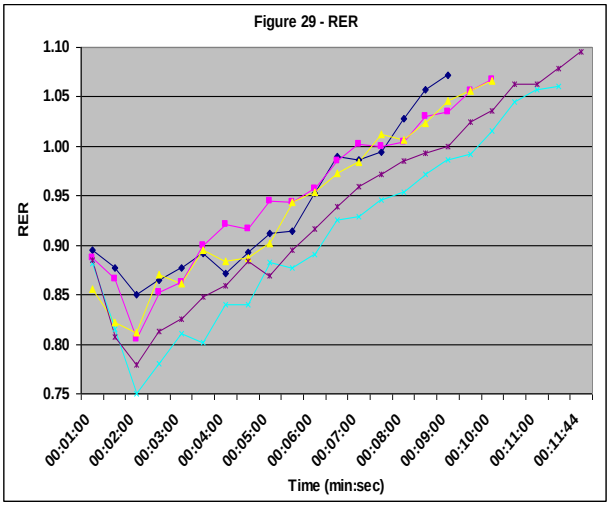
Trained individual 2

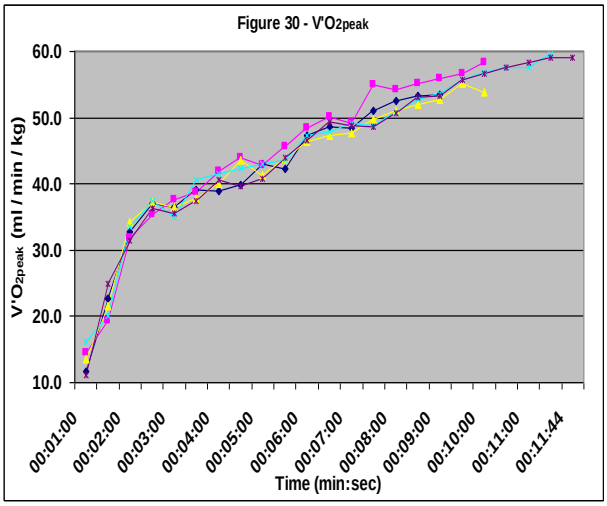


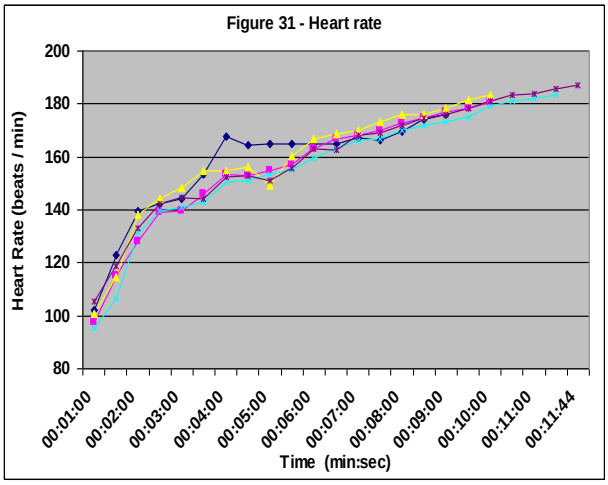


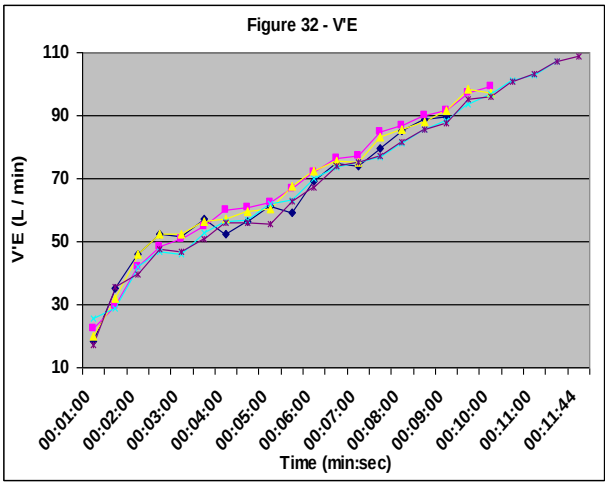


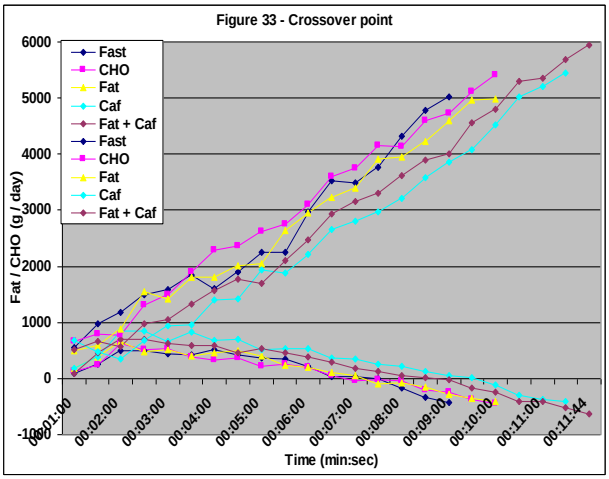


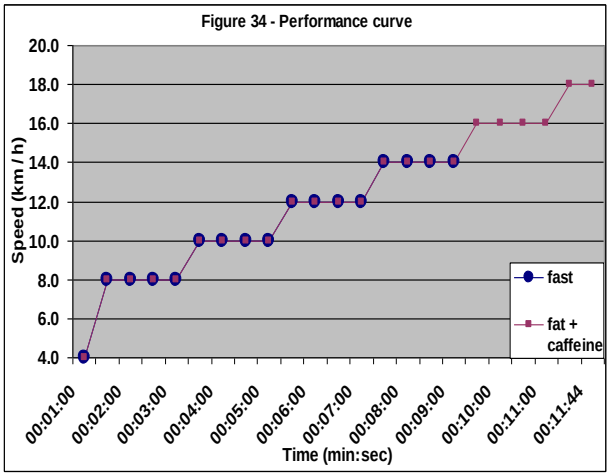












Figures 25-34: The compiled results in graphic format for the various interventions and their corresponding variables.

- Figures 25 and 26 respectively indicate that the Caffeine trial (C) shows the highest fat and lowest carbohydrate oxidation capacities at all exercise intensity levels (4-16km/h) compared to the Carbohydrate trial (Ch) which shows the lowest fat and highest carbohydrate oxidation capacities. Compared to the Carbohydrate trial (Ch), the Caffeine trial (C) coincided with a decrease in RER values (Fig. 29) which were rather caused by a larger decrease in the V'CO_2 (Fig. 28) relative to the decrease in the V'O_2 (Fig. 27).

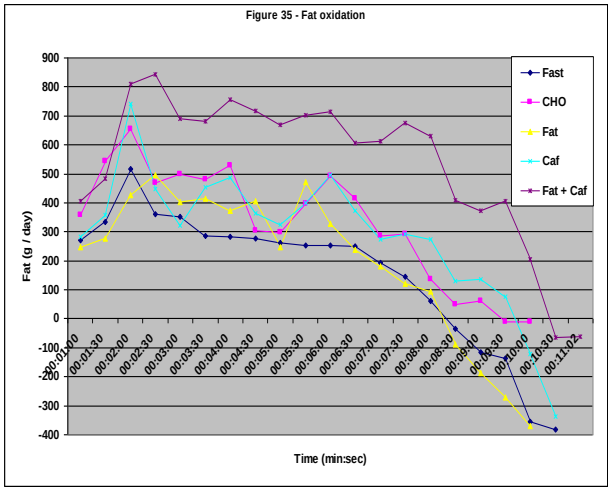
- This finding corresponds with minor or no distinct alterations in HR values (Fig. 31), with no differences in the heart rate values recorded at the point of

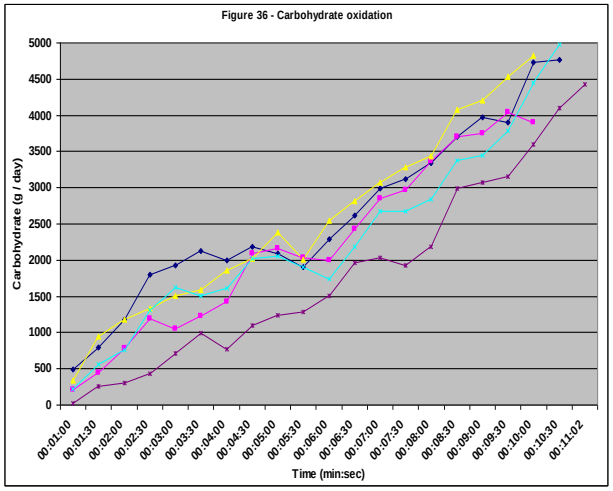
voluntary fatigue. Minor alterations are also visible in the V'O_{2peak} (Fig. 30) and VE (Fig. 32).

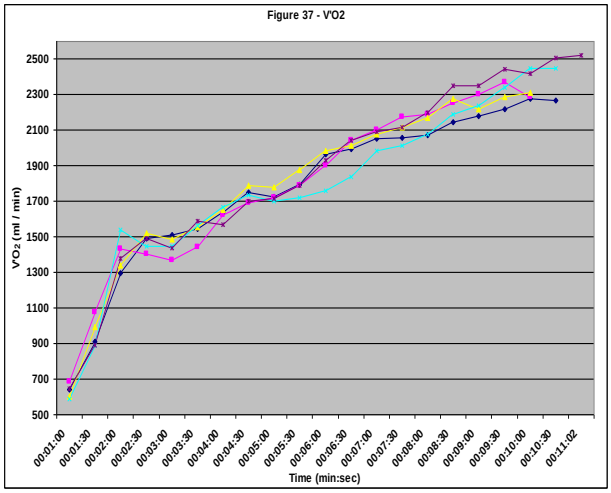
- Figure 33 show that no FCCP exist for the Fasting (Fa), Fat (F) and Carbohydrate (Ch) trials and exist at 8 km/h for the Caffeine (C) and Fat and Caffeine (FC) trials.

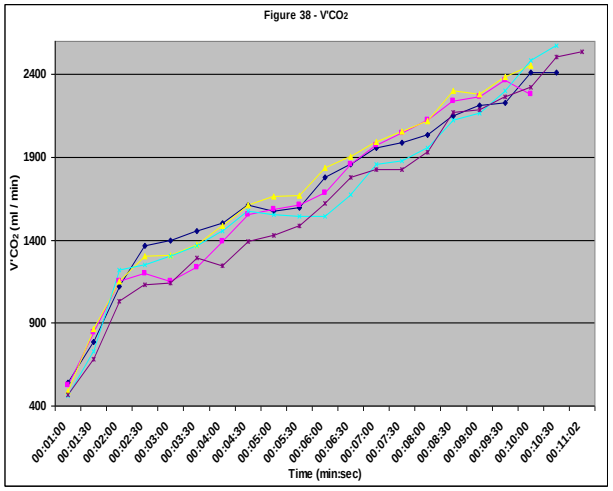
- When comparing the best to the worst result on performance the Fat and Caffeine trial (FC) shows the better result compared to the Fasting trial (Fig. 34). On the Fat and Caffeine trial (FC) the participant ran 19 seconds longer at an intensity of 14 km/h, continued an additional 120 seconds at 16 km/h and even continued for an additional 44 seconds at a treadmill speed of 18 km/h- see Appendix C.

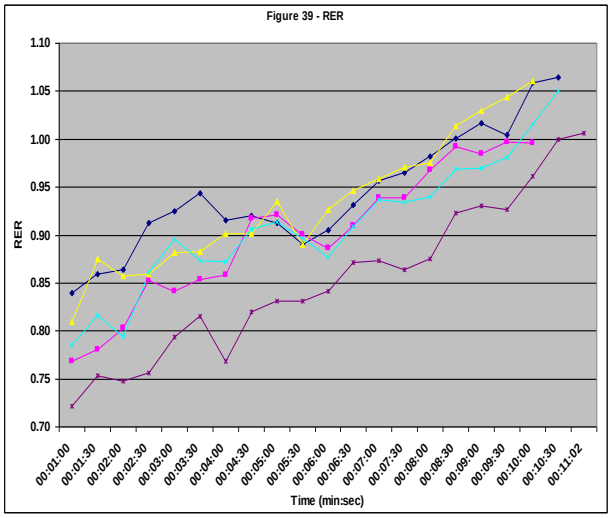
Trained individual 3

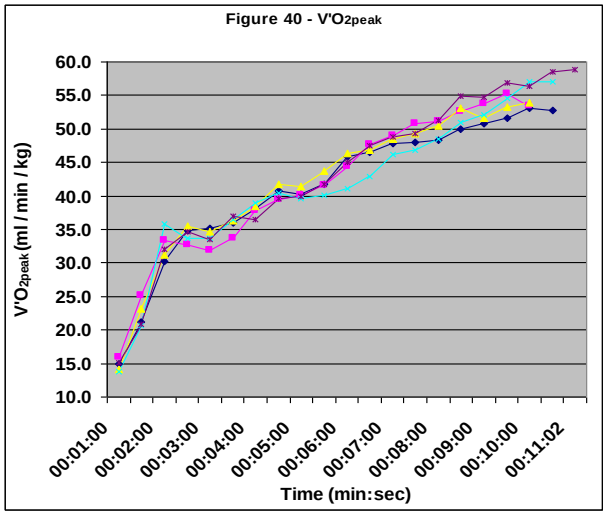


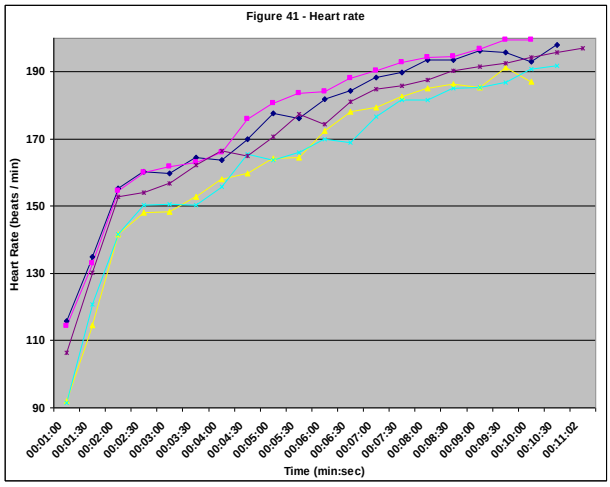


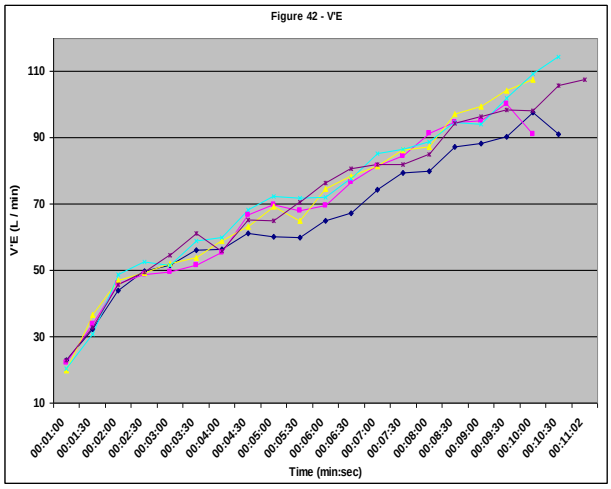


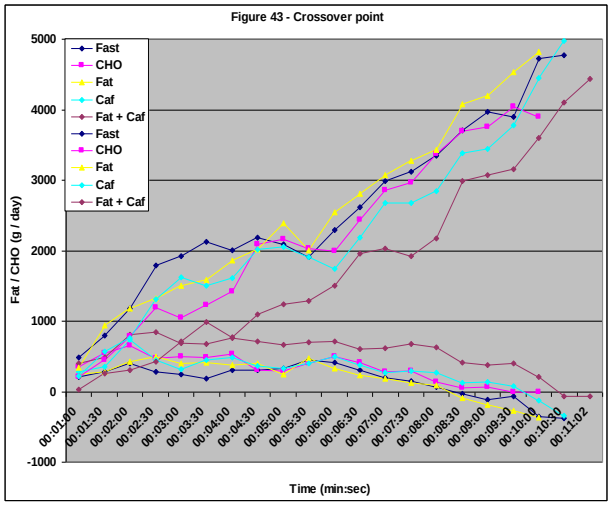


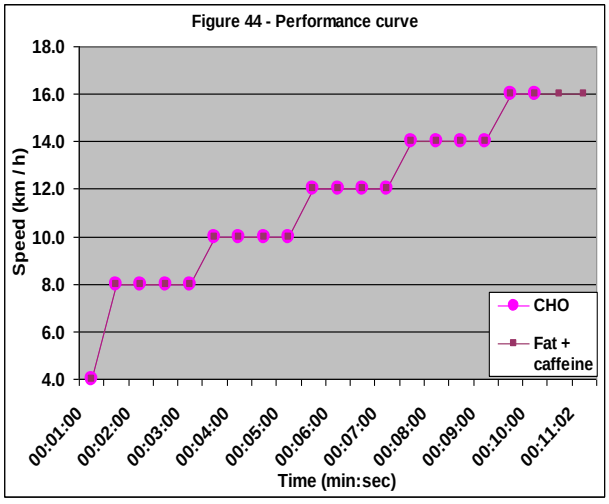












Figures 35-44: The compiled results in graphic format for the various interventions and their corresponding variables.

- Figures 35 and 36 respectively show that the Fat and Caffeine trial (FC) produced the highest fat and lowest carbohydrate oxidation capacities at all exercise intensity (4-16km/h) levels compared to the Fat (F) or Fasting (Fa) trials showing the lowest fat and highest carbohydrate oxidation capacities. The combination of Fat and Caffeine intake (FC) coincided with a decrease in the RER values (Fig. 39) which were rather caused by a decrease in the $V'CO_2$ values (Fig. 38) in combination with no distinct alterations in the $V'O_2$ values (Fig. 37) between these interventions.

- The highest HR values (Fig. 41) are recorded for the Carbohydrate trial (Ch) compared to Fat (F) and Caffeine (C) trials showing the lowest values throughout the trial run. At the point of voluntary fatigue the Carbohydrate trial (Ch) shows a higher HR value compared to the Fat trial (F).

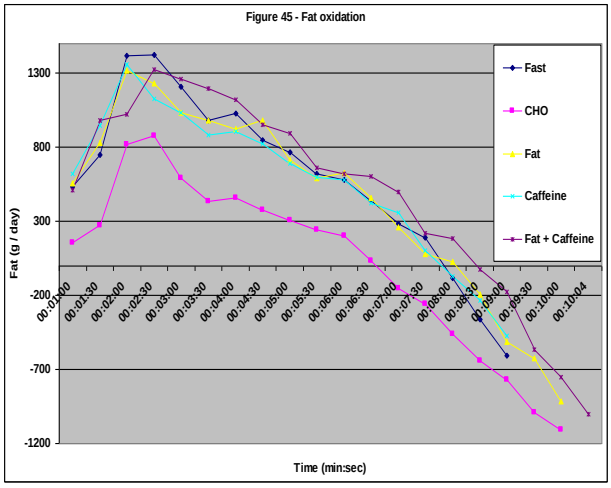
- The combination of Fat and Caffeine (FC) show a higher $V'O_{2peak}$ value (Fig. 40) compared to the Fasting (Fa), Fat (F) or Carbohydrate (Ch) trials.

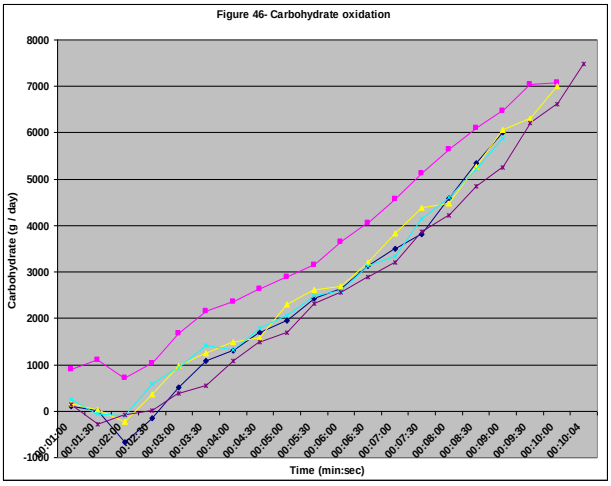
- The Fasting trial (Fa) coincides with a decrease in the V'E (Fig. 42) at all exercise intensity levels excluding exercise at a relatively low intensity (8km/h) level. The highest value obtained during the entire course of the exercise regimes recorded for the Caffeine trial (C) (114 L/min) can be compared to the lowest value being recorded for the Fasting (Fa) and Carbohydrate (Ch) trials (91L/min).

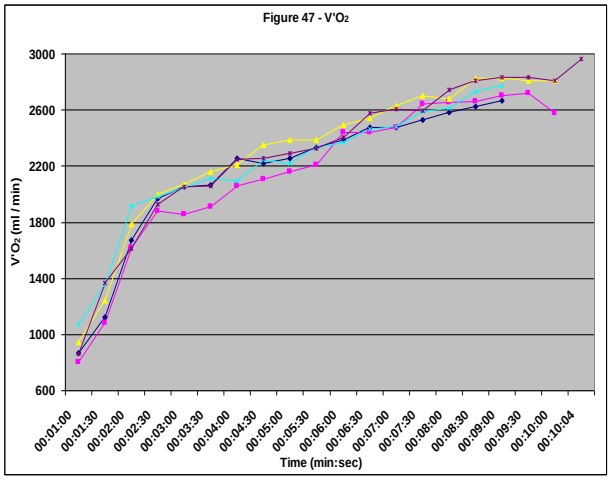
- No FCCP (Fig. 43) exist for the Fasting (Fa) and the Fat (F) trials, whereas the FCCP occur at 4 km/h for the Caffeine trial (C) and at 8 km/h for the Carbohydrate (Ch) and Fat and Caffeine (FC) trials.

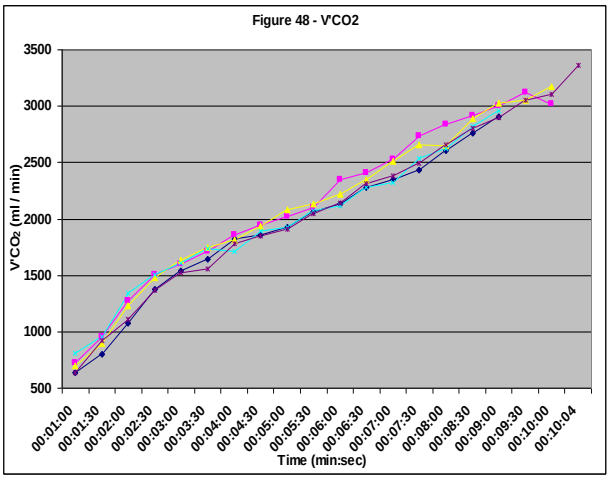
- On comparing the best and worst performance values (Fig. 44) the participant ran 82 seconds longer at an intensity of 16km/h on the Fat and Caffeine (FC) trial compared to the Carbohydrate trial-see Appendix C.

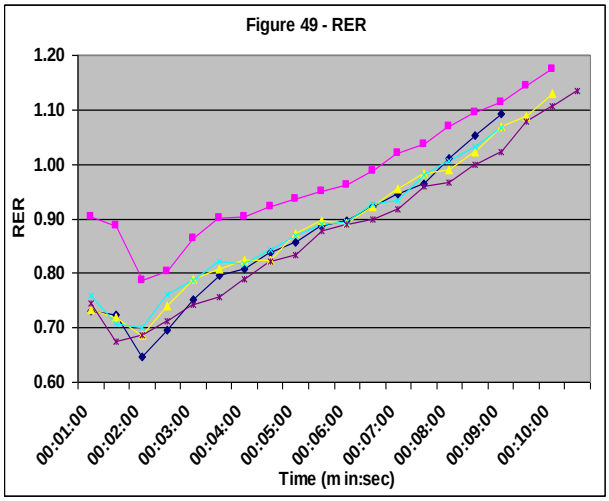
Trained individual 4

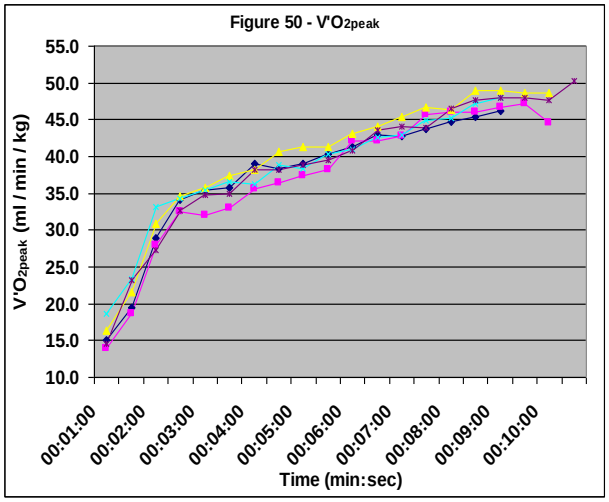


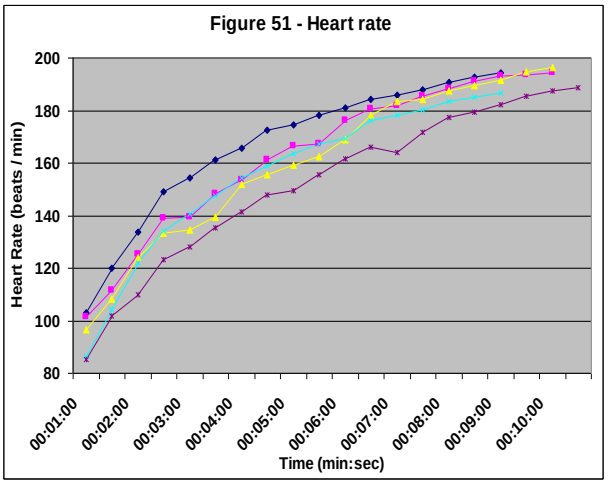


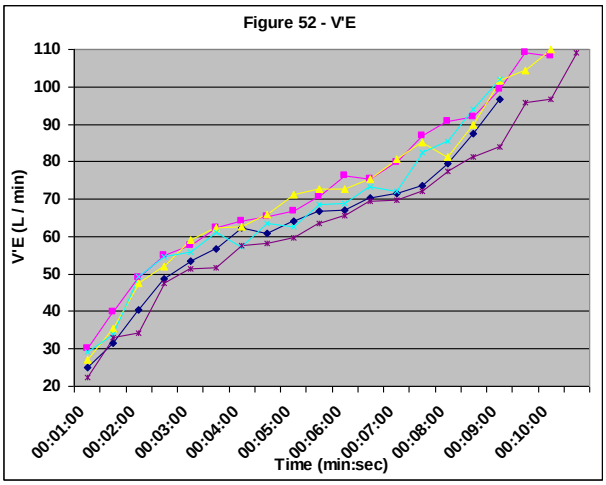


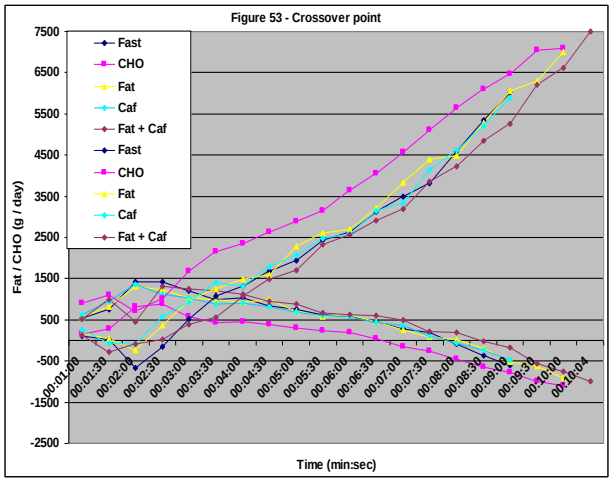


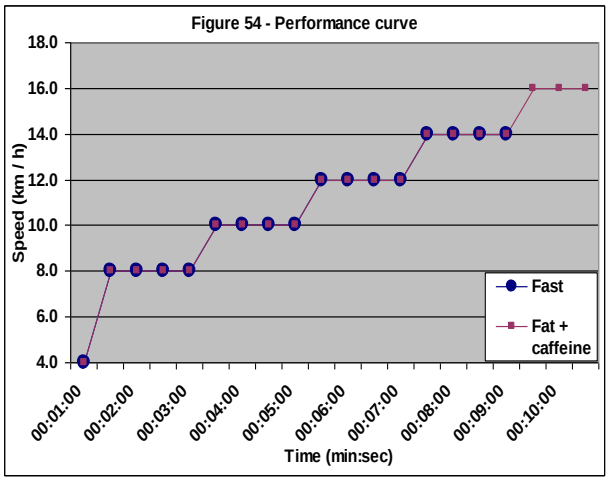












Figures 45-54: The compiled results in graphic format for the various interventions and their corresponding variables.

- Figures 45 and 46 respectively indicate that the Fasting (F) trial show the highest fat and lowest carbohydrate oxidation capacity values at low exercise intensity levels (4-8 km/h). Beyond 8 km/h the Fat and Caffeine trial (FC) shows the highest fat and lowest carbohydrate oxidation capacities compared to the Carbohydrate trial (Ch) showing the lowest fat oxidation and the highest carbohydrate oxidation capacity values throughout the entire course of the exercise regime.

- The $V'O_2$ (Fig. 47) and $V'CO_2$ (Fig. 48) are fairly constant between all interventions during the entire exercise regime.

- Throughout the entire course of the exercise regime the Carbohydrate trial (Ch) shows the highest RER values (Fig. 49) compared to any of the other interventions, with the Fat and Caffeine trial (FC) showing the lowest RER values compared to the other interventions.

- The Fat and Caffeine trial (FC) shows a trend (50.2/46.1=8.2%) towards a higher $V'O_{2peak}$ value (Fig. 50) compared to the Fasting trial (Fa) presenting the lowest $V'O_{2peak}$ value at maximum effort (16 km/h)-see Appendix C.

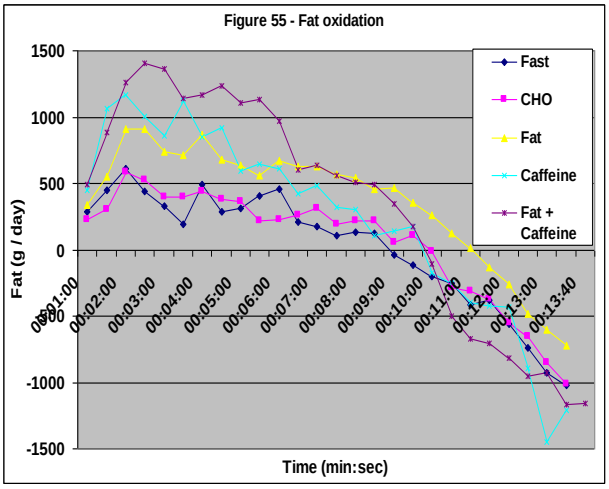
- Higher HR values (Fig. 51) are observed for the Fasting trial (Fa) compared to the Fat and Caffeine trial (FC) showing the lowest values throughout the entire course of the exercise regime. No differences in the HR values are recorded between these interventions at the point of voluntary fatigue.

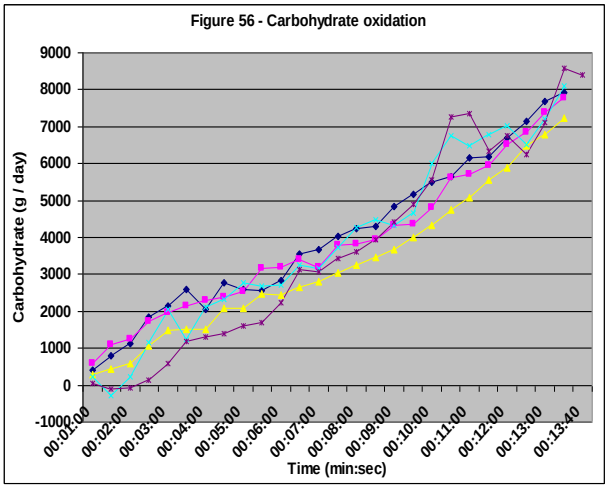
- Figure 52 reveals that the Fat and Caffeine trial (FC) show the lowest V'E values throughout the trial run compared to either the Carbohydrate (Ch) or the Fat (F) trial.

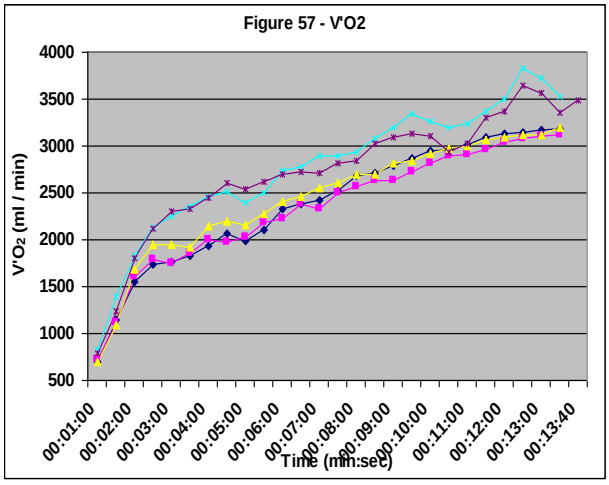
- The FCCP for the Fat (F), Carbohydrate (Ch) and Caffeine (C) trials occurred at 8 km/h whereas the FCCP occurred at 10 km/h for the Fasting (Fa) and Fat and Caffeine (FC) trials.

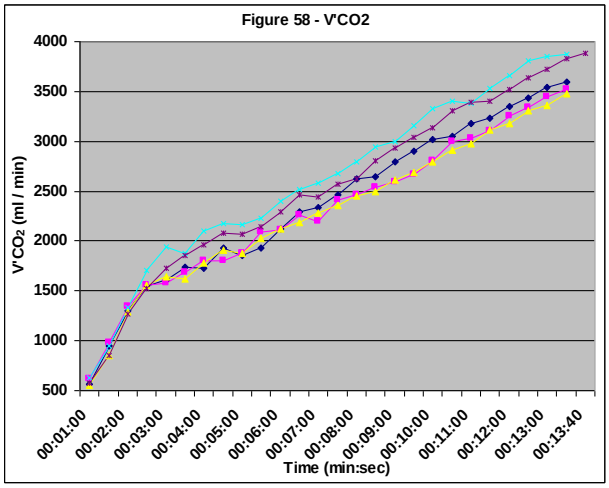
- On comparing the best and worst performance results (Fig. 54), the Fat and Caffeine trial (FC) enabled the participant to run 6 seconds longer at 14 km/h and 64 seconds longer at 16 km/h compared to the Fasting trial (F)-see Appendix C.

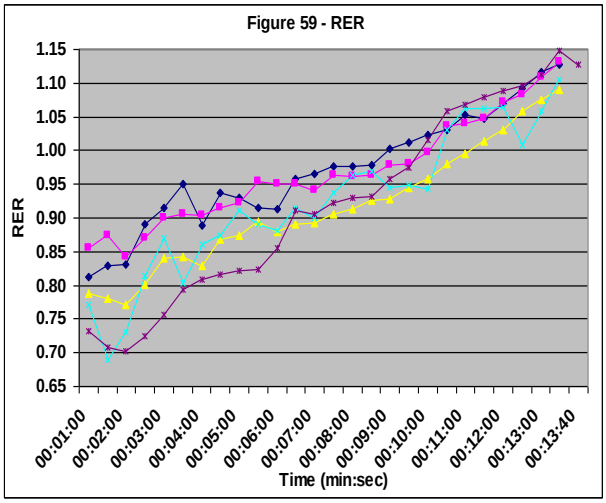
Trained individual 5

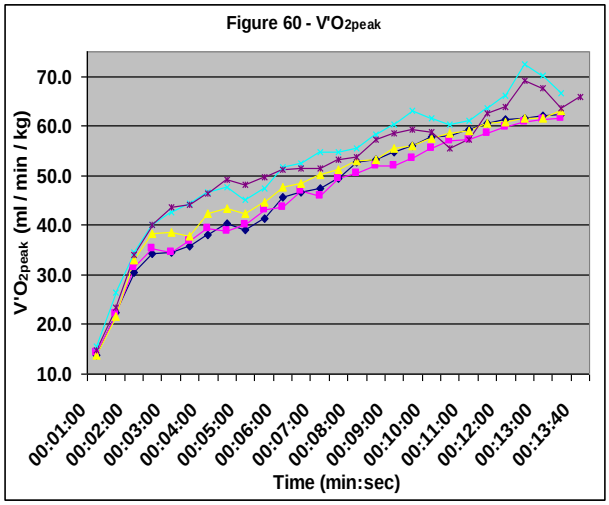


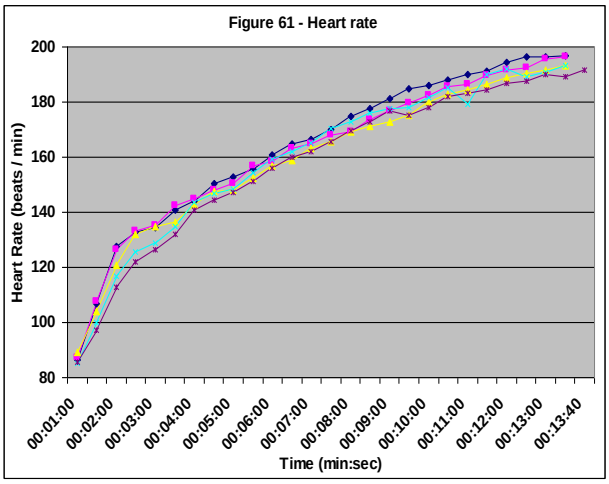


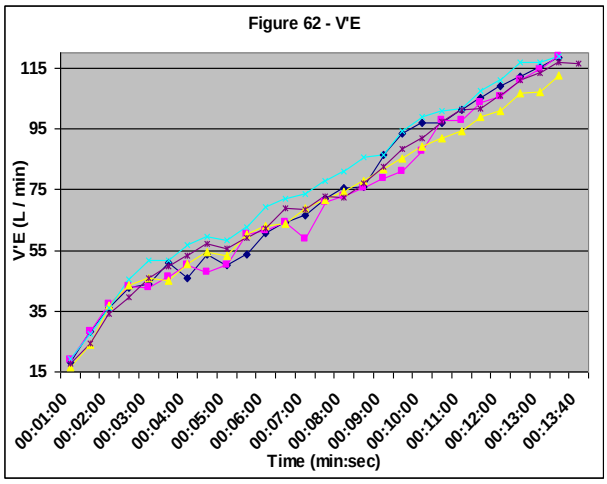


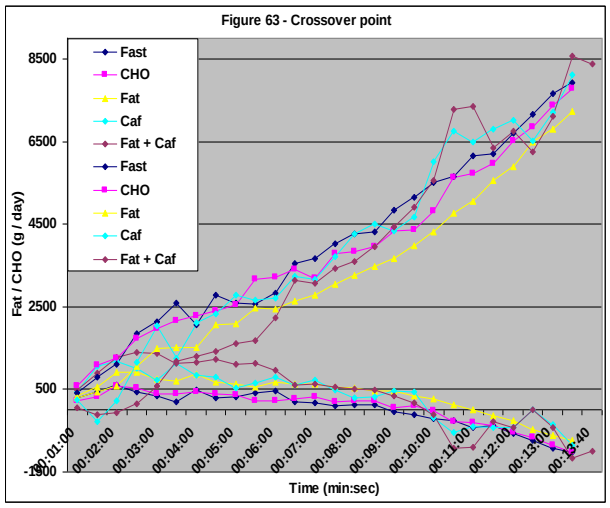


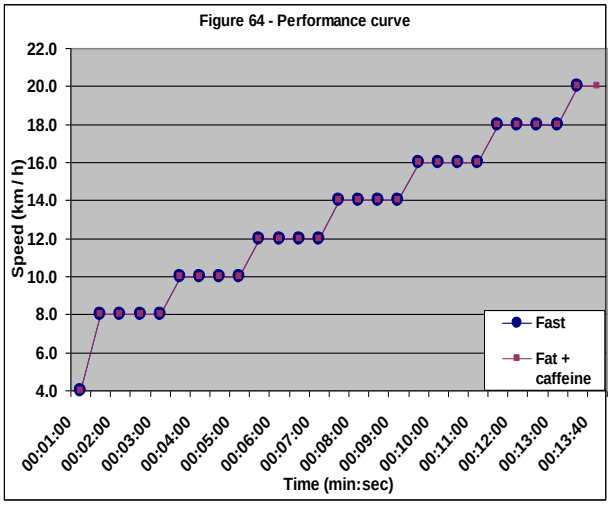












Figures 55-64: The compiled results in graphic format for the various interventions and their corresponding variables.

- The findings indicate that during lower exercise intensities (8-12 km/h) the Fat and Caffeine trial (FC) show the highest fat (Fig. 55) and lowest carbohydrate (Fig. 56) oxidation capacities compared to either the Carbohydrate (Ch) or Fasting (Fa) trials showing the lowest fat oxidation and the highest carbohydrate oxidation capacities. At this exercise intensity level the Caffeine trial (C) also shows the lowest RER values (Fig. 59) compared to either the Fasting (Fa) or Carbohydrate (Ch) trials.

- The Fat trial (F) shows increased levels of fat oxidation coinciding with a decrease in carbohydrate oxidation compared to the Fat and Caffeine trial (FC)

at exercise intensities beyond 12km/h. At an exercise intensity of 14 km/h the Fasting trial (F) and at 16 km/h the Fat and Caffeine trial (FC) show the highest RER values compared to the Fat trial (Fig. 59).

- Throughout the entire course of the exercise regime the Caffeine (C) as well as the Caffeine and Fat (FC) trials shows increased levels of oxygen consumption values (Fig. 57) compared to any of the other interventions. The same notion prevails for carbon dioxide production (Fig. 58).

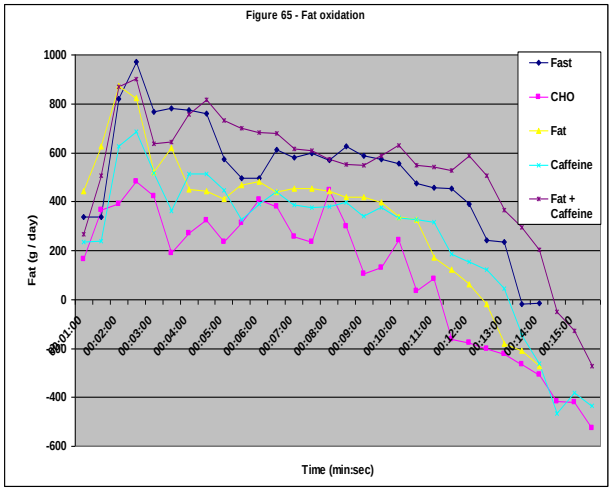
- The Caffeine trial (C) shows the highest $V'O_{2peak}$ value (Fig. 60) compared to any of the other interventions excluding the Fat and Caffeine trial (FC).

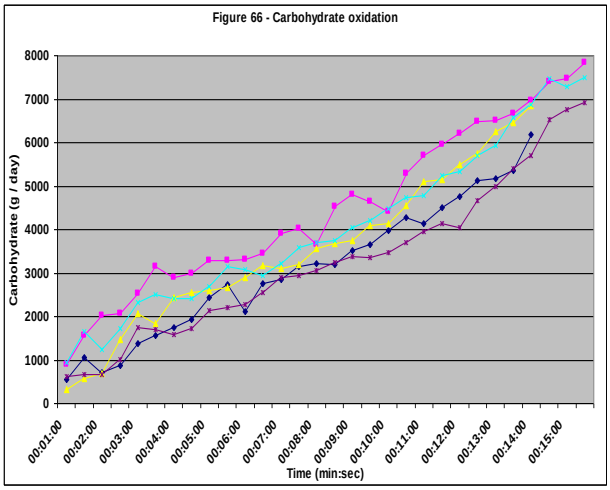
- Minor alterations are observed for HR (Fig. 61) and V'E (Fig. 62) for all the interventions throughout the entire exercise regime.

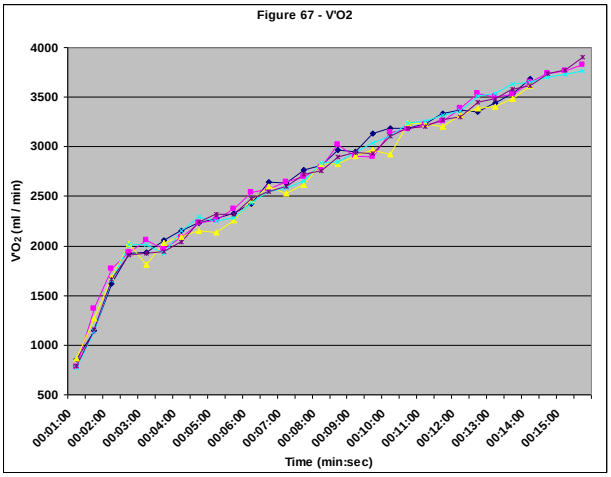
- There exist no FCCP (Fig. 63) for the Fasting (Fa) and Carbohydrate (Ch) trials, whereas a FCCP exist at 8 km/h for the Fat trial (F) and at 10 km/h for the Caffeine (C) and Fat and Caffeine (FC) trials.

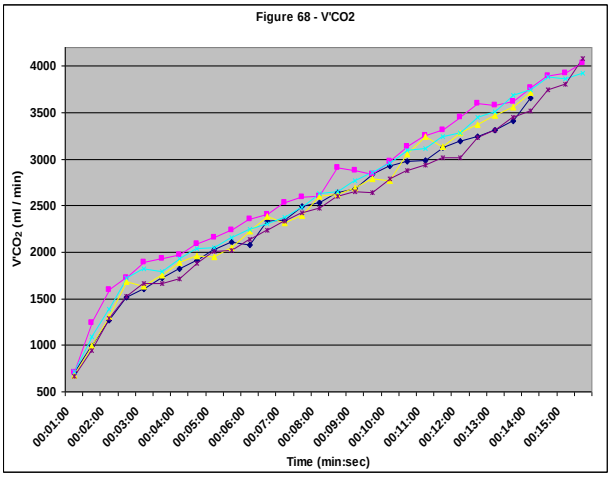
- On comparing the best (FC) to the worst (Fa) result on performance the participant ran 31 seconds longer at an intensity of 20km/h (Fig. 64)-see Appendix C.

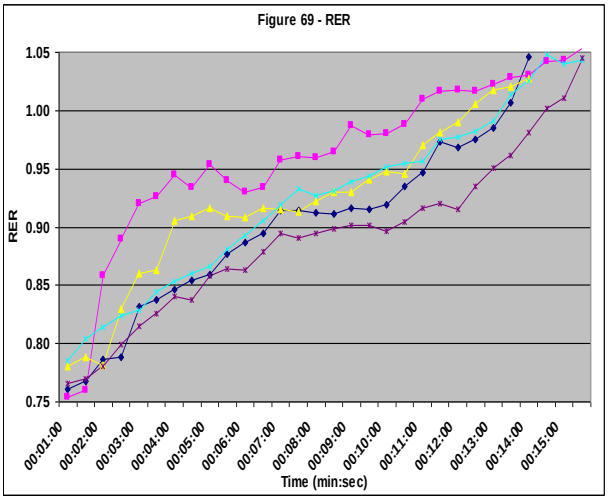
Trained individual 6

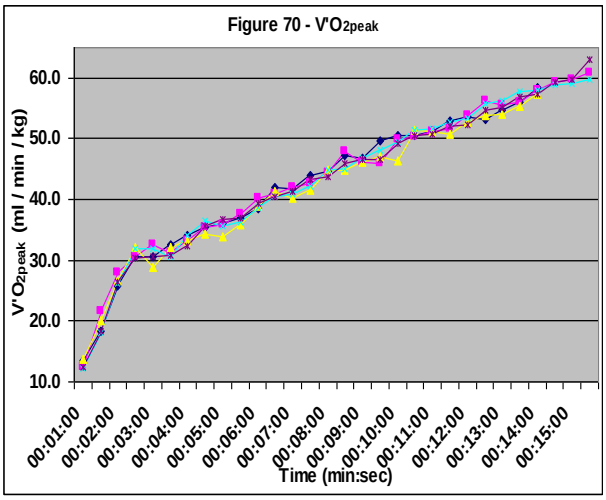


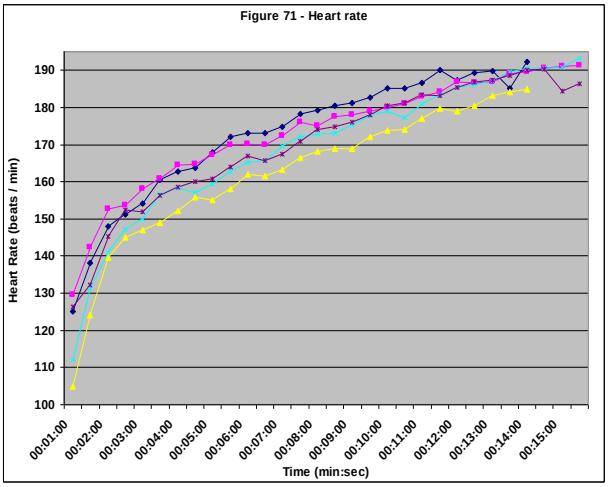


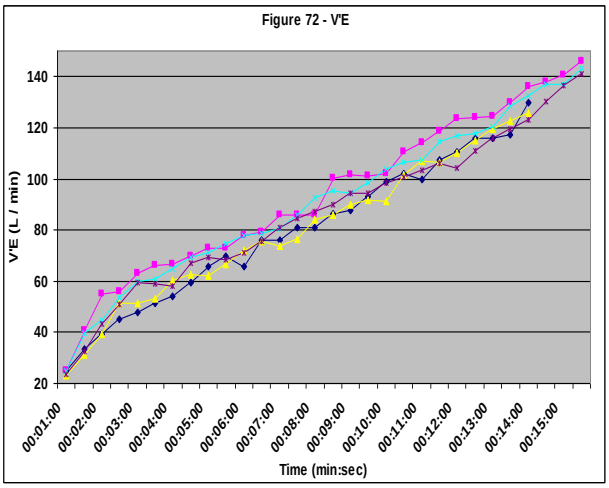


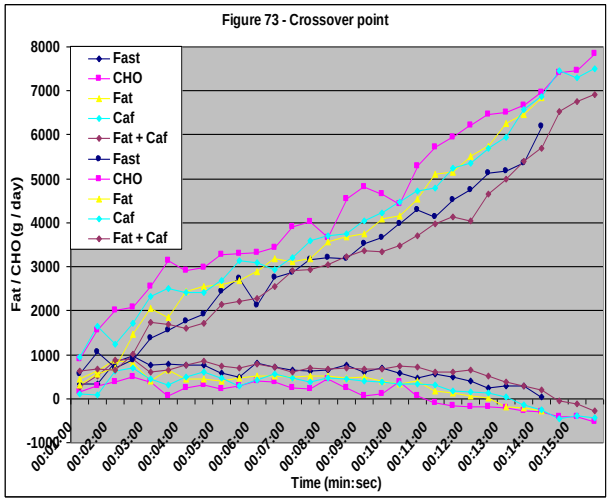


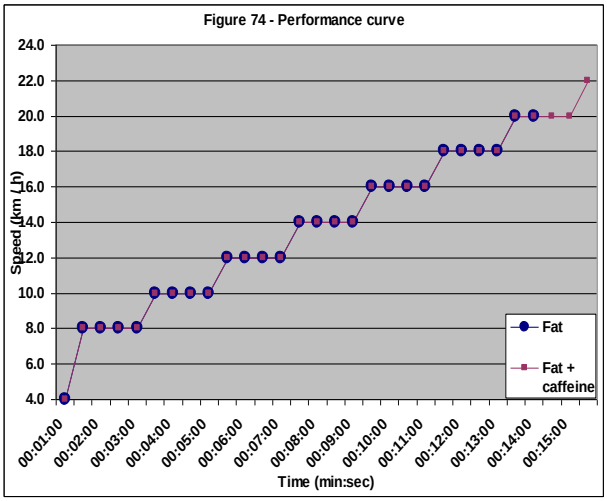












Figures 65-74: The compiled results in graphic format for the various interventions and their corresponding variables.

- The findings reveal that the Fat and Caffeine (FC) as well as the Fasting (Fa) trials show the highest fat (Fig. 65) and lowest carbohydrate (Fig. 66) oxidation capacity values compared to the Carbohydrate trial (Ch), which shows the lowest fat oxidation and the highest carbohydrate oxidation capacity values.

- The $V'O_2$ (Fig. 67) shows fairly constant values and the $V'CO_2$ (Fig. 68) shows minor alterations in the specific values during the entire exercise regime. Throughout the entire course of the exercise regime the Carbohydrate trial (Ch) shows the highest RER values (Fig. 69) compared to any of the other

interventions, with the Fat and Caffeine trial (FC) showing the lowest RER values.

- Minor differences in the $V'O_{2peak}$ are observed between any of the interventions throughout the entire course of the exercise regime (Fig. 70), although the Fat and Caffeine trial (FC) produced the highest $V'O_{2peak}$ value.

- Figure 71 indicates that the Fasting trial (Fa) shows the highest HR values compared to the Fat trial (F), which shows the lowest values throughout the trial run. No differences in the heart rate values are recorded between these interventions at the point of voluntary fatigue.

- Minor alterations are observed for V'E (Fig. 72) throughout the trial run.

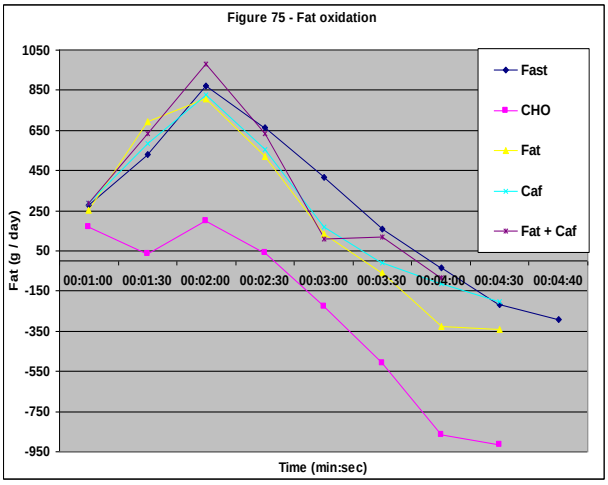
- The FCCP (Fig. 73) does not exist for the Caffeine (C) and Carbohydrate (Ch) trials and occurs at 8 km/h for all the other interventions.

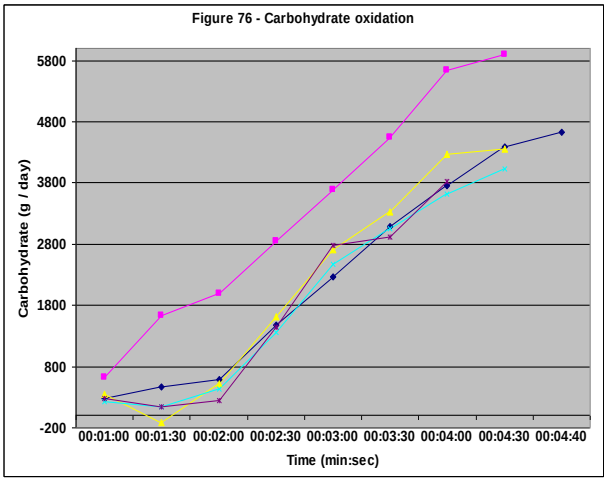
- When comparing the best and worst performance results, the Fat and Caffeine trial (FC) shows the better result compared to the Fasting trial (Fa)-see fig. 74. On the Fat and Caffeine trial (FC) the participant ran 72 seconds longer at an intensity of 20km/h and could continue for an additional 30 seconds at a treadmill speed of 22km/h-see Appendix C.

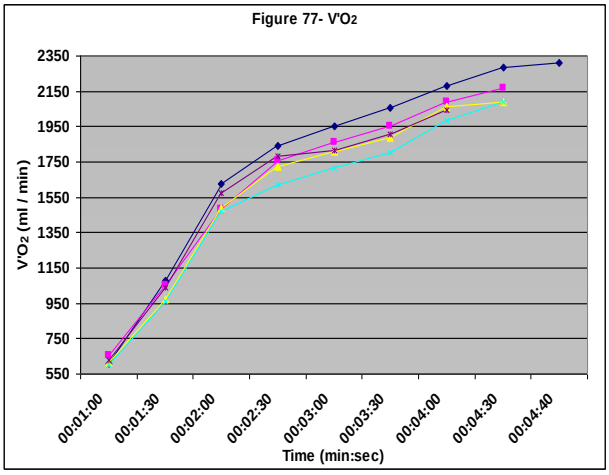
4.4.2. Untrained subjects

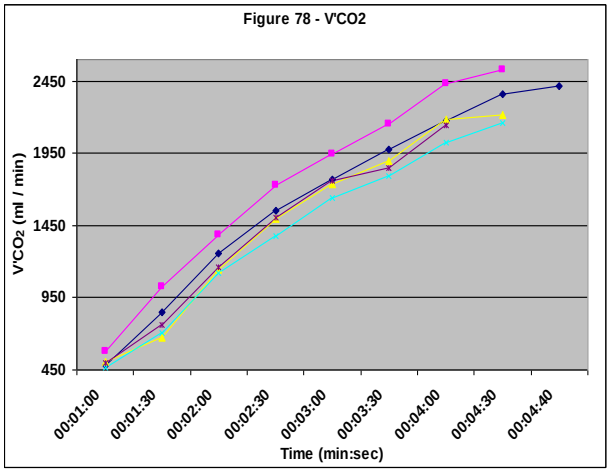
The following graphs show data from the group of untrained subjects.

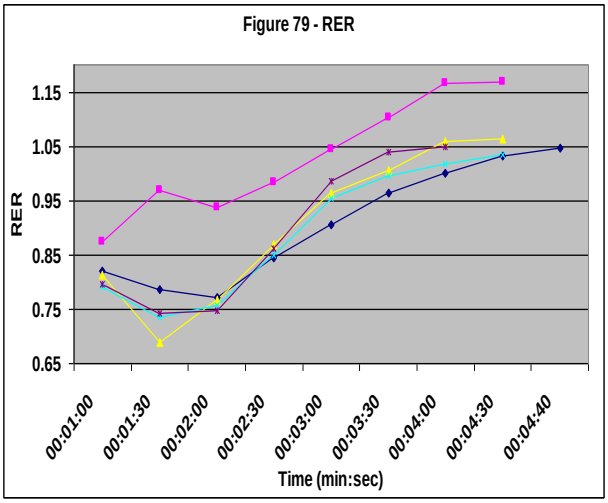
Untrained individual 1

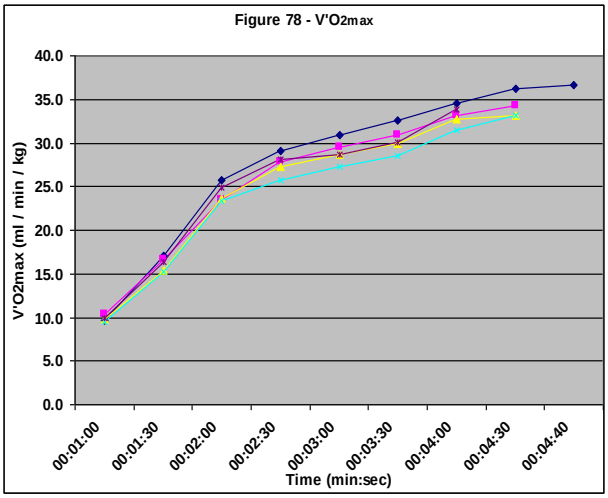


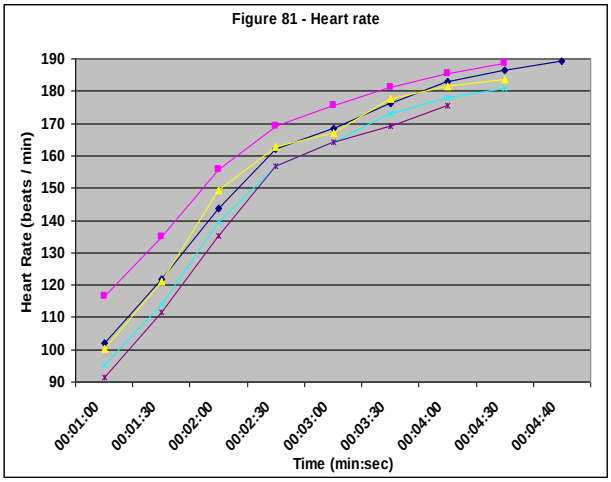


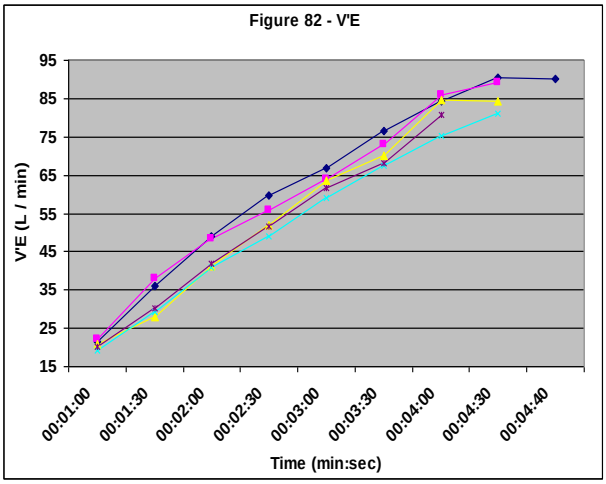


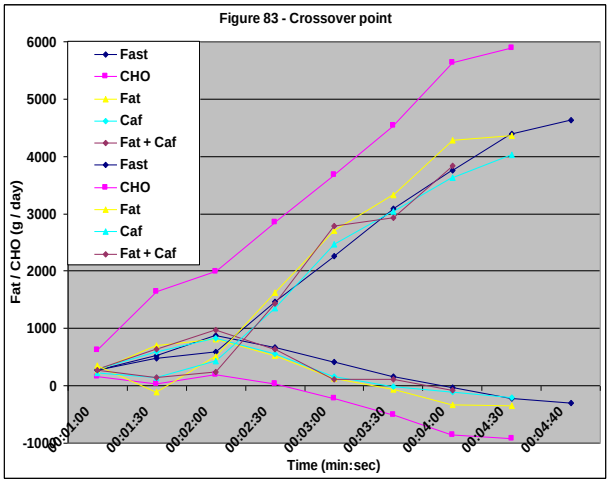


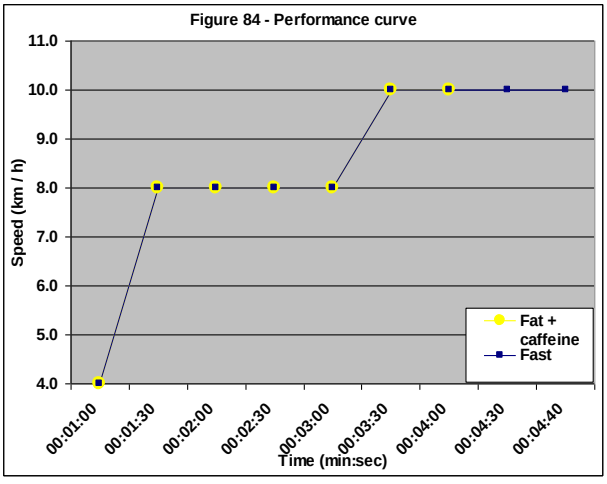












Figures 75-84: The compiled results in graphic format for the various interventions and their corresponding variables.

- Figures 75 and 76 respectively indicate that the Carbohydrate trial (Ch) shows the lowest fat and the highest carbohydrate oxidation capacity values compared to any of the other interventions during the entire course of the exercise regime.

- The Fasting trial (Fa) shows the highest and the Caffeine trial (C) the lowest oxygen consumption capacity values ($CV'O_2$) beyond an exercise intensity of 8 km/h until voluntary fatigue sets in-see fig. 77. The Carbohydrate trial (Ch) shows the highest and the Caffeine trial (C) the lowest carbon dioxide production ($CV'CO_2$) capacity values (Fig. 78) throughout the entire exercise regime.

- Figure 79 shows that the Carbohydrate trial (Ch) produced the highest RER values throughout the entire course of the exercise regime compared to any of the other interventions.

- The highest $V'O_{2peak}$ (Fig. 80) value is evident in the Fasting trial (Fa) compared to any of the other interventions.

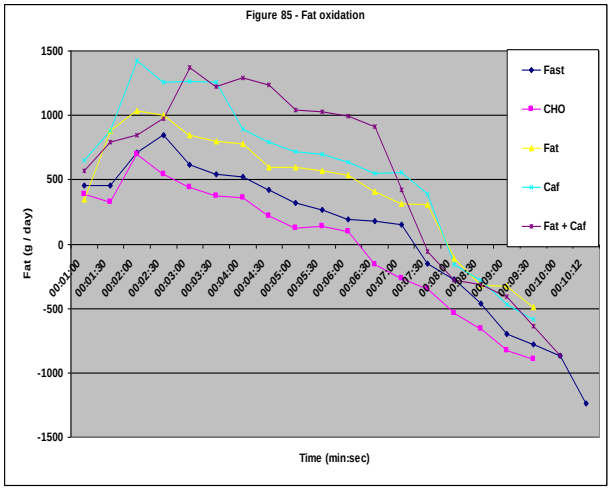
- The Carbohydrate trial (Ch) coincided with higher HR values (Fig. 81) being recorded compared to the Fat and Caffeine trial (FC), which shows the lowest values throughout the entire exercise regime. The largest difference in the maximum heart rate values recorded (point of voluntary fatigue) lies between the Fasting (Fa) and Fat and Caffeine (FC) trials-189 vs. 175 bpm respectively-see Appendix C.

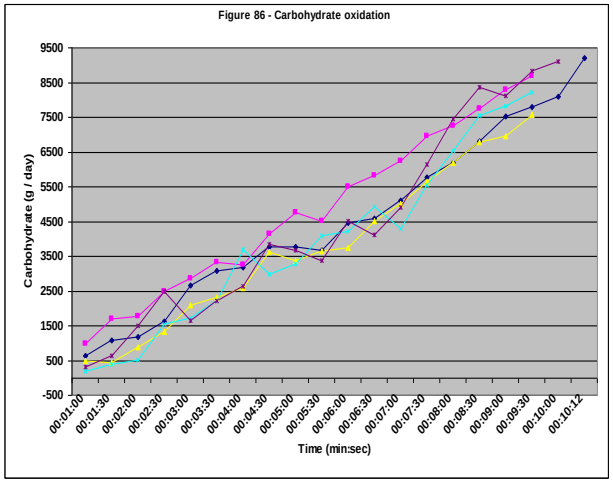
- Figure 82 indicates that the Fasting trial (Fa) show the highest V'E values, with the Caffeine (C) trial showing the lowest values throughout the entire exercise regime.

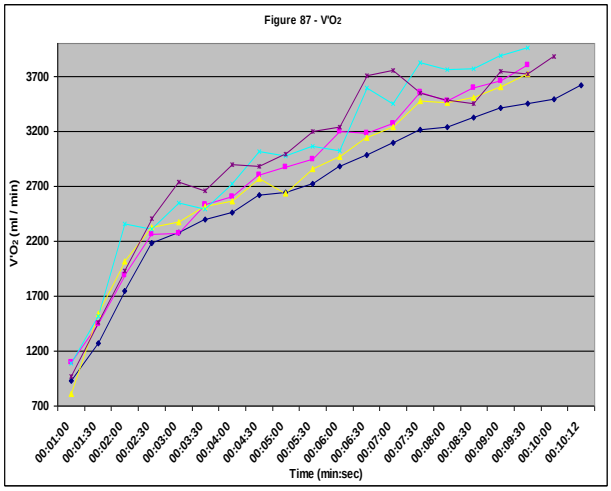
- There is no FCCP (Fig. 83) for the Carbohydrate trial (Ch). The FCCP for the Fasting trial (Fa) occurred at 4 km/h, whereas all of the other interventions occur at 8 km/h.

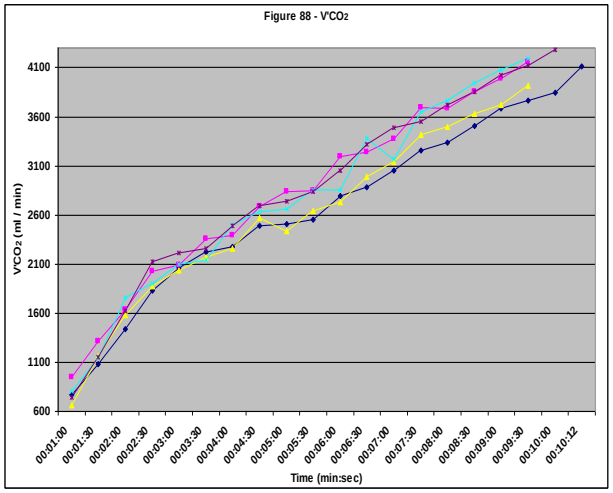
- When comparing the best and worst performance results, the Fasting trial (Fa) shows the better result compared to the Fat and Caffeine trial (FC)-see figure 84. On the Fasting trial the participant ran 47 seconds longer at an intensity of 10km/h compared to the Fat and Caffeine trial-see Appendix C.

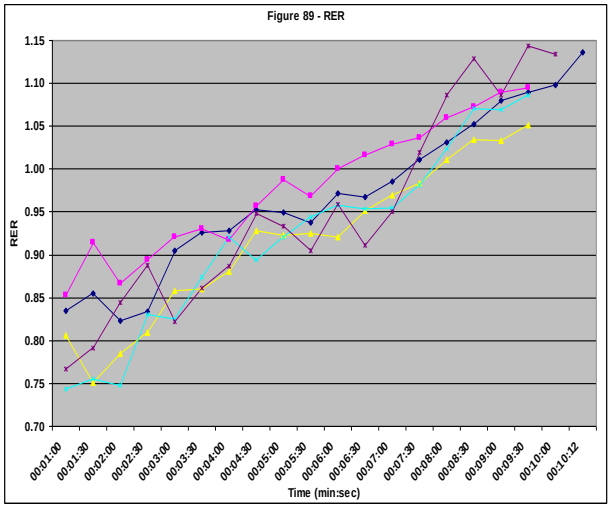
Untrained individual 2

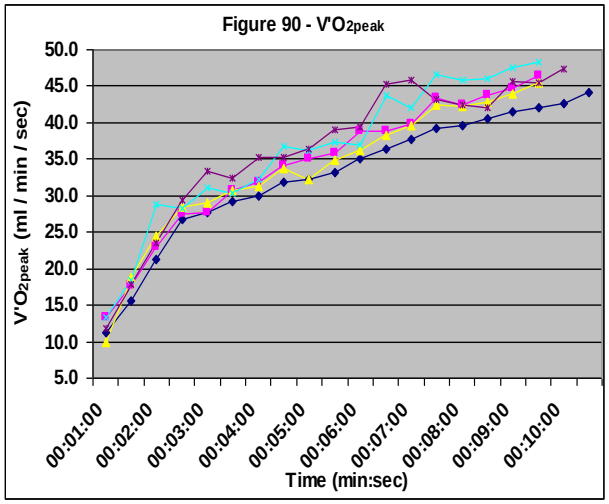


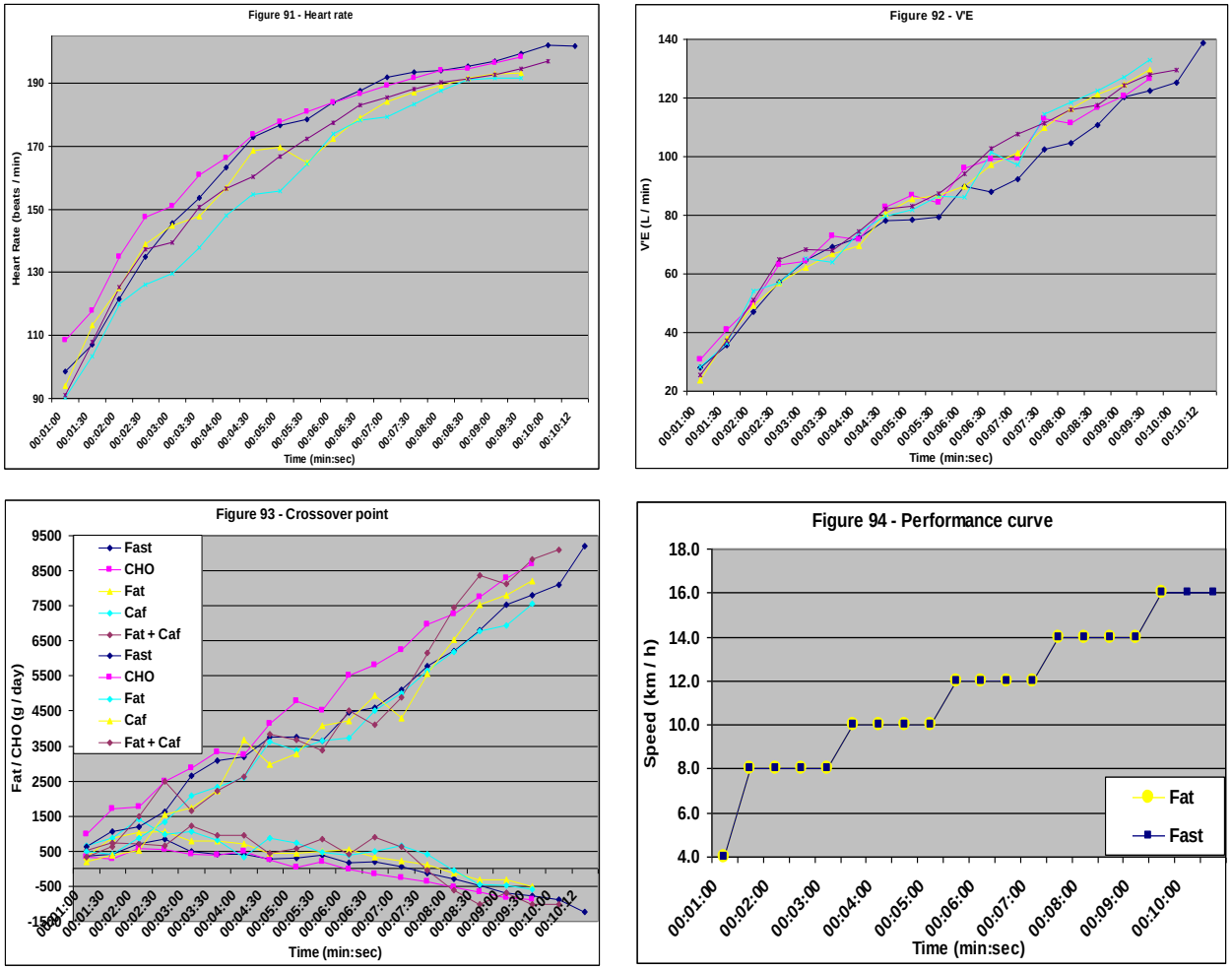












Figures 85-94: The compiled results in graphic format for the various interventions and their corresponding variables.

- Figures 85 and 86 respectively indicate that the Carbohydrate trial (Ch) shows the lowest fat and the highest carbohydrate oxidation capacity values compared to any of the other interventions throughout the entire exercise regime.

- The Fasting trial (Fa) shows the lowest oxygen consumption capacity values ($CV'O_2$) beyond an exercise intensity of 8 km/h compared to any of the other interventions (see Fig. 87). Similar findings are recorded for the carbon dioxide production capacity ($CV'CO_2$) values (Fig. 88) beyond 12 km/h.

- The RER values fluctuate throughout the entire trial between the various interventions (Fig. 89).

- The highest $V'O_{2peak}$ value (Fig. 90) is evident in the Caffeine trial (C) compared to the lowest $V'O_{2peak}$ value recorded for the Fasting trial (Fa).

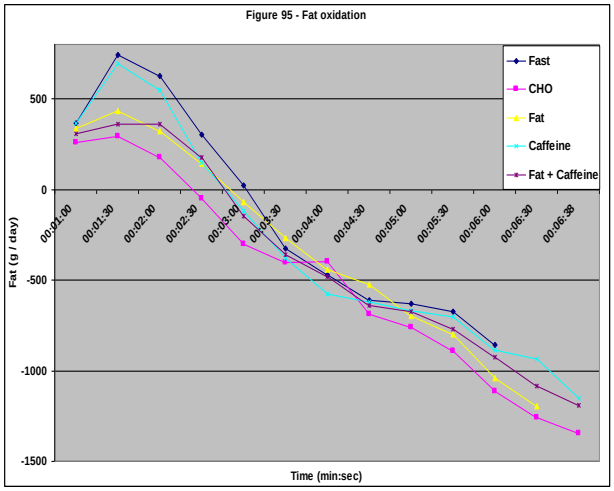
- The Carbohydrate (Ch) and Fasting (Fa) trials show higher HR values (Fig. 91) compared to the Caffeine trial (C), which shows the lowest values throughout the entire exercise regime. A difference in the HR values is recorded between these interventions at the point of voluntary fatigue.

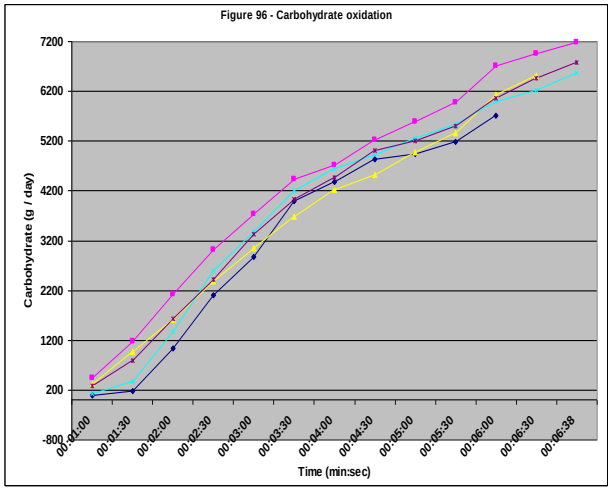
- Minor fluctuations are observed for V'E (Fig. 92) between the various interventions, but a tendency towards a decrease exists in the Fasting trial (Fa) at exercise intensities in the range of 12km/h.

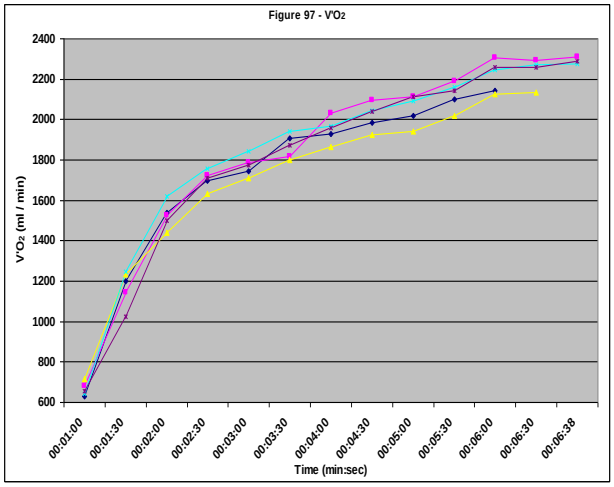
- There exist no FCCP (Fig. 93) for the Fasting (Fa) and Carbohydrate (Ch) trials and exist at 8 km/h for the other interventions.

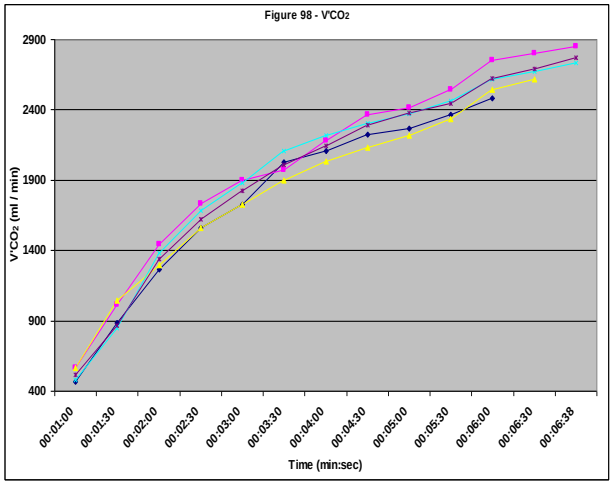
- When comparing the best and worst performance results, the Fasting trial (Fa) shows the better result compared to the Fat trial (F)-see figure 94. In the Fasting trial the participant ran 58 seconds longer at an intensity of 16km/h-before the onset of voluntary fatigue Appendix C.

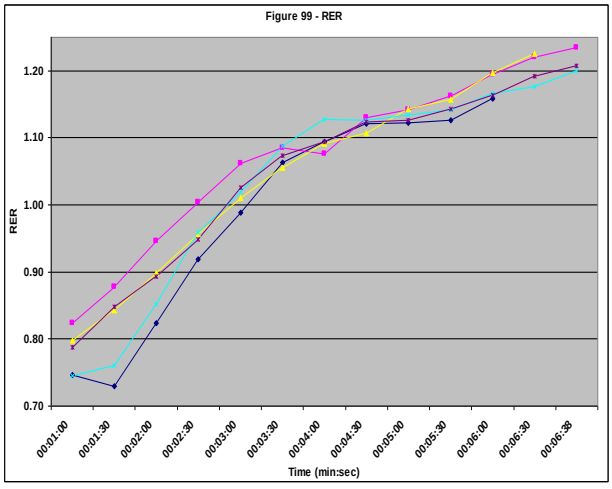
Untrained individual 3

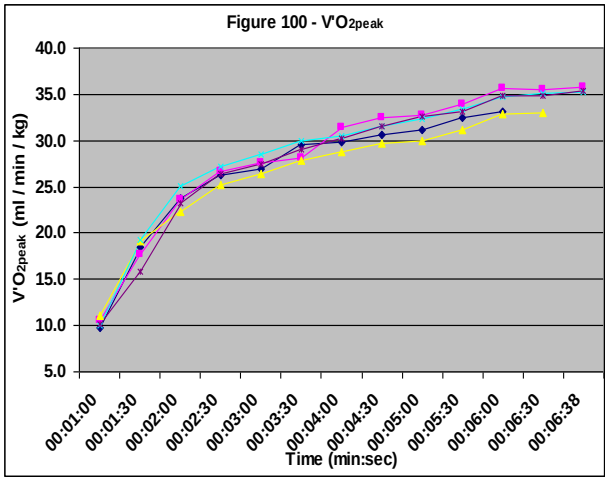


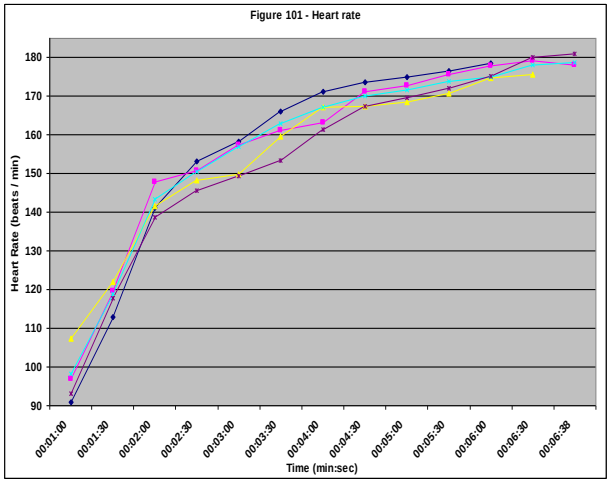


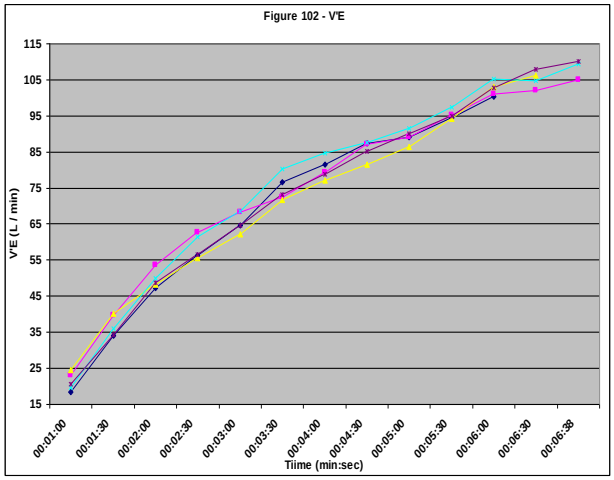


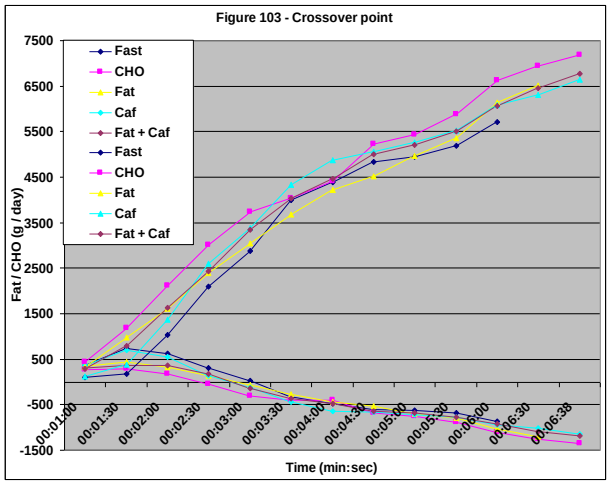


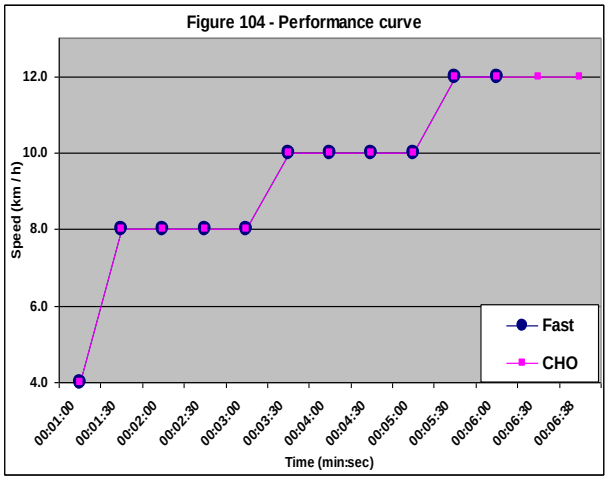












Figures 95-104: The compiled results in graphic format for the various interventions and their corresponding variables.

- Figures 95 and 96 respectively indicate that the Fasting trial (Fa) shows the highest fat and the lowest carbohydrate oxidation capacity values compared to the Carbohydrate trial (Ch) during exercise intensities ranging between 4-8 km/h. The Carbohydrate trial (Ch) shows the highest carbohydrate oxidation capacity values compared to the any of the other interventions.

- The Fat trial (F) showed the lowest oxygen consumption capacity ($CV'O_2$) values (Fig. 97) beyond an exercise intensity of 8 km/h compared to any of the other interventions. The same notion applies for the carbon dioxide production

capacity ($CV'CO_2$) values (Fig. 98) recorded for the various interventions beyond 8 km/h.

- During low exercise intensities (<10 km/h) the RER values for the Fasting trial (Fa) are lower compared to the Carbohydrate trial (Ch). Beyond 10km/h the RER values shows minor fluctuations between the various interventions -see Figure 99.

- The lowest $V'O_{2peak}$ (Fig. 100) values are observed for the Fat (F) and Fasting (Fa) trials compared to the higher values observed for the other interventions.

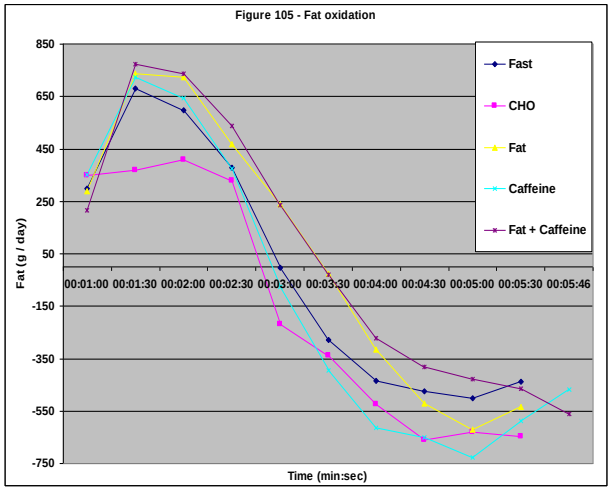
- The Fat and Caffeine trial (FC) shows lower HR values (Fig. 101) during exercise intensities in the range of 8-10 km/h compared to the Fasting (Fa) or Caffeine (C) trials. No differences in the heart rate values are recorded between these interventions at the point of voluntary fatigue.

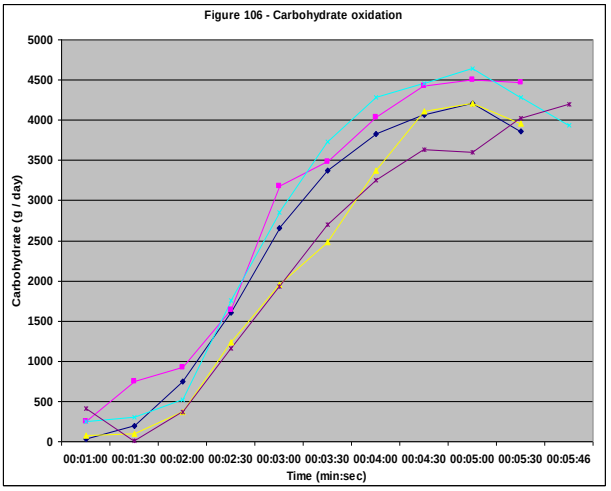
- Minor fluctuations are observed for V'E (Fig. 102) between the various interventions but a trend towards a decrease is observed in the Fat trial (F) compared to the Caffeine trial (C) at exercise intensities in the range of 10-12km/h.

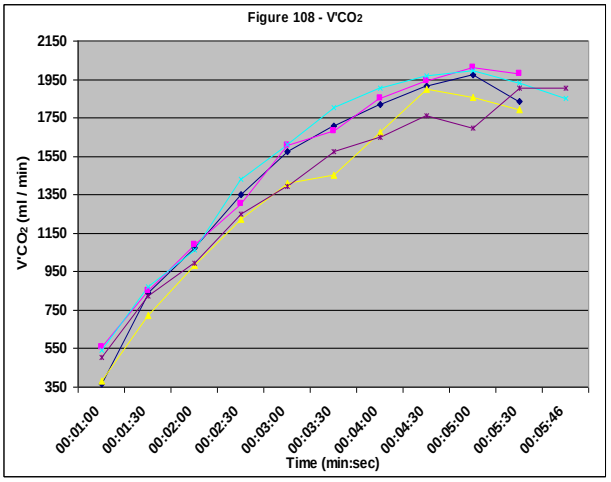
- There exist no FCCP (Fig. 103) for the Fat (F), Carbohydrate (Ch) and Fat and Caffeine (FC) trials and occurs at 8 km/h for the Fasting (Fa) and Caffeine (C) trials.

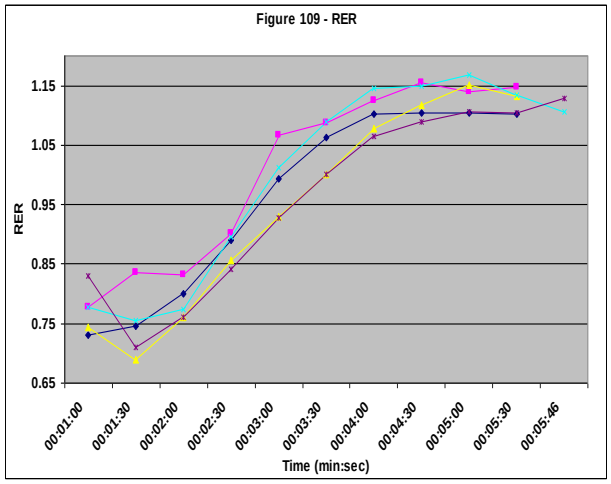
- When comparing the best and worst performance results (Fig. 104), the Carbohydrate trial (Ch) shows the better result compared to the Fasting trial (Fa). On the Carbohydrate trial (Ch) the participant ran 37 seconds longer at an intensity of 12km/h- see Appendix C.

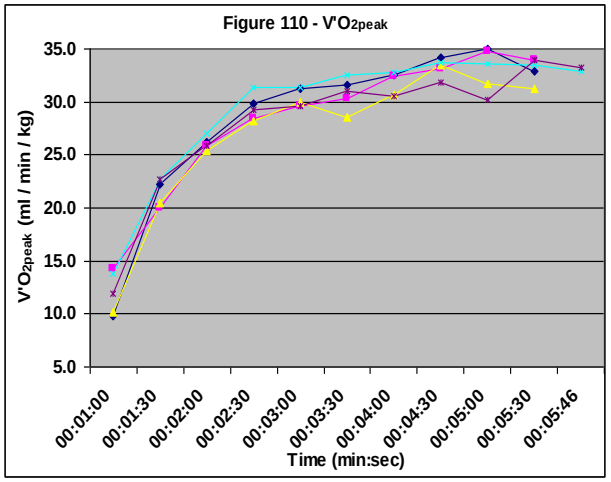
Untrained individual 4

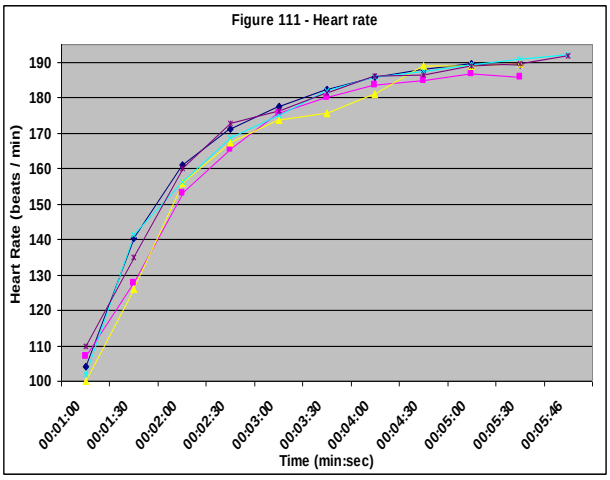


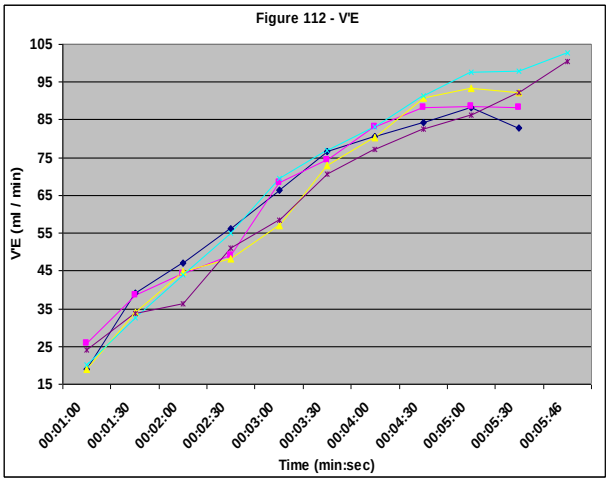


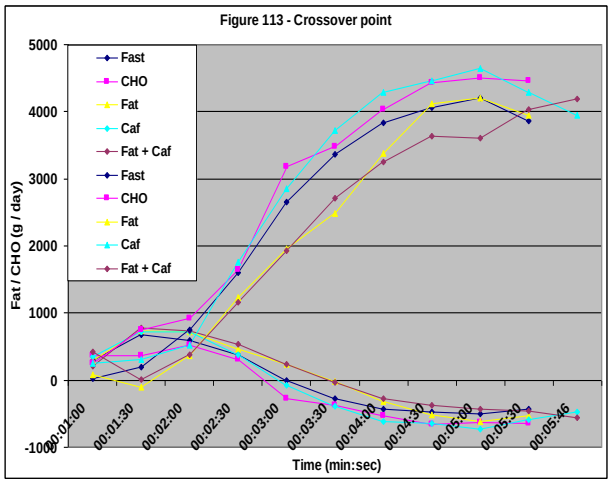


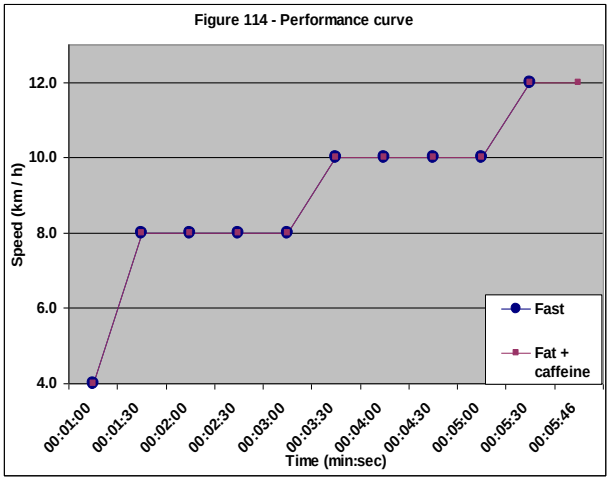












Figures 105-114: The compiled results in graphic format for the various interventions and their corresponding variables.

- Figures 105 and 106 respectively indicate that the Fat (F) and Fat and Caffeine (FC) trials show the highest fat and the lowest carbohydrate oxidation capacity values compared to the Carbohydrate trial (Ch) during exercise intensities ranging between 4-10 km/h. In accordance with this exercise intensity range, the Carbohydrate trial (Ch) show the highest carbohydrate oxidation capacity values compared to the any of the other interventions.

- At exercise intensities beyond 10 km/h, the Caffeine (C) trial shows the lowest fat and the highest carbohydrate oxidation capacity values, with the Carbohydrate trial (Ch) showing higher fat and lower carbohydrate oxidation

capacity values. This tendency is also cited for the RER values (Fig. 109) where the Carbohydrate trial (Ch) show the highest RER values between exercise intensities ranging between 4-10 km/h compared to the Fat (F) and Fat and Caffeine (FC) trials. Clear of this exercise intensity range, the Caffeine (C) and Carbohydrate (Ca) trials both show higher RER values compared to the Fat and Caffeine (FC) trial.

- The Fat (F) and Fat and Caffeine (FC) trials show the lowest oxygen consumption capacity ($CV'O_2$) values (Fig. 107) throughout the entire course of the exercise regime compared to any of the other interventions. The same notion applies for the carbon dioxide production capacity ($CV'CO_2$) values (Fig. 108).

- The lowest $V'O_{2peak}$ (Fig. 110) value is observed for the Fat (F) and Caffeine (C) trials compared to the highest $V'O_{2peak}$ value observed for the Fasting trial- 31.2 and 35 ml/min/kg respectively.

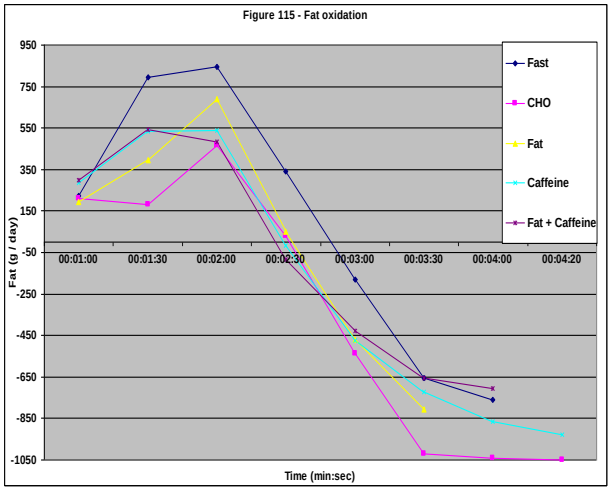
- Minor fluctuations are observed for HR (Fig. 111) values between the various interventions, but a trend towards a decrease is observed in the Fat trial (F) compared to the other interventions at exercise intensities in the range of 10-12 km/h. No differences in the HR values are recorded between these interventions at the point of voluntary fatigue.

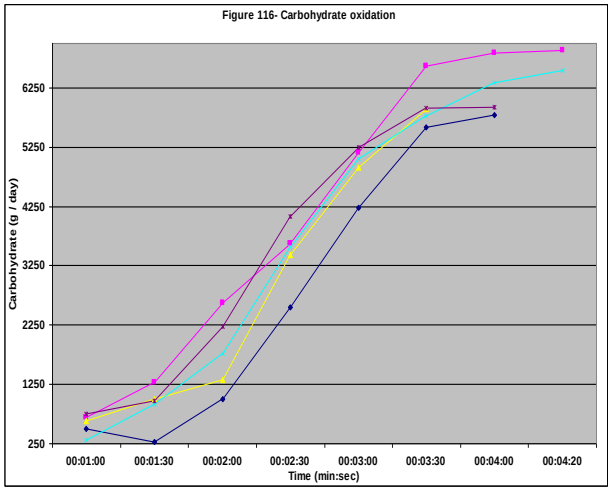
- Figure 112 shows that at exercise intensities ranging between 8-12 km/h, the Fat (F) and Fat and Caffeine (FC) trials show the lowest V'E values compared to the other interventions. Beyond 10 km/h, the Caffeine trial (C) shows the highest V'E values compared to any of the other interventions.

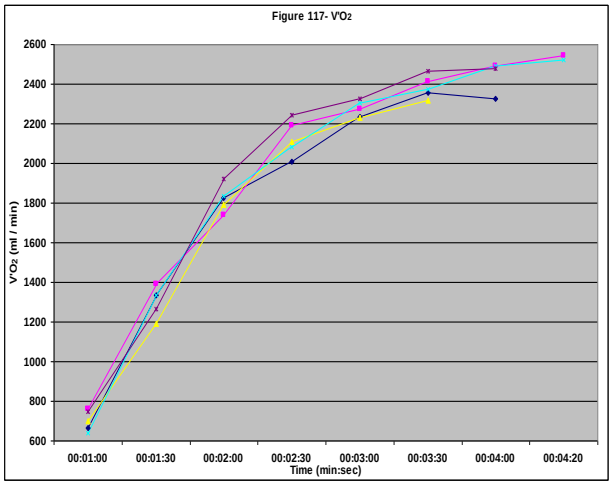
- The FCCP (Fig. 113) occurs at 8 km/h for all the interventions.

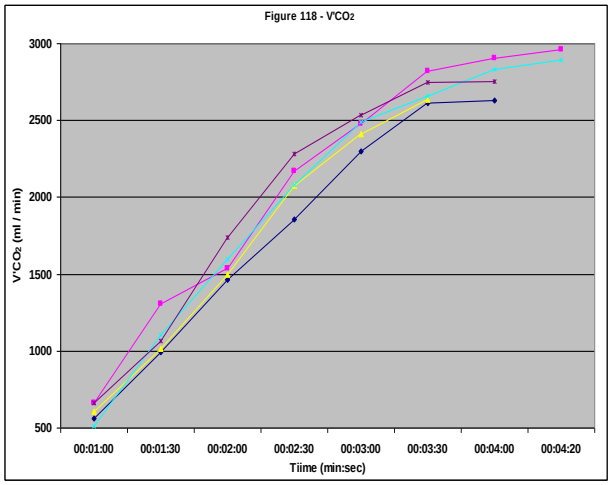
- When comparing the best and worst performance results (Fig. 114), the Fat and Caffeine trial (FC) shows the better result compared to the Fasting trial (Fa). On the Fat and Caffeine trial (FC) the participant ran 36 seconds longer at an intensity of 12km/h- see Appendix C.

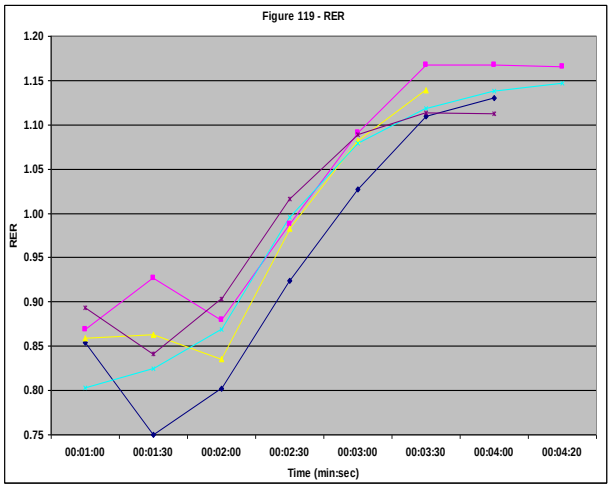
Untrained individual 5

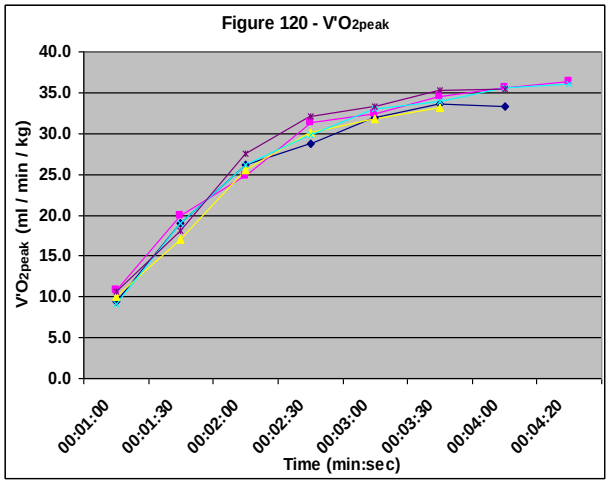


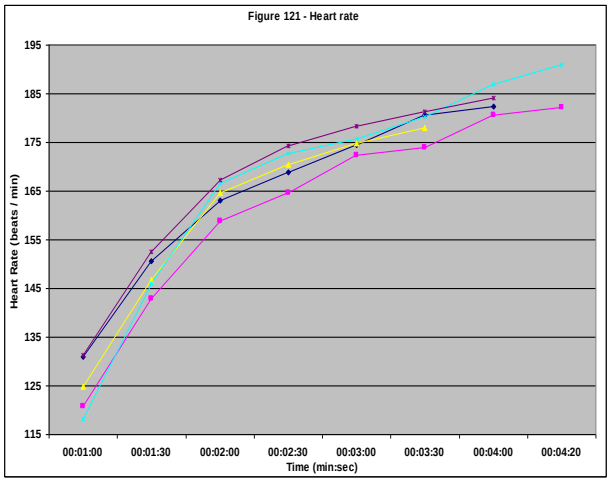


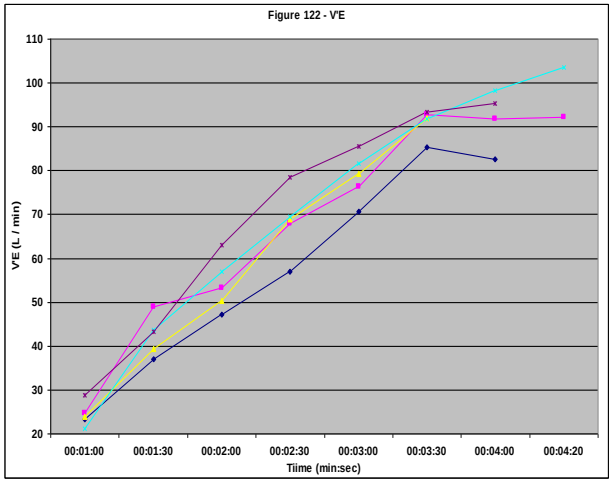


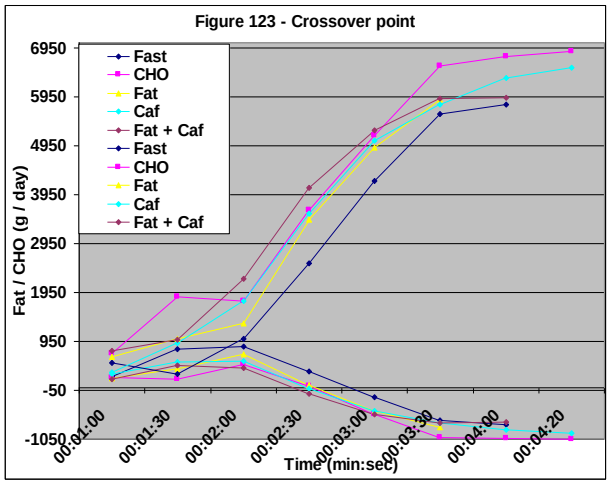


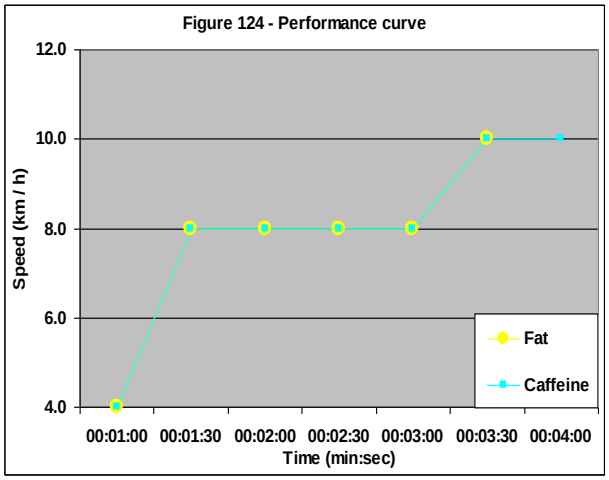












Figures 115-124: The compiled results in graphic format for the various interventions and their corresponding variables.

- Figures 115 and 116 respectively indicate that the Fasting trial (Fa) shows the highest fat and the lowest carbohydrate oxidation capacity values of all the interventions throughout the entire exercise regime. At exercise intensities ranging between 4-8 km/h, the Carbohydrate trial (Ch) shows the lowest fat and highest carbohydrate oxidation values. Beyond exercise intensities of 10 km/h, the Carbohydrate trial (Ch) shows the highest carbohydrate oxidation values compared to the Fasting trial (Fa).

- The Fat and Caffeine (FC) trial shows the highest oxygen consumption capacity ($CV'O_2$) values (Fig. 117) from exercise intensities beyond 8km/h

compared to the Fasting trial (Fa) which shows the lowest $V'O_2$ values. The same notion applies for the carbon dioxide production capacity ($CV'CO_2$) values (Fig. 118).

- Figure 119 indicates that the Fasting trial (Fa) shows the lowest RER values of all the interventions. At exercise intensities ranging between 4-8 km/h the Carbohydrate trial (Ch) shows the highest RER values, whilst at exercise intensities ranging between 8-10 km/h, the Fat and Caffeine (FC) trial show the highest RER values. Beyond 10 km/h the Carbohydrate trial (Ch) again shows the highest RER values.

- The highest $V'O_{2peak}$ (Fig. 120) value for any of the interventions is observed for the Carbohydrate trial (Ch) compared to the Fat trial (F) which showed the lowest $V'O_{2peak}$ values.

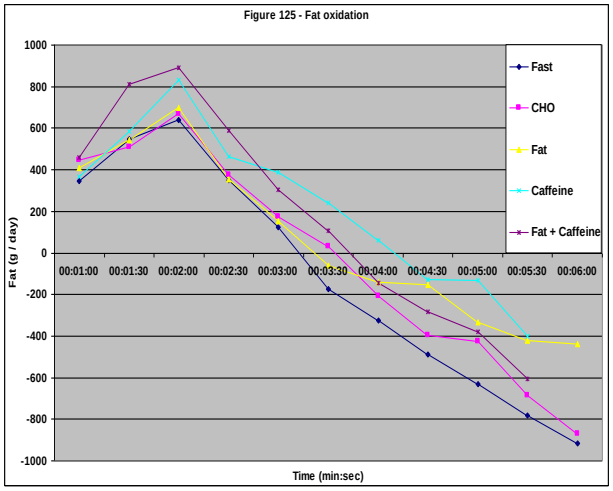
- Figure 121 indicates that the Fat and Caffeine trial (FC) shows the highest HR values compared to the Carbohydrate trial (Ch) throughout the entire exercise regime.

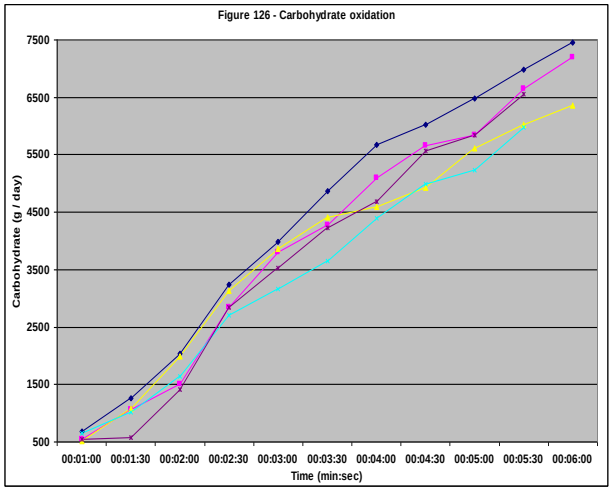
- Figure 122 shows that at exercise intensities ranging between 8-10 km/h, the Fat and Caffeine (FC) trial shows the highest V'E values compared to the Fasting trial (Fa) which shows the lowest V'E values throughout the entire exercise regime.

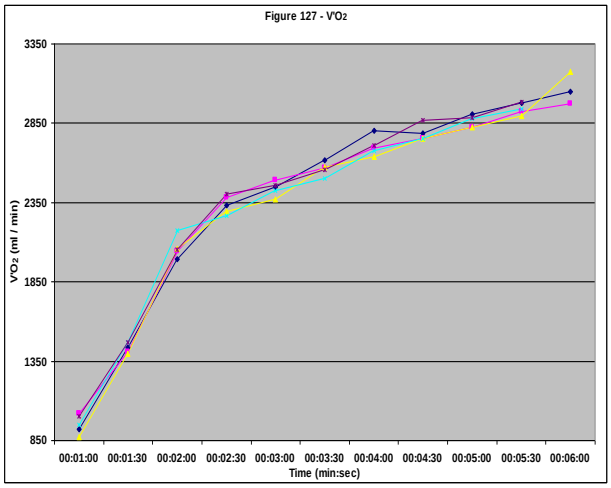
- There exist no FCCP (Fig. 123) for all the interventions except for the Fasting trial (Fa) which occurs at 8 km/h.

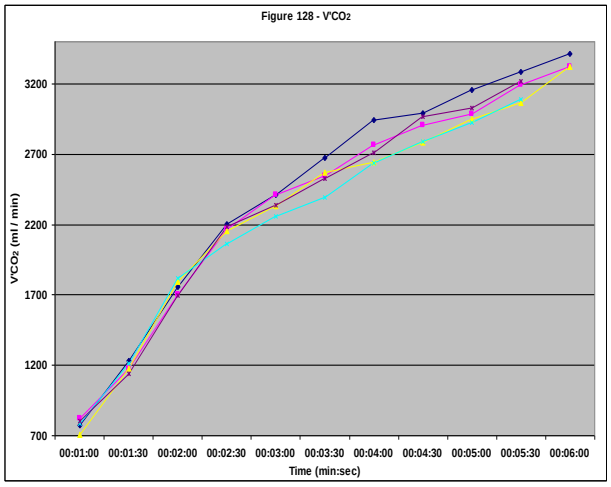
- When comparing the best and worst performance results (Fig. 124), the Caffeine trial (C) produced the better result compared to the Fat trial (F). On the Caffeine trial (C) the participant ran 57 seconds longer at an intensity of 10 km/h- see Appendix C.

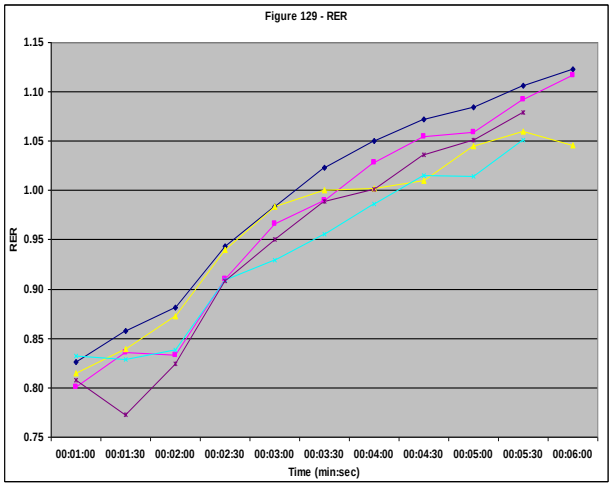
Untrained individual 6

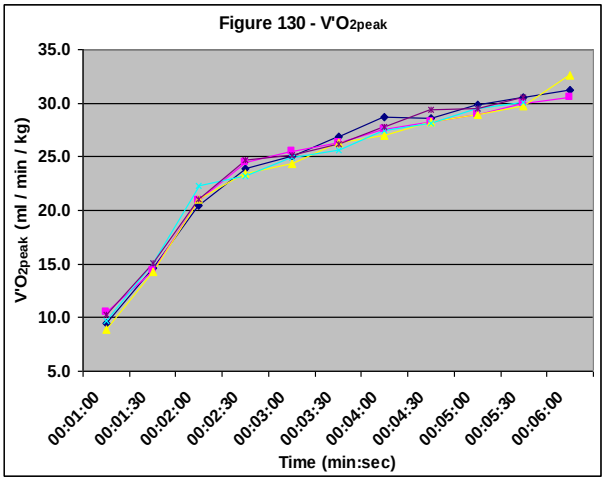


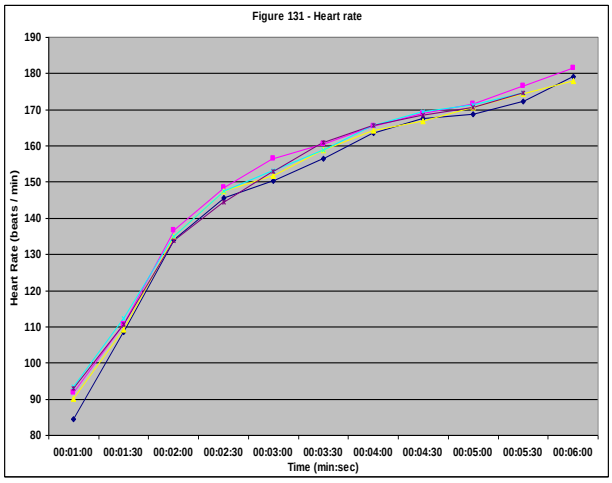


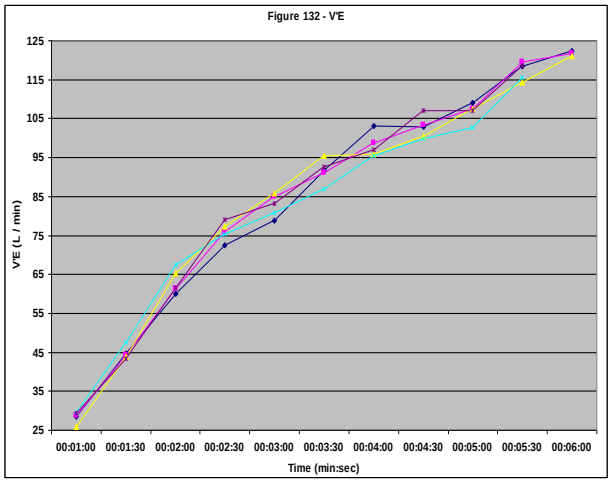


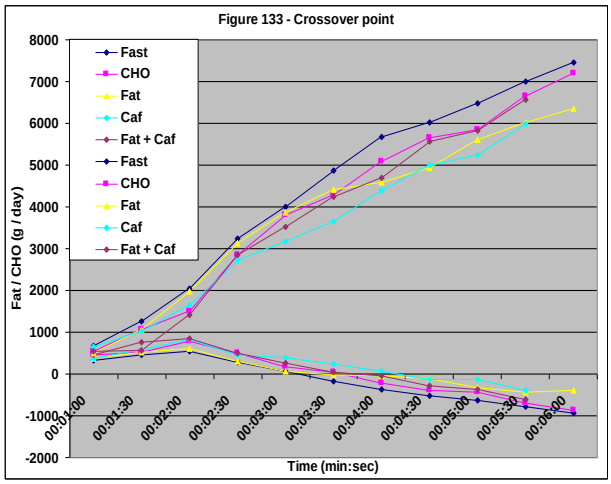


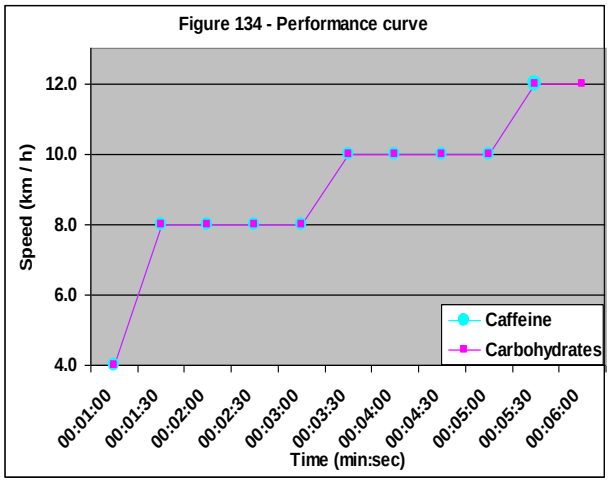












Figures 125-134: The compiled results in graphic format for the various interventions and their corresponding variables.

- Figure 125 indicates that the Fat and Caffeine trial (FC) shows the highest fat oxidation capacity values for exercise intensities ranging between 4-10 km/h compared to the Fasting trial (Fa) which shows the lowest fat oxidation capacity values for the entire course of the exercise regime. Beyond 10 km/h the Caffeine trial (C) shows the highest fat oxidation values.

- Figure 126 indicates that the Fasting trial (Fa) shows the highest carbohydrate oxidation capacity values throughout the entire course of the exercise regime compared to the Caffeine trial (C) which shows the lowest carbohydrate oxidation capacity values at exercise intensities beyond 8 km/h.

- Figure 127 indicates that there are minor alterations in the $V'O_2$ between any of the interventions throughout the entire course of the exercise regime. In contrast to this notion, the Fasting trial (Fa) shows the highest $V'CO_2$ values compared to the Caffeine (C) and Fat (F) trials which show the lowest $V'CO_2$ values beyond exercise intensities of 8 km/h (Fig. 128).

- Figure 129 indicates that the Fasting trial (Fa) show the highest RER values of all the interventions throughout the entire course of the exercise regime. At exercise intensities ranging between 4-8 km/h, the Fat and Caffeine trial (FC) show the lowest RER values. Beyond 8 km/h, the Caffeine trial (C) shows the lowest RER values.

- The highest $V'O_{2peak}$ (Fig. 130) value is observed for the Fat trial (F) and the lowest for the Caffeine trial (C). No difference in maximum heart rate is noted between any of the interventions.

- Figure 131 indicates that the Carbohydrate trial (Ch) shows a trend towards higher HR values compared to the Fasting trial (Fa).

- Figure 132 shows that minor deviations exist between the various interventions for the V'E values.

- There exist no FCCP (Fig. 133) for any of the interventions except for the Fat and Caffeine trial (FC) where it occurs at 8 km/h.

- When comparing the best and worst performance results (Fig. 134), the Carbohydrate trial (Ch) shows the better result compared to the Caffeine trial (C). On the Carbohydrate trial (Ch) the participant ran 41 seconds longer at an intensity of 12 km/h- see Appendix C.

CHAPTER 5

INTERPRETATION AND DISCUSSION OF METHODOLOGY AND RESULTS

5.1. INTRODUCTION

The primary aim of this investigation was to evaluate the effect of various "nutrient intake interventions" prior to exercise (training/competition) on macronutrient (fat and carbohydrate) metabolism and various physiological variables in man. The secondary aim of this investigation was to investigate whether nutrient intake within the hours prior to training influenced peak treadmill running velocity (indicative of athletic performance capacity).

It is commonly known amongst researchers that a negative correlation between percentage of body fat and health risks associated with being obese or overweight exist (Kerr et al., 2002:407). New information on how to improve fat oxidation by the synchronization of training and nutritional manipulating strategies to alter body composition could provide groundbreaking results on unresolved research questions such as:

- "Why combine diet and physical activity in the same international research society?" (Baranowski, 2004:2-19).
- "Diet and exercise for weight loss?" (Volek et al., 2005:1-9).

Athletes are subjected to evaluation protocols to identify areas of strengths and weaknesses to optimize performance ability. Should the intake of a pre-exercise meal within hours prior to training influence metabolism during training, and

should this aspect not form part of the inclusion criteria for the testing protocol, the implications would render “fitness testing results” invalid in the sense that the findings of one athlete cannot be compared to the same athlete at a later stage (re-testing) or to any other athletes at any stage of testing.

Furthermore, due to genetic predisposition and/or the current physiological profile images, it could be possible that all athletes will not perform to the same extent if these athletes are subjected to the same foodstuff within the hours prior to training/competition. The question can also be asked how nutrient intake within the hours prior to competition could influence structural and metabolic adaptation processes in the long-term.

5.2. METHODOLOGY OF INTERVENTIONS

Before the results of this study are discussed it would be appropriate to argue the matters on: (1) why these specific nutritional interventions were incorporated in this study and (2) the methods implemented to measure change/alterations in specified metabolic and systemic variables.

5.2.1. Interventions

The interventions of the investigation will be discussed in the following paragraphs.

5.2.1.1.Fasting

Refer to Chapter 2, section 2.2.4. p 55-59 for a more detailed background discussion on the topic.

It is known that the ingestion of foodstuff creates an anabolic metabolism compared to the fasting state where a catabolic metabolism reigns. Surely when

exercise comes into play, energy systems (catabolic events) are activated to maintain homeostasis.

Due to the following views, fasting *per se* could be considered to be a nutritional intervention:

- Fasting (8 hours) has a significant effect on the utilization of fuels during sub-maximal exercise (Aragon-Vargas, 1993:255) and performance (Jeukendrup et al., 1998:373).
- Since insulin activity by means of capillary wall LPL promotes fat storage and inhibits HSL activity in adipocytes and muscle tissue, the intake of carbohydrate or protein foodstuffs could affect the catabolic processes-see 2.1.5.1.(a).

In accordance with this data, researchers have been trying to determine the relationship between fasting and exercise performance for many years.

5.2.1.2. Fat intake

Refer to Chapter 2, section 2.2.1. p 36-45 for a more detailed background discussion on the topic.

It has been proposed that the capability to sustain exercise may be extended if the provision of fats is immediately made available before exercise, as the FFA oxidation rate is directly correlated to the fatty acid serum concentration level (Ferreira et al., 2003:422). A number of interventions have been used to increase fatty acid availability before or during exercise including fasting, caffeine ingestion, ingestion of medium chain triglyceride solutions and the ingestion of long chain triglyceride solutions (Hawley, 2002:1485-1486).

The present protocol design is formulated in such a manner that it investigates whether the same mechanism(s) are involved between these aforementioned interventions (i.e. fasting, caffeine ingestion, ingestion of medium chain

triglyceride solutions and the ingestion of long chain triglyceride solutions). For this reason olive oil was incorporated in the protocol design since olive oil contains high levels of MCTs and LCTs free of caffeine.

According to Bucci (1993:21) MCTs have been shown to possess at least four properties of vital interest to exercise performance: (1) they are a readily available energy source; (2) they mobilize body fat stores; (3) they increase the metabolic rate; and (4) they spare lean muscle mass. Vistisen et al. (2003:2435) suggests that an additional property may qualify by means of the sparing effect on glycogen utilization during exercise, which has previously been connected with fatigue. This investigation could substantiate some of these views (1 and 3) and/or provide new perspectives on the manipulating effects of nutrient intake on metabolism and other physiological variables implemented in the methodological approach.

There is no clear evidence what the dosage or the time interval prior to testing should be. Ferreira et al. (2003:423-424) concluded that the intake of 30g of MCT does not seem to spare muscle glycogen or even enhance performance. The ingestion of 25-30g of MCTs at any one time is the maximum amount which can be tolerated by the digestive system of a human in order to prevent nausea and diarrhea (Horowitz et al., 2000:219; Ferreira et al., 2003:424). By mere speculation the author of this investigation believes that the prospective value of fat as a readily accessible source of energy can be achieved by ingesting less (5 ml compared to 25g) of olive oil when implemented in the fasting state.

Hawley (2002:1486) believes that by raising the concentration of fatty acids in the blood prior to exercise, fat could become a source of energy for contracting skeletal muscle during sub-maximal exercise. More recently Hawley et al. (2006:713–714) suggest that fats are an alternative source of energy compared to carbohydrates at certain exercise intensities, which could lead to an improvement in performance.

The author of the current investigation believes that although the aforementioned views/proposals could be true, they should be identified as generalizations, since fat cannot be utilized to the same extent between individuals with different muscle fiber characteristics or 'fitness levels'.

5.2.1.3. Caffeine intake

Refer to Chapter 2, section 2.2.2. p.45-51 for a more detailed background discussion on the topic.

Caffeine has been reported to enhance exercise performance by enhancing fat oxidation and therefore sparing muscle glycogen, particularly during the early stages of prolonged high intensity exercise. Numerous mechanisms have been proposed to explain this caffeine-induced sparing of muscle glycogen (Hadjicharalambous et al., 2006:876):

- Caffeine may reduce the muscle glycogenolytic rate by inhibiting glycogen phosphorylase activity, which is the flux-generating step for muscle glycogenolysis;
- Caffeine can enhance free fatty acid mobilization by stimulating the release of epinephrine, which increases the potential for fat oxidation; and
- Caffeine may indirectly promote fat oxidation and carbohydrate sparing by inhibiting adenosine receptors in adipose tissue, which otherwise inhibit free fatty acid mobilization from adipocytes.

The proposed effects of caffeine relate to the following:

- Increase work time to exhaustion
- Decrease lactate levels
- Reduced rate of glycogen oxidation
- Improved mobilization of free fatty acids and in effect glycolysis and glucose uptake is limited
- Increased muscle tension and contractile state
- Assist impulse transmissions

- Increase membrane extractability which may help in the recruitment of motor units spreading the tension demand over a larger muscle mass
- Increase the tidal volume of lungs
- Enhance inspiratory muscle endurance and lower sense of effort.

None of these effects have been confirmed but with further research a true understanding of caffeine's actions will undoubtedly be revealed.

The following question arises unequivocally:
Do all individuals respond in a similar fashion or to the same extent (work time to exhaustion) towards caffeine intake by means of these proposed mechanisms?
The author of this investigation feels that not all individuals will respond to caffeine ingestion in a similar fashion or to the same extent, owing to interindividual differences demarcated by muscle fiber type distribution and/or the current 'fitness' level of the individual.

Current studies in this field used minute amounts of caffeine (1-2 mg/kg). In numerous studies, coffee was used whilst others have used caffeine. They all showed, however, that small amounts of caffeine are efficient in improving exercise performance significantly and these smaller amounts (90mg) are not associated with any unnecessary side effects (Doherty and Smith, 2004:626). When caffeine is ingested, 100% of all the caffeine is absorbed and peak levels are seen in the blood after 15-120 minutes after an oral dose of about 250mg.

The present protocol design adheres to these proposed requirements to validate the time of caffeine intake as well as the amount of caffeine ingested.

5.2.1.4. Carbohydrate intake

Refer to Chapter 2, section 2.2.3. p 51-55 for a more detailed background discussion on the topic.

Over the past 25 years, research has accentuated the role of carbohydrates in endurance exercise and the advice that the ingestion of a high carbohydrate diet prior to exercise as well as the continual carbohydrate supplementation during exercise has been accepted by various athletes.

Work in the late 20th century, however, suggests that carbohydrates ingested prior to exercise was damaging to exercise performance, resulted in pre-exercise carbohydrate intake being avoided by athletes for many years. This effect was attributed to the following:

- A momentary increase in blood glucose levels following carbohydrate ingestion, thus bringing about a rapid release of insulin, which results in a decline in blood glucose concentration. More recent studies have shown that the intake of smaller amounts of carbohydrates just before exercise results in no increase of plasma insulin during exercise, no rebound hypoglycemia as well as an enhancement of performance. This has resulted in the intake of carbohydrate solutions before exercise becoming more widespread (Maughan et al., 1997:168).

- The inhibition of free fatty acid release. The ingestion of 50–100g of carbohydrate in the hours before exercise will inhibit lipolysis and will also reduce fat oxidation by about 30-40%. The exact mechanism by which glucose and/or insulin reduce fat oxidation at an intramuscular level is still subject to debate (Jeukendrup, 2003:1272).

- The premature development of fatigue.

The metabolic and performance benefits resulting from pre-exercise nutritional supplementation in the hour before exercise are uncertain (Kirwan et al., 1998:56). Data from this study show that ingestion of a breakfast cereal with a moderate GI 45 min before exercise can augment exercise time to exhaustion.

However, data on metabolic and exercise performance measures related to pre-exercise dietary carbohydrate intake are ambiguous. Investigators have accounted decreased, unchanged, or enhanced exercise performance after pre-exercise carbohydrate meals. Furthermore, qualitative and quantitative differences in blood glucose responses may have been disregarded for some types and sources of carbohydrate. Some studies exploring the effects of pre-exercise carbohydrate feeding with different GIs have shown a positive effect on physiological parameters, with the low GI food resulting in favorable substrate levels during exercise. The effects of dietary fiber, however and the influence of the GI of a meal on exercise performance, necessitate further investigation under conditions of controlled diet and activity levels (Kirwan et al., 1998:53). Some studies have been restricted by the lack of control of previous physical activity and dietary intake, both of which may have a profound effect on the metabolic and performance outcomes (Kirwan et al., 1998:53).

The present protocol design also incorporated carbohydrate ingestion as a variable not only to validate the “lack of control of previous physical activity and dietary intake both having a profound effect on the metabolic and performance outcomes”, but also to evaluate discrepancies on carbohydrate intake prior to training or competition on metabolism and/or athletic performance.

5.2.1.5. The combined intake of fat(oil) and caffeine

Refer to Chapter 2, section2.2.5. p 59-62 for a more detailed background discussion on the topic.

Very little information regarding the simultaneous intake of fat and caffeine exist. The study of Hadjicharalambous et al. (2006:885-886) was designed to distinguish between the putative metabolic and CNS effects of pre-exercise caffeine ingestion. This was achieved by having the participants exercise after elevating their circulating plasma [FFA] with a high fat meal and subsequently co-ingesting caffeine or placebo. Assuming that perception of effort reflects, in part

at least, CNS responses, the results presented here indicate a differentiation between the putative metabolic effects and the CNS actions of caffeine during constant load exercise, as perception of effort was reduced after caffeine ingestion (despite an elevation in cardiopulmonary and metabolic responses), but there were no differences in substrate utilization and no improvement in exercise performance.

The present protocol design made no provision for collecting information on CNS effects or the RPE. The combination of fat plus caffeine could shed light on caffeine's effect on metabolism if fat intake as a variable *per se* is indicated as the norm.

5.3. METHODOLOGY OF TESTING AND ANALYSIS

Refer to Chapter 2, section 2.3. p 63-72 for a more detailed background discussion on the topic.

5.3.1. Indirect Calorimetry

During exercise, direct calorimeters such as the Atwater-Rose calorimeter are of diminutive use for several reasons. Firstly, such devices are very costly. Secondly, the heat generated by an ergometer, if it is electrically powered, may far surpass that of the subject. Thirdly, body temperature augments during exercise because not all the heat produced is liberated from the body. Therefore, the sensors in the walls of the calorimeter do not pick up all the heat formed. Finally, the body sweats during exercise, which also affects the calorimeter and alters body mass. Changes in body mass and the unequal distribution of heat within the body make the use of indirect calorimetry in exercise very complicated (Brooks et al., 2000:47–48). For this reason the use of indirect calorimetry during exercise is required.

Although breath-by-breath data collection is currently the most accepted method, it is imperative to recognize that the confidence with which these “metabolic” indicators can be calculated depends on the combined measurement errors for each of the determined variables. If great care is not taken with the calibration of the sensors, these errors can be additive and vast. It is suggested that all breaths collected should be used in the processing of the data. However, invalid breaths caused by swallowing, coughing and so on, should not be included. For the concluding tabular and graphic report, 30–60 second intervals for averaging data are recommended although 20-second intervals may be acceptable (Casaburi et al., 2003:221). In the present protocol design 30-second intervals for averaging were calculated for each intervention and participant. The concluding tabular reports appear in Appendix C and the corresponding graphical reports can be depicted in Chapter 4.

Various studies have provided conflicting results, with some reporting a significant change with repeated testing and others reporting no significant change. A number of reasons may explain these discrepancies, which include whether repeated testing was undertaken within a short period of time (i.e. four tests within 7-10 days). An additional factor that may influence the reproducibility of measurements is the time of testing. Preferably, repeated testing should be undertaken at the same time of day, as significant diurnal variation in results has been reported. Furthermore the testing protocol, procedure and instructions for the patient must be rigidly controlled, as these have been shown to significantly affect performance (Casaburi et al., 2003:223-224). The present protocol design adhered to all three of these prerequisites to address the possible issues involved in repeatability.

5.3.2. Statistical analysis

Descriptive statistics is used to:

- Compare the effects of the various interventions on metabolic variables and the $V'O_{2peak}$ for each individual.

- Highlight major differences or similarities on the effects of the various interventions on metabolic variables and the $V'O_{2peak}$ between the participants.

5.4. DISCUSSION OF RESULTS

The discussion of the relative results of all the subjects for all the interventions, will be handled on the assumption that each individual, trained or untrained, must be discussed on an individual basis. The discussion of the group of trained individuals will be followed by the group of untrained individuals.

5.4.1 Group of trained individuals

The results from the group of trained individuals will follow in the following paragraphs.

Trained individual 1

Fat oxidation

- It is known that fasting is a way to increase fat utilization, to spare muscle glycogen and as a result improve exercise performance (Jeukendrup and Gleeson, 2004:142). In general, the Fasting trial (Fa) produced the highest fat oxidation values at low exercise intensities (4-8 km/h)- see figure 15. As from exercise intensities ranging between 10 km/h until voluntary fatigue sets in, the combination between Fat and Caffeine (FC) produced the lowest fat oxidation results compared to any of the other interventions. It has been reported that caffeine enhances fat oxidation (Jeukendrup and Gleeson, 2004:145), but recently it has been noted that caffeine actually improves short, high intensity exercise, which is fueled from carbohydrates (glucose and glycogen). Caffeine's facilitating effect on neuromuscular activity is thought to be liable for any progress in short duration, high intensity exercise

(http://www.pponline.co.uk). The present findings also show that caffeine (C) *per se* (or in combination with fat-FC) has no stimulating properties on fat oxidation but Caffeine in combination with Fat (FC) actually increase carbohydrate oxidation when compared to any of the other interventions at any stage of the exercise regime. In addition to this, it has also been proposed by several other studies (McNaughton, 1986; Flinn et al., 1990; Jackman et al., 1996), that an enhancement in high intensity/incremental exercise performance after caffeine ingestion suggests CNS stimulation. Thus the decrease in fat oxidation which is visible in the Fat and Caffeine (FC) trial could also be ascribed to the effect that caffeine has on carbohydrate metabolism. Furthermore, it can be stated that the Caffeine (C) did not produce high fat oxidation rates. The Fat trial (F) also produced low fat oxidation rates, thus the combination between Fat and Caffeine (FC) did not produce favourable fat oxidation results in this participant.

- It is clear from the graph that the range in which the fatty acid thresholds (FAT) for all the interventions occurs is between the 2nd and 3rd minute, which is evident at an exercise intensity of 8 km/h and occurs between 53–62% V'O_{2peak}. In accordance to Robergs and Roberts (2000:114-115), this study will rather make use of the concept V'O_{2peak} instead of V'O_{2max} (refer to section 2.3.9). Jeukendrup and Gleeson (2004:134) indicates that maximal fat oxidation occurs between 62-63 %V'O_{2max} (refer to figure 8), whereas Achten and Jeukendrup (2004a:716) indicate that maximal fat oxidation occurs in trained individuals at around 59-64% V'O_{2max} and Van Loon (2004:1183) states that maximal fat oxidation occurs at 40-65% V'O_{2max} (refer to section 2.2.1.5). Accordingly this trained individual's maximal fat oxidation range falls in this range of values indicated by the above-mentioned authors.

- This does not imply that fat oxidation is similar between the interventions for this individual. This implies that work load and/or percentages of the V'O_{2peak} are not descriptive of the exact amounts of fat being oxidized. At an exercise

intensity level of 8 km/h the athlete could burn fat at a rate of 1378 g/24hrs in the Fasting trial (Fa) compared to 809 g/24hrs during the Fat trial (F) which equates to a 41% difference between these two interventions.

Carbohydrate oxidation

- It stands to reason that the intervention that produced the weakest fat oxidation results will produce the best carbohydrate oxidation results, since fat and carbohydrate are basically the only two energy sources in the human body (Jeukendrup and Gleeson, 2004:137). Figure 16 indicates that at low exercise intensities ranging between 4-8 km/h, the Fasting trial (Fa) show the lowest carbohydrate oxidation values, relative to the highest fat oxidation values for this exercise intensity range. At exercise intensities beyond 8 km/h until voluntary fatigue sets in, the Fat and Caffeine trial (FC) show the highest carbohydrate oxidation values, relative to lowest fat oxidation values for this exercise intensity range. This can most likely be explained by the fact that caffeine (or one of caffeine's by products) could directly influence skeletal muscle and manipulate the transmission of neural signals in regions between the brain and neuromuscular junction (Hadjicharalambous et al., 2006:876). In contrast, fasting produced the lowest carbohydrate oxidation results. This is due to the fact that fasting produced the highest fat oxidation results, and due to the fact that fat and carbohydrate together constitute most, if not all, of the energy provision (Jeukendrup and Gleeson, 2004:137).

$V'O_2$, $V'CO_2$, V'E and $V'O_{2peak}$

- At low exercise intensities ranging between 4-8 km/h, the Fasting trial (Fa) shows a decrease in RER (Fig. 19) which is rather caused by an increase in the $V'O_2$ relative to the increased $V'CO_2$.

- As from exercise intensities ranging between 10km/h until voluntary fatigue sets in, an increase in the $V'O_2$, $V'CO_2$, RER, V'E and $V'O_{2peak}$ is seen for the Fat

and Caffeine trial (FC) when compared to the corresponding values for any of the other interventions–see figures 17, 18, 19 and 22 respectively

- It should also be noted that the carbohydrate threshold for the Fat and Caffeine trial (FC) was reached at 6:30 at an exercise intensity of 12 km/h (earliest of all interventions), which means from that point onwards this trained individual continue running with energy derived from anaerobic metabolism, i.e. glycolysis. The increased $V'CO_2$ levels could have originated from two pathways: (1) during intense exercise, when glycolysis is stimulated, the rate of pyruvate entry into the mitochondria for aerobic metabolism is decreased which means that pyruvate is rather converted to lactate. During high intensity exercise the rate of lactate production exceeds the rate of lactate removal and some of the lactate that accumulates in the muscles is transferred to the blood, which causes the $[H^+]$ to increase. The increased $[H^+]$ leads to decreased plasma pH levels In order to raise the pH level to neutral again, chemical buffers need to buffer the excess H^+ which accumulates in the blood. The most common chemical buffer is bicarbonate. During this process, hydrogen ions combine with bicarbonate to form carbonic acid. A raise in plasma CO_2 due to the buffering action instantly stimulate ventilation to eliminate excess CO_2, which is also evident in the increased V'E values in the Fat and Caffeine trial (FC) (Astorino, 2000:213-214; McArdle et al., 2001:159) or (2) when pyruvate is converted to Acetyl-CoA via pyruvate dehydrogenase to enter the Krebs cycle, the reaction releases CO_2 (see figure 2).

- The Fasting trial (Fa) shows the lowest V'E value at the point of voluntary fatigue compared to the Fat and Caffeine trial (FC), i.e. 146 L/min vs 185 L/min respectively, which shows a 21% difference between these two interventions.

Heart rate

- In addition to this, it should be noted that the intervention which produced the highest $V'O_2$ value at the point of fatigue also produced the highest $V'O_{2peak}$

value, i.e. the Fat and Caffeine trial (FC). The highest $V'O_{2peak}$ value was reached at 59.1 ml/min/kg. The lowest VO_{2max} value was 52 ml / min / kg, which was evident during the Fasting trial (Fa). This means that, should the participant comply with the FC diet, a 12% increase in the $V'O_{2peak}$ would be evident, compared to the Fasting state (Fa).

- The heart rate (HR) (Fig. 21) for the Fasting trial (Fa) shows lower values compared to any of the other interventions during exercise intensities ranging between 4-10 km/h. No differences in the heart rate values are recorded beyond 10 km/h up to the point of voluntary fatigue between any of the interventions.

FCCP for all interventions

- Results of indirect calorimetry studies show that in a resting post-absorptive individual, 60% of energy is obtained from fat oxidation, 35% from carbohydrate oxidation and 5% from proteins. At the onset of exercise, however, and as exercise intensity increases, the relative contribution of fats decline and that of carbohydrate increases. Thus high intensity exercise is accomplished by "crossover" to dependence on carbohydrate oxidation and utilization (Brooks et al., 2000:133).

- For the Fat trial (F) no FCCP (Fig. 23) exists, whereas the FCCP for all other interventions occurred at a treadmill speed of 8 km/h. This result reveals that the crossover concept does not exist in every individual as indicated by various researchers such as Brooks et al. (2000:133).

Performance curve

- The performance curve provides an indication of the intervention which enabled the individual to run for longer at a specific treadmill speed and/or longer and faster at a specified treadmill speed. When comparing the best and worst performance results (Fig. 24), the Fat trial (F) shows that the participant ran 1

minute longer at an intensity of 16 km/h and could continue an additional minute at 18 km/h compared to the Fasting trial (Fa). This relates to a figure of a 12.2% increase in time to exhaustion between the Fasting trial (Fa) and the Fat trial (F). These results, which are specific to this particular trained individual, thus show that in contrast to the views advocated by other researchers (Maughan et al., 1997:168; Jeukendrup et al., 1997:836: Arkinstall et al., 2004:2275;. Johnson et al., 2004:151; Jeukendrup et al., 2004:1555–1556; http://www.sportsdieticians.com) which encourage the intake of dietary carbohydrate prior to exercise to enhance performance, the intake of Fat (F) prior to exercise enhanced this trained individual's endurance performance (721 seconds) compared to Caffeine (C) (667 seconds), Caffeine and Fat (FC) (667 seconds), Fasting (Fa) (601 seconds) or carbohydrate intake (Ch) (660 seconds).

Trained individual 2

Fat oxidation

- Figure 25 indicates that the Caffeine trial (C) show the highest fat capacities at all exercise intensity levels (4-16km/h) compared to the Carbohydrate trial (Ch) showing the lowest fat oxidation capacities. This is a typical example where caffeine ingestion enhances fat oxidation as depicted by Jeukendrup and Gleeson (2004:145) and Robergs and Roberts (2000:253). The decrease in fat oxidation as depicted by the Carbohydrate trial (Ch) is, according to Jeukendrup and Gleeson (2004:137), due to increased plasma insulin concentrations (which reduced lipolysis and caused a distinct decrease in FA availability for energy provision).

- In addition to this it should be noted that at the fatty acid thresholds (FAT) which occur for all the interventions at 8 km/h, the Fasting trial (Fa) showed a decrease of 37% in fat oxidation values compared to the Caffeine trial (C).

- It is clear from the graph that the range in which the fatty acid thresholds (FAT) for all the interventions occurs is between the 2nd and 3rd minute, which is evident at an exercise intensity of 8 km/h and occurs between 53–69% $V'O_{2peak}$. Jeukendrup and Gleeson (2004:134) indicates that maximal fat oxidation occurs between 62-63 %$V'O_{2max}$ (refer to figure 8), whereas Achten and Jeukendrup (2004a:716) indicate that maximal fat oxidation occurs in trained individuals at around 59-64% $V'O_{2max}$ and Van Loon (2004:1183) states that maximal fat oxidation occurs at 40-65% $V'O_{2max}$ (refer to section 2.2.1.5). Accordingly this trained individual's maximal fat oxidation range falls in the values indicated by the above-mentioned authors. It should be noted that the fatty acid threshold (FAT) for the Fasting trial (Fa) occurred at 69% $V'O_{2peak}$ which means that this intervention enabled the trained individual to oxidize fats at a much higher exercise intensity.

Carbohydrate oxidation

- Figure 26 indicates that the Carbohydrate trial (Ch) shows the highest carbohydrate oxidation values compared to the Caffeine trial (C) which shows the lowest carbohydrate oxidation values. This is explained by the fact that carbohydrate ingestion, prior to exercise increases insulin levels, and as a result enhances carbohydrate oxidation during exercise (Jeukendrup and Gleeson, 2004:109). In contrast to this, it appears that the Caffeine trial (C) was inclined towards increasing fat oxidation rather than carbohydrate oxidation which is seen during high intensity exercise.

$V'O_2$, $V'CO_2$, RER, V'E and $V'O_{2peak}$

- Compared to the Carbohydrate trial (Ch), the Caffeine trial (C) coincided with a decrease in the RER (Fig. 29) which was rather caused by a larger decrease in the $V'CO_2$ (Fig. 28) which is relative to the decrease in the $V'O_2$ (Fig 27).

- The high levels of CO_2 produced during the Carbohydrate trial (Ch) could be ascribed to either the formation of lactate or from the reaction where pyruvate is

converted to Acetyl-CoA, which releases CO_2 (refer to trained individual 1–$V'O_2$ *etc*.), but no distinct difference in V'E is noted (Fig. 32). In contrast to this finding, the Caffeine trial (C) showed the lowest $V'CO_2$ and RER values which might be due to the fact that this intervention stimulated fat oxidation throughout the entire course of the exercise regime. A lower (RER) value in exercising athletes indicates a relative increase in fat oxidation. (Bergman and Brooks, 1999:480).

- The highest $V'O_{2peak}$ was reached with the Caffeine trial (C), i.e. 59.7 ml/min/kg, with the Fasting trial (Fa) producing the lowest $V'O_{2peak}$ value of 53.4 ml/min/kg. This indicates a 10.55% improvement between the Fasting trial (Fa) and the Caffeine trial (C).

Heart rate

- Minor or no distinct alterations are evident in HR (Fig. 31), with only a 6% difference in maximal heart rate values between the Fasting trial (Fa) and the Fat and Caffeine trial (FC).

FCCP for all interventions

- Figure 33 shows that no FCCP exist for the Fasting (Fa), Fat (F) and Carbohydrate (Ch) trials and exist at 8 km/h for the Caffeine (C) and Fat and Caffeine (FC) trials. For only two of the five interventions, a FCCP existed, which again relates to the argument that a FCCP does not exist for each and every individual.

Performance curve

- When comparing the best and worst performance results (Fig. 34), the Fat and Caffeine trial (FC) shows the better result compared to the Fasting trial (Fa). On the Fat and Caffeine trial (FC) the participant ran 19 seconds longer at an intensity of 14 km/h, continued an additional 120 seconds at 16 km/h and even continued for an additional 44 seconds at a treadmill speed of 18 km/h. There

exists a 26% increase in the total time until volitional fatigue sets in between the Fasting trial (Fa) and the Fat and Caffeine trial (FC). Thus it is evident from these results that the combination of Fat and Caffeine (FC) significantly improved performance, which is in contrast to numerous other researchers and authors which state that carbohydrate intake prior to exercise will enhance performance (Hawley and Hopkins, 1995:245; Maughan et al., 1997:168; Hawley et al., 2006:713; http://www.sportsdieticians.com).

Trained individual 3

Fat oxidation

- Figure 35 indicates that the Fat and Caffeine trial (FC) shows the highest fat oxidation values, compared to the Fat (F) and Fasting (Fa) trials showing the lowest fat oxidation values. From this can be deduced that the addition of fat to caffeine showed synergistic effects (i.e. the FC trial) on the increased levels of fat oxidation compared to only the Fat (F) or Caffeine (C) trials. In contrast to this specific finding, Hadjicharalambous et al. (2006:885) observed that it is possible that the pre–exercise high fat meal showed no synergistic effects on the increased levels of fat oxidation previously attributed to the sole effect of caffeine intake. In addition to this, Hadjicharalambous et al. (2006:885) also states that plasma [FFA] is elevated before exercise by fat ingestion and that even though increased lipolysis was evident after caffeine ingestion (i.e. FC), the addition of caffeine did not further enhance free fatty acid utilization.

- In addition to this it should be noted that at the fatty acid thresholds (FAT) which occur for all the interventions at 8 km/h, the Fat trial (F) showed a decrease of 41% in fat oxidation values compared to the Fat and Caffeine trial (FC).

- It is clear from the graph that the range in which the fatty acid thresholds (FAT) for all the interventions occurs is between the 2nd and 3rd minute, which is

evident at an exercise intensity of 8 km/h and occurs between 57–66% $V'O_{2peak.}$ Jeukendrup and Gleeson (2004:134) indicates that maximal fat oxidation occurs between 62-63 $\%V'O_{2max}$ (refer to figure 8), whereas Achten and Jeukendrup (2004a:716) indicate that maximal fat oxidation occurs in trained individuals at around 59-64% $V'O_{2max}$ and Van Loon (2004:1183) states that maximal fat oxidation occurs at 40-65% $V'O_{2max}$ (refer to section 2.2.1.5). Accordingly this trained individual's maximal fat oxidation range falls in the values indicated by the above-mentioned authors.

Carbohydrate oxidation

- Figure 36 indicates that the Fat and Caffeine trial (FC) show the lowest carbohydrate oxidation values compared to the Fat (F) and Fasting (Fa) trials which show the highest carbohydrate oxidation values. Due to the fact that the Fat and Caffeine (FC) trial shows the highest fat oxidation values, it is logical that this combination would have the lowest carbohydrate oxidation values.

$V'O_2$, $V'CO_2$, RER, V'E and $V'O_{2peak}$

- The Fat and Caffeine trial (FC) coincided with a decrease in the RER values (Fig. 39) which was rather caused by a decrease in the $V'CO_2$ values (Fig. 38) in combination with no distinct alterations in the $V'O_2$ values (Fig. 37) between these interventions.

- In contrast to this the Fat (F) and Fasting (Fa) trial coincided with an increase in RER values (Fig. 39) rather caused by an increase in the $V'CO_2$ values (Fig. 38) in combination with no distinct alterations in the $V'O_2$ values (Fig. 37) between these interventions. The increased $V'CO_2$ values are most probably caused by increased lactate levels or by the action where pyruvate is converted to Acetyl-CoA (refer to trained individual 1). In addition to this it should also be noted that in only these two interventions, i.e. Fat (F) and Fasting (Fa), the carbohydrate threshold (i.e. RER = 1.0) occurred at 8:30 and at an exercise intensity of 14 km/h, which is an indication that this trained individual had to run

from anaerobic glycolysis from an earlier stage until the point of volitional fatigue had been reached. This in turn could indicate that the increased $V'CO_2$ levels are most probably due to the increased lactate production which occurs during glycolysis.

- The Fasting trial (Fa) coincides with a decrease in the V'E (Fig. 42) at all exercise intensity levels excluding exercise at a relatively low intensity (8km/h) level. The highest value obtained during the entire course of the exercise regime recorded for the Caffeine trial (C) (114 L/min) can be compared to the lowest value being recorded for the Fasting (Fa) and Carbohydrate (Ch) trials (91L/min) which indicates that a 20% difference between these interventions exists.

- The Fat and Caffeine trial (FC) show the highest $V'O_{2peak}$ value (Fig. 40) compared to the Fasting (Fa), Fat (F) or Carbohydrate (Ch) trials. The Fat and Caffeine trial (FC) show a $V'O_{2peak}$ value of 58.8 ml/min/kg, whereas the Fasting trial (Fa) show a $V'O_{2peak}$ of 53.1 ml/min/kg. This indicates that there is a 9.69% difference in the $V'O_{2peak}$ between the Fasting (Fa) and Fat and Caffeine (FC) trials.

Heart rate

- The highest HR values (Fig. 41) are recorded for the Carbohydrate trial (Ch) compared to Fat (F) and Caffeine (C) trials showing the lowest values throughout the trial run. At the point of volitional fatigue the Carbohydrate trial (Ch) shows a higher HR value compared to the Fat trial (F), i.e. 199 bpm vs 187 bpm, which indicates a 6% difference between these interventions.

FCCP for all interventions

- No FCCP (Fig. 43) exists for the Fasting (Fa) and the Fat (F) trials, whereas the FCCP occur at 4 km/h for the Caffeine trial (C) and at 8 km/h for the Carbohydrate (Ch) and Fat and Caffeine (FC) trials. Again it should be noted

that for two of the five interventions, no FCCP exist, which contradicts authors such as Brooks et al. (2000:133).

Performance curve

- On comparing the best and worst performance values (Fig. 44) the participant ran 82 seconds longer at an intensity of 16km/h on the Fat and Caffeine (FC) trial compared to the Carbohydrate trial (Ch). This indicates that there is a 12.3% improvement in performance when this trained individual ingested a combination of Fat and Caffeine (FC) compared to the Carbohydrate trial (Ch). This finding is in sharp contrast to findings presented by various authors which claim that carbohydrate ingestion prior to exercise will improve performance (Hawley and Hopkins 1995:245; Maughan et al., 1997:168; Hawley et al., 2006:713; http://www.sportsdieticians.com).

Trained individual 4

Fat oxidation

- Figure 45 indicates that the Fasting (F) trial produced the highest fat oxidation capacity values at low exercise intensity levels (4-8 km/h). Beyond 8 km/h the Fat and Caffeine trial (FC) shows the highest fat oxidation capacities compared to the Carbohydrate trial (Ch) showing the lowest fat oxidation throughout the entire course of the exercise regime. Through research it has become apparent that fasting can be a way to increase the utilization of fat and in the process sparing glycogen and improving performance (Jeukendrup et al., 1998:373). In addition to this it is thus noticeable that the addition of caffeine to fat showed synergistic effects (i.e. the FC trial) on the increased levels of fat oxidation compared to the Fat (F) or Caffeine (C) trials only, which contradicts the findings of Hadjicharalambous et al. (2006:885).

- In addition to this it should be noted that at the fatty acid thresholds (FAT) which occur for all the interventions at 8 km/h, the Carbohydrate trial (Ch)

showed a decrease of 38% in fat oxidation values compared to the Fasting trial (Fa). This corresponds to findings of Achten and Jeukendrup (2003:1021) which indicated that the ingestion of carbohydrates prior to exercise in a graded exercise test decreases maximal fat oxidation by 28% compared to fasting.

- It is clear from the graph that the range in which the fatty acid thresholds (FAT) for all the interventions occurs is between the 2nd and 3rd minute, which is evident at an exercise intensity of 8 km/h and occurs between 63-74% V'$O_{2peak.}$ Jeukendrup and Gleeson (2004a:134) indicates that maximal fat oxidation occurs between 62-63 %V'O_{2max} (refer to figure 8), whereas Achten and Jeukendrup (2004:716) indicate that maximal fat oxidation occurs in trained individuals at around 59-64% V'O_{2max} and Van Loon (2004:1183) states that maximal fat oxidation occurs at 40-65% V'O_{2max} (refer to section 2.2.1.5). Accordingly this trained individual's maximal fat oxidation range falls for only some of the intervention in the ranges depicted above.

Carbohydrate oxidation

- Figure 46 indicates that the Fasting (F) trial shows the lowest carbohydrate oxidation capacity values at low exercise intensity levels (4-8 km/h). Beyond 8 km/h the Fat and Caffeine trial (FC) shows lowest carbohydrate oxidation capacities compared to the Carbohydrate trial (Ch), which shows the highest carbohydrate oxidation capacity values throughout the entire course of the exercise regime. The high carbohydrate oxidation values seen in the Carbohydrate trial (Ch) can be explained by the fact that carbohydrate ingestion prior to exercise increases insulin levels and as a result enhances carbohydrate oxidation during exercise (Jeukendrup and Gleeson, 2004:109). It appears that in the Fat and Caffeine trial (FC), caffeine rather improved fat oxidation instead of high intensity exercise.

$V'O_2$, $V'CO_2$, V'E, $V'O_{2peak}$ and RER

- The $V'O_2$ (Fig. 47) and $V'CO_2$ (Fig. 48) are fairly constant between all interventions during the entire exercise regime.

- Throughout the entire course of the exercise regime the Carbohydrate trial (Ch) shows the highest RER values (Fig. 49) compared to any of the other interventions, with the Fat and Caffeine trial (FC) showing the lowest RER values compared to the other interventions. This can, to a certain extent, be attributed to the Carbohydrate trial (Ch) producing the highest carbohydrate oxidation values and that the Fat and Caffeine trial (FC) produced the highest fat oxidation values (refer to table 2).

- The Fat and Caffeine trial (FC) shows a trend towards a higher $V'O_{2peak}$ value (Fig. 50) compared to the Fasting trial (Fa) which presents the lowest $V'O_{2peak}$ value at maximum effort (16 km/h). The Fat and Caffeine trial (FC) produced a $V'O_{2peak}$ value of 50.2 ml/min/kg, with the Fasting trial (Fa) showing a $V'O_{2peak}$ of 46.1 ml/min/kg. This equates to an 8.16% difference between these interventions.

Heart rate

- Higher HR values (Fig. 51) are observed for the Fasting trial (Fa) compared to the Fat and Caffeine trial (FC) showing the lowest values throughout the entire course of the exercise regime. No significant differences in the HR values are recorded between these interventions at the point of voluntary fatigue.

FCCP for all interventions

- The FCCP for the Fat (F), Carbohydrate (Ch) and Caffeine (C) trials occurred at 8 km/h whereas the FCCP occurred at 10 km/h for the Fasting (Fa) and Fat and Caffeine (FC) trials. This finding indicates that through Fasting (Fa) and the ingestion of Fat and Caffeine (FC) this trained individual was able to shift the FCCP to the next exercise intensity (i.e. 8 km/h vs 10 km/h), which means that

this individual was able to oxidize fats at a higher exercise intensity compared to the Fat (F), Carbohydrate (Ch) and Caffeine (C) trials.

Performance curve

- On comparing the best and worst performance results (Fig. 54), the Fat and Caffeine trial (FC) enabled the participant to run 6 seconds longer at 14 km/h and 64 seconds longer at 16 km/h compared to the Fasting trial (F). This indicates that there exists an 11.6% difference between the Fasting trial (F) and the Fat and Caffeine trial (FC). This contradicts the findings of Hadjicharalambous et al. (2006:885) which show that some of the participants in their study did not improve performance on the fat and caffeine trial.

Trained individual 5

Fat oxidation

- The findings indicate that during lower exercise intensities (8-12 km/h) the Fat and Caffeine trial (FC) show the highest fat oxidation capacities compared to either the Carbohydrate (Ch) or Fasting (Fa) trials showing the lowest fat oxidation capacities. The addition of caffeine to fat showed synergistic effects (i.e. the FC trial) on the increased levels of fat oxidation compared to the Fat (F) or Caffeine (C) trials only, which is also in contrast to the findings of Hadjicharalambous et al. (2006:885).

- The Fat trial (F) shows increased levels of fat compared to the Fat and Caffeine trial (FC) and any of the other interventions at exercise intensities beyond 12km/h. It should be noted that at 6:30 and at an exercise intensity of 8 km/h, the amount of fat oxidized by the Fat trial (F) was 626g/day compared to the Fasting trial (Fa) which produced the lowest fat oxidation values at 207g/day. This equates to a 67% difference between these two interventions. This indicates that this specific trained individual's intake of Fat (F) prior to exercise can increase fat oxidation although exercise intensities are high (above

12km/h). In addition to this it is also noticeable that the Fat trial (F) reached a carbohydrate threshold (i.e. RER =1.0) at the highest exercise intensity of all the other interventions, i.e. 18 km/h (F) vs 14 km/h (Fa) and 16 km/h (Ch, Ca, FC). This finding stands in clear contrast to a variety of researchers who state that the addition of fat increases fat oxidation during the early stages of exercise (Brooks and Mercier, 1994:2253; Achten and Jeukendrup, 2004a:716; Venables et al., 2004:167; Van Loon, 2004:1183).

- In addition to this it should be noted that at the fatty acid thresholds (FAT) which occur for all the interventions at 8 km/h, the Carbohydrate trial (Ch) showed a decrease of 58% in fat oxidation values compared to the Fat and Caffeine trial (FC) which showed the highest fat oxidation values.

- It is clear from the graph that the range in which the fatty acid thresholds (FAT) for all the interventions occurs is between the 2nd and 3rd minute, which is evident at an exercise intensity of 8 km/h and occurs between 48-61% V'$O_{2peak.}$ Jeukendrup and Gleeson (2004:134) indicates that maximal fat oxidation occurs between 62-63 %V'O_{2max} (refer to figure 8), whereas Achten and Jeukendrup (2004a:716) indicate that maximal fat oxidation occurs in trained individuals at around 59-64% V'O_{2max} and Van Loon, (2004:1183) states that maximal fat oxidation occurs at 40-65% V'O_{2max} (refer to section 2.2.1.5). Accordingly this trained individual's maximal fat oxidation range falls more or less in the ranges indicated by the above-mentioned authors.

Carbohydrate oxidation

- The findings indicate that during lower exercise intensities (8-12 km/h) the Fat and Caffeine trial (FC) shows the lowest carbohydrate (Fig. 56) oxidation capacities. The Fat trial (F) shows decreased levels of carbohydrate oxidation compared to the Fat and Caffeine trial (FC) at exercise intensities beyond 12km/h, compared to the Carbohydrate (Ch) and Fasting (Fa) trials which show the highest carbohydrate oxidation values throughout the entire course of the

exercise regime. Thus the notion that the ingestion of carbohydrates prior to exercise in order to increase carbohydrate oxidation can be substantiated by a variety of researchers (Jeukendrup et al., 1997:836; Maughan et al., 1997:168; Arkinstall et al., 2004:2275; Johnson et al., 2004:151; Jeukendrup et al., 2004:1555–1556; Hawley et al., 2006:713; http://www.sportsdieticians.com).

$V'O_2$, $V'CO_2$, V'E, $V'O_{2peak}$ and RER

- Throughout the entire course of the exercise regime the Caffeine (C) as well as the Caffeine and Fat (FC) trials shows increased levels of oxygen consumption values (Fig. 57) compared to any of the other interventions. The same notion prevails for carbon dioxide production (Fig. 58). In addition to this, it appears that both the Fat and Caffeine (FC) and Caffeine (C) trials produced the highest $V'O_{2peak}$ values throughout the test, with the Caffeine trial (C) producing the highest $V'O_{2peak}$ value of 72.4 ml/min/kg. The Carbohydrate trial (Ch) shows the lowest $V'O_{2peak}$ value, 61.5 ml/min/kg. This indicates that there is a 15.06% difference between these two interventions.

- At an exercise intensity of 8 km/h, the Caffeine trial (C) also shows the lowest RER values (Fig. 59) compared to either the Fasting (Fa) or Carbohydrate (Ch) trials, whereas at an exercise intensity of 14 km/h the Fat trial (F) shows the lowest RER values. At 14km/h the Fasting trial (F) and at 16 km/h the Fat and Caffeine trial (FC) shows the highest RER values compared to the Fat trial (Fig. 59). These findings can be connected to the interventions which produced the highest and lowest fat and carbohydrate oxidation values at specific exercise intensities.

- Minor alterations were observed for V'E values between the interventions for the entire course of the exercise regime.

Heart rate

- Heart rate is fairly constant, with no significant alterations between the interventions

FCCP for all interventions

- There exists no FCCP (Fig. 63) for the Fasting (Fa) and Carbohydrate (Ch) trials, whereas a FCCP exists at 8 km/h for the Fat trial (F) and at 10 km/h for the Caffeine (C) and Fat and Caffeine (FC) trials. Once again it can be seen that a FCCP is not discernible in each and every individual, irrespective of prior nutritional intake.

Performance curve

- On comparing the best (FC trial) to the worst (Fa trial) result on performance the participant ran 31 seconds longer at an intensity of 20km/h, which indicates a 3.8% difference between these two interventions (Fig. 64). This stands in contrast to the study done by Hadjicharalambous et al. (2006:884) which indicates that the combination of fat and caffeine showed no differences in substrate utilization and no improvement in exercise performance was discernible compared to either the fat or caffeine trials.

Trained individual 6

Fat oxidation

- The findings reveal that the Fat and Caffeine (FC) as well as the Fasting (Fa) trials show the highest fat (Fig. 65) oxidation capacity values compared to the Carbohydrate trial (Ch), which shows the lowest fat oxidation capacity values. It has been proposed by various researchers that fasting can increase fat oxidation values during exercise (Horowitz et al., 1997:774; Jeukendrup et al., 1998:373; Horowitz and Klein, 2000:559). In addition to Fasting (Fa), the Fat and Caffeine trial (FC) also showed high fat oxidation values which is in contrast to what Hadjicharalambous et al. (2006:885) have found.

- In addition to this it should be noted that at the fatty acid thresholds (FAT) which occur for all the interventions at 8 km/h, the Carbohydrate trial (Ch) showed a decrease of 50% in fat oxidation values compared to the Fasting trial (Fa) which showed the highest fat oxidation values. This corresponds with findings of Achten and Jeukendrup (2003:1021) which indicates that the ingestion of carbohydrates prior to exercise in a graded exercise test decreases maximal fat oxidation by 28%.

- It is clear from the graph that the range in which the fatty acid thresholds (FAT) for all the interventions occurs is between the 2nd and 3rd minute, which is evident at an exercise intensity of 8 km/h and occurs between 47-54% $V'O_{2peak}$. Jeukendrup and Gleeson (2004:134) indicates that maximal fat oxidation occurs between 62-63 $\%V'O_{2max}$ (refer to figure 8), whereas Achten and Jeukendrup (2004a:716) indicate that maximal fat oxidation occurs in trained individuals at around 59-64% $V'O_{2max}$ and Van Loon (2004:1183) states that maximal fat oxidation occurs at 40-65% $V'O_{2max}$ (refer to section 2.2.1.5). Accordingly this trained individual's maximal fat oxidation range falls more or less in the ranges indicated by the above-mentioned authors.

Carbohydrate oxidation

- Figure 66 reveals that the Carbohydrate trial (Ch) produced the highest carbohydrate oxidation values, with the Fat and Caffeine trial (FC) showing the lowest values. Thus the notion that the ingestion of carbohydrates prior to exercise in order to increase carbohydrate oxidation can be substantiated by a variety of researchers (Jeukendrup et al., 1997:836; Maughan et al., 1997:168; Arkinstall et al., 2004:2275; Johnson et al., 2004:151; Jeukendrup et al., 2004:1555–1556; Hawley et al., 2006:713; http://www.sportsdieticians.com).

$V'O_2$, $V'CO_2$, V'E, $V'O_{2peak}$ and RER

- The $V'O_2$ (Fig. 67) shows fairly constant values and the $V'CO_2$ (Fig. 68) shows minor alterations in the specific values during the entire exercise regime.

Throughout the entire course of the exercise regime the Carbohydrate trial (Ch) shows the highest RER values (Fig. 69) compared to any of the other interventions, with the Fat and Caffeine trial (FC) showing the lowest RER values, which could be explained by their respective roles in carbohydrate and fat oxidation.

- Even though minor differences were noted in the $V'O_{2peak}$ throughout the entire course of the exercise regime, the Fat and Caffeine trial (FC) produced the highest $V'O_{2peak}$ value at 62.9 ml/min/kg. The Fat trial (F) produced the lowest $V'O_{2peak}$ value at 57.4 ml/min/kg, which indicates that there is a 8.74% difference between these interventions (Fig. 70).

- Minor alterations are observed for V'E (Fig. 72) throughout the trial run.

Heart rate

- Figure 71 indicates that the Fasting trial (Fa) shows the highest HR values compared to the Fat trial (F), which shows the lowest values throughout the trial run. No differences in the heart rate values are recorded between these interventions at the point of voluntary fatigue.

FCCP for all interventions

- The FCCP (Fig. 73) does not exist for the Caffeine (C) and Carbohydrate (Ch) trials and this occurs at 8 km/h for all the other interventions. From this finding it appears that the different nutritional interventions adhered to prior to the exercise regime, did however influence this trained individual's capacity to utilize fuels at specific exercise intensities.

Performance curve

- When comparing the best and weakest performance results, the Fat and Caffeine trial (FC) shows the better result compared to the Fasting trial (Fa)- see fig. 74. On the Fat and Caffeine trial (FC) the participant ran 72 seconds

longer at an intensity of 20km/h and could continue for an additional 30 seconds at a treadmill speed of 22km/h. There exists an 11% improvement in performance time between the Fasting trial (Fa) and the Fat and Caffeine trial (FC).This finding contradicts the findings of Hadjicharalambous et al. (2006:884), where the combination of fat and caffeine did not improve performance.

5.4.2. Group of untrained individuals

Untrained individual 1

Fat oxidation

- Figure 75 indicates that the Carbohydrate trial (Ch) shows the lowest fat oxidation capacity values compared to any of the other interventions during the entire course of the exercise regime. Horowitz et al. (1997:774) claims that total fat oxidation can be reduced by about 30% over an 8 hour period when carbohydrate are ingested before exercise. Reducing lipolysis and impairing daily fat oxidation by ingesting carbohydrate before exercise increases the likelihood of being in a positive lipid balance and may thus increase fat deposition. Due to the sensitivity of lipolysis to even small elevations in insulin, it appears that to maintain high rates of fat oxidation at rest and during subsequent exercise, people should not eat even small amounts of carbohydrates before exercise. The reduction in lipolysis and fat oxidation after carbohydrate ingestion necessitates a compensatory increase in carbohydrate oxidation to maintain energy production during exercise.

- In addition to this it should be noted that at the fatty acid thresholds (FAT) which occur for all the interventions at 8 km/h, the Carbohydrate trial (Ch) showed a decrease of 80% in fat oxidation values compared to the Fat and Caffeine trial (FC) which showed the highest fat oxidation values.

- It is clear from the graph that the range in which the fatty acid thresholds (FAT) for all the interventions occurs during the 2nd minute, which is evident at an exercise intensity of 8 km/h and occurs between 68-77% V'$O_{2peak.}$ Achten and Jeukendrup (2004a716) indicate that maximal fat oxidation occurs in untrained individuals, at around 47-52% V'O_{2max} (refer to section 2.2.1.5). It can thus be seen that this untrained individual's maximal fat oxidation occurs at a much higher % of V'O_{2peak}.

Carbohydrate oxidation

- It stands to reason that the intervention that produced the weakest fat oxidation results will produce the best carbohydrate oxidation results, since fat and carbohydrate are the only two energy sources in the human body (Jeukendrup and Gleeson, 2004:137). Since the Carbohydrate trial (Ch) produced the weakest fat oxidation results, it produced the best carbohydrate oxidation results. Carbohydrate ingestion prior to incremental exercise increases carbohydrate oxidation as substantiated by Jeukendrup and Gleeson (2004:137).

$V'O_2$, $V'CO_2$, V'E, $V'O_{2peak}$ and RER

- The Fasting trial (Fa) shows the highest and the Caffeine trial (C) the lowest oxygen consumption capacity values ($CV'O_2$) beyond an exercise intensity of 8 km/h until voluntary fatigue sets in (Fig. 77). The Carbohydrate trial (Ch) shows the highest and the Caffeine trial (C) the lowest carbon dioxide production ($CV'CO_2$) capacity values (Fig. 78) throughout the entire exercise regime. In addition to these findings, the Caffeine trial (C) also shows the lowest V'E and $V'O_{2peak}$ value(s) of all the interventions. The increase in $V'CO_2$ values seen in the Carbohydrate trial (Ch) can be due to: (1) an increase in lactate production during glycolysis; (2) pyruvate which is converted to Acetyl-CoA and in the process release CO_2; (3) the Krebs cycle which releases CO_2. Due to the fact that the carbohydrate threshold (i.e. RER = 1.0) for the Carbohydrate trial (Ch) is reached sooner than in any of the other trials, it indicates that this untrained individual was required to run from anaerobic glycolysis from an earlier treadmill speed. Thus the increased CO_2 levels are most probably due to an increase in lactate accumulation in the blood.

- Figure 79 shows that the Carbohydrate trial (Ch) produced the highest RER values throughout the entire course of the exercise regime compared to any of the other interventions. This can be linked to the Carbohydrate trial (Ch) which produced the highest $V'CO_2$ and carbohydrate oxidation values.

- The Fasting trial (Fa) shows the highest $V'O_{2peak}$ value at 36.6 ml/min/kg compared to both the Fat (F) and Caffeine (C) trials which produced a concomitant $V'O_{2peak}$ value of 33.1 ml/min/kg. This indicates that there is a 9.56% improvement between these interventions.

Heart rate

- The Carbohydrate trial (Ch) coincided with higher HR values (Fig. 81) being recorded compared to the Fat and Caffeine trial (FC), which shows the lowest values throughout the entire exercise regime. The largest difference in the maximum heart rate values recorded (point of voluntary fatigue) lies between the Fasting (Fa) and Fat and Caffeine (FC) trials 189 vs. 175 bpm respectively. This equates to a 7.4% difference between maximal heart rates of the various interventions. This might be due to the individual's performance during these tests. The Fasting trial (Fa) produced the best endurance performance, which might explain the high heart rates at the end of the test, with the combination of Fat and Caffeine (FC) producing the worst endurance performance, which might explain the lowest heart rate at volitional fatigue.

FCCP for all the interventions

- There is no FCCP (Fig. 83) for the Carbohydrate trial (Ch). The FCCP for the Fasting trial (Fa) occurred at 4 km/h, whereas all of the other interventions occur at 8 km/h. The addition of nutritional interventions to the equation disrupts the theory that a crossover concept occurs between 48-53% $V'O_{2max}$ (Brooks and Mercier, 1994:2253).

Performance curve

- When comparing the best and weakest performance results, the Fasting trial (Fa) shows the better result compared to the Fat and Caffeine trial (FC)-see figure 84. On the Fasting trial (Fa) the participant ran 47 seconds longer at an intensity of 10km/h compared to the Fat and Caffeine trial (FC). The improvement of endurance performance by means of Fasting (Fa) has been

emphasized by researchers such as Koubi et al. (1991:1337) and Jeukendrup et al. (1998:373).

Untrained individual 2

Fat oxidation

- Figure 85 indicates that the Carbohydrate trial (Ch) shows the lowest fat oxidation capacity values compared to any of the other interventions undertaken throughout the entire exercise regime. The Caffeine (C) and Fat and Caffeine (FC) trials produced the highest fat oxidation values of all the interventions. According to the results it seems that the addition of Fat to Caffeine (FC) increased fat oxidation even further than which either the Caffeine trial (C) or Fat trial (F) achieved individually. This contradicts what Hadjicharalambous et al. (2006) have found in their study, i.e. that the addition of caffeine to fat will not increase fat oxidation to a greater extent.

- In addition to this, it should be noted that at the fatty acid thresholds (FAT) which occur in all the interventions at 8 km/h, the Carbohydrate trial (Ch) showed a decrease of 51% in fat oxidation values compared to the Caffeine trial (C) which showed the highest fat oxidation values.

- The graph indicates that the range in which the fatty acid thresholds (FAT) occurs between the 2nd and 3rd minute for all the interventions. This becomes evident at an exercise intensity of 8 km/h and occurs between 50-71% V'O_{2peak}. Achten and Jeukendrup (2004a:716) indicate that maximal fat oxidation occurs in untrained individuals at around 47-52% V'O_{2max} (refer to section 2.2.1.5). It can thus be deducted that this untrained individual's maximal fat oxidation value relevant for the Carbohydrate (Ch) and Fat (F) trials occurs within this range; but during the other interventions, maximal fat oxidation occurred at a much higher % V'O_{2peak}.

Carbohydrate oxidation

- According to figure 86, the Carbohydrate trial (Ch) displays the highest carbohydrate oxidation capacity values compared to any of the other interventions throughout the entire exercise regime. This is similar to what other researchers have found in their studies that the ingestion of carbohydrates prior to exercise will increase carbohydrate oxidation (Jeukendrup et al., 1997:836; Maughan et al., 1997:168; Arkinstall et al., 2004:2275; Johnson et al., 2004:151; Jeukendrup et al., 2004:1555–1556; Hawley et al., 2006:713 http://www.sportsdieticians.com).

$V'O_2$, $V'CO_2$, V'E, $V'O_{2peak}$ and RER

- The Fasting trial (Fa) shows the lowest oxygen consumption capacity values ($CV'O_2$) beyond an exercise intensity of 8 km/h, compared to any of the other interventions (see Fig. 87). Similar findings are recorded for the carbon dioxide production capacity ($CV'CO_2$) values (Fig. 88) as well as the V'E (Fig. 92) beyond 12 km/h. In addition to this the RER values fluctuated to such an extent between the various interventions that a clear result is not possible (Fig. 89).

- The highest $V'O_{2peak}$ value (Fig. 90) is evident in the Caffeine trial (C) compared to the lowest $V'O_{2peak}$ value recorded for the Fasting trial (Fa). The Caffeine trial (C) produced a $V'O_{2peak}$ of 48.3 ml/min/kg whereas the Fasting trail (Fa) produced a $V'O_{2peak}$ value of 44.1 ml/min/kg. This equates to an 8.7% difference between these two interventions.

Heart rate

- The Carbohydrate (Ch) and Fasting (Fa) trials show higher HR values (Fig. 91) compared to the Caffeine trial (C), which shows the lowest values throughout the entire exercise regime. A 5% difference in the HR values is recorded between the Caffeine trial (C) and the Fasting trial (Fa) at the point of voluntary fatigue. This stands in contradiction to what Bridge and Jones (2006:437) have claimed. They indicate that caffeine ingestion and succeeding exercise results

in an increase in mean heart rate of 4 beats per minute, which means that athletes are able to work at a higher percentage of their maximal heart rate. The higher mean heart rates could actually be attributed to the fact that caffeine is a stimulant by way of caffeine's effect on central perceptions of effort.

FCCP for all interventions

- There exists no FCCP (Fig. 93) for the Fasting (Fa) and Carbohydrate (Ch) trials and this exists at 8 km/h for the other interventions.

Performance curve

- When comparing the best and weakest performance results, the Fasting trial (Fa) shows the better result compared to the Fat trial (F)-see figure 94. In the Fasting trial (Fa) the participant ran 58 seconds longer at an intensity of 16km/h before the onset of voluntary fatigue. This equates to a 9.5% improvement in performance time between these two interventions. This might aid the cause of researchers who have been trying to prove the relationship between fasting and performance for years.

Untrained individual 3

Fat oxidation

- Figure 95 indicates that the Fasting trial (Fa) shows the highest fat oxidation capacity values compared to the Carbohydrate trial (Ch) during exercise intensities ranging between 4-8 km/h. From 8 km/h onwards, fat oxidation reveals negative values which correspond to higher carbohydrate oxidation values which occur at an RER of 1.0. The increased fat oxidation values in the Fasting trial (Fa) have been stipulated by various researchers (Horowitz et al., 1997:773,774; Jeukendrup et al., 1998:373).

- In addition to this, it should be noted that at the fatty acid thresholds (FAT) which occur for all the interventions at 8 km/h, the Carbohydrate trial (Ch)

showed a decrease of 61% in fat oxidation values compared to the Fasting trial (Fa) which showed the highest fat oxidation values. This not only corresponds to the findings of Achten and Jeukendrup (2003:1021) which indicates that the ingestion of carbohydrates prior to exercise in a graded exercise test decreases maximal fat oxidation by 28%; it, in fact surpasses it.

- It is clear from the graph that the range in which the fatty acid thresholds (FAT) for all the interventions occurs at 1:30, which is evident at an exercise intensity of 8 km/h and occurs between 45-58% $V'O_{2peak.}$ Achten and Jeukendrup (2004a:716) indicate that maximal fat oxidation occurs in untrained individuals at around 47-52% $V'O_{2max}$ (refer to section 2.2.1.5). It can thus be seen that this untrained individual's maximal fat oxidation occurs within this range for the Carbohydrate (Ch) and Fat and Caffeine (FC) trials whereas it occurs at a slightly higher % $V'O_{2peak}$ for the other interventions.

Carbohydrate oxidation

- From figure 96 can be deduced that the Fasting trial (Fa) shows the lowest carbohydrate oxidation capacity values during exercise intensities ranging between 4-8 km/h. The Carbohydrate trial (Ch) shows the highest carbohydrate oxidation capacity values compared to the any of the other interventions. This corresponds with what other researchers have found in their studies, namely, that the ingestion of carbohydrates prior to exercise will increase carbohydrate oxidation (Jeukendrup et al., 1997,836; Maughan et al., 1997:168; Arkinstall et al., 2004:2275); Johnson et al., 2004:151; Jeukendrup et al., 2004:555–1556; Hawley et al., 2006:713; http://www.sportsdieticians.com).

$V'O_2$, $V'CO_2$, V'E, $V'O_{2peak}$ and RER

- It has been found that for the Fat trial (F) there exists a correlation between the following variables at exercise intensities beyond 8 km/h: lowest $V'O_2$ (Fig. 97), lowest $V'CO_2$ (Fig. 98), lowest V'E values (Fig. 102) and the lowest $V'O_{2peak}$ value (Fig. 100).

- During low exercise intensities (<10 km/h) the RER values (Fig. 99) for the Fasting trial (Fa) are lower compared to the Carbohydrate trial (Ch). The increased RER at these exercise intensities is to a greater extent due to the increased V'CO_2 levels rather than the concomitant increase in V'O_2 levels. The increased CO_2 levels are most probably due to increased lactate levels in the Carbohydrate trial (Ch) which points to the fact that the carbohydrate threshold was reached sooner in this intervention than in any of the other interventions. This in effect indicates that glycolysis was stimulated at an earlier exercise intensity level. Beyond 10km/h the RER values shows minor fluctuations between the various interventions.

- The Carbohydrate trial (Ch) show the highest V'O_{2peak} value compared to the Fat (F) and Fasting (Fa) trials. The highest V'O_{2peak} was reached at 35.7 ml/min/kg for the Carbohydrate trial (Ch) and the lowest V'O_{2peak} was reached at 33.0 ml/min/kg for the Fat trial (F). This indicates a 7.56% improvement between these two interventions.

Heart rate

- The Fat and Caffeine trial (FC) shows lower HR values (Fig. 101) during exercise intensities in the range of 8-10 km/h compared to the Fasting (Fa) or Caffeine (C) trials. This contradicts to what Bridge and Jones (2006:437) found in their studies. They found that caffeine produced an increase in the mean heart rate of 4 bpm. No differences in the heart rate values are recorded between these interventions at the point of voluntary fatigue.

FCCP for all interventions

- There exists no FCCP (Fig. 103) for the Fat (F), Carbohydrate (Ch) and Fat and Caffeine (FC) trials and the FCCP occurs at 8 km/h for the Fasting (Fa) and Caffeine (C) trials. In comparison with previous individuals, it is noted that the FCCP varies for each individual and each intervention.

Performance curve

- When comparing the best and weakest performance results (Fig. 104), the Carbohydrate trial (Ch) shows the better result compared to the Fasting trial (Fa). On the Carbohydrate trial (Ch) the participant ran 37 seconds longer at an intensity of 12km/h. This equates to a 9.3% improvement in performance time between these two interventions. This finding corresponds with what other researchers have found in their studies (Maughan et al., 1997:168; Jeukendrup et al., 1997:836; Jeukendrup et al., 2004:1555–1556; Hawley et al., 2006:713).

Untrained individual 4

Fat oxidation

- Figure 105 indicates that the Fat (F) and Fat and Caffeine (FC) trials show the highest fat oxidation capacity values compared to the Carbohydrate trial (Ch) during exercise intensities ranging between 4-10 km/h. This indicates that in contrast to Hadjicharalambous et al. (2006:884), the addition of Caffeine (C) to Fat (F) enhanced fat oxidation to a greater extent than what Fat (F) or Caffeine (C) could achieve individually.

- In addition to this it should be noted that at the fatty acid thresholds (FAT) which occur for all the interventions at 8 km/h, the Carbohydrate trial (Ch) showed a decrease of 47% in fat oxidation values compared to the Fat and Caffeine trial (FC) which showed the highest fat oxidation values.

- It is clear from the graph that the range in which the fatty acid thresholds (FAT) for all the interventions falls, occurs between 1:30 and 2:30, which is evident at an exercise intensity of 8 km/h and occurs between 61-75% $V'O_{2peak.}$ Achten and Jeukendrup (2004a:716) indicate that maximal fat oxidation occurs in untrained individuals, at around 47-52% $V'O_{2max}$ (refer to section 2.2.1.5). It can thus be seen that this untrained individual's maximal fat oxidation occurs at a much higher % $V'O_{2peak}$ for all the interventions.

Carbohydrate oxidation

- Figure 106 indicates that the Fat (F) and Fat and Caffeine (FC) trials show the lowest carbohydrate oxidation capacity values compared to the Carbohydrate trial (Ch). The increased carbohydrate oxidation in the Carbohydrate trial (Ch) is evident in many of the individuals and is substantiated by a variety of authors (Jeukendrup et al., 1997:836; Maughan et al., 1997:168 Arkinstall et al., 2004:2275; Johnson et al., 2004:151; Jeukendrup et al., 2004:1555–1556; Hawley et al., 2006:713; http://www.sportsdieticians.com).

- At exercise intensities beyond 10 km/h, the Caffeine (C) trial shows the highest carbohydrate oxidation capacity values, which is also noted by other authors. Used in fairly small quantities before exercise, caffeine can enhance performance in both short, high intensity effort as well as in endurance exercise. Well-controlled research studies have indicated that caffeine is an efficient ergogenic aid for a wide selection of exercise modes and intensities. (http://www.medicdirectsport.com).

$V'O_2$, $V'CO_2$, V'E, $V'O_{2peak}$ and RER

- The Fat (F) and Fat and Caffeine (FC) trials show the lowest oxygen consumption capacity (Fig 107) and V'E values (Fig. 112) throughout the entire course of the exercise regime, compared to any of the other interventions. The Fat (F) and Fat and Caffeine (FC) trials show the lowest carbon dioxide production capacity values (Fig 108) compared to any of the other interventions. In contrast to this, the Caffeine trial (C) produced the highest $V'O_2$, $V'CO_2$ values. Beyond 10 km/h, the Caffeine trial (C) shows the highest V'E values compared to any of the other interventions.

- The increase in $V'CO_2$ and V'E levels during the Caffeine trial (Ca) can be linked to increase carbohydrate oxidation values at high exercise intensity levels. The carbohydrate threshold is also reached earlier in the Caffeine trial (Ca) compared to the other interventions, except for the Carbohydrate trial (Ch)

which occurs at the same time. This indicates that the increased CO_2 levels and the subsequent increase in V'E are due to the increase in lactate which is formed in the Caffeine trial (C).

- The RER values (Fig. 109) indicate that the Carbohydrate trial (Ch) shows the highest RER values between exercise intensities that range between 4-10 km/h, compared to the Fat (F) and Fat and Caffeine (FC) trials. Clear of this exercise intensity range, the Caffeine (C) and Carbohydrate (Ca) trials both show higher RER values compared to the Fat and Caffeine (FC) trial. This exact notion is also visible during carbohydrate oxidation.

- The lowest $V'O_{2peak}$ (Fig. 110) value is observed for the Fat (F) and Caffeine (C) trials compared to the highest $V'O_{2peak}$ value observed for the Fasting trial. The Fasting trial (Fa) produced a $V'O_{2peak}$ of 35.0 ml/min/kg, whereas the Fat trial (F) produced a $V'O_{2peak}$ of 33.5 ml/min/kg. This equates a 4.28% improvement between these two interventions.

Heart rate

- Minor fluctuations are observed for HR (Fig. 111) values between the various interventions, but a trend towards a decrease is observed in the Fat trial (F) compared to the other interventions at exercise intensities in the range of 10-12 km/h. No differences in the HR values are recorded between these interventions at the point of voluntary fatigue.

FCCP for all interventions

- The FCCP (Fig. 113) occurs at 8 km/h for all the interventions. This is the first individual where the FCCP occurs for all the interventions and this occurs at exactly the same exercise intensity, i.e. 8 km/h.

Performance curve

- When comparing the best and weakest performance results (Fig. 114), the Fat and Caffeine trial (FC) shows the better result compared to the Fasting trial (Fa). On the Fat and Caffeine trial (FC) the participant ran 36 seconds longer at an intensity of 12km/h. Once again this is in contrast to what Hadjicharalambous et al. (2006:884) found in their study.

Untrained individual 5

Fat oxidation

- Figure115 indicates that the Fasting trial (Fa) shows the highest fat oxidation capacity values of all the interventions discernible throughout the entire exercise regime. This finding is substantiated by a variety of researchers (Koubi et al., 1991:1337; Horowitz et al., 1997:773 & 774; Jeukendrup et al., 1998:373). At exercise intensities ranging between 4-8 km/h, the Carbohydrate trial (Ch) shows the lowest fat oxidation values. The latter finding has been substantiated by various researchers (Horowitz et al., 1997:773; Bergman and Brooks, 1999:480).

- In addition to this it should be noted that at the fatty acid thresholds which occur for all the interventions at 8 km/h, the Carbohydrate trial (Ch) showed a decrease of 45% in fat oxidation values compared to the Fasting trial (Fa) which showed the highest fat oxidation values. This finding corresponds to the findings of Achten and Jeukendrup (2003:1021) which indicated that the ingestion of carbohydrates prior to exercise in a graded exercise test decreases maximal fat oxidation by 28% compared to fasting.

- It is clear from the graph that the range in which the fatty acid thresholds (FAT) for all the interventions falls, occurs between 1:30 and 2:30, which is evident at an exercise intensity of 8 km/h and occurs between 51-78% $V'O_{2peak.}$ Achten and Jeukendrup (2004a:716) indicate that maximal fat oxidation occurs in

untrained individuals, at around 47-52% $V'O_{2max}$ (refer to section 2.2.1.5). It can thus be seen that this untrained individual's maximal fat oxidation occurs in this range only for the Fat and Caffeine trial (FC). Maximal fat oxidation occurs at a much higher $\%V'O_{2peak}$ for some of the interventions.

Carbohydrate oxidation

- Figure 116 indicates that the Fasting trial (Fa) shows the lowest carbohydrate oxidation capacity values of all the interventions throughout the entire exercise regime. At exercise intensities ranging between 4-8 km/h, the Carbohydrate trial (Ch) shows the highest carbohydrate oxidation values. Beyond exercise intensities of 10 km/h, the Carbohydrate trial (Ch) shows the highest carbohydrate oxidation values compared to the Fasting trial (Fa).

$V'O_2$, $V'CO_2$, V'E, $V'O_{2peak}$ and RER

- The Fat and Caffeine (FC) trial shows the highest oxygen consumption capacity ($CV'O_2$) values (Fig. 117) from exercise intensities beyond 8km/h compared to the Fasting trial (Fa) which shows the lowest $V'O_2$ values. The same notion applies for the carbon dioxide production capacity (Fig 118) and V'E values (Fig. 122). The increased CO_2 and V'E levels shown in the Fat and Caffeine trial (FC) are most probably due to the accumulation of lactate. The lactate accumulation is due to enhanced stimulation of glycolysis, and this in turn can be attributed to the fact that the carbohydrate threshold has been reached sooner than any of the other interventions except for the Caffeine trial (C).

- Figure 119 indicates that the Fasting trial (Fa) shows the lowest RER values of all the interventions, which is due to the following: (1) this intervention producing the highest fat oxidation values of all the interventions and (2) it also produced the lowest $V'CO_2$ levels of all the interventions. At exercise intensities ranging between 4-8 km/h the Carbohydrate trial (Ch) shows the highest RER values, which is explained by the fat that carbohydrate oxidation

was stimulated in this time frame, and this can in turn be due to the fact that $V'CO_2$ was stimulated to the same extent. At exercise intensities ranging between 8-10 km/h, the Fat and Caffeine (FC) trial shows the highest RER values, which is also evident in the enhanced carbohydrate oxidation values and enhanced $V'CO_2$ levels for this time frame.

- The highest $V'O_{2peak}$ (Fig. 120) value for any of the interventions is observed for the Carbohydrate trial (Ch) compared to the Fat trial (F) which showed the lowest $V'O_{2peak}$ values. The highest $V'O_{2peak}$ value was 36.3 ml/min/kg for the Carbohydrate trial (Ch) and the lowest $V'O_{2peak}$ value was 33.1 ml/min/kg which equates an 8.81% improvement between these two interventions.

- The intervention with the highest $V'O_2$ near the end of the test produced the highest $V'O_{2max}$. The highest $V'O_{2max}$ was reached with the Carbohydrate trial (Ch). The highest $V'O_{2max}$ value was reached at 36.3 ml/min/kg. The lowest $V'O_{2max}$ value was 33.1 ml/min/kg, which was evident during the Fat trial (F). This means that the there was an 8.8 % improvement between the two interventions.

Heart rate

- Figure 121 indicates that the Fat and Caffeine trial (FC) shows the highest HR values compared to the Carbohydrate trial (Ch) throughout the entire exercise regime. There exists a 6.8% difference between maximum heart rates of the Caffeine (C) and Fat (F) trials.

FCCP for all interventions

- There exists no FCCP (Fig. 123) for all the interventions except for the Fasting trial (Fa) which occurs at 8 km/h. This is once again an example where the crossover concept does not occur in all individuals.

Performance curve

- When comparing the best and weakest performance results (Fig. 124), the Caffeine trial (C) produced the better result compared to the Fat trial (F). On the Caffeine trial (C) the participant ran 57 seconds longer at an intensity of 10 km/h. This finding, where the Caffeine trial (C) improves performance, is substantiated by (Bridge and Jones, 2006:436; http://www.medicdirectsport.com; http://www.pponline.co.uk).

Untrained individual 6

Fat oxidation

- Figure 125 indicates that the Fat and Caffeine trial (FC) shows the highest fat oxidation capacity values for exercise intensities ranging between 4-10 km/h, compared to the Fasting trial (Fa) which shows the lowest fat oxidation capacity values for the entire course of the exercise regime. Beyond 10 km/h the Caffeine trial (C) shows the highest fat oxidation values. The finding that Caffeine (C) improves fat oxidation is similar to what various researchers have found in their studies (Nehlig and Debry, 1994:216-217; Jeukendrup et al., 1998:372–373; Hadjicharalambous et al., 2006:876). The finding that the Fat and Caffeine trial (FC) improved fat oxidation is in contrast with what Hadjicharalambous et al. (2006:884) found in their study: the combination of fat and caffeine (FC) showed no differences in substrate utilization, compared to either the fat (F) or caffeine (C) trials. Furthermore, Hadjicharalambous et al. (2006:885) also claim that the addition of caffeine to fat will not enhance fat oxidation even further. This is clearly not the case with the present finding, where the addition of fat to caffeine improved fat oxidation values to a greater extent than that which either the Fat (F) or Caffeine (C) trial showed individually.

- In addition to this it should be noted that at the fatty acid thresholds (FAT) which occur for all the interventions at 8 km/h, the Fasting trial (Fa) showed a

decrease of 28% in fat oxidation values compared to the Fat and Caffeine trial (FC) which showed the highest fat oxidation values. This could be attributed to the fact that the Fasting (Fa) produced the highest carbohydrate oxidation values of all the interventions.

- It is clear from the graph that the range in which the fatty acid thresholds for all the interventions falls, occurs during the 2nd minute, which is evident at an exercise intensity of 8 km/h and occurs between 65-74% V'$O_{2peak.}$ Achten and Jeukendrup (2004a:716) indicate that maximal fat oxidation occurs in untrained individuals at around 47-52% V'O_{2max} (refer to section 2.2.1.5). It can thus be seen that this untrained individual's maximal fat oxidation occurs at a much higher % V'O_{2peak} for all the interventions.

Carbohydrate oxidation

Figure 126 indicates that the Fasting trial (Fa) shows the highest carbohydrate oxidation capacity values throughout the entire course of the exercise regime, compared to the Caffeine trial (C) which shows the lowest carbohydrate oxidation capacity values at exercise intensities beyond 8 km/h. In contrast to what has been stated in the literature on this aspect (which states that Fasting (Fa) improves fat oxidation) this untrained individual produced the highest carbohydrate oxidation values with the Fasting trial (Fa) (Koubi et al., 1991:1337; Horowitz et al., 1997:773 & 774; Jeukendrup et al., 1998:373). It stands to reason that the intervention which showed the best fat oxidation values will produce the worst carbohydrate oxidation values, since fat and carbohydrate are basically the only two energy sources in the human body (Jeukendrup and Gleeson, 2004:137). Owing to the fact that Caffeine (Ca) produced the highest fat oxidation from exercise intensities beyond 8 km/h, it is apparent that this intervention produced the lowest carbohydrate oxidation values.

$V'O_2$, $V'CO_2$, V'E, $V'O_{2peak}$ and RER

- Figure 127 indicates that there are minor alterations in the $V'O_2$ between any of the interventions throughout the entire course of the exercise regime. In contrast to this notion the Fasting trial (Fa) produced the highest $V'CO_2$ (Fig. 128) and RER (Fig. 129) values of all the interventions. This could be related to the Fasting trial (Fa) producing the highest carbohydrate oxidation values compared to any of the other interventions. In contrast to this, the Fat (F) and Caffeine (C) trials show the lowest $V'CO_2$ values beyond exercise intensities of 8 km/h. In addition to this the Fat (F) and Caffeine (C) trials also show the lowest RER values for an exercise intensity range of 4-8 km/h. Beyond 8 km/h the Caffeine trial (C) shows the lowest RER values. This is linked to the fat oxidation capacity.

- The highest $V'O_{2peak}$ (Fig. 130) value is observed for the Fat trial (F) and the lowest for the Caffeine trial (C). The Fat trial (F) showed a $V'O_{2peak}$ of 32.5 ml/min/kg whereas the Caffeine trial (C) showed a $V'O_{2peak}$ value of 30.1 ml/min/kg, which equates to a 7.38% difference between these two interventions.

- Figure 132 shows that minor deviations exist between the various interventions for the V'E values.

Heart rate

- Figure 131 indicates that even though minor alterations are noted between the interventions, the Carbohydrate trial (Ch) shows a trend towards higher HR values compared to the Fasting trial (Fa). No difference in maximum heart rate is noted between any of the interventions.

FCCP for all interventions

- There exists no FCCP (Fig. 133) for any of the interventions except for the Fat and Caffeine trial (FC) where it occurs at 8 km/h. This indicates the effect that pre-exercise nutrient intake as well as inter-individuality has on FCCP.

Performance curve

- When comparing the best and weakest performance results (Fig. 134), the Carbohydrate trial (Ch) shows the better result compared to the Caffeine trial (C). On the Carbohydrate trial (Ch) the participant ran 41 seconds longer at an intensity of 12 km/h, which equates to an 11.4% improvement in performance time.

5.5. INTEGRATED DISCUSSION OF RESULTS

The integrated discussion on the impact of the rationale for the research project (see section 1.3.) according to the objectives (see section 1.4) is subdivided into three categories:

- The Athletic Industry
- The Well-being Industry
- Scientific relevance

5.5.1. The athletic industry

In the exploration for tactics to advance athletic performance, current interest has focused on numerous nutritional actions to promote athletic performance and gain a ‘competitive edge’. Athletes are also subjected to evaluation protocols with the view to identifying areas of strengths and weaknesses.

The present investigation suggests that the:

- Hypothesis (see section 1.6, p. 5)

  "Pre-exercise nutrient intake and the timing thereof prior to a graded exercise test until voluntary fatigue sets in, manipulates metabolism (fat and/or carbohydrate oxidation) during exercise"

and the

- Postulation, namely: (see section 1.7, p. 5)
  - Fasting, fat intake, caffeine intake, fat in combination with caffeine intake and carbohydrate intake prior to a graded exercise test influence macronutrient metabolism in different ways.
  - Owing to genetic predisposition and/or the current physiological profile images of an individual, not all individuals will respond to the aforementioned interventions to the same extent

bear truth.

By implication the present findings could impact the athlete's performance ability and render "fitness testing results" invalid in the sense that the finds of one athlete cannot be compared to the same athlete at a later stage (re-testing) or any other athletes at any stage of testing unless the nutritional intake prior to testing is well-controlled.

5.5.1.1.Performance

Figure 135 indicates that pre-exercise nutrient intake affects the outcome on graded exercise performance in all subjects studied. Time margins during which voluntary fatigue sets in was increased by 120, 183, 82, 69, 31, and 102 seconds [97.83(56.55; 139.11) population mean] for the trained subjects and 47, 58, 37, 36, 57 and 41 seconds [46.0(38.22; 53.78) population mean] for the untrained subjects when the best performance according to any of the interventions for a specific subject is compared to the worst performance according to any of the interventions for the same subject.

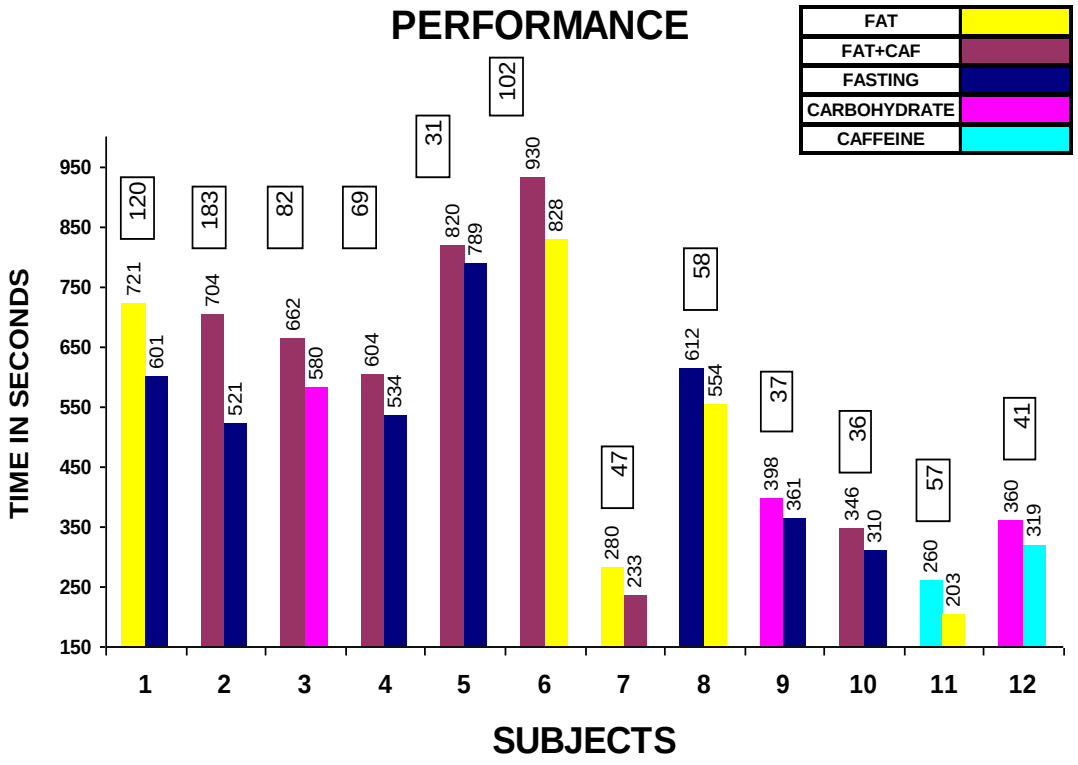


Figure 135-The effect of pre-exercise nutrient intake on running time to exhaustion. The colour coding is descriptive towards the intervention and the time delay until voluntary fatigue sets in is indicated in the square box above the graphs for each and every trained (1-6) and untrained (7-12) subject.

It is important to take the reproducibility of the variables measured during clinical exercise testing for appropriate interpretation of the results into consideration. These values could be misleading since sources of error in the reproducibility of measured values during exercise testing can occur (Casaburi et al., 2003:223-224)-see section 2.3.10. Although care was taken to ensure that factors which may contribute to alteration of measured exercise responses were meticulously controlled and pilot studies conducted to determine the effect of methodological error, i.e. repeatability, shows that the largest error that could occur for repeated re-testing is 32 seconds. The actual values relate to figures of 88, 151, 50, 37, 0, and 70 seconds [66(24.92; 107.18) population mean] for the trained subjects and

15, 26, 5, 4, 25 and 9 seconds [14.16(6.20; 22.12) population mean] for the untrained subjects.

These results accordingly indicate that nutritional intake within 8 hours prior to exercise testing show:

- A 100% (61.0-100%) population proportion for the trained subjects to decrease/increase performance according to the specified exercise regime where the best performance according to any of the interventions for a specific subject is compared to the worst performance according to any of the interventions for the same subject.
- A 100% (61.0-100%) population proportion for the untrained subjects to decrease/increase performance according to the specified exercise regime where the best performance according to any of the interventions for a specific subject is compared to the worst performance according to any of the interventions for the same subject.
- A 100% (75.7-100%) population proportion for all of the subjects included in this study to decrease/increase performance according to the specified exercise regime where the best performance according to any of the interventions for a specific subject is compared to the worst performance according to any of the interventions for the same subject.

The present protocol design supplies no information on the actual effect(s) of these interventions on athletic performance during competition. It is highly probable that the results could have a serious impact on athletic performance but this need to be evaluated by means of further research which is beyond the scope of this protocol design.

Figure 135 also indicates that the FC intervention increased the limit of tolerance with 88, 151, 50, 37, 0 and 70 seconds [66(24.92-107.18) population mean] in 5 of the 6 trained subjects [83.3%(43.6 ; 97%) population proportion] compared to any of the other interventions. Very little information regarding the simultaneous

intake of fat and caffeine exists. Due to the protocol design the findings of Hadjicharalambous et al. (2006) cannot be used to compare or validate the findings of the present investigation.

Compared to the weakest performance for any of the other interventions, the Carbohydrate intervention (Ch) increased the limit of tolerance in two of the untrained subjects (5 and 9 seconds- corrected values) and this phenomenon was not discernible in any of the trained subjects. It could also be stated that the ingestion of Carbohydrate (Ch) in one of the trained subjects was responsible for one of the weakest performances (50 seconds- corrected value) when compared to the FC trial. Within these perspectives the present results support the notion outlined in section 2.2.3.2, which indicates that carbohydrate ingestion prior to exercise could be damaging to exercise performance. More recent studies have shown, however, that the intake of smaller amounts of carbohydrates within the immediate moment of the onset of exercise does not result in an increase in plasma insulin levels during exercise, and no rebound hypoglycemia and an enhancement of performance occurs. As a result of this, the intake of carbohydrate solutions before exercise has become more widespread (Maughan et al., 1997:168). The present protocol design, however, does not cater for any comments on this issue.

The results of this investigation (figure 136) indicate that the combination of Fat and Caffeine intake shows increased values on:

- Performance [(119, 75, 5, 11 and 102 seconds- corrected values) (62.4(20.7 ; 104.1) population mean] in 5 of the 6 trained subjects [83.3% (43.6% ; 97.0%) population proportion] when compared to Fat intake.
- Performance [(31, 54, 65, 26 and 17 seconds- corrected values) (38.6(22.5 ; 54.7) population mean] in 5 of the 6 trained subjects [83.3%(43.6% ; 97.0%) population proportion] when compared to Caffeine intake

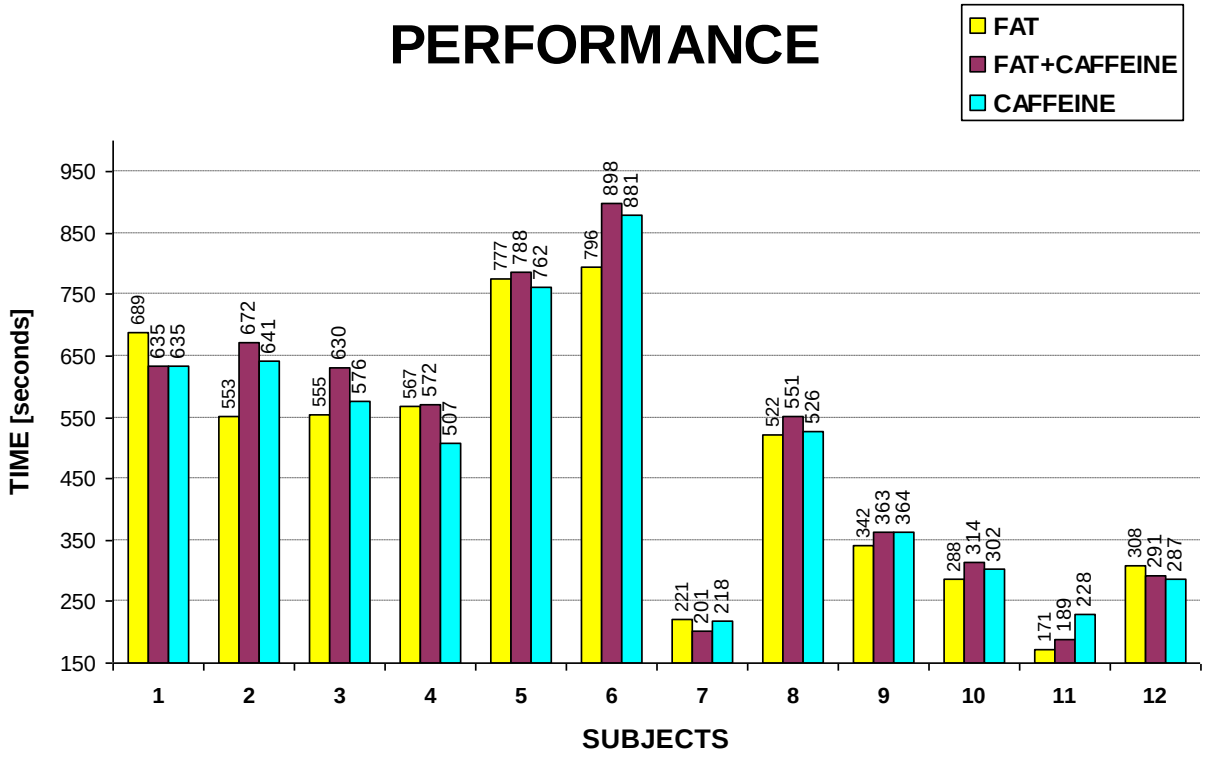


Figure 136-The effect of Fat (F), Caffeine (C) and Fat and Caffeine (FC) intake on performance. The colour coding is descriptive towards the intervention and the time delay until voluntary fatigue sets in is indicated in the square box above the graphs for each and every trained (1-6) and untrained (7-12) subject.

The findings of figure 136 show the synergistic properties embedded in the combination of Fat and Caffeine (FC) intake on strenuous short-term endurance exercise modalities in trained individuals when compared to either Caffeine (C) or Fat (F) intake. The implementation of this newly-gained knowledge (appears to be the first statement of its kind) could outclass the claims made for Caffeine intake (see sections 2.2.2.4 p. 49-50; 2.2.2.5 p.50-51) and Fat intake (see section 2.2.1 p. 36-45) on athletic performance by other researchers.

The synergistic properties embedded in the combination of Fat and Caffeine (FC) intake on strenuous short-term endurance exercise modalities in untrained individuals when compared to either Caffeine (C) [(25, 12 and 4 seconds-corrected values) (13.67(5.2 ; 22.2) population mean] or Fat (F) [(29, 21, 26 and

18 seconds- corrected values) (23.5(19.6 ; 27.5) population mean] intake appears to be less prominent in comparison to trained individuals. Bridge and Jones (2006:436) suggest that the ergogenic advantage of caffeine should be considered on an individual basis for competitive athletes. This notion is supported by the findings of this investigation since not all trained subjects responded towards Caffeine (C) and/or Fat and Caffeine (FC) intake to same manner or extent.

To find out if repeating trends exist for specific variables between subjects in terms of best performance irrespective of the kind of intervention at stake, a table (see table 4) was drawn up on which visual trends [higher trends (>), lower trends (<) or no trends (=)] for each and every subject were noted according to the graphs presented in sections 4.4.1 and 4.4.2. The 4-12 km/h zone was taken into account and the best performance was compared to the weakest performance irrespective of the intervention at stake.

Table 4-Visual trends between the interactions of the variables for each and every subject when comparing the best with the worst performances recorded for a specific intervention between 4 and 12 km/h.

		INTERVENTION BEST:WORST PERFORMANCE	FAT [g/day]	CHO [g/day]	V'O2 [ml/min]	V'CO2 [ml/min]	RER	V'E [L/min]	HR [bpm]	FCCP
TRAINED	SUBJECT 1	F : Fa	<	=	=	=	<	=	≥	<
	SUBJECT 2	FC : Fa	>	<	=	=	<	=	<	≥
	SUBJECT 3	FC : Ca	>	<	≤	≤	<	=	=	>
	SUBJECT 4	FC : Fa	>	=	=	=	≤	<	<	≥
	SUBJECT 5	FC : Fa	>	<	>	>	<	=	<	>
	SUBJECT 6	FC : F	>	=	=	=	=	=	<	=
UNTRAINED	SUBJECT 7	Fa : FC	=	=	>	=	=	=	>	=
	SUBJECT 8	Fa : F	<	≥	≤	=	>	>	=	<
	SUBJECT 9	Ca : Fa	<	>	≥	=	>	=	=	<
	SUBJECT 10	FC : Fa	>	>	≤	=	<	<	=	=
	SUBJECT 11	C : Fa	=	≥	=	=	>	=	<	=
	SUBJECT 12	Ca : C	<	≥	=	=	=	=	=	=

It appears that in the trained subjects an increase in fat oxidation coincides with a decrease in carbohydrate oxidation and the RER-refer to the red areas indicated in table 4. A shift in the FCCP towards higher exercise intensity levels also seems to be evident.

Before these variables could be judged to be decisive, factors affecting performance ability, the opposite of these findings should also be affirmative for the untrained subjects. This appears to be the case, since trends towards decreased values were noted for fat oxidation and increased values for carbohydrate oxidation and the RER values-refer to the blue areas indicated in table 4.

In order to validate these findings the relative values for these specific variables for the trained subjects were taken from Appendix C and pooled by means of the RER ≤1 (implicating treadmill speeds from 4km/h to 12km/h- see Table 5.

Table 5-Effect of pre-exercise nutrient intake comparing the best to the worst performances in the trained subjects pooled from 4km/h to 12km/h for the specific variables indicated in the grey rectangles. The red rectangles show the calculated amount of substrate oxidized during this time period (7 minutes). The blue rectangles show the average values for the specific variables. The pink rectangles show the percentage change between the values for the various interventions for specific variables-negative values indicate a decrease and positive values indicate an increase.

TRAINED SUBJECTS

SUBJECT 1

		BEST PERFORMANCE				WORST PERFORMANCE			
		FAT				FASTING			
Time [min]	Speed [km/h]	CHO [g/day]	FAT [g/day]	RER	HR [bpm]	CHO [g/day]	FAT [g/day]	RER	HR [bpm]
0:01:00	4.0	689	311	0.85	98	415	476	0.79	108
0:01:30		1248	552	0.85	136	255	765	0.75	113
0:02:00	8.0	1575	809	0.84	152	276	1378	0.73	115
0:02:30		1903	715	0.85	158	1146	1239	0.79	118
0:03:00		2706	684	0.89	161	1630	1043	0.81	124
0:03:30		3369	383	0.93	160	2594	744	0.88	136
0:04:00	10.0	3458	437	0.93	160	2981	705	0.89	144
0:04:30		3116	607	0.91	166	3481	546	0.92	150
0:05:00		4306	360	0.95	164	3983	484	0.94	159
0:05:30		4475	249	0.96	163	4395	273	0.96	162
0:06:00	12.0	4845	170	0.97	169	4181	492	0.93	171
0:06:30		5314	96	0.98	173	5473	73	0.99	174
0:07:00		5450	107	0.98	177	5702	26	0.99	178
		42453	5480	0.91	156.79	36512	8164	0.87	142
		206	27	Average values		177	40	Average values	
		g substrate oxidised in 7 minutes				g substrate oxidised in 7 minutes			
		14	-49	4.41	9.19				
		PERCENTAGE CHANGE							

SUBJECT 2

		BEST PERFORMANCE				WORST PERFORMANCE			
		FAT PLUS CAFFEINE				FASTING			
Time [min]	Speed [km/h]	CHO [g/day]	FAT [g/day]	RER	HR [bpm]	CHO [g/day]	FAT [g/day]	RER	HR [bpm]
0:01:00	4.0	510	91	0.88	105	494	175	0.94	104
0:01:30		669	461	0.81	119	588	386	0.88	123
0:02:00	8.0	570	703	0.78	133	889	659	0.85	139
0:02:30		967	692	0.81	142	1547	482	0.87	142
0:03:00		1051	633	0.83	145	1425	513	0.88	144
0:03:30		1325	583	0.85	144	1808	395	0.89	156
0:04:00	10.0	1568	579	0.86	153	1815	464	0.88	159
0:04:30		1778	464	0.88	153	2008	494	0.89	128
0:05:00		1692	535	0.87	151	2046	488	0.91	131
0:05:30		2112	462	0.89	156	2645	237	0.91	159
0:06:00	12.0	2481	393	0.92	163	2947	196	0.95	157
0:06:30		2942	292	0.94	163	3224	110	0.97	165
0:07:00		3160	186	0.96	168	3402	53	0.99	167
		20825	6074	0.87	145.73	24838	4573	0.91	144
		101	30	Average values		121	22	Average values	
		g substrate oxidised in 7 minutes				g substrate oxidised in 7 minutes			
		-15	-25	-4.67	1.49				
		PERCENTAGE CHANGE							

SUBJECT 3

		BEST PERFORMANCE				WORST PERFORMANCE			
		FAT PLUS CAF				CARBOHYDRATE			
Time [min]	Speed [km/h]	CHO [g/day]	FAT [g/day]	RER	HR [bpm]	CHO [g/day]	FAT [g/day]	RER	HR [bpm]
0:01:00	4.0	26	406	0.72	106	214	356	0.77	114
0:01:30		261	484	0.75	130	443	542	0.78	133
0:02:00	8.0	309	811	0.75	153	783	656	0.80	155
0:02:30		420	842	0.76	154	1192	469	0.85	160
0:03:00		708	691	0.79	157	1054	500	0.84	162
0:03:30		992	681	0.82	162	1232	480	0.85	163
0:04:00	10.0	776	755	0.77	167	1424	529	0.86	166
0:04:30		1099	717	0.82	165	2095	303	0.92	176
0:05:00		1244	669	0.83	171	2156	298	0.92	181
0:05:30		1289	702	0.83	177	2032	397	0.90	184
0:06:00	12.0	1509	713	0.84	174	1996	493	0.89	184
0:06:30		1959	608	0.87	181	2431	416	0.91	188
0:07:00		2035	614	0.87	185	2852	285	0.94	190
		12634	8694	0.80	160	19903	5724	0.86	166
		61	42	Average values		97	28	Average values	
		g substrate oxidised in 7 minutes				g substrate oxidised in 7 minutes			
		-58	-34	7.75	3.48				
		PERCENTAGE CHANGE							

SUBJECT 4

		BEST PERFORMANCE				WORST PERFORMANCE			
		FAT PLUS CAFFEINE				FASTING			
Time [min]	Speed [km/h]	CHO [g/day]	FAT [g/day]	RER	HR [bpm]	CHO [g/day]	FAT [g/day]	RER	HR [bpm]
0:01:00	4.0	138	513	0.74	85	106	533	0.73	103
0:01:30		282	983	0.68	102	20	749	0.72	120
0:02:00	8.0	77	1021	0.69	110	-665	1421	0.65	134
0:02:30		12	1324	0.71	123	-153	1423	0.70	149
0:03:00		384	1261	0.74	128	509	1210	0.75	154
0:03:30		553	1198	0.76	135	1079	984	0.80	161
0:04:00	10.0	1077	1120	0.79	141	1314	1026	0.81	166
0:04:30		1491	952	0.82	148	1686	846	0.84	172
0:05:00		1698	894	0.83	150	1947	764	0.86	174
0:05:30		2329	661	0.88	155	2429	621	0.89	178
0:06:00	12.0	2565	620	0.89	162	2626	580	0.90	181
0:06:30		2899	604	0.90	166	3123	442	0.92	184
0:07:00		3200	501	0.92	164	3501	284	0.95	186
		15987	11652	0.80	136.13	17522	10882	0.81	159
		78	57	Average values		85	53	Average values	
		g substrate oxidised in 7 minutes				g substrate oxidised in 7 minutes			
		-10	-7	-1.47	-16.61				
		PERCENTAGE CHANGE							

SUBJECT 5

		BEST PERFORMANCE				WORST PERFORMANCE			
		FAT PLUS CAF				FASTING			
Time [min]	Speed [km/h]	CHO [g/day]	FAT [g/day]	RER	HR [bpm]	CHO [g/day]	FAT [g/day]	RER	HR [1/min]
0:01:00	4.0	56	494	0.73	86	402	289	0.81	86
0:01:30		-116	881	0.71	97	791	447	0.83	107
0:02:00	8.0	-62	1264	0.70	113	1116	610	0.83	128
0:02:30		146	1403	0.72	122	1854	438	0.89	133
0:03:00		575	1361	0.76	127	2136	333	0.92	134
0:03:30		1171	1138	0.79	132	2593	196	0.95	141
0:04:00	10.0	1310	1166	0.81	141	2062	492	0.89	144
0:04:30		1410	1234	0.82	144	2785	283	0.94	150
0:05:00		1605	1109	0.82	147	2601	308	0.93	153
0:05:30		1691	1129	0.82	151	2577	403	0.92	156
0:06:00	12.0	2223	970	0.86	156	2824	458	0.91	161
0:06:30		3146	607	0.91	160	3536	207	0.96	165
0:07:00		3068	633	0.91	162	3670	178	0.96	167
		16223	13389	0.80	133.65	28947	4642	0.90	140
		79	65	Average values		141	23	Average values	
		g substrate oxidised in 7 minutes				g substrate oxidised in 7 minutes			
		-78	65	-13.30	-4.94				
		PERCENTAGE CHANGE							

SUBJECT 6

		BEST PERFORMANCE				WORST PERFORMANCE			
		FAT PLUS CAF				FAT			
Time [min]	Speed [km/h]	CHO [g/day]	FAT [g/day]	RER	HR [bpm]	CHO [g/day]	FAT [g/day]	RER	HR [1/min]
0:01:00	4.0	625	268	0.77	126	320	443	0.78	105
0:01:30		670	508	0.77	132	568	625	0.79	124
0:02:00	8.0	659	871	0.78	145	685	874	0.78	140
0:02:30		1021	900	0.80	152	1462	825	0.83	145
0:03:00		1744	637	0.81	152	2058	521	0.86	147
0:03:30		1696	643	0.83	156	1848	620	0.86	149
0:04:00	10.0	1590	756	0.84	159	2427	450	0.91	152
0:04:30		1722	816	0.84	160	2559	445	0.91	156
0:05:00		2140	731	0.86	161	2599	411	0.92	155
0:05:30		2215	702	0.86	164	2675	468	0.91	158
0:06:00	12.0	2276	684	0.86	167	2889	483	0.91	162
0:06:30		2547	681	0.88	166	3174	439	0.92	162
0:07:00		2907	617	0.89	167	3106	454	0.92	163
		21814	8814	0.83	154.49	26371	7058	0.87	148
		106	43	Average values		128	34	Average values	
		g substrate oxidised in 7 minutes				g substrate oxidised in 7 minutes			
		-21	-20	-4.54	4.49				
		PERCENTAGE CHANGE							

- Indicative of -49, 25, 34, 7, 65, and 20% [17%(-13 ; 47%) population mean] indicative of a trend towards increased levels for fat oxidation
- 14, -19, -58, -10, -78 and -21% [28.67%(1.85 ; 55.5%)- population mean] indicative of decreased levels for carbohydrate oxidation
- 4.41, -4.67, -7.75, -1.47, -13.3 and -4.54% [-4.55%(-9.3 ; +0.2%) population mean] indicative of a trend towards a decrease in the RER
- 9.19, 1.49, -3.48, -16.61, -4.49 and 4.49% [-1.56%(-8.7 ; +5.6%) population mean] indicative of no change for HR

These findings indicate that a trend towards increased levels of fat oxidation (-13 to 47%) coinciding with decreased levels of carbohydrate oxidation (1.85 ; 55.5%) as well as a trend towards a decrease in the RER (-9.3 -0.2%) will enhance incremental treadmill running up to the point of exhaustion in 83.3% of trained subjects [(43.6% ; 97.0%) population proportion] if trained subjects consume fat in combination with caffeine.

In order to validate these findings for the untrained subjects, the relative values for these specific variables were taken from Appendix C and pooled by means of the RER ≤1 (implicating treadmill speeds from 4km/h to 8km/h in most of untrained subjects)-see Table 6.

Table 6-Effect of pre-exercise nutrient intake comparing the best to the worst performances in the untrained subjects pooled from 4km/h to 12km/h for the specific variables indicated in the grey rectangles. The red rectangles show the calculated amount of substrate oxidized during this time period (7 minutes). The blue rectangles show the average values for the specific variables. The pink rectangles show the percentage change between the values for the various interventions for specific variables-negative values indicate a decrease and positive values indicate an increase.

UNTRAINED SUBJECTS

		SUBJECT 7								SUBJECT 8							
		BEST PERFORMANCE				WORST PERFORMANCE				BEST PERFORMANCE				WORST PERFORMANCE			
		FASTING				FAT PLUS CAF				FASTING				FAT			
Time [min]	Speed [km/h]	CHO [g/day]	FAT [g/day]	RER	HR [1/min]	CHO [g/day]	FAT [g/day]	RER	HR [1/min]	CHO [g/day]	FAT [g/day]	RER	HR [1/min]	CHO [g/day]	FAT [g/day]	RER	HR [1/min]
0:01:00	4.0	277	277	0.82	102	271	290	0.80	92	641	454	0.84	99	482	350	0.81	94
0:01:30	8.0	472	532	0.79	122	147	634	0.74	111	1068	452	0.86	107	426	878	0.75	113
0:02:00	8.0	586	874	0.77	144	244	978	0.75	135	1186	713	0.82	121	871	1035	0.79	125
0:02:30	8.0	1473	663	0.84	162	1441	636	0.86	157	1620	847	0.83	135	1337	1004	0.81	139
0:03:00	8.0	2269	419	0.91	169	2788	107	0.99	164	2649	614	0.90	146	2086	847	0.86	145
0:03:30	10.0									3080	540	0.93	154	2317	798	0.86	148
0:04:00	10.0									3181	523	0.93	163	2593	781	0.88	157
0:04:30	10.0									3770	421	0.95	173	3629	599	0.93	169
0:05:00	10.0									3765	322	0.95	177	3382	595	0.92	170
0:05:30	12.0									3661	266	0.94	179	3659	571	0.93	165
0:06:00	12.0									4470	193	0.97	184	3743	534	0.92	172
0:06:30	12.0									4582	179	0.97	188	4514	408	0.95	179
0:07:00	12.0									5093	150	0.99	192	5009	312	0.97	184
		5077	2765	0.32	53.71	4891	2645	0.32	51	38774	5673	0.91	155.07	34049	8713	0.87	151
		25	13	Average values		24	13	Average values		188	28	Average values		166	42	Average values	
		g substrate oxidised in 7 minutes				g substrate oxidised in 7 minutes				g substrate oxidised in 7 minutes				g substrate oxidised in 7 minutes			
		4	4	-0.13	5.58					12	-54	4.22	2.80				
		PERCENTAGE CHANGE								PERCENTAGE CHANGE							

		SUBJECT 9								SUBJECT 10							
		BEST PERFORMANCE				WORST PERFORMANCE				BEST PERFORMANCE				WORST PERFORMANCE			
		CARBOHYDRATE				FASTING				FAT PLUS CAFFEINE				FASTING			
Time [min]	Speed [km/h]	CHO [g/day]	FAT [g/day]	RER	HR [1/min]	CHO [g/day]	FAT [g/day]	RER	HR [1/min]	CHO [g/day]	FAT [g/day]	RER	HR [1/min]	CHO [g/day]	FAT [g/day]	RER	HR [1/min]
0:01:00	4.0	441	261	0.82	97	97	366	0.75	91	416	215	0.83	110	30	298	0.73	104
0:01:30	8.0	1175	291	0.88	128	172	741	0.73	113	9	775	0.71	135	200	679	0.75	140
0:02:00	8.0	2121	175	0.95	148	1037	628	0.82	141	372	739	0.76	160	746	599	0.80	161
0:02:30	8.0	3012	-51	1.00	151	2103	303	0.92	153	1162	537	0.84	173	1605	380	0.89	171
0:03:00	8.0	3734	-300	1.06	157	2878	20	0.99	158	1932	237	0.93	176	2658	-3	0.99	178
0:03:30	10.0																182
0:04:00	10.0																186
0:04:30	10.0																188
0:05:00	10.0																190
0:05:30	12.0																
0:06:00	12.0																
0:06:30	12.0																
0:07:00	12.0																
		10484	376	0.36	52	6287	2058	0.32	50	3891	2502	0.31	58.02	5239	1954	0.32	115
		51	2	Average values		31	10	Average values		19	12	Average values		25	9	Average values	
		g substrate oxidised in 7 minutes				g substrate oxidised in 7 minutes				g substrate oxidised in 7 minutes				g substrate oxidised in 7 minutes			
		40	-448	10.76	2.35					-35	-22	-2.18	-90.88				
		PERCENTAGE CHANGE								PERCENTAGE CHANGE							

		SUBJECT 11								SUBJECT 12							
		BEST PERFORMANCE				WORST PERFORMANCE				BEST PERFORMANCE				WORST PERFORMANCE			
		CAFFEINE				FASTING				CARBOHYDRATE				CAFFEINE			
Time [min]	Speed [km/h]	CHO [g/day]	FAT [g/day]	RER	HR [1/min]	CHO [g/day]	FAT [g/day]	RER	HR [1/min]	CHO [g/day]	FAT [g/day]	RER	HR [1/min]	CHO [g/day]	FAT [g/day]	RER	HR [1/min]
0:01:00	4.0	698	212	0.87	121	505	222	0.85	131	545	446	0.80	92	651	365	0.83	93
0:01:30	8.0	1284	182	0.93	143	274	794	0.75	151	1058	508	0.84	111	1013	586	0.83	112
0:02:00	8.0	2616	468	0.88	159	1004	846	0.80	163	1506	667	0.83	137	1646	831	0.84	135
0:02:30	8.0	3630	31	0.99	165	2547	342	0.92	169	2838	374	0.91	148	2707	462	0.91	147
0:03:00	8.0	5151	-535	1.09	172	4226	-182	1.03	174	3804	172	0.97	156	3169	388	0.93	153
0:03:30	10.0									4281	33	0.99	161	3655	243	0.96	159
0:04:00	10.0									5089	-209	1.03	166	4396	61	0.99	166
0:04:30	10.0																169
0:05:00	10.0																171
0:05:30	12.0																
0:06:00	12.0																
0:06:30	12.0																
0:07:00	12.0																
		13379	358	0.37	58.43	8557	2022	0.34	61	19121	1992	0.49	74.63	17238	2935	0.48	101
		65	2	Average values		42	10	Average values		93	10	Average values		84	14	Average values	
		g substrate oxidised in 7 minutes				g substrate oxidised in 7 minutes				g substrate oxidised in 7 minutes				g substrate oxidised in 7 minutes			
		36	-465	8.32	-3.75					10	-47	1.31	-34.66				
		PERCENTAGE CHANGE								PERCENTAGE CHANGE							

Table 6 shows that the percentage change observed for the various variables for a specific trained subject in terms of the population setting are:

- 4, -54, -448, 22, -465 and -47% [164%(-17.5 ; -347%) population mean] which indicates a trend towards decreased levels of fat oxidation
- 4, 12, 40, -35, 36, and 10% [11.16%(-10.4 ; 32.7%) population mean] which indicates a trend towards increased levels for carbohydrate oxidation
- -0.13, 4.22, 10.76, -2.18, 1.31 and 8.32% [3.76%(-0.23 ; 7.75%) population mean] which indicates a trend towards increased RER levels.
- 5.58, 2.80, 2.35, 0.02, -3.75 and -34.66% [4.61%(-16.65 ; 7.43%) population mean] which indicates no change in HR.

In terms of metabolism, one can deduce from these findings that the stimulation of the aerobic lipolytic power systems (formation of Acetyl-CoA from lipids) during the onset of exercise shifts the aerobic glycolytic systems (formation of Acetyl-CoA from carbohydrates) and the anaerobic lactic power system (formation of lactate from pyruvate) to a higher exercise intensity level. The combined effect is responsible for improvement of exercise ability. Perhaps these views describe the undefined “second wind stage” attained during physical activity.

5.5.1.2. Fitness testing

Due the diverse effects of pre-exercise nutrient intake on metabolism and performance, the present results could render many (if not all) “fitness testing results” invalid in the sense that the findings of one athlete cannot be compared to the same athlete at a later stage (re-testing) or any other athletes at any stage of testing and/or re-testing, unless the nutritional intake prior to testing is ‘well controlled’.

> "Well controlled" implies that the trained subject be evaluated in a similar nutritional status prior to testing and re-testing. This however does not imply that the particular trained subject performed at optimal levels unless the best nutritional status for the specific athlete is determined in advance. Until this is achieved, no results obtained from testing procedures of any one trained subject can be compared (extrapolated) to any of the results obtained for another trained subject (or any prescribed norm for that matter) in order to identify areas of strength or weaknesses for many strenuous test-protocols implemented in practice-refer to Appendix D".

5.5.2. Well-being industry

It is commonly known amongst researchers that a positive correlation exists between percentage of body fat and many disease states (Kerr et al., 2002:407). Interventions aimed at improving the metabolism of fat could potentially reduce the symptoms of metabolic diseases such as obesity, type 2 diabetes, the metabolic syndrome etc. and may have clinical significance. In order to reach this objective an understanding of the factors that enhance or reduce fat oxidation is vital. Exercise duration and intensity are very important regulators of fat metabolism. Fat oxidation is maximal at low to moderate intensity exercise but as the intensity of exercise increases too much, less reliance on fat metabolism is evident and more reliance on carbohydrate metabolism becomes clear. Maximal rates of fat oxidation have been noted in trained individuals at around 59–64% $V'O_{2max}$, whilst in untrained individuals, maximal fat oxidation occurs around 47–52% $V'O_{2max}$ (Achten and Jeukendrup, 2004a:716). For a more detailed discussion see section 2.1.5.1. with special reference to (e) p. 32-34

A specific fat-zone should be demarcated from the onset of exercise until the FCCP is reached. The highest values obtained for fat oxidation within the fat-zone demarcate the fatty acid threshold (FAT) and is indicative of the maximal fat oxidation capacity.

Figures 8, 9, 10 indicate that maximal fat oxidation rates occur during relatively low exercise intensities and decreases with increasing exercise intensities and

this is in agreement with the findings of other researchers (Brooks and Mercier, 1994:2253; Brooks et al., 2000:133; Venables et al., 2004:167; Van Loon, 2004:1183; Achten and Jeukendrup, 2004a:716). By implication this means that the more strenuous the exercise, the lower the subject's ability to burn fat.

Table 7 purports to show that a generalized approach to subject individuals to a specified heart rate based on population norms will not yield optimum results if the goal is set to maximize fat oxidation: The present findings indicate that at the FAT heart rates for individuals could vary with 37, 14, 13, 27, 11, and 15 bpm [19.5(11.3 ; 27.7) population mean] in the trained subjects and 21, 20, 9, 27, 15 and 3 bpm [15.83(8.85 ; 22.81) population mean] in the untrained subjects. Although the difference between these heart rate values that were established for the various interventions for any of the subjects appears to be relatively low, dramatic consequences on the amounts of fat oxidized will ensue if heart rate is used as a descriptive tool for optimal fat utilization: Table 7 indicates that the various interventions have a significant impact on the maximum amount of fat utilized (FAT) irrespective of heart rate- refer to subject 1 for example: In the fasting state (Fa) the FAT shows a value of 1378 gfat/24hrs and occurs at a heart rate of 115 bpm compared to a value of 809 gfat/24hrs achieved at a heart rate of 152 bpm when fat (F) is consumed prior to the running test. The corresponding values for the $\%V'O_{2peak}$ values are 56 and 53% respectively.

Table 7-The interactions between various variables at the FAT for trained and untrained subjects. FOC-fatty acid oxidation capacity; HR-heart rate; $\%V'O_2$-$\%V'O_{2peak}$; Fa-fasting; Ch-carbohydrate; F-fat; C-caffeine and FC-Fat in combination with caffeine.

		Fa			Ch			F			C			FC		
	SUBJECT	HR	FOC	%VO2	HR	FOC	%VO2	HR	FOC	%VO2	HR	FOC	%VO2	HR	FOC	%VO2
TRAINED	1	115	1378	56	144	1149	59	152	809	53	128	1140	53	139	1244	64
	2	142	542	69	128	633	55	138	659	64	131	856	56	133	703	53
	3	155	518	57	155	656	60	148	496	65	142	741	63	154	842	59
	4	149	1423	73	139	879	69	124	1321	64	122	1362	69	123	1324	65
	5	128	610	49	127	589	51	132	911	61	117	1168	52	122	1403	61
	6	151	970	59	154	483	50	140	874	46	147	686	54	152	900	48
UNTRAINED	7	144	874	70	156	196	69	149	806	71	140	829	70	135	978	73
	8	135	847	61	135	697	50	125	1035	54	120	1425	59	140	1375	71
	9	113	741	56	120	291	50	122	435	58	119	692	55	118	360	45
	10	140	679	64	153	408	74	126	739	61	141	649	67	135	775	67
	11	163	846	77	159	468	68	165	690	77	167	537	73	152	544	51
	12	134	640	65	137	667	70	135	699	65	135	831	74	134	892	69

The present findings also indicate that the %V'O_{2peak} that demarcate a training level for optimal fat combustion is affected by the pre-exercise meal.

- When the %V'O_{2peak} is specified for individuals the present findings indicate that a small variation in the % V'O_{2peak} could have significant impact on fat oxidation. This can be expressed as follows:

    - The findings on subject 6 for example show that the FAT is recorded at a value of 50% V'O_{2peak} and 46% V'O_{2peak} respectively corresponds with values of 483 and 874gfat/24hrs (Fasting vs. Fat trials). By implication a 8% difference in the % V'O_{2peak} brings about a 44.73% difference in the amount of fat oxidized.

    - The findings on subject 4 for example show that the FAT is recorded at a value of 73%V'O_{2peak} and 69% V'O_{2peak} respectively corresponding with values of 1423 and 879g fat/24hrs (Fasting vs. Carbohydrate trials)-see Table 3. By implication a 5.48% difference in the %V'O_{2peak} brings about a 38.23% difference in the amount of fat oxidized.

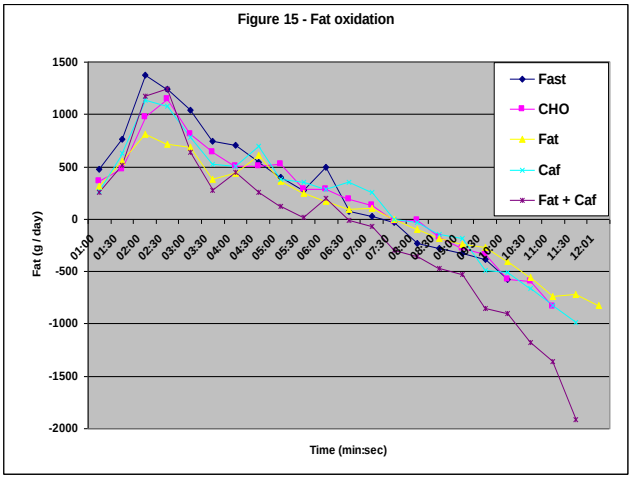
    - Similar trends are evident in both the trained and untrained subjects that were subjected to this regimen. The reasons for this could possibly be explained by the uncontrolled actions of nutrient intake on metabolism.

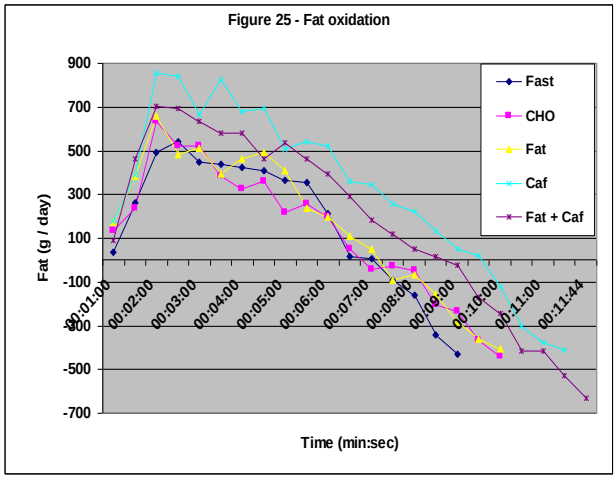
The findings presented here indicate that the effect of pre-exercise nutritional interventions on the %V'O_{2peak} at the FAT show values ranging from 46-73 %V'O_{2peak} for trained and 45-77 V'O_{2peak} for untrained subjects. Others investigators have reported maximal rates of fat oxidation of in 59-64% V'O_{2max}

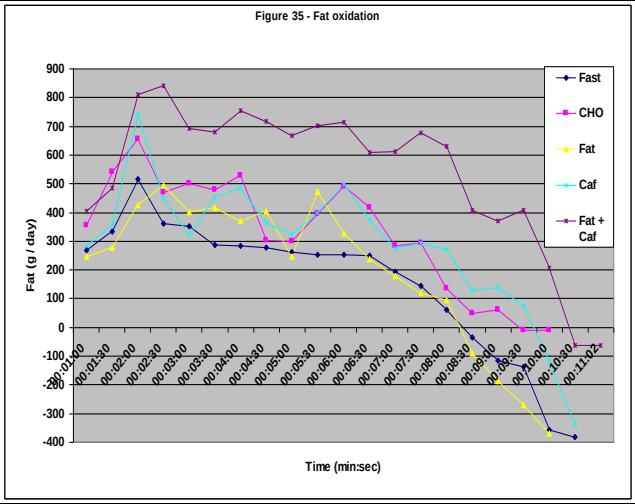
in trained individuals and 47-52% $V'O_{2max}$ in untrained individuals (Achten and Jeukendrup, 2004a:716).

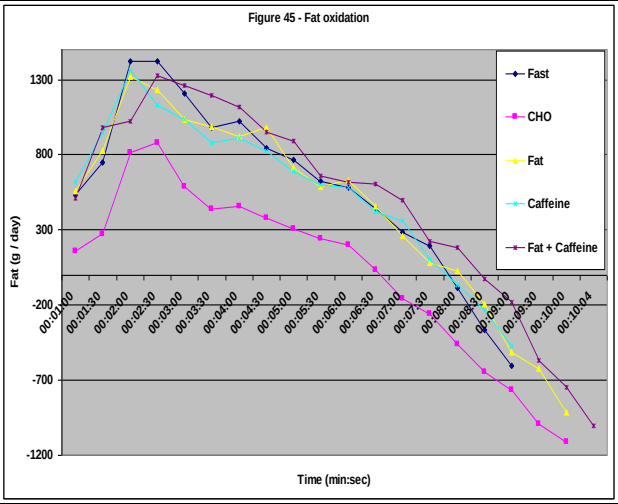
Furthermore, these findings reveal that when the highest value obtained for fat oxidation is compared to the lowest value for any of the interventions for a specific subject (expressed in terms of a percentage), pre-exercise nutrient intake significantly alters the fat oxidation capacity (amounts and rates) within the fat-zone for both the trained subjects [(41, 36, 41, 38, 70 and 50%) {46(35.8 ; 56.15)} population mean] and untrained [(79, 51, 60, 47 44 and 28%) {51.5(37.8 ; 65.2)} population mean] subjects.

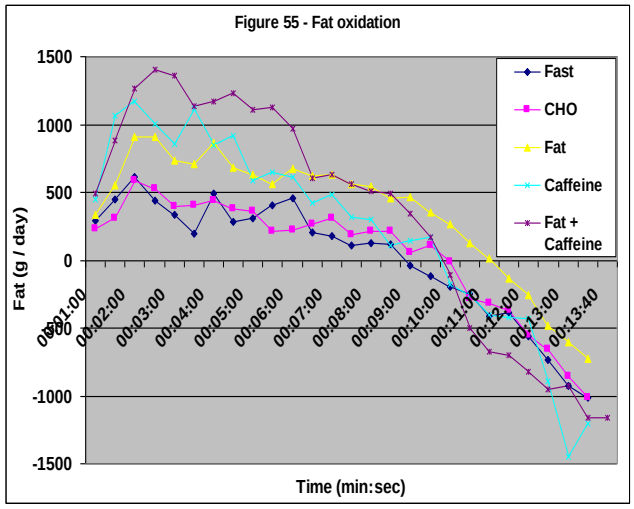
FASTING | CARBO | FAT | CAFFEINE | FAT + CAF

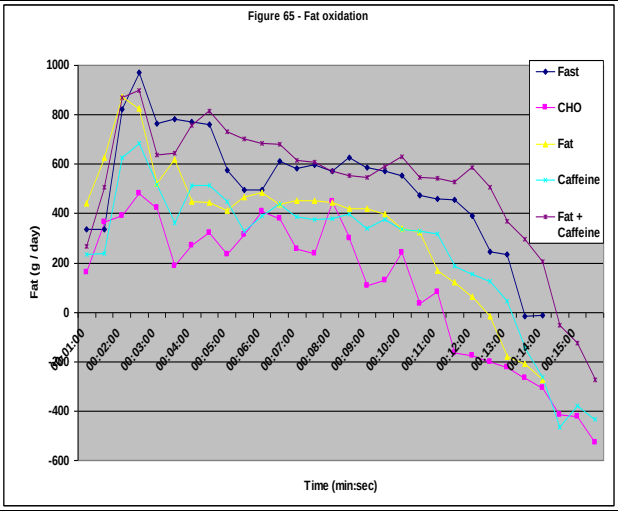












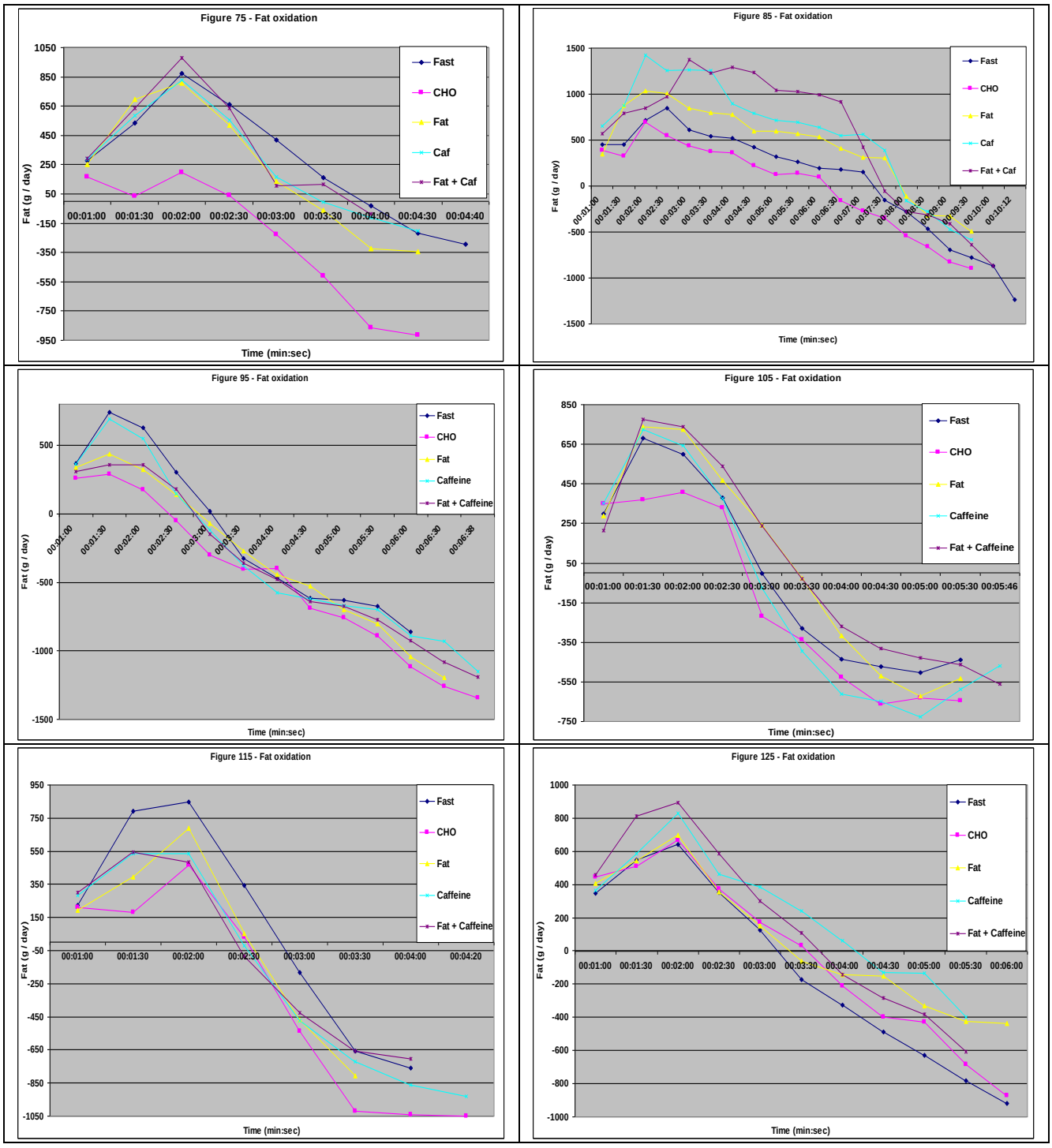


Figure 137 & 138-Graphic illustration of the fat utilization capacity values (Chapter 4 & Appendix C) of the trained and untrained subjects for the various interventions throughout the entire trial run respectively. The colour coding is descriptive for the intervention.

Figures 137 and 138 serve to show that when the highest value obtained for fat oxidation is compared to the lowest value for any of the interventions for a specific subject (Appendix C) and is expressed in terms of a percentage, the intake of Carbohydrate (Ch) prior to exercise decreases fat oxidation rates and values within the fat-zone for both the trained subjects [(41, 36, 41, 38, 70 and 50%); {46(35.8 ; 56.15)} population mean] and untrained [(79, 51, 60, 47 44 and 28%) {51.5(37.8 ; 65.2)} population mean] subjects. These findings are in agreement with the results reported by Achten and Jeukendrup (2004a:716), which show that the ingestion of carbohydrates prior to exercise training significantly reduces the rate of fat oxidation compared to fasting conditions, whereas fasting for longer than 6 hours maximizes fat oxidation.

The ingestion of carbohydrates prior to exercise in a graded exercise test decreases maximal fat oxidation by 28% compared to fasting (Achten and Jeukendrup, 2003:1021). An average figure of 19.34% can be reported for the present study in trained and an average figure of 41.06% in untrained subjects (=an average value of 30.2% for the pooled data)-see Appendix C.

The fat-carbohydrate crossover point (FCCP) occurs between 48 and 53% of the $V'O_{2max}$, (Achten and Jeukendrup, 2003:1021) and is consistent with the 50% $V'O_{2max}$ value reported by Brooks and colleagues (Brooks and Mercier, 1994:2253). These statements require the need for comment. The reader gains the impression that all athletes have a FCCP that occurs between 48 and 53% of the $V'O_{2max}$. Table 8 indicates that in 5 of the 6 trained and untrained subjects no FCCP exist for specific interventions. Only one of the trained subjects (subject 4) and one of the untrained subjects (subject 10) showed a FCCP for all of the interventions implemented in the present study. All trained subjects showed a FCCP in the FC-trial – refer to table 8.

Table 8-The effect of the various interventions on the FCCP for trained and untrained subjects. Treadmill speed at the FCCP is also indicated.

	THE FAT - CARBOHYDRATE CROSSOVER POINT					
	SUBJECT	Fa	Ca	F	C	FC
TRAINED	1	8 km/h	8 km/h	None	8 km/h	8 km/h
	2	None	None	None	8 km/h	8 km/h
	3	None	8 km/h	None	4 km/h	8 km/h
	4	**10 km/h**	**8 km/h**	**8 km/h**	**8 km/h**	10 km/h
	5	None	None	8 km/h	10 km/h	10 km/h
	6	8 km/h	None	8 km/h	None	8 km/h
UNTRAINED	7	4 km/h	None	8 km/h	8 km/h	8 km/h
	8	None	None	8 km/h	8 km/h	8 km/h
	9	8 km/h	None	None	8 km/h	None
	10	**8 km/h**	**8 km/h**	**8 km/h**	**8 km/h**	**8 km/h**
	11	8 km/h	None	None	None	None
	12	None	None	None	None	8 km/h

Tables 9 A and B show the number of days that it would take to burn 1 kilogram of fat should the trained subjects (A) or untrained subjects (B) exercise at the desired fatty acid threshold for 30 minutes daily.

SUBJECT 1		FASTING										
Time [min]	Speed [km/h]	V'O2 [ml/min]	V'CO2 [ml/min]	VO2max	RER	HR [1/min]	CHO [g/day]	FAT [g/day]	V'E [L/min]	CHO [g/30 min session	FAT [g/30 min session	FAT [Days to burn 1 kg fat]
0:01:00	4.0	969	761	13.3	0.79	108	415	476	27	9	10	100.7557
0:01:30	8.0	1282	955	17.6	0.75	113	255	765	33	5	16	62.77245
0:02:00		2154	1575	29.5	0.73	115	276	1378	53	6	29	34.8373
0:02:30		2458	1937	33.7	0.79	118	1146	1239	63	24	26	38.75054
0:03:00		2462	2021	33.7	0.81	124	1630	1043	65	34	22	46.00812
CARBOHYDRATE												
Time [min]	Speed [km/h]	V'O2 [ml/min]	V'CO2 [ml/min]	VO2max	RER	HR [1/min]	CHO [g/day]	FAT [g/day]	V'E [L/min]	CHO [g/30 min session	FAT [g/30 min session	FAT [Days to burn 1 kg fat]
0:01:00	4.0	771	609	10.6	0.79	100	342	365	22	7	8	131.528
0:01:30	8.0	1117	908	15.3	0.81	115	663	480	33	14	10	100.0569
0:02:00		1838	1425	25.2	0.78	130	711	975	47	15	20	49.24914
0:02:30		2352	1867	32.2	0.79	144	1178	1149	62	25	24	41.7694
0:03:00		2364	2019	32.4	0.86	153	2028	810	65	42	17	59.28213
FAT												
Time [min]	Speed [km/h]	V'O2 [ml/min]	V'CO2 [ml/min]	VO2max	RER	HR [1/min]	CHO [g/day]	FAT [g/day]	V'E [L/min]	CHO [g/30 min session	FAT [g/30 min session	FAT [Days to burn 1 kg fat]
0:01:00	4.0	895	755	12.3	0.85	98	689	311	26	14	6	154.5064
0:01:30	8.0	1554	1315	21.3	0.85	136	1248	552	45	26	12	86.95652
0:02:00		2103	1758	28.8	0.84	152	1575	809	57	33	17	59.33862
0:02:30		2160	1853	29.6	0.85	158	1903	715	65	40	15	67.1068
0:03:00		2577	2284	35.3	0.89	161	2706	684	73	56	14	70.13556
CAFFEINE												
Time [min]	Speed [km/h]	V'O2 [ml/min]	V'CO2 [ml/min]	VO2max	RER	HR [1/min]	CHO [g/day]	FAT [g/day]	V'E [L/min]	CHO [g/30 min session	FAT [g/30 min session	FAT [Days to burn 1 kg fat]
0:01:00	4.0	719	599	9.9	0.84	107	501	263	21	10	5	182.7991
0:01:30	8.0	1326	1055	18.1	0.80	119	798	630	35	17	13	76.17948
0:02:00		2080	1599	28.5	0.77	128	729	1140	50	15	24	42.12374
0:02:30		2416	1961	33.1	0.81	137	1464	1078	59	31	22	44.53379
0:03:00		2532	2196	34.7	0.87	140	2375	787	65	49	16	60.95536
FAT + CAFFEINE												
Time [min]	Speed [km/h]	V'O2 [ml/min]	V'CO2 [ml/min]	VO2max	RER	HR [1/min]	CHO [g/day]	FAT [g/day]	V'E [L/min]	CHO [g/30 min session	FAT [g/30 min session	FAT [Days to burn 1 kg fat]
0:01:00	4.0	915	796	12.5	0.87	105	849	259	28	18	5	185.0157
0:01:30	8.0	1175	951	16.1	0.80	106	679	515	36	14	11	93.1436
0:02:00		2241	1744	30.7	0.78	127	912	1179	57	19	25	40.70326
0:02:30		2680	2156	36.7	0.80	139	1518	1244	66	32	26	38.58521
0:03:00		2627	2351	36.0	0.89	145	2898	641	74	60	13	74.93414

Table 9 A-The effect of pre-exercise nutrient intake on the fatty acid threshold (FAT) for one trained subject (subject 1). The values in the three last columns were calculated from the relative data values for this subject supplied in Appendix C.

SUBJECT 8		FASTING										
Time [min]	Speed [km/h]	V'O2 [ml/min]	V'CO2 [ml/min]	VO2max	RER	HR [1/min]	CHO [g/day]	FAT [g/day]	V'E [L/min]	CHO [g/30 min session	FAT [g/30 min session	FAT [Days to burn 1 kg fat]
0:01:00	4.0	930	772	11.3	0.84	99	641	454	28	13	9	105.8289
0:01:30	8.0	1270	1084	15.5	0.86	107	1068	452	36	22	9	106.2143
0:02:00		1744	1438	21.3	0.82	121	1186	713	47	25	15	67.36842
0:02:30		2187	1826	26.7	0.83	135	1628	847	57	34	18	56.647
0:03:00		2275	2061	27.8	0.90	146	2649	614	65	55	13	78.13348
CARBOHYDRATE												
Time [min]	Speed [km/h]	V'O2 [ml/min]	V'CO2 [ml/min]	VO2max	RER	HR [1/min]	CHO [g/day]	FAT [g/day]	V'E [L/min]	CHO [g/30 min session	FAT [g/30 min session	FAT [Days to burn 1 kg fat]
0:01:00	4.0	1100	949	13.4	0.85	108	979	387	31	20	8	124.0728
0:01:30	8.0	1442	1312	17.6	0.91	118	1699	325	41	35	7	147.541
0:02:00		1887	1637	23.0	0.87	135	1763	697	50	37	15	68.84188
0:02:30		2261	2024	27.6	0.89	147	2492	546	63	52	11	87.91209
FAT												
Time [min]	Speed [km/h]	V'O2 [ml/min]	V'CO2 [ml/min]	VO2max	RER	HR [1/min]	CHO [g/day]	FAT [g/day]	V'E [L/min]	CHO [g/30 min session	FAT [g/30 min session	FAT [Days to burn 1 kg fat]
0:01:00	4.0	812	662	9.9	0.81	94	482	350	24	10	7	137.1211
0:01:30	8.0	1539	1166	18.8	0.75	113	426	878	38	9	18	54.66404
0:02:00		2015	1577	24.6	0.79	125	871	1035	49	18	22	46.36647
0:02:30		2329	1877	28.4	0.81	139	1337	1004	57	28	21	47.78637
0:03:00		2372	2034	28.9	0.86	145	2086	847	62	43	18	56.6706
CAFFEINE												
Time [min]	Speed [km/h]	V'O2 [ml/min]	V'CO2 [ml/min]	VO2max	RER	HR [1/min]	CHO [g/day]	FAT [g/day]	V'E [L/min]	CHO [g/30 min session	FAT [g/30 min session	FAT [Days to burn 1 kg fat]
0:01:00	4.0	1090	809	13.3	0.74	90	192	654	28	4	14	73.42256
0:01:30	8.0	1516	1143	18.5	0.76	103	388	877	36	8	18	54.70936
0:02:00		2357	1759	28.7	0.75	120	514	1425	54	11	30	33.68224
0:02:30		2313	1901	28.2	0.83	126	1543	1254	57	32	26	38.26642
FAT + CAFFEINE												
Time [min]	Speed [km/h]	V'O2 [ml/min]	V'CO2 [ml/min]	VO2max	RER	HR [1/min]	CHO [g/day]	FAT [g/day]	V'E [L/min]	CHO [g/30 min session	FAT [g/30 min session	FAT [Days to burn 1 kg fat]
0:01:00	4.0	969	745	11.8	0.77	91	321	568	25	7	12	84.46243
0:01:30	8.0	1466	1149	17.9	0.79	108	635	795	37	13	17	60.40499
0:02:00		1932	1624	23.6	0.84	125	1494	847	51	31	18	56.65002
0:02:30		2405	2125	29.3	0.89	137	2485	974	65	52	20	49.30663
0:03:00		2737	2218	33.4	0.82	140	1648	1375	68	34	29	34.90728

Table 9 B-The effect of pre-exercise nutrient intake on the fatty acid threshold (FAT) for one untrained subject (subject 8). The values in the three last columns were calculated from the relative data values for this subject supplied in Appendix C.

Depending on the quantity and quality of the nutrient intake within the hours prior to training, the values indicated in blue show that diverse differences in the number of 30-minute training sessions exist between the various interventions for the same subject:

- 34.83, 41.76, 59.33, 42.13 and 38. 59 for the trained subject.
- 56.65, 68.84, 46.37, 33.68, and 34.91 for the untrained subject.

Accordingly, nutrient intake prior to a training session determines the number of training sessions required to burn 1 kg of fat.

Cognisance should be taken of the fact that training at a prescribed treadmill speed of heart rate is deceptive: comparing similar heart rates (or training at a specified and constant treadmill speed) for each intervention with the number of 30-minute sessions (values indicated in blue) required to burn 1 kilogram of fat clearly indicate significant differences for the various interventions in both trained and untrained subjects.

In practice it can easily be observed how many individuals do not respond to prescribed exercise or dietary regimes. The lack of control of dietary intake and exercise intensity could explain why few positive results on fat mass reduction are observed in the industry. The results presented here not only indicate the importance of why it is necessary to combine diet and physical activity for weight loss and athletic performance but also make a sincere contribution towards solving problems within the well-being and athletic industries.

New information on how to improve fat oxidation by the synchronization of training and nutritional manipulating strategies to alter body composition could provide groundbreaking results on such unresolved research questions:

- “Why combine diet and physical activity in the same international research society? “(Baranowski, 2004:2-19).
- “Diet and exercise for weight loss?” (Volek et al., 2005:1-9).

The present exercise regime does not take into account what the metabolic response towards the FAT or FCCP for prolonged and sustained exercise at low exercise intensity levels would be. The possibility exists that prolonged and sustained exercise at low exercise intensities could alter metabolism in such a way that the FAT and/or the FCCP improves.

5.5.3. Scientific relevance

The intentions of the present protocol design were not to dissect various metabolic pathways or physiological systems at work with the view to maintaining homeostasis during increased levels of physical activity. However, information on some of the variables and their interactions during incremental work loads could support, clarify, question or introduce new aspects on metabolism in trained and/or untrained subjects.

The remarks that follow are specific for the conditions and procedures described in the present protocol design and include reference to the number of subjects included in the investigation, the exercise mode, specific types of nutrient interventions as well as the time of intake of specific types of nutrients. Furthermore, these remarks relate to a general formative approach across the entire exercise regime and do not necessarily imply truth at a specific stage during the exercise regime.

5.5.3.1. New philosophies and arguments

1. Synchronization of exercise and nutrition

New information on how to improve fat oxidation by the synchronization of training and nutritional manipulating strategies to alter body composition could provide groundbreaking results on such unresolved research questions as:

- “Why combine diet and physical activity in the same international research society?” (Baranowski, 2004:2-19).
- “Diet and exercise for weight loss?” (Volek et al., 2005:1-9).

The present results indicate that:

- Maximal fat oxidation rates occur during relatively low exercise intensities and decreases with increasing exercise intensities and is in agreement with the findings of other researchers (Brooks and Mercier, 1994:2253; Brooks et al., 2000:133; Venables et al., 2004:167; Van Loon, 2004:1183; Achten and Jeukendrup, 2004a:716). By implication this means that the more strenuous the exercise, the lower the subject's ability to burn fat.

- The intake of nutrients within the hours prior to exercise not only influence metabolism but also affect exercise performance:

    - Pre-exercise nutrient intake or fasting (8 hours) prior to low and moderate exercise intensities influence fat metabolism during exercise.

    - The pre-exercise meal affects strenuous short-term endurance performance. The possibility exists that athletic performance in various sporting codes could also be affected by the intake of a pre-exercise meal but needs clarification during competitive field analysis.

    - The metabolic responses between individuals are not the same for a specific foodstuff when physical activity is involved.

These findings unequivocally answer the questions raised above, which indicate why diet and physical activity should be combined not only for the purpose of 'the same international research society, but also for weight loss.

2. Calorie intake

Peer reviewed literature justifies by appeal to restrict calorie intake (Kerr et al., 2002:407) to reduce body fat. Calorie restriction, however, coincides with an

increase in the quantity and activity of lipoprotein lipase (the main enzyme responsible for storing of fat), a decrease in the basal metabolic rate (BMR-further reducing one's ability to burn fat throughout the day) and an increase in the conversion of carbohydrate and protein in an indirect manner towards fat storage. These facts refute the views on calorie restriction in order to reduce fat reserves. This investigation will supply information on the actual effect of nutrient intake in combination with exercise on the energy yielding processes.

When viewed within the framework of energy capacities and the role that nutrients fulfill in the process of energy release, these refute and dispel the common myth of the expression "a calorie is a calorie" proposed by Bray (2003:1853). According to this, there appears to be exclusive reference to the first law of thermodynamics (in any transformation the total energy in the system can be accounted for by the heat added to the system, the work done by the system on its environment and the change in energy content of all the components of the system) (Fine and Feinman, 2004:1).

In biological systems the first law does not say what the relative distribution between these effects will be for any process. In fact, the first law does not even allow scientists to say whether the process will occur at all (Fine and Feinman, 2003:209) (Fine and Feinman, 2004:9). Within this perspective, the tenet "a calorie is a calorie" implies that those diets of equal caloric content will result in identical weight change independent of macronutrient composition. The essence of the second law of thermodynamics [entropy (S)-a measure of disorder in all processes] is that it guarantees inefficiency in all metabolic processes. Variation of efficiency is, however not excluded. In fact, the laws of thermodynamics are silent on the existence of variable efficiency (Fine and Feinman, 2004:1). If efficiency can vary (as in the example of oxidative uncoupling) then the tenet "a calorie is a calorie" is no longer a true statement and also violates the second law of thermodynamics (Fine and Feinman, 2004:9).

The present protocol design yields information to substantiate these arguments and philosophies.

3. Nutritional ergogenic interventions

The findings of this investigation indicate the following:

- Any of the specified nutritional interventions implemented in this investigation affect metabolism and strenuous short duration endurance performance in all subjects.
- Any of the specified nutritional interventions do not affect metabolism and strenuous short duration endurance performance in all individuals to the same extent.
- Information relating to pre-exercise nutritional manipulations on exercise metabolism during graded exercise tests appears to be absent (Achten and Jeukendrup, 2003:1022). The given results indicate that the combination of fat and caffeine intake increase the limit of tolerance towards strenuous short duration endurance exercise in 5 of the 6 trained subjects compared to Fasting (Fa), Fat (F), Carbohydrate (Ch) or Caffeine (C) intake.

5.5.3.2. General comments on metabolic aspects and processes

Fat oxidation

It has been said that fat oxidation is inhibited by carbohydrate ingestion (Jeukendrup and Gleeson, 2004:137) prior to exercise. The results given here support this notion. Furthermore that fat oxidation is stimulated by fasting, fat or caffeine (Jeukendrup and Gleeson, 2004:142,239,258).

- The results presented here indicate that the synergistic properties embedded in the combination of Fat and Caffeine (FC) intake on strenuous short-term endurance exercise outclass Fasting (Fa), Fat (F),

Caffeine (C) or Carbohydrates (Ch) and appears to be more prominent in trained than in untrained subjects.

- In a specific subject lower oxygen consumption rates often coincide with higher fat oxidation rates and are influenced by the pre-exercise nutrient intake.
- Compared to any of the other interventions, Caffeine intake (C) produced the maximum fat oxidation values in only two of the subjects.

Carbohydrate oxidation

It has long been accepted that there is a close correlation between dietary carbohydrate intake, muscle glycogen concentration, and endurance capacity. For this reason, it is recommended that individuals training for sports in which carbohydrate is the most profoundly metabolized fuel should consume a diet rich in carbohydrate (Hawley et al., 2006:713). High pre-exercise muscle glycogen content after a carbohydrate rich diet would also support a larger rate of glycogenolysis during subsequent exercise, whereas low pre-exercise muscle glycogen content after a diet low in carbohydrate would decrease rates of glycogenolysis (Arkinstall et al., 2004: 2275).

- It appears from the results presented here that the ingestion of Carbohydrates (Ch) prior to the graded exercise tests increased subsequent carbohydrate oxidation in three of the trained and three of the untrained individuals when compared to any of the other interventions. These results support the findings of Arkinstall et al. (2004:2275). However, Fasting (Fa) also produced the highest carbohydrate oxidation rates in three of individuals (two trained and one untrained subject). Accordingly Carbohydrate (Ch) intake is not the only means by which carbohydrate oxidation can be increased in all individuals. Fat (F) also produced high carbohydrate oxidation results, but this was only observable in one trained subject.

Oxygen consumption ($V'O_2$)

An increase in oxygen consumption relates to an increase in the Electron Transport Chain (ETC) activity levels and/or oxygen availability (cardio-pulmonary delivering capacity and blood flow volumes due to muscular contraction rates) in the active musculature.

- Pre-exercise nutrient intake influences oxygen uptake.

- The combination of fat and caffeine (FC) intake showed increased values for the $V'O_2$ in four subjects (two trained and two untrained individuals). Caffeine (C) intake increased the $V'O_2$ in four of the individuals (one trained and three untrained individuals). Fasting (Fa) increased the $V'O_2$ in three of the individuals (one trained and two untrained subjects). Carbohydrate (Ch) intake showed increased values for the $V'O_2$ in three subjects (two trained and one untrained). Lastly, Fat (F) only increased the $V'O_2$ in two of the trained subjects.

- When comparing the highest to the lowest $V'O_{2peak}$ values for any of the interventions for a specific subject, the pre-exercise meal could alter the $V'O_{2peak}$ values with 12, 10.55, 9.69, 8.16, 15.06 and 8.74% (10.7±1.89) for the trained subjects and 9.56, 8.7, 7.56, 4.28, 8.81 and 7.38% (7.72±1.31) for the untrained subjects. The T-Test indicate a difference between the trained and untrained subjects (P=0.0447).

$V'CO_2$

The increased $V'CO_2$ levels could have originated from three metabolic pathways: (1) during intense exercise, when glycolysis is stimulated, the rate of pyruvate entry into the mitochondria for aerobic metabolism is decreased and more pyruvate is converted to lactate. During high intensity exercise the rate of lactate production exceeds the rate of lactate removal in the active musculature and some of the lactate that accumulates in the muscles is transferred to the

blood, which causes the [H^+] to increase. Chemical buffers need to buffer the excess H^+ which accumulates in the blood. The most common chemical buffer is bicarbonate. During this process, hydrogen ions combine with bicarbonate to form carbonic acid. A raise in plasma CO_2 due to the buffering action instantly stimulate ventilation to eliminate excess CO_2 (Astorino, 2000:213-214; McArdle et al., 2001:159); (2) when pyruvate is converted to Acetyl-CoA via pyruvate dehydrogenase to enter the Krebs cycle, the reaction releases CO_2 or (3) during the Krebs cycle, CO_2 is released. This protocol design cannot distinguish between the contributions of these pathways towards CO_2 production.

- Carbohydrate (Ch) intake showed increased V'CO_2 values in six of the trained and three of the untrained subjects. By mere speculation it is proposed that Carbohydrate (Ch) intake stimulates the glycolytic system to a larger extent since Carbohydrate (Ch) intake only showed increased values for the V'O_2 in three subjects (two trained and one untrained).

- Compared to all of the other interventions, the combination of Fat and Caffeine (FC) intake showed increased V'CO_2 values for three individuals, two trained and one untrained individual. Caffeine (C) intake also showed increased V'CO_2 values for three subjects (one trained and two untrained subjects). Fasting (Fa) showed increased V'CO_2 values, with only two individuals responding positively, one trained and one untrained subject. Individuals responded the least to Fat (F) intake in order to increase V'CO_2 levels, with only one trained subject responding.

RER

RER is the ratio between V'CO_2 production and V'O_2 consumption. RER values of ± 0.7, symbolizes pure fat oxidation, with an RER of 0.85 symbolizing a mixture of fat and carbohydrates and an RER of equal to and more than 1.0 symbolizes pure carbohydrate oxidation. The following interventions produced the highest RER values throughout the graded exercise test:

- Compared to all of the other interventions, Carbohydrate (Ch) intake coincided with the highest RER values. Nine individuals, four trained and five untrained individuals showed increased RER values. This could largely be attributed to Carbohydrate (Ch) intake showing increased $V'CO_2$ values in six of the trained and three of the untrained subjects rather than the increase in the $V'O_2$ values in three subjects (two trained and one untrained).

5.6. LIMITING FACTORS

Possible limitations identified in the present investigation are the following:

1. Specificity of the protocol design

- The results presented here relate to specific conditions and procedures and include reference to subjects forming part and parcel of a specific sub-population; exercise mode; specific types and volumes of nutrient interventions; time of intake of specific types of nutrients prior to exercise.

2. The number of subjects

- The number of subjects included in the investigation is not representative of the population. The present findings are confined to a specific sub-population.

- Increasing the number of subjects to validate the findings in terms of the general population is a meaningless endeavor and prospect. The genetic and physiological profile images of individuals are not alike and trends or differences observed for the general population cannot be extrapolated to any single individual in the problem solving process. This approach can only predict the percentage error that could occur.

3. Performance

- The given findings indicate that performance is defined in terms of graded incremental treadmill running until the point of voluntary fatigue. The notion prevails that other exercise modes could show alternative results.

- The findings presented here do not mimic any sporting code. In accordance, the present findings cannot be extrapolated to competitive field analysis or athletic performance.

4. Nutritional intake

- The given findings are confined to specific foodstuff quantities and qualities. Although defined in terms of ‘fat’, ‘carbohydrate’, ‘caffeine’ and ‘fasting’, these terminologies should not be viewed as single entities but rather as a composite of many substances where fat’, ‘carbohydrate’, ‘caffeine’ and ‘fasting’ dominate in terms of quantities. The effect of the main substrate or other substrates that comprise the intervention could influence the outcomes *per se*, in combination or synergistically. From a logistical point of view the protocol design presented here incorporated inexpensive every day foodstuffs and procedures that individuals use/could use to bring about the required change.

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

6.1. INTRODUCTION

The questions that formed the basis of this investigation are:

1. Does pre-exercise nutrient intake within the hours prior to exercise manipulate macronutrient metabolism?
2. Does pre-exercise nutrient intake within the hours prior to exercise affect performance?

6.2. CONCLUSION

The findings presented here suggest that all athletes do not respond to Fasting (Fa), Fat (F), Caffeine (C), Fat in combination with Caffeine (FC) and Carbohydrate (Ch) intake prior to a graded exercise test to the same extent.

These findings also suggest that pre-exercise nutrient intake within the hours prior to exercise could have significant impact on the well being status (obesity) or physical performance ability of some individuals.

It was furthermore found that carbohydrate and fat manipulating strategies over periods of days/weeks to enhance athletic performance should not be considered the only prevailing order of thought.

In order to combat obesity or increase performance it seems to be evident that exercise and nutrition sciences need to be synchronized.

Genetic predisposition and/or the current physiological profile ('fitness') images of an individual could possibly explain the altered responses between individuals towards pre-exercise nutrient intake.

6.3. RECOMMENDATIONS

It would be appropriate to consider the following recommendations:

6.3.1. The athletic industry

Three domains could be addressed:

6.3.1.1 Performance

Athletes should be subjected to various pre-exercise nutritional interventions to determine which intervention renders the best performance results specific for the sporting code. In this process and in many instances competitive field analysis could yield better results compared to laboratory analysis.

6.3.1.2. Muscular and systemic adaptations

It remains to be seen which variables and to what extent specific variables actually impact metabolism relative to a specific sporting code. Indirect calorimetry could yield information that could be more appropriate to meet the energy demands for improvements in short-term responses, mid and long-term adaptations in the active musculature in combination with systemic responses/adaptations compared to generalized non-specific concepts propagated in peer reviewed scientific literature.

6.3.1.3. Evaluation ("fitness testing")

Owing to the diverse effects of pre-exercise nutrient intake on metabolism and performance, the results presented here could render many (if not all) "fitness testing results" invalid in the sense that the findings of one athlete cannot be compared to the same athlete at a later stage (re-testing) or any other athletes at

any stage of testing and/or re-testing unless the nutritional intake prior to testing is ‘thoroughly controlled’.

‘Thoroughly controlled’ implicates actions where trained subject should be evaluated in a similar nutritional status prior to testing and re-testing. This, however, does not imply that the particular trained subject performed at optimal levels, unless the best nutritional status for the specific athlete is determined in advance. Until this is achieved, no results obtained from testing procedures of any one trained subject can be compared (extrapolated) to any of the results obtained for another trained subject (or any prescribed norm for that matter) in order to identify areas of strength or weaknesses for many strenuous test-protocols implemented in practice- refer to Appendix D.

6.3.2. The well-being industry

Many scientists are aware of the facts that a positive correlation exists between percentage of body fat and many disease states (Kerr et al., 2002:407). Up to date generalizations (many argue that adequate nutrition for well-being is readily obtained by adhering to a well-balanced diet) have been the only means to support individuals with weight loss programs. The notion lies within the perspective that the body will extract what is needed and excrete the remainder. Furthermore, one should also distinguish between weight loss and fat loss.

Peer reviewed literature justify by appeal to restrict calorie intake (Kerr et al., 2002:407) to reduce body fat. Calorie counting is restricted to laboratory enquiries since the human body is not a bomb- calorimetry meter and "a calorie is not a calorie" in the human body.

According to the objectives of this research, the newly founded perspectives could explain current contrasting research results presented in the peer reviewed scientific literature on weight loss. Irrespective of the effect of pre-exercise nutrient intake on fat metabolism, the findings presented here also indicate that heart rate and work load (treadmill speed) cannot always be implemented as the

criteria to reflect the fatty acid threshold (FAT) or fat-carbohydrate crossover point (FCCP). Once again, indirect calorimetry could yield reliable information to address the issues on fat loss.

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SUMMARY

KEY WORDS

- Performance
- Metabolism
- Indirect calorimetry
- Fat intake
- Caffeine intake
- Fatty acid oxidation
- Pre-exercise nutrient intake
- Fasting
- Carbohydrate intake
- Fat and caffeine intake
- Well-being and sporting industries
- Trained individuals
- Untrained individuals
- Physical work capacity
- Fitness testing results

In the exploration for methods to advance athletic performance, current interest has focused on numerous nutritional actions which may hypothetically promote fatty acid oxidation, ease the rate of muscle glycogen depletion and ultimately improve exercise capacity. Numerous factors are known to influence the selection of fuel for exercise, and there can be noteworthy interactions between several of them. These factors include: substrate availability, nutritional status, diet, mode, intensity, duration of exercise, muscle fiber type composition, physical fitness, the effect of training, drugs, and hormones and environmental factors (temperature and altitude). Furthermore, dietary manipulating strategies aimed at improving the metabolism of fat could have clinical significance in terms of body composition and obesity. An understanding of the factors that enhance or reduce fat oxidation is vital.

One of the unanswered questions which served as basis for this investigation is the following: Does pre–exercise nutrient intake within the hours prior to exercise have an effect on macronutrient metabolism of man during subsequent exercise?
Not only will newly-gained knowledge in this domain serve those individuals who would like to promote well-being (correct body composition by reducing fat mass) but will also provide constructive information for athletes on fuel utilization to improve athletic performance. Accordingly, the primary purpose of this

investigation was to evaluate the effect of pre-exercise nutrient intake on fat and carbohydrate metabolism during exercise in man. The secondary purpose of this investigation was to investigate whether nutrient intake within the hours prior to training influence the physical work capacity (PWC). Newly-gained perspectives on these research objectives may also provide information for other researchers who wish to explore this field of study further and this could also explain contrasting research results presented in the peer reviewed scientific literature on weight loss or athletic performance that has been presented to date.

In a double blind cross-over protocol design fasting, fat intake, caffeine intake, fat in combination with caffeine intake and carbohydrate intake prior to a graded exercise test served as interventions to validate the effect of pre-exercise nutrient intake on metabolism. Indirect calorimetry by means of an automated computerized breath-by-breath analysis system (Jaeger: Oxycon Pro; Masterscreen CPX Ergospirometry-Germany) coupled to a Technogym short range radio telemetry heart rate analyser was used when 12 subjects (6 trained and 6 untrained) were subjected to a graded incremental treadmill running test up to the point of voluntary fatigue. A Technogym RUNRACE 1200HC treadmill was used for this purpose.

The findings presented here suggest that all trained subjects and untrained subjects did not respond to the various interventions to the same extent. Various foodstuffs ingested at specific time intervals prior to exercise influence fat and carbohydrate oxidation and the PWC significantly. The findings revealed by this investigation also suggest that trends towards increased levels of fat oxidation (-13 to 47%) coinciding with decreased level of carbohydrate oxidation (1.85- 55.5%) and a trend towards a decrease in the RER (-9.3 -0.2%) during the initial phases [(first 7 minutes of the exercise regime); (treadmill speeds between 4-12 km/h)] coincided with an improvement in the PWC.
In 83.3% [(43.6%-97.0%) population proportion] of trained subjects an increase in the PWC is observed when fat, in combination with caffeine, is consumed

compared to the intervention responsible for the worst PWC. Pertaining to the aforementioned variables (and in many cases also the interventions) the opposite seems to rule for the untrained group of subjects.

Depending on the quantity, quality and time-intake of foodstuffs within the hours prior to exercise, it affects fat oxidation rates significantly. Furthermore, training at a prescribed treadmill speed or heart rate to promote fat loss, is deceptive for both trained and untrained individuals.

Conclusion

Although exercise duration and intensity are very important regulators of fat metabolism, the findings of this investigation suggest that pre-exercise nutrient intake within the hours prior to exercise affects metabolism during subsequent exercise and could impact on the well-being status (obesity), physical work capacity and the validity of “fitness testing results” when individuals are subjected to numerous evaluation protocols. The rationale of this investigation also indicates the importance why it is necessary to combine diet and exercise in the well-being and sporting industries.

OPSOMMING

SLEUTELWOORDE

- Prestasie
- Metabolisme
- Indirekte Kalorimetrie
- Vetinname
- Kaffeieinname
- Vetsuuroksidasie
- Inname van voedingstowwe voor oefening
- Vas
- Koolhidraatinname
- Vet en kaffeieninname
- Welstand- en sportindustrië
- Geoefende mense
- Ongeoefende mense
- Fisieke werksvermoë
- Fisieke evalueringstoetse

In die strewe na metodes om sportprestasie te bevorder is die huidige belangstelling gefokus op verskeie voedingskundige aspekte wat hipoteties vetsuuroksidasie bevorder en glikogeensparend intree om die oefenkapasiteit te bevorder. Dit is bekend dat verskeie faktore die seleksie van brandstowwe vir verhoogde fisieke aktiweiteit beïnvloed, ook dat daar interaksies tussen verskeie van hierdie faktore onderling kan wees. Hierdie faktore sluit die volgende in: substraatbeskikbaarheid, voedingstatus, dieet, duurte van oefening, spierveseltipering, tipe oefening, fiksheid, effek van oefening, farmakologiese agente, hormone en omgewingsinvloede (temperatuur en hoogte bo seespieël). Verder kan dieet-manipulerende strategië wat beoog om die vetmetabolisme te verbeter ook klinies relevant wees in terme van liggaamsamestelling en obesiteit. Dit is dus noodsaaklik om die faktore wat vetoksidasie bevorder of demp te verstaan.

Een van die onopgelosde vraagstukke wat as die basis vir die huidige ondersoek dien is die volgende: Bëinvloed die inname van voedingstowwe in die ure voordat

oefening ‘n aanvang neem die makronutriënt metabolisme gedurende oefening by die mens?

Nuwe kennis in hierdie terrein sal nie net insig verskaf vir individue wat hul welstand status wil bevorder nie maar ook konstruktiewe informasie rakende brandstofverbruik aan atlete kon verskaf om prestasie te bevorder. Dienooreenkomstig was die primêre doel van hierdie ondersoek om die effek van voedingstofinname voor oefening op die vet- en koolhidraatmetabolisme te ondersoek. Die sekondêre doel van hierdie ondersoek was om te kyk of die inname van voedingstowwe voor oefening ‘n aanvang neem, die fisieke werksvermoë beïnvloed. Nuutgefonde perspektiewe m.b.t. die huidige navorsingsdoelwitte kan ook nuwe inligting aan ander navorsers verskaf om hierdie studieveld vêrder te verken of om weersprekende navorsingsresultate rakende gewigsverlies of sportprestasie in geakrediteerde joernale uit te klaar.

Die invloed van vas, kaffeieninname, vetinname, vet en kaffeieninname en koolhidraatinname is op ‘n dubbelblind-oorkruis manier as intervensies aangewend om die effek van voedingstofinname voordat oefening ‘n aanvang neem op die metabolisme te ondersoek. Deur gebruik te maak van indirekte kalorimetrie is 12 mense (6 geoefende en 6 ongeoefende mense) aan ‘n standaard geoutomatiseerde en gerekenariseerde “asem vir asem” analitiese sisteem (Jaeger: Oxycon Pro; Masterscreen CPX Ergospirometry-Germany) gekoppel met ‘n harttempo analiseerder (Technogym short range radio telemetry heart rate analyser) aan ‘n gegradeerde trapmeultoets onderwerp. ‘n Technogym RUNRACE 1200HC-trapmeul was hiervoor gebruik.

Die bevindinge wys daarop dat alle geoefende en ongeoefende individue nie tot dieselfde mate teenoor die verskeie intervensies reageer en vetoksidasie en die fisieke werksvermoë betekenisvol beïnvloed nie. Die huidige bevindinge suggereer ook dat neigings tot ‘n verhoging in vetverbranding (-13 to 47%) en ‘n verlaging in die RER (-9.3 -0.2%) gepaard gegaan het met ‘n afname in

koolhidraatverbranding (1.85- 55.5%) gedurende die eerste fases [(eerste 7 minute); (trapmeulspoed tussen 4-12 km/h)] van die oefenprotokol by geoefende individue met ‘n verbetering in die fisieke werksvermoë gepaard gegaan het. In 83.3% [(43.6%-97.0%) populasie proporsie] van die geoefende gevalle is ‘n toename in die fisieke werksvermoë aangetoon indien vet in kombinasie met kaffeïen ingeneem word en met dié intervensie wat die slegste fisieke werksvermoë opgelewer het, vergelyk word. Die teendeel m.b.t. van voorafgenoemde veranderlikes (en baie van die intervensies) geld vir die onongeoefende groep persone. Afhangende van die kwantiteit, kwaliteit en die tydsduur van inname van kossoorte voordat oefening ‘n aanvang neem, kan vetoksidasie betekenisvol beïnvloed word. Dit blyk verder dat ‘n voorgeskrewe trapmeulspoed of harttempo by beide groepe individue misleidend tot die werklikheid staan indien dit die intensie is om vetverbranding te bevorder.

Gevolgtrekking

Alhoewel die intensiteit en duurte van oefening belangrike reguleerders van die vetmetabolisme kon wees, stel die huidige bevindinge voor dat voedingstofinname voordat oefening ‘n aanvang neem, die metabolisme tot so ‘n mate beïnvloed dat dit impak op die welstandstatus (obesiteit) van die individu, fisieke werkverrigtingsvermoë en die geldigheid van fisieke evalueringstoetse waaraan sportlui onderwerp word, kon uitoefen. Die rasionaal van die huidige resultate dui ook op die noodsaaklikheid om oefening en voeding gelyktydig en gesinkroniseerd in die welstand- en sportindustrië aangespreek moet word.

APPENDIX A

INFORMED CONSENT

Study: The effect of pre-exercise nutrient intake on metabolism

Dear participant

Please note the following information/conditions which pertain to this study:

1. Participating this study is voluntary, therefore any information will be handled confidentially
2. The participant will be introduced to and familiarized with treadmill running prior to testing. The participant will perform a graded exercise test to the point of volitional fatigue on a treadmill on five occasions spread over a period of 5 days (wash-out period of 24 hours between interventions
3. At 08h00 on five (5) consecutive days the following interventions will be implemented prior to participation in a scheduled treadmill run:
    - Post absorptive state (Fasting state of 8 hours)
    - Olive oil (5ml) and peanut butter (30ml) ingestion 120 minutes prior to testing in the post absorptive state
    - Caffeine ingestion (150 mg) 90 minutes prior to testing in the post absorptive state
    - Olive oil (5ml) and peanut butter (30ml) ingestion 120 minutes prior to testing followed by caffeine ingestion (150 mg) 90 minutes prior to testing in the post absorptive state
    - Carbohydrate ingestion, 4 slices white bread, apricot jam + 340ml coke, 240 minutes prior to testing
4. On completion of the test, the participant will be supplied with a report sheet to which would indicate the variables measured

Thank you for contributing towards the success of the study

I ……Date of Birth ……..,

give permission to take part in the research project as explained to me.

Patient …… Date……

Witness ……... Date ……...

APPENDIX B

A typical example of a print-out report indicating the absolute breath-by breath raw data values for the entire treadmill regime for a specific intervention and participant

Time [min]	Speed [km/h]	V'O2 [ml/min]	V'CO2 [ml/min]	V'O2peak	RER	HR [L/min]	CHO [g/day]	FAT [g/day]	V'E [L/min]
00:00:01	0.6	184	155	2.9	0.78	106	110	41	9
00:00:05	1.8	298	248	4.7	0.74	104	185	92	9
00:00:08	2.9	583	479	9.3	0.72	108	355	226	16
00:00:10	4	509	408	8.1	0.73	111	246	217	16
00:00:12	4	651	516	10.3	0.73	113	295	299	19
00:00:15	4	907	751	14.4	0.74	118	614	350	25
00:00:17	4	790	670	12.5	0.73	123	622	264	24
00:00:19	4	652	561	10.4	0.73	127	554	193	23
00:00:22	4	941	833	14.9	0.73	132	960	233	29
00:00:24	4	1004	917	15.9	0.73	135	1193	183	30
00:00:27	4	716	671	11.4	0.72	136	945	79	24
00:00:28	4	972	900	15.4	0.73	135	1229	145	34
00:00:30	4	929	890	14.7	0.72	135	1346	66	34
00:00:32	4	583	564	9.3	0.72	136	865	17	23
00:00:34	4	616	596	9.8	0.72	137	915	20	22
00:00:37	4	422	398	6.7	0.73	138	556	28	20
00:00:38	4	1157	1118	18.4	0.73	139	1746	65	36
00:00:41	4	701	658	11.1	0.73	140	929	74	23
00:00:43	4	1315	1206	20.9	0.71	140	1605	235	39
00:00:49	4	606	573	9.6	0.72	138	821	51	22
00:00:50	4	397	371	6.3	0.78	138	496	35	17
00:00:52	4	2668	2504	42.4	0.94	139	3634	369	70
00:00:56	4	943	855	15	0.91	140	1079	186	25
00:00:57	4	86	75	1.4	0.88	142	48	-3	12
00:01:01 T	4	916	846	14.5	0.73	137	1139	142	26
00:01:04 T	5	674	624	10.7	0.78	134	841	91	20
00:01:06 T	6.1	1440	1278	22.9	0.78	134	1504	366	38
00:01:08 T	7.2	1286	1104	20.4	0.78	135	1121	412	35
00:01:10 T	8	1363	1175	21.6	0.74	136	1219	427	37
00:01:12 T	8	1374	1171	21.8	0.74	139	1150	464	38
00:01:14 T	8	517	442	8.2	0.73	140	415	154	21
00:01:16 T	8	1533	1284	24.3	0.74	144	1154	576	40
00:01:17 T	8	2126	1779	33.7	0.81	145	1602	815	57
00:01:20 T	8	2006	1785	31.8	0.76	146	2141	508	59
00:01:22 T	8	1558	1405	24.7	0.76	148	1769	341	51
00:01:23 T	8	1405	1299	22.3	0.78	148	1780	228	48
00:01:25 T	8	1569	1491	24.9	0.74	149	2230	160	51
00:01:27 T	8	1562	1483	24.8	0.75	150	2209	164	50
00:01:29 T	8	1179	1363	18.7	0.78	150	3107	-477	36
00:01:31 T	8	1797	1653	28.5	0.80	150	2237	320	59
00:01:32 T	8	1687	1569	26.8	0.80	152	2199	257	53

00:01:34 T	8	1502	1393	23.8	0.80	153	1927	237	51
00:01:36 T	8	1775	1630	28.2	0.81	153	2193	322	55
00:01:38 T	8	1770	1615	28.1	0.82	152	2122	348	53
00:01:40 T	8	1691	1525	26.8	0.82	152	1918	375	52
00:01:41 T	8	1946	1775	30.9	0.82	152	2331	388	58
00:01:43 T	8	1804	1616	28.6	0.90	153	1989	427	58
00:01:45 T	8	1931	1799	30.6	0.93	153	2538	292	59
00:01:47 T	8	1590	1435	25.2	0.90	152	1811	347	52
00:01:48 T	8	1871	1664	29.7	0.89	152	1990	474	56
00:01:50 T	8	1614	1408	25.6	0.87	153	1551	470	51
00:01:51 T	8	1913	1665	30.4	0.87	153	1816	575	59
00:01:53 T	8	1873	1682	29.7	0.90	154	2088	435	55
00:01:55 T	8	1815	1640	28.8	0.90	154	2079	398	54
00:01:57 T	8	1639	1444	26	0.88	154	1658	444	51
00:01:58 T	8	1847	1620	29.3	0.88	154	1829	523	55
00:02:00 T	8	1868	1651	29.6	0.88	153	1928	497	53
00:02:02 T	8	1868	1645	29.7	0.88	153	1891	513	52
00:02:04 T	8	2003	1728	31.8	0.86	153	1814	640	57
00:02:05 T	8	1920	1666	30.5	0.87	154	1796	588	57
00:02:07 T	8	1605	1401	25.5	0.87	154	1546	466	46
00:02:09 T	8	2029	1784	32.2	0.88	155	2040	566	55
00:02:11 T	8	2077	1896	33	0.91	155	2503	411	59
00:02:13 T	8	1999	1796	31.7	0.90	155	2236	464	60
00:02:15 T	8	1910	1744	30.3	0.91	154	2299	375	57
00:02:17 T	8	1984	1766	31.5	0.89	154	2122	500	57
00:02:19 T	8	2343	2227	37.2	0.95	153	3349	254	70
00:02:21 T	8	1574	1417	25	0.90	153	1766	354	46
00:02:23 T	8	1791	1571	28.4	0.88	154	1771	507	51
00:02:25 T	8	2014	1766	32	0.88	154	1998	572	58
00:02:26 T	8	1964	1750	31.2	0.89	153	2112	491	57
00:02:28 T	8	1995	1767	31.7	0.89	153	2078	526	59
00:02:30 T	8	1824	1632	29	0.89	153	1998	437	54
00:02:31 T	8	1675	1478	26.6	0.88	154	1705	451	51
00:02:33 T	8	2507	2236	39.8	0.89	154	2715	630	70
00:02:35 T	8	2214	2015	35.1	0.91	155	2636	454	65
00:02:36 T	8	2058	1879	32.7	0.91	156	2483	405	64
00:02:38 T	8	2018	1854	32	0.92	157	2498	371	64
00:02:39 T	8	2073	1909	32.9	0.92	157	2600	368	64
00:02:41 T	8	1969	1809	31.2	0.92	158	2440	359	64
00:02:43 T	8	1713	1607	27.2	0.94	158	2313	229	52
00:02:44 T	8	2124	1969	33.7	0.93	158	2742	346	67
00:02:46 T	8	2018	1874	32	0.93	159	2617	322	63
00:02:50 T	8	2284	2045	36.3	0.90	158	2520	552	64
00:02:51 T	8	2067	1854	32.8	0.90	159	2296	489	62
00:02:53 T	8	2499	2281	39.7	0.91	159	3018	500	71
00:02:55 T	8	2037	1888	32.3	0.93	159	2621	334	63
00:02:56 T	8	2031	1887	32.2	0.93	159	2641	321	65

00:02:58 T	8	1815	1705	28.8	0.94	160	2470	237	60
00:02:59 T	8	1982	1894	31.5	0.96	160	2889	185	63
00:03:01 T	8	2131	2029	33.8	0.95	160	3067	218	70
00:03:02 T	9	1920	1836	30.5	0.91	160	2803	176	64
00:03:04 T	9	1998	1912	31.7	0.91	160	2929	180	65
00:03:06 T	9.8	1831	1747	29.1	0.91	160	2652	174	60
00:03:07 T	9.8	1926	1822	30.6	0.91	159	2698	223	60
00:03:09 T	9.8	2004	1925	31.8	0.91	159	2978	164	63
00:03:11 T	9.8	2193	2142	34.8	0.91	159	3471	97	71
00:03:13 T	9.8	1916	1897	30.4	0.91	159	3180	18	67
00:03:14 T	9.8	2033	2049	32.3	0.91	160	3595	-69	70
00:03:16 T	9.8	1870	1869	29.7	0.91	160	3209	-27	65
00:03:17 T	9.8	1895	1877	30.1	0.93	160	3153	14	66
00:03:19 T	9.8	2011	1999	31.9	0.93	160	3387	2	67
00:03:20 T	9.8	1986	1962	31.5	0.93	161	3274	30	69
00:03:22 T	9.8	1977	1986	31.4	0.93	162	3458	-52	66
00:03:23 T	9.8	2105	2076	33.4	0.91	162	3450	43	72
00:03:25 T	9.8	1998	1966	31.7	0.92	163	3247	49	69
00:03:26 T	9.8	1928	1889	30.6	0.98	163	3082	67	66
00:03:28 T	9.8	2031	1981	32.2	0.98	163	3201	92	68
00:03:29 T	9.8	1836	1786	29.1	0.97	163	2860	93	63
00:03:31 T	9.8	1934	1866	30.7	0.96	163	2921	137	67
00:03:32 T	9.8	2017	1948	32	0.97	164	3062	139	67
00:03:35 T	9.8	2125	2065	33.7	0.97	164	3308	114	67
00:03:36 T	9.8	2151	2035	34.1	0.95	164	3016	253	69
00:03:38 T	9.8	2152	2052	34.2	0.95	164	3113	215	69
00:03:39 T	9.8	2085	1969	33.1	0.94	165	2905	251	67
00:03:41 T	9.8	2057	1933	32.6	0.94	165	2808	271	66
00:03:42 T	9.8	2177	2042	34.6	0.94	165	2947	300	69
00:03:44 T	9.8	2085	1954	33.1	0.94	165	2818	287	67
00:03:45 T	9.8	1910	1809	30.3	0.95	165	2687	217	64
00:03:47 T	9.8	1865	1762	29.6	0.94	165	2596	221	62
00:03:49 T	9.8	2053	2047	32.6	1.00	166	3495	-13	62
00:03:51 T	9.8	2035	1906	32.3	0.94	166	2736	285	65
00:03:52 T	9.8	1958	1823	31.1	0.93	165	2566	300	62
00:03:54 T	9.8	2214	2019	35.1	0.91	165	2661	444	67
00:03:55 T	9.8	2489	2287	39.5	0.92	164	3092	463	74
00:03:57 T	9.8	2277	2119	36.1	0.93	164	2986	355	72
00:03:58 T	9.8	1995	1840	31.7	0.92	163	2513	348	63
00:04:00 T	9.8	2105	1924	33.4	0.91	163	2552	411	65
00:04:01 T	9.8	2038	1854	32.3	0.91	163	2417	418	63
00:04:03 T	9.8	1943	1755	30.8	0.90	164	2228	427	61
00:04:04 T	9.8	2258	2041	35.8	0.90	164	2602	499	67
00:04:06 T	9.8	2528	2260	40.1	0.89	164	2770	623	74
00:04:07 T	9.8	2403	2225	38.1	0.93	164	3090	402	72
00:04:09 T	9.8	2433	2271	38.6	0.93	164	3233	365	76

00:04:10 T	9.8	2328	2179	36.9	0.94	165	3130	332	73
00:04:12 T	9.8	2191	2069	34.8	0.94	165	3050	268	71
00:04:13 T	9.8	2366	2240	37.6	0.95	165	3333	276	74
00:04:15 T	9.8	2316	2197	36.8	0.95	165	3289	259	73
00:04:16 T	9.8	2096	1999	33.3	0.95	165	3030	208	68
00:04:18 T	9.8	2095	2005	33.3	0.96	166	3071	191	63
00:04:20 T	9.8	2199	2095	34.9	0.95	166	3174	222	72
00:04:21 T	9.8	2166	2065	34.4	0.95	166	3128	218	69
00:04:23 T	9.8	2123	2025	33.7	0.95	166	3071	211	67
00:04:24 T	9.8	2323	2191	36.9	0.94	165	3222	291	73
00:04:26 T	9.8	1983	1886	31.5	0.95	165	2836	207	65
00:04:27 T	9.8	2187	2061	34.7	0.94	166	3018	278	68
00:04:29 T	9.8	2500	2351	39.7	0.94	166	3425	335	77
00:04:30 T	9.8	2185	2078	34.7	0.95	166	3129	231	71
00:04:32 T	9.8	2252	2149	35.8	0.95	166	3268	222	72
00:04:33 T	9.8	2202	2110	35	0.96	166	3245	196	72
00:04:35 T	9.8	1594	1536	25.3	0.96	167	2390	112	57
00:04:36 T	9.8	2625	2503	41.7	0.95	167	3804	269	79
00:04:38 T	9.8	2272	2159	36.1	0.95	167	3245	246	73
00:04:40 T	9.8	2245	2139	35.6	0.95	168	3244	226	73
00:04:41 T	9.8	2103	2025	33.4	0.96	168	3161	159	71
00:04:43 T	9.8	2342	2267	37.2	0.97	168	3591	153	78
00:04:44 T	9.8	1415	1352	22.5	0.96	167	2054	122	48
00:04:46 T	9.8	2192	2030	34.8	0.93	167	2818	364	63
00:04:47 T	9.8	2400	2189	38.1	0.91	167	2891	482	72
00:04:49 T	9.8	2798	2588	44.4	0.92	167	3584	482	84
00:04:50 T	9.8	2375	2229	37.7	0.94	168	3225	327	79
00:04:51 T	9.8	2143	2064	34	0.96	168	3223	162	73
00:04:53 T	9.8	2293	2208	36.4	0.96	168	3445	179	69
00:04:54 T	9.8	2361	2259	37.5	0.96	168	3463	220	79
00:04:56 T	9.8	2343	2252	37.2	0.96	168	3498	192	76
00:04:57 T	9.8	2480	2387	39.4	0.96	167	3726	197	82
00:04:58 T	9.8	2534	2451	40.2	0.97	167	3878	173	84
00:05:00 T	9.8	2249	2209	35.7	0.98	167	3637	68	78
00:05:01 T	9.8	2249	2209	35.7	0.98	167	3640	67	74
00:05:03 T	10.8	2200	2145	34.9	0.97	167	3462	105	73
00:05:04 T	11.9	2173	2096	34.5	0.96	168	3287	158	73
00:05:06 T	11.9	2125	2061	33.7	0.97	168	3280	126	68
00:05:07 T	12.1	2081	1990	33	0.96	168	3045	191	69
00:05:09 T	12.1	2325	2214	36.9	0.95	169	3350	240	73
00:05:11 T	12.1	2095	2048	33.3	0.98	169	3331	84	64
00:05:12 T	12.1	2258	2150	35.8	0.95	169	3251	233	72
00:05:14 T	12.1	2333	2163	37	0.93	169	3013	384	73
00:05:15 T	12.1	2464	2326	39.1	0.94	170	3432	306	75
00:05:17 T	12.1	2290	2170	36.4	0.95	170	3234	263	71
00:05:18 T	12.1	2353	2212	37.3	0.94	170	3219	314	72
00:05:22 T	12.1	3005	2741	47.7	0.91	171	3626	612	79
00:05:23 T	12.1	2840	2579	45.1	0.91	171	3356	605	83

00:05:25 T	12.1	2536	2394	40.3	0.94	171	3528	318	78
00:05:26 T	12.1	2504	2341	39.7	0.94	172	3354	366	77
00:05:28 T	12.1	2361	2179	37.5	0.92	172	2992	413	73
00:05:29 T	12.1	2413	2247	38.3	0.93	172	3174	375	74
00:05:31 T	12.1	2720	2547	43.2	0.94	171	3668	392	80
00:05:32 T	12.1	2376	2181	37.7	0.92	171	2940	444	78
00:05:34 T	12.1	2531	2368	40.2	0.94	171	3396	369	77
00:05:37 T	12.1	2341	2283	37.2	0.98	171	3692	112	71
00:05:38 T	12.1	2464	2273	39.1	0.92	170	3115	436	73
00:05:40 T	12.1	2440	2239	38.7	0.92	170	3014	460	71
00:05:41 T	12.1	2517	2305	40	0.92	170	3083	486	76
00:05:42 T	12.1	2308	2116	36.6	0.92	169	2841	437	72
00:05:44 T	12.1	2427	2236	38.5	0.92	169	3051	436	71
00:05:46 T	12.1	2583	2332	41	0.90	169	2968	581	74
00:05:47 T	12.1	2674	2416	42.4	0.90	170	3087	597	77
00:05:48 T	12.1	2677	2439	42.5	0.91	170	3211	548	82
00:05:50 T	12.1	2702	2484	42.9	0.92	170	3372	500	84
00:05:51 T	12.1	2684	2492	42.6	0.93	170	3495	437	86
00:05:52 T	12.1	2634	2451	41.8	0.93	171	3457	417	85
00:05:53 T	12.1	2985	2826	47.4	0.95	171	4212	358	93
00:05:55 T	12.1	2493	2352	39.6	0.94	171	3467	312	82
00:05:56 T	12.1	2436	2318	38.7	0.95	171	3503	257	80
00:05:58 T	12.1	2333	2234	37	0.96	170	3433	212	74
00:05:58 T	12.1	2843	2673	45.1	0.94	170	3902	383	85
00:05:59 T	12.1	2145	1961	34.1	0.91	170	2605	418	68
00:06:01 T	12.1	2610	2450	41.4	0.94	169	3554	360	83
00:06:02 T	12.1	2527	2380	40.1	0.94	169	3484	330	82
00:06:04 T	12.1	2563	2415	40.7	0.94	169	3542	331	81
00:06:05 T	12.1	2661	2492	42.2	0.94	169	3590	382	85
00:06:07 T	12.1	2299	2172	36.5	0.94	169	3205	281	70
00:06:08 T	12.1	2609	2459	41.4	0.94	169	3615	334	79
00:06:10 T	12.1	2390	2243	37.9	0.94	168	3251	327	73
00:06:11 T	12.1	2776	2588	44.1	0.93	168	3675	429	84
00:06:12 T	12.1	2476	2359	39.3	0.95	169	3576	255	79
00:06:14 T	12.1	2349	2216	37.3	0.94	169	3262	293	73
00:06:15 T	12.1	2231	2032	35.4	0.91	169	2667	453	68
00:06:16 T	12.1	2591	2357	41.1	0.91	170	3080	541	75
00:06:18 T	12.1	2836	2596	45	0.92	170	3475	553	81
00:06:19 T	12.1	2660	2447	42.2	0.92	170	3325	490	80
00:06:20 T	12.1	2571	2393	40.8	0.93	171	3379	403	77
00:06:22 T	12.1	2494	2314	39.6	0.93	171	3232	410	76
00:06:23 T	12.1	2701	2514	42.9	0.93	171	3553	425	80
00:06:25 T	12.1	2597	2427	41.2	0.93	171	3474	384	80
00:06:26 T	12.1	2767	2603	43.9	0.94	171	3806	369	85
00:06:28 T	12.1	2630	2483	41.8	0.94	172	3666	329	86
00:06:29 T	12.1	2630	2509	41.8	0.95	172	3822	265	84
00:06:31 T	12.1	2506	2358	39.8	0.94	172	3444	331	78

00:06:32 T	12.1	2648	2505	42	0.95	172	3719	320	83
00:06:34 T	12.1	2635	2592	41.8	0.98	173	4289	77	79
00:06:35 T	12.1	2430	2289	38.6	0.94	173	3358	312	78
00:06:37 T	12.1	2877	2699	45.7	0.94	173	3909	405	91
00:06:38 T	12.1	2546	2396	40.4	0.94	172	3502	336	84
00:06:39 T	12.1	2565	2410	40.7	0.94	172	3506	347	81
00:06:40 T	12.1	2866	2755	45.5	0.96	171	4287	242	91
00:06:42 T	12.1	2379	2240	37.8	0.94	171	3279	308	72
00:06:43 T	12.1	2697	2539	42.8	0.94	171	3719	354	85
00:06:45 T	12.1	2844	2737	45.1	0.96	171	4278	230	88
00:06:46 T	12.1	2337	2241	37.1	0.96	171	3458	205	78
00:06:48 T	12.1	2653	2552	42.1	0.96	171	3981	216	85
00:06:49 T	12.1	2717	2613	43.1	0.96	172	4075	223	87
00:06:50 T	12.1	2565	2430	40.7	0.95	172	3625	299	87
00:06:52 T	12.1	2527	2391	40.1	0.95	172	3554	301	83
00:06:53 T	12.1	2629	2534	41.7	0.96	172	3974	201	85
00:06:54 T	12.1	2876	2763	45.7	0.96	173	4295	246	94
00:06:55 T	12.1	2900	2778	46	0.96	173	4285	267	96
00:06:57 T	12.1	2614	2567	41.5	0.98	173	4236	83	85
00:06:58 T	12.1	2534	2461	40.2	0.97	173	3936	150	88
00:06:59 T	12.1	3120	3030	49.5	0.97	174	4855	191	102
00:07:01 T	12.1	2682	2597	42.6	0.97	174	4125	177	94
00:07:02 T	12.9	2646	2589	42	0.98	174	4230	108	89
00:07:03 T	12.9	2398	2344	38.1	0.98	174	3815	102	80
00:07:05 T	13.8	2493	2440	39.6	0.98	174	3982	101	81
00:07:06 T	13.8	2686	2622	42.6	0.98	175	4256	127	83
00:07:07 T	13.8	2301	2178	36.5	0.95	175	3238	269	78
00:07:08 T	13.8	2689	2534	42.7	0.94	175	3719	349	83
00:07:10 T	13.8	2987	2845	47.4	0.95	176	4320	315	90
00:07:11 T	13.8	2742	2693	43.5	0.98	176	4439	92	87
00:07:13 T	13.8	2549	2480	40.5	0.97	176	3989	138	81
00:07:14 T	13.8	2641	2541	41.9	0.96	176	3963	214	83
00:07:16 T	13.8	2813	2704	44.6	0.96	177	4209	236	88
00:07:17 T	13.8	2602	2460	41.3	0.95	177	3648	316	85
00:07:18 T	13.8	2540	2436	40.3	0.96	177	3764	224	84
00:07:20 T	13.8	2581	2503	41	0.97	177	3991	160	79
00:07:21 T	13.8	2883	2758	45.8	0.96	176	4239	274	95
00:07:23 T	13.8	2869	2767	45.5	0.96	176	4351	218	92
00:07:23 T	13.8	2660	2533	42.2	0.95	176	3839	278	94
00:07:25 T	13.8	2668	2538	42.3	0.95	176	3831	288	84
00:07:26 T	13.8	2739	2606	43.5	0.95	176	3935	296	85
00:07:28 T	13.8	2953	2861	46.9	0.97	177	4557	193	90
00:07:29 T	13.8	2775	2656	44	0.96	177	4086	259	89
00:07:31 T	13.8	2660	2571	42.2	0.97	176	4063	187	85
00:07:32 T	13.8	2767	2666	43.9	0.96	176	4176	217	87
00:07:34 T	13.8	2787	2635	44.2	0.95	175	3911	340	91
00:07:35 T	13.8	2644	2497	42	0.94	175	3692	327	87
00:07:36 T	13.8	2799	2639	44.4	0.94	175	3886	358	89

00:07:38 T	13.8	2735	2675	43.4	0.98	175	4364	117	82
00:07:40 T	13.8	2721	2549	43.2	0.94	174	3673	390	83
00:07:41 T	13.8	2480	2271	39.4	0.92	174	3036	479	79
00:07:42 T	13.8	2694	2480	42.8	0.96	174	3377	493	82
00:07:43 T	13.8	2942	2717	46.7	0.96	174	3749	517	87
00:07:45 T	13.8	2493	2307	39.6	0.96	174	3193	424	77
00:07:46 T	13.8	2863	2592	45.4	0.96	175	3339	628	88
00:07:47 T	13.8	2849	2563	45.2	0.96	175	3224	665	84
00:07:49 T	13.8	2954	2679	46.9	0.97	175	3472	639	89
00:07:50 T	13.8	2824	2574	44.8	0.97	175	3397	576	84
00:07:51 T	13.8	3368	3077	53.5	0.97	175	4100	676	98
00:07:52 T	13.8	2677	2482	42.5	0.97	176	3459	447	79
00:07:53 T	13.8	3015	2778	47.9	0.97	176	3801	547	91
00:07:55 T	13.8	3086	2856	49	0.97	176	3968	530	97
00:07:56 T	13.8	2722	2498	43.2	0.98	176	3373	514	89
00:07:57 T	13.8	2583	2440	41	0.98	176	3610	318	78
00:08:01 T	13.8	3831	3488	60.8	0.91	177	4597	803	96
00:08:02 T	13.8	3095	2767	49.1	0.89	177	3400	769	96
00:08:03 T	13.8	3288	3029	52.2	0.92	177	4148	600	101
00:08:04 T	13.8	3061	2837	48.6	0.93	177	3959	515	98
00:08:05 T	13.8	3128	2915	49.7	0.93	177	4138	490	101
00:08:07 T	13.8	2871	2701	45.6	0.94	176	3945	386	91
00:08:08 T	13.8	3002	2855	47.6	0.95	176	4312	329	94
00:08:09 T	13.8	3160	2996	50.2	0.95	176	4490	368	104
00:08:10 T	13.8	3140	2998	49.8	0.95	176	4583	316	104
00:08:11 T	13.8	3146	3037	49.9	0.97	176	4790	236	106
00:08:13 T	13.8	2989	2890	47.4	0.97	177	4577	211	106
00:08:14 T	13.8	3100	3040	49.2	0.98	177	5003	115	108
00:08:15 T	13.8	2861	2802	45.4	0.98	177	4591	114	101
00:08:16 T	13.8	3087	3043	49	0.99	177	5074	77	107
00:08:17 T	13.8	2873	2826	45.6	0.98	177	4683	85	103
00:08:18 T	13.8	2853	2815	45.3	0.99	178	4703	62	100
00:08:19 T	13.8	2925	2861	46.4	0.98	178	4673	126	103
00:08:20 T	13.8	3075	3049	48.8	0.99	178	5159	34	108
00:08:22 T	13.8	2678	2656	42.5	0.99	178	4491	25	98
00:08:23 T	13.8	2890	2882	45.9	1.00	179	4944	-10	103
00:08:24 T	13.8	2872	2837	45.6	0.99	179	4755	54	99
00:08:25 T	13.8	2644	2616	42	0.99	179	4395	40	90
00:08:26 T	13.8	3022	2938	48	0.97	179	4722	175	101
00:08:28 T	13.8	2877	2806	45.7	0.98	179	4549	143	93
00:08:29 T	13.8	2991	2930	47.5	0.98	180	4806	118	97
00:08:30 T	13.8	2915	2855	46.3	0.98	180	4677	117	98
00:08:32 T	13.8	3041	3038	48.3	1.00	180	5238	-23	99
00:08:33 T	13.8	2925	2877	46.4	0.98	180	4766	88	100
00:08:34 T	13.8	3101	3060	49.2	0.99	180	5114	71	108
00:08:35 T	13.8	2750	2735	43.6	0.99	179	4659	6	98
00:08:36 T	13.8	2854	2810	45.3	0.98	179	4667	78	95
00:08:38 T	13.8	2812	2797	44.6	0.99	179	4767	7	94

00:08:39 T	13.8	2760	2696	43.8	0.98	179	4384	127	96
00:08:40 T	13.8	2791	2751	44.3	0.99	179	4578	70	95
00:08:41 T	13.8	2817	2738	44.7	0.97	178	4394	163	98
00:08:42 T	13.8	2990	2878	47.5	0.96	178	4499	244	101
00:08:43 T	13.8	3035	2971	48.2	0.98	178	4864	126	106
00:08:44 T	13.8	2936	2902	46.6	0.99	178	4872	52	103
00:08:46 T	13.8	2617	2569	41.5	0.98	178	4233	87	95
00:08:47 T	13.8	3076	3036	48.8	0.99	177	5081	66	107
00:08:48 T	13.8	2877	2828	45.7	0.98	177	4677	90	101
00:08:49 T	13.8	2780	2745	44.1	0.99	177	4589	57	98
00:08:50 T	13.8	2736	2686	43.4	0.98	178	4425	93	97
00:08:52 T	13.8	2928	2862	46.5	0.98	178	4663	133	98
00:08:53 T	13.8	3139	3122	49.8	0.99	178	5323	12	101
00:08:54 T	13.8	2973	2891	47.2	0.97	178	4650	169	104
00:08:55 T	13.8	2966	2906	47.1	0.98	177	4769	116	102
00:08:56 T	13.8	3114	3072	49.4	0.99	177	5135	71	111
00:08:57 T	13.8	3133	3129	49.7	1.00	177	5387	-18	114
00:08:58 T	13.8	3016	3015	47.9	1.00	177	5206	-28	112
00:08:59 T	13.8	2968	3022	47.1	1.02	177	5448	-161	110
00:09:01 T	13.8	2874	2896	45.6	1.01	176	5093	-82	104
00:09:02 T	14.8	2800	2797	44.4	1.00	176	4815	-22	104
00:09:03 T	14.8	2676	2661	42.5	0.99	176	4531	6	99
00:09:04 T	15.8	2867	2867	45.5	1.00	176	4947	-27	102
00:09:05 T	15.8	2805	2762	44.5	0.98	177	4590	74	100
00:09:06 T	15.8	2802	2750	44.5	0.98	177	4528	98	99
00:09:07 T	15.8	2892	2841	45.9	0.98	177	4692	94	102
00:09:08 T	15.8	3102	3040	49.2	0.98	178	4993	121	109
00:09:10 T	15.8	2927	2886	46.5	0.99	178	4811	71	108
00:09:11 T	15.8	2956	2925	46.9	0.99	178	4922	46	107
00:09:12 T	15.8	2636	2593	41.8	0.98	178	4294	75	99
00:09:13 T	15.8	2641	2613	41.9	0.99	179	4395	37	95
00:09:14 T	15.8	2636	2572	41.8	0.98	179	4172	125	94
00:09:15 T	15.8	2745	2670	43.6	0.97	179	4292	153	91
00:09:17 T	15.8	3055	2976	48.5	0.97	179	4808	163	100
00:09:17 T	15.8	2768	2641	43.9	0.95	180	4024	280	96
00:09:19 T	15.8	3250	3136	51.6	0.96	180	4941	247	107
00:09:20 T	15.8	3118	3024	49.5	0.97	180	4834	198	110
00:09:21 T	15.8	3043	2972	48.3	0.98	180	4834	144	107
00:09:22 T	15.8	2806	2764	44.5	0.99	181	4598	72	97
00:09:23 T	15.8	3012	2976	47.8	0.99	181	4991	58	101
00:09:25 T	15.8	2991	2953	47.5	0.99	181	4943	62	100
00:09:26 T	15.8	3025	3006	48	0.99	180	5112	17	104
00:09:28 T	15.8	2846	2764	45.2	0.97	180	4426	171	99
00:09:29 T	15.8	2862	2777	45.4	0.97	180	4441	176	97
00:09:29 T	15.8	2880	2767	45.7	0.96	180	4304	245	99
00:09:31 T	15.8	2917	2804	46.3	0.98	180	4367	246	100
00:09:32 T	15.8	2833	2744	45	0.98	180	4363	187	97

00:09:34 T	15.8	2869	2873	45.5	1.00	180	4980	-40	88
00:09:35 T	15.8	3068	2938	48.7	0.96	180	4532	285	97
00:09:37 T	15.8	3010	2837	47.8	0.97	179	4171	392	93
00:09:38 T	15.8	2729	2569	43.3	0.97	179	3764	358	83
00:09:39 T	15.8	2815	2638	44.7	0.97	179	3809	402	95
00:09:40 T	15.8	3303	3080	52.4	0.97	179	4386	513	103
00:09:41 T	15.8	3077	2903	48.8	0.97	179	4284	394	103
00:09:43 T	15.8	3222	3044	51.1	0.98	180	4514	403	107
00:09:43 T	15.8	3158	3021	50.1	0.96	180	4644	304	106
00:09:44 T	15.8	2934	2812	46.6	0.96	180	4346	266	96
00:09:46 T	15.8	3472	3354	55.1	0.97	180	5305	257	116
00:09:47 T	15.8	2960	2875	47	0.97	180	4610	177	104
00:09:50 T	15.8	3713	3052	58.9	0.98	181	2500	1579	101
00:09:51 T	15.8	3382	3095	53.7	0.98	181	4145	669	102
00:09:52 T	15.8	3488	3237	55.4	0.98	181	4544	580	106
00:09:53 T	15.8	3639	3412	57.8	0.98	180	4950	521	115
00:09:55 T	15.8	3284	3138	52.1	0.98	180	4808	327	109
00:09:56 T	15.8	3188	3045	50.6	0.98	180	4665	316	108
00:09:57 T	15.8	2987	2878	47.4	0.98	180	4515	235	102
00:09:58 T	15.8	3109	2996	49.4	0.98	179	4698	247	104
00:09:59 T	15.8	3137	3032	49.8	0.98	179	4799	226	107
00:10:00 T	15.8	2772	2637	44	0.95	179	3986	299	95
00:10:01 T	15.8	3276	3153	52	0.96	179	4933	270	106
00:10:02 T	15.8	2990	2869	47.5	0.96	179	4446	265	103
00:10:04 T	15.8	3168	3038	50.3	0.96	179	4703	287	106
00:10:05 T	15.8	3299	3164	52.4	0.96	179	4906	297	109
00:10:05 T	15.8	3371	3267	53.5	0.97	180	5208	225	114
00:10:07 T	15.8	3178	3085	50.4	0.97	180	4939	197	110
00:10:08 T	15.8	3331	3301	52.9	0.99	180	5579	44	118
00:10:09 T	15.8	2981	2980	47.3	1.00	180	5145	-27	106
00:10:10 T	15.8	3289	3297	52.2	1.00	180	5731	-48	116
00:10:11 T	15.8	2879	2874	45.7	1.00	181	4941	-17	105
00:10:12 T	15.8	3063	3074	48.6	1.00	181	5353	-54	107
00:10:13 T	15.8	2973	2920	47.2	0.98	181	4822	99	102
00:10:14 T	15.8	3398	3341	53.9	0.98	182	5534	111	116
00:10:16 T	15.8	3242	3203	51.5	0.99	182	5371	66	114
00:10:17 T	15.8	3251	3253	51.6	1.00	182	5628	-33	114
00:10:18 T	15.8	3245	3229	51.5	1.00	182	5513	10	116
00:10:19 T	15.8	2813	2823	44.7	1.00	182	4915	-53	100
00:10:20 T	15.8	3555	3577	56.4	1.01	183	6277	-82	122
00:10:22 T	15.8	3274	3288	52	1.00	183	5743	-63	118
00:10:23 T	15.8	3305	3379	52.5	1.02	183	6152	-210	116
00:10:24 T	15.8	2898	2892	46	1.00	182	4969	-15	107
00:10:25 T	15.8	3183	3172	50.5	1.00	182	5433	-1	110
00:10:26 T	15.8	3237	3212	51.4	0.99	182	5448	31	114
00:10:27 T	15.8	3331	3341	52.9	1.00	182	5818	-54	117
00:10:28 T	15.8	3229	3253	51.3	1.01	182	5719	-86	119

00:10:30 T	15.8	2225	2247	35.3	1.01	182	3966	-83	77
00:10:31 T	15.8	3641	3681	57.8	1.01	183	6539	-128	119
00:10:32 T	15.8	3273	3227	52	0.99	183	5384	83	109
00:10:34 T	15.8	3300	3275	52.4	0.99	183	5558	31	112
00:10:35 T	15.8	3224	3218	51.2	1.00	183	5536	-15	111
00:10:35 T	15.8	3054	3050	48.5	1.00	182	5252	-19	104
00:10:37 T	15.8	3523	3539	55.9	1.00	182	6188	-69	118
00:10:38 T	15.8	3118	3146	49.5	1.01	182	5556	-98	109
00:10:39 T	15.8	3098	3076	49.2	0.99	182	5224	24	108
00:10:40 T	15.8	3298	3290	52.4	1.00	183	5653	-10	115
00:10:41 T	15.8	3299	3283	52.4	1.00	183	5605	11	116
00:10:43 T	15.8	3319	3328	52.7	1.00	183	5790	-51	119
00:10:44 T	15.8	3112	3097	49.4	1.00	183	5287	7	111
00:10:44 T	15.8	3167	3201	50.3	1.01	184	5673	-111	114
00:10:46 T	15.8	3508	3590	55.7	1.02	184	6556	-230	123
00:10:47 T	15.8	3253	3320	51.6	1.02	184	6017	-191	116
00:10:48 T	15.8	3275	3354	52	1.02	184	6128	-221	120
00:10:49 T	15.8	3073	3102	48.8	1.01	185	5483	-101	113
00:10:50 T	15.8	3297	3368	52.3	1.02	185	6122	-203	124
00:10:51 T	15.8	3047	3135	48.4	1.03	184	5786	-243	114
00:10:55 T	15.8	3690	3627	58.6	0.98	184	6005	126	128
00:10:56 T	15.8	3196	3184	50.7	1.00	184	5451	0	112
00:10:58 T	15.8	3583	3648	56.9	1.02	183	6578	-185	126
00:10:59 T	15.8	3063	3106	48.6	1.01	183	5547	-134	117
00:11:00 T	15.8	3298	3390	52.4	1.03	183	6245	-252	120
00:11:01 T	15.8	2920	2979	46.4	1.02	183	5390	-171	107
00:11:02 T	16.4	3335	3403	52.9	1.02	183	6167	-194	117
00:11:03 T	16.4	3193	3222	50.7	1.01	184	5693	-101	111
00:11:04 T	17.5	3081	3047	48.9	0.99	184	5120	54	109
00:11:05 T	17.5	3162	3156	50.2	1.00	184	5429	-15	112
00:11:07 T	17.9	3363	3377	53.4	1.00	184	5895	-62	116
00:11:08 T	17.9	3077	3101	48.8	1.01	184	5460	-88	108
00:11:09 T	17.9	3458	3494	54.9	1.01	183	6190	-115	120
00:11:10 T	17.9	3095	3035	49.1	0.98	183	4991	117	111
00:11:11 T	17.9	3371	3380	53.5	1.00	183	5880	-51	118
00:11:12 T	17.9	3226	3234	51.2	1.00	183	5620	-47	115
00:11:13 T	17.9	3294	3353	52.3	1.02	184	6043	-172	121
00:11:14 T	17.9	3177	3190	50.4	1.00	184	5568	-61	121
00:11:16 T	17.9	3475	3544	55.2	1.02	184	6419	-198	132
00:11:16 T	17.9	3578	3688	56.8	1.03	184	6840	-296	136
00:11:17 T	17.9	2857	3003	45.3	1.05	185	5797	-383	122
00:11:19 T	17.9	3110	3258	49.4	1.05	185	6249	-388	118
00:11:20 T	17.9	3023	3139	48	1.04	185	5911	-311	112
00:11:21 T	17.9	3396	3507	53.9	1.03	185	6531	-300	122
00:11:22 T	17.9	3126	3223	49.6	1.03	186	5978	-265	115
00:11:23 T	17.9	3407	3478	54.1	1.02	187	6309	-201	124
00:11:25 T	17.9	3101	3116	49.2	1.01	185	5450	-67	112

00:11:25 T	17.9	3375	3440	53.6	1.02	185	6220	-187	123
00:11:26 T	17.9	3245	3288	51.5	1.01	185	5866	-135	118
00:11:28 T	17.9	3505	3627	55.6	1.03	184	6783	-326	130
00:11:29 T	17.9	3239	3303	51.4	1.02	184	5974	-183	122
00:11:30 T	17.9	3335	3431	52.9	1.03	184	6330	-261	124
00:11:31 T	17.9	3166	3249	50.2	1.03	185	5964	-231	117
00:11:32 T	17.9	3381	3420	53.7	1.01	185	6072	-122	120
00:11:33 T	17.9	3357	3379	53.3	1.01	185	5936	-84	122
00:11:34 T	17.9	3557	3655	56.5	1.03	185	6729	-266	128
00:11:35 T	17.9	3332	3346	52.9	1.00	185	5842	-63	119
00:11:37 T	17.9	3580	3671	56.8	1.03	190	6731	-250	133
00:11:37 T	17.9	3482	3569	55.3	1.02	190	6536	-240	135
00:11:38 T	17.9	3543	3689	56.2	1.04	189	6988	-382	138
00:11:40 T	17.9	3331	3478	52.9	1.04	188	6628	-386	129
00:11:41 T	17.9	3059	3118	48.6	1.02	188	5636	-173	118
00:11:42 T	17.9	3170	3244	50.3	1.02	188	5916	-208	120
00:11:43 T	17.9	3684	3832	58.5	1.04	187	7247	-388	136
00:11:44 T	17.9	3198	3324	50.8	1.04	186	6277	-337	122
00:11:45 T	17.9	3473	3638	55.1	1.05	186	6981	-429	130
00:11:46 T	17.9	3245	3370	51.5	1.04	186	6351	-334	121
00:11:47 T	17.9	3369	3502	53.5	1.04	186	6613	-352	124
00:11:48 T	17.9	3271	3353	51.9	1.02	187	6137	-227	118
00:11:49 T	17.9	3433	3544	54.5	1.03	187	6593	-298	119
00:11:50 T	17.9	3248	3243	51.5	1.00	187	5585	-18	113
00:11:52 T	17.9	3426	3404	54.4	0.99	187	5792	25	118
00:11:53 T	17.9	3418	3403	54.3	1.00	188	5820	8	121
00:11:55 T	17.9	3112	3216	49.4	1.03	187	5991	-281	103
00:11:56 T	17.9	3116	3037	49.5	0.97	187	4914	164	114
00:11:56 T	17.9	3805	3751	60.4	0.99	187	6266	100	130
00:11:58 T	17.9	2976	2990	47.2	1.00	187	5221	-62	101
00:11:59 T	17.9	4029	3971	64	0.99	187	6629	111	140
00:11:59 T	17.9	3712	3718	58.9	1.00	186	6455	-43	141
00:12:01 T	17.9	3608	3587	57.3	0.99	186	6117	21	125
00:12:02 T	17.9	2962	2985	47	1.01	186	5253	-85	88
00:12:04 T	17.9	3855	3787	61.2	0.98	186	6266	137	127
00:12:05 T	17.9	3935	3922	62.5	1.00	186	6731	3	131
00:12:06 T	17.9	3713	3772	58.9	1.02	187	6772	-172	129
00:12:07 T	17.9	3754	3890	59.6	1.04	187	7302	-360	134
00:12:08 T	17.9	3484	3600	55.3	1.03	187	6711	-311	124
00:12:09 T	17.9	3533	3649	56.1	1.03	187	6796	-310	126
00:12:10 T	17.9	3461	3574	54.9	1.03	186	6650	-302	122
00:12:11 T	17.9	3610	3711	57.3	1.03	186	6843	-275	126
00:12:12 T	17.9	3363	3433	53.4	1.02	186	6224	-197	119
00:12:13 T	17.9	3561	3646	56.5	1.02	186	6663	-236	126
00:12:14 T	17.9	3281	3339	52.1	1.02	186	6020	-172	118
00:12:16 T	17.9	3525	3531	56	1.00	187	6133	-44	120
00:12:17 T	17.9	3635	3688	57.7	1.01	187	6598	-157	128

00:12:17 T	17.9	3649	3754	57.9	1.03	187	6930	-283	129
00:12:19 T	17.9	3550	3669	56.4	1.03	187	6843	-317	127
00:12:20 T	17.9	3691	3840	58.6	1.04	187	7269	-392	133
00:12:21 T	17.9	3212	3290	51	1.02	186	6010	-217	128
00:12:23 T	17.9	2302	2283	36.5	0.99	186	3856	17	67
00:12:24 T	17.9	3815	3758	60.6	0.98	187	6260	110	131
00:12:25 T	17.9	4007	4074	63.6	1.02	187	7330	-191	136
00:12:26 T	17.9	3599	3661	57.1	1.02	187	6592	-180	124
00:12:27 T	17.9	3796	3855	60.3	1.02	187	6917	-172	144
00:12:28 T	17.9	3703	3772	58.8	1.02	187	6812	-196	140
00:12:29 T	17.9	3338	3441	53	1.03	186	6381	-280	122
00:12:30 T	17.9	3587	3724	56.9	1.04	186	7016	-362	127
00:12:31 T	17.9	3428	3536	54.4	1.03	186	6567	-292	121
00:12:32 T	17.9	3611	3723	57.3	1.03	186	6909	-301	125
00:12:34 T	17.9	3498	3606	55.5	1.03	186	6686	-290	122
00:12:34 T	17.9	3515	3595	55.8	1.02	185	6553	-224	123
00:12:35 T	17.9	3388	3442	53.8	1.02	185	6177	-160	125
00:12:37 T	17.9	3738	3813	59.3	1.02	185	6906	-209	133
00:12:38 T	17.9	3642	3780	57.8	1.04	186	7118	-365	129
00:12:39 T	17.9	2820	2888	44.8	1.02	186	5275	-196	95
00:12:40 T	17.9	3514	3541	55.8	1.01	186	6241	-97	122
00:12:41 T	17.9	3384	3439	53.7	1.02	186	6177	-163	120
00:12:43 T	17.9	3712	3774	58.9	1.02	186	6786	-179	128
00:12:44 T	17.9	3360	3440	53.3	1.02	187	6280	-222	123
00:12:44 T	17.9	3515	3520	55.8	1.00	187	6104	-40	125
00:12:46 T	17.9	3391	3443	53.8	1.02	187	6171	-156	123
00:12:47 T	17.9	3543	3614	56.2	1.02	187	6550	-202	126
00:12:48 T	17.9	3361	3425	53.4	1.02	188	6188	-184	119
00:12:49 T	17.9	3587	3684	56.9	1.03	188	6776	-264	123
00:12:50 T	17.9	3291	3338	52.2	1.01	188	5963	-142	115
00:12:51 T	17.9	3873	3930	61.5	1.01	188	7042	-169	132
00:12:52 T	17.9	3376	3348	53.6	0.99	188	5670	39	120
00:12:53 T	17.9	3787	3838	60.1	1.01	187	6855	-154	137
00:12:55 T	17.9	3591	3689	57	1.03	187	6793	-268	131
00:12:56 T	17.9	3502	3592	55.6	1.03	187	6585	-246	128
00:12:56 T	17.9	3220	3271	51.1	1.02	187	5865	-152	121
00:12:58 T	17.9	3667	3772	58.2	1.03	187	6967	-285	134
00:12:59 T	17.9	3388	3499	53.8	1.03	188	6517	-300	124
00:13:00 T	17.9	3671	3790	58.3	1.03	188	7053	-317	132
00:13:01 T	17.9	3458	3583	54.9	1.04	188	6719	-332	127
00:13:02 T	18.5	3527	3654	56	1.04	188	6853	-338	129
00:13:03 T	18.5	3466	3553	55	1.03	188	6507	-240	126
00:13:04 T	19.6	3587	3714	56.9	1.04	189	6954	-337	131
00:13:05 T	19.6	3311	3414	52.6	1.03	189	6333	-279	126
00:13:07 T	19.8	3366	3421	53.4	1.02	189	6142	-162	122
00:13:07 T	19.8	3524	3574	55.9	1.01	189	6391	-150	128
00:13:08 T	19.8	3674	3754	58.3	1.02	189	6825	-221	134

00:13:10 T	19.8	3635	3751	57.7	1.03	189	6974	-310	132
00:13:11 T	19.8	3573	3700	56.7	1.04	188	6928	-336	131
00:13:11 T	19.8	3616	3718	57.4	1.03	188	6857	-276	135
00:13:13 T	19.8	3640	3777	57.8	1.04	188	7107	-361	139
00:13:14 T	19.8	3473	3613	55.1	1.04	189	6833	-368	133
00:13:15 T	19.8	3453	3519	54.8	1.02	189	6361	-190	127
00:13:16 T	19.8	3549	3658	56.3	1.03	189	6785	-294	134
00:13:17 T	19.8	3668	3837	58.2	1.05	189	7346	-440	134
00:13:18 T	19.8	3387	3528	53.8	1.04	190	6688	-370	125
00:13:19 T	19.8	3671	3831	58.3	1.04	190	7300	-419	134
00:13:20 T	19.8	3562	3685	56.5	1.03	190	6891	-329	133
00:13:22 T	19.8	3696	3817	58.7	1.03	190	7111	-323	138
00:13:22 T	19.8	3674	3775	58.3	1.03	190	6954	-275	138
00:13:23 T	19.8	3581	3672	56.8	1.03	189	6730	-249	133
00:13:25 T	19.8	3555	3692	56.4	1.04	189	6957	-361	132
00:13:26 T	19.8	3329	3383	52.8	1.02	189	6077	-161	126
00:13:26 T	19.8	3621	3713	57.5	1.03	189	6807	-252	137
00:13:28 T	19.8	2235	2239	35.5	1.00	190	3877	-40	69
00:13:29 T	19.8	4251	4161	67.5	0.98	190	6825	189	147
00:13:31 T	19.8	3973	3957	63.1	1.00	190	6778	11	138
00:13:32 T	19.8	3935	3960	62.5	1.01	191	6960	-91	145
00:13:32 T	19.8	3846	3934	61	1.02	191	7178	-244	141
00:13:34 T	19.8	3548	3667	56.3	1.03	191	6842	-319	136
00:13:35 T	19.8	3698	3849	58.7	1.04	191	7289	-395	138
00:13:36 T	19.8	3509	3655	55.7	1.04	190	6933	-384	131
00:13:37 T	19.8	3725	3859	59.1	1.04	190	7239	-355	137
00:13:38 T	19.8	3396	3488	53.9	1.03	190	6417	-253	128
00:13:39 T	19.8	3568	3641	56.6	1.02	190	6603	-206	127
00:13:40 T	19.8	3549	3632	56.3	1.02	189	6626	-229	131
00:13:41 T	19.8	3814	3924	60.5	1.03	189	7249	-295	136
00:13:42 T	19.8	3688	3724	58.5	1.01	189	6597	-119	136
00:13:43 T	19.8	3612	3677	57.3	1.02	189	6637	-189	136
00:13:44 T	19.8	3414	3460	54.2	1.01	189	6179	-143	142
00:13:46 T	19.8	3799	4002	60.3	1.05	189	7773	-521	148
00:13:46 T	19.8	3502	3618	55.6	1.03	188	6745	-311	136
00:13:47 T	19.8	3609	3763	57.3	1.04	188	7159	-405	137
00:13:49 T	19.8	3190	3242	50.6	1.02	189	5819	-154	123
00:13:50 T	19.8	3822	3953	60.7	1.03	189	7391	-348	136
00:13:50 T	19.8	3780	3888	60	1.03	189	7178	-291	139
00:13:52 T	19.8	3792	3937	60.2	1.04	189	7421	-382	138
00:13:53 T	19.8	3565	3713	56.6	1.04	189	7039	-387	133
00:13:54 T	19.8	3744	3852	59.4	1.03	190	7116	-291	136
00:13:55 T	19.8	3687	3835	58.5	1.04	190	7257	-390	137
00:13:56 T	19.8	3856	4027	61.2	1.04	190	7690	-447	144
00:13:57 T	19.8	3657	3822	58	1.05	190	7307	-431	138
00:13:58 T	19.8	3762	3950	59.7	1.05	189	7623	-486	142
00:13:59 T	19.8	3220	3308	51.1	1.03	189	6087	-244	121

00:14:01 T	19.8	3813	3943	60.5	1.03	189	7364	-344	139
00:14:01 T	19.8	3703	3844	58.8	1.04	191	7245	-373	138
00:14:02 T	19.8	3891	4090	61.8	1.05	190	7911	-512	142
00:14:04 T	19.8	3690	3830	58.6	1.04	190	7211	-368	137
00:14:05 T	19.8	4020	4208	63.8	1.05	190	8070	-485	148
00:14:05 T	19.8	3746	3916	59.5	1.05	191	7485	-441	144
00:14:07 T	19.8	3715	3921	59	1.06	191	7648	-530	141
00:14:08 T	19.8	3702	3918	58.8	1.06	191	7682	-553	144
00:14:09 T	19.8	3530	3683	56	1.04	192	7010	-400	131
00:14:10 T	19.8	3785	3919	60.1	1.04	192	7343	-355	145
00:14:11 T	19.8	3715	3909	59	1.05	191	7579	-502	141
00:14:12 T	19.8	3618	3810	57.4	1.05	191	7394	-495	138
00:14:13 T	19.8	3733	3975	59.3	1.06	191	7893	-617	137
00:14:14 T	19.8	3654	3795	58	1.04	191	7154	-371	138
00:14:15 T	19.8	3848	4066	61.1	1.06	191	7948	-558	144
00:14:17 T	19.8	2227	2253	35.3	1.01	190	3993	-93	69
00:14:18 T	19.8	4504	4555	71.5	1.01	190	8097	-151	148
00:14:19 T	19.8	4257	4336	67.6	1.02	191	7837	-221	148
00:14:20 T	19.8	4090	4263	64.9	1.04	191	8105	-450	148
00:14:21 T	19.8	3577	3764	56.8	1.05	191	7297	-484	136
00:14:22 T	19.8	3880	4071	61.6	1.05	190	7849	-495	143
00:14:23 T	19.8	3700	3881	58.7	1.05	190	7475	-470	139
00:14:25 T	19.8	3824	4011	60.7	1.05	190	7723	-483	141
00:14:25 T	19.8	3462	3610	55	1.04	190	6864	-389	134
00:14:26 T	19.8	3715	3882	59	1.04	190	7416	-435	131
00:14:28 T	19.8	3680	3772	58.4	1.02	191	6911	-253	142
00:14:29 T	19.8	3782	3944	60	1.04	191	7500	-421	141
00:14:30 T	19.8	3681	3821	58.4	1.04	191	7195	-368	141
00:14:31 T	19.8	3707	3886	58.8	1.05	191	7476	-465	135
00:14:32 T	19.8	3733	3878	59.3	1.04	191	7314	-380	139
00:14:33 T	19.8	3636	3730	57.7	1.03	192	6846	-258	129
00:14:34 T	19.8	3813	3971	60.5	1.04	192	7529	-411	136
00:14:35 T	19.8	4058	4188	64.4	1.03	192	7790	-343	152
00:14:37 T	19.8	3611	3846	57.3	1.06	193	7636	-599	125
00:14:38 T	19.8	3742	3837	59.4	1.03	193	7033	-258	143
00:14:39 T	19.8	3697	3883	58.7	1.05	193	7497	-481	133
00:14:40 T	19.8	3593	3692	57	1.03	192	6803	-271	137
00:14:41 T	19.8	3620	3675	57.5	1.02	192	6589	-164	129
00:14:42 T	19.8	3736	3837	59.3	1.03	191	7062	-275	136
00:14:43 T	19.8	3873	4023	61.5	1.04	191	7590	-394	137
00:14:44 T	19.8	3639	3806	57.8	1.05	191	7285	-435	133
00:14:45 T	19.8	3915	4083	62.1	1.04	191	7767	-436	139
00:14:46 T	19.8	3606	3745	57.2	1.04	191	7058	-366	137
00:14:47 T	19.8	3825	4002	60.7	1.05	190	7667	-460	138
00:14:48 T	19.8	4051	4192	64.3	1.03	190	7848	-372	156
00:14:49 T	19.8	3783	3914	60.1	1.03	190	7318	-346	146
00:14:50 T	19.8	3695	3890	58.7	1.05	190	7544	-502	139

00:14:51 T	19.8	3784	3990	60.1	1.05	190	7769	-530	140
00:14:52 T	19.8	3889	4110	61.7	1.06	191	8037	-565	143
00:14:53 T	19.8	4256	4424	67.6	1.04	191	8364	-438	166
00:14:54 T	19.8	3490	3639	55.4	1.04	191	6918	-391	135
00:14:55 T	19.8	3926	4086	62.3	1.04	191	7744	-419	148
00:14:56 T	19.8	3645	3822	57.9	1.05	191	7351	-458	141
00:14:57 T	19.8	3834	4071	60.9	1.06	190	8038	-605	161
00:14:58 T	19.8	3768	3949	59.8	1.05	190	7592	-469	147
00:14:59 T	19.8	3606	3821	57.2	1.06	190	7508	-550	140
00:15:00 T	19.8	3690	3936	58.6	1.07	190	7842	-627	140
00:15:01 T	20.4	3669	3876	58.2	1.06	191	7571	-531	138
00:15:02 T	20.4	3698	3923	58.7	1.06	191	7733	-577	138
00:15:03 T	21.6	4014	4192	63.7	1.04	191	8003	-462	159
00:15:04 T	21.6	3495	3624	55.5	1.04	191	6808	-343	134
00:15:05 T	21.6	3955	4150	62.8	1.05	190	8002	-504	147
00:15:06 T	21.9	3762	3943	59.7	1.05	190	7580	-468	140
00:15:07 T	21.9	3875	4061	61.5	1.05	190	7808	-481	142
00:15:08 T	21.9	3666	3831	58.2	1.04	191	7316	-428	143
00:15:09 T	21.9	3797	3984	60.3	1.05	191	7676	-483	144
00:15:10 T	21.9	3695	3803	58.7	1.03	191	7031	-291	132
00:15:11 T	21.9	4082	4202	64.8	1.03	192	7777	-321	147
00:15:12 T	21.9	4439	4633	70.5	1.04	192	8840	-502	162
00:15:13 T	21.9	3822	4019	60.7	1.05	192	7783	-509	143
00:15:14 T	21.9	4158	4425	66	1.06	192	8778	-677	157
00:15:15 T	21.9	3969	4198	63	1.06	191	8225	-585	146
00:15:16 T	21.9	4011	4227	63.7	1.05	191	8222	-554	160
00:15:17 T	21.9	4062	4315	64.5	1.06	191	8530	-644	166
00:15:19 T	21.9	3356	3540	53.3	1.05	191	6890	-475	119
00:15:19 T	21.9	4393	4554	69.7	1.04	192	8558	-419	168
00:15:20 T	21.9	4142	4436	65.7	1.07	192	8915	-745	169
00:15:22 T	21.9	3952	4230	62.7	1.07	192	8486	-704	163
00:15:22 T	21.9	3735	4043	59.3	1.08	192	8287	-777	154
00:15:23 T	21.9	4001	4339	63.5	1.08	193	8927	-850	168
00:15:25 T	21.9	2125	2238	33.7	1.05	193	4330	-304	69

APPENDIX D

Participants

Fourteen athletes (provincial netball players) were randomly selected to perform an “aerobic assessment exercise test” [the multistage shuttle test - (Bleep test)]. It was done over a period of 8 days. At 10h00 on Mondays and Thursdays, the following specific interventions were implemented respectively in a double blind cross-over protocol fashion;

1. Olive oil ingestion (5 ml) 120 minutes prior to testing plus one cup of strong black, sugarless caffeinated coffee 90 minutes prior to testing.
2. Carbohydrate ingestion (200g high GI CHO)-240 minutes prior to testing
3. Fasting period-4 hours prior to testing. The last, preference, meal intake was at 20h00.

On three separate occasions the interventions were implemented in a double blind cross-over study design.

Exercise regime

The subjects were exposed to a multistage shuttle run (Bleep test) until voluntary fatigue (demarcated as the onset of recovery) set in. The test was conducted on a 20-meter marked off non-slippery, flat surface. Athletes run back and forth between the clearly marked lines (0m–20m), touching the line with one foot at the precise moment that a sound signal is emitted from a tape recorder.

Statistical analysis

The population proportion analysis was used. The confidence interval [C. I. for *p* (population proportion)] is stated for the 95% confidence level.

Results

Table 10 lists the levels, stages, and total distance covered (TDC) for 14 provincial netball players subjected to the Bleep-Test. Improvement in the total distance covered (ITDC) and the $VO_{2max\text{-}diff}$ (according to the norms described for

the Bleep Test) relates to the difference between the best and worst performances between any of the interventions for a specific subject.

Table 10-Levels, stages, total distance covered (D in meters) and $VO2_{max\ diff}$ for each subject respectively when subjected to Fasting (Fa), Fat and Caffeine (FC) and Carbohydrate (Ca) ingestion.

SUBJECTS (n)	MEASUREMENTS						CALCULATIONS	
	C&F	D (m)	CARB	D (m)	F	D (m)	ITDC (m)	$VO_{2max\ DIFF}$ ($ml O_2$/kg BW)
1	8.5	1320	8.1	1240	8.7	1360	120	1.3
2	9.1	1460	8.1	1240	9.1	1460	220	3.4
3	9.6	1560	9.6	1560	10.5	1760	200	2.8
4	8.1	1240	8.4	1300	8.7	1360	120	1.3
5	11.3	1940	11.3	1940	11.4	1960	20	0.6
6	9.1	1460	10.5	1760	9.8	1600	300	4.1
7	8.3	1280	8.1	1240	8.1	1240	40	0.6
8	9.9	1620	9.9	1620	10.6	1780	160	3.4
9	9.2	1480	10.1	1680	10.1	1680	200	3.5
10	8.1	1240	9.6	1560	7.7	1160	400	6.7
11	9.5	1540	8.5	1320	9.1	1460	220	3.4
12	8.5	1320	8.5	1320	8.1	1240	80	0.6
13	11.9	2060	12.1	2140	10.3	1720	420	6.9
14	8.1	1240	9.1	1460	7.7	1160	300	5.4

These results indicate that nutritional intake prior to participating in the Bleep test affected 85.7% [95% CI level: 60.1% ; 96.0%) population proportion] of the players in terms of the total distance covered.

This means that nutritional intake prior to participating in the Bleep test has an 85.7% chance to influence the outcome (levels, stages) of this test when applied to these netball players. Unless nutrient intake prior to testing is monitored, the present results indicate that at least 60.1% up to 96.0% of netball players in the population could show perspectives on levels, stages and total distance covered irrespective of training status, adaptations and/or genetic endowment during the trial period. Furthermore, the “total distance covered” does not take into account that the actual work load performed for various subjects is disguised since

alterations in exercise intensity (stage levels) are not reflected in the total distance covered.

Although the intention with the Bleep Test is to predict/determine the $VO_{2max,}$ for any individual, the present results show that the average change in the VO_{2max} for pooled data coincides with a difference of 3.14±1.67 mlO_2/kgBW. This figure corresponds with significant changes in levels, stages and total distance covered for each subject subjected to the various interventions. These perspectives question the validity and reliability of the Bleep Test to determine the VO_{2max} if nutrient-intake prior to testing is ignored.

The present protocol design supplies no information on the actual effect(s) of nutrient intake prior to performing the Bleep Test on netball performance during competition.