CHAPTER 1: BACKGROUND AND STUDY OBJECTIVES

1. INTRODUCTION

The two main fuels for muscle metabolism are carbohydrates and fat. A limited carbohydrate store is available in the body, but this is not the case with fat. Fat constitutes approximately 15% of the average lean man's body weight, and 25% of the average woman's body weight [1].

Dietary carbohydrate has certainly been one of the most studied ergogenic aids for athletic performance. The basis for this interest being the repeated observation that the depletion or reduction of bodily carbohydrate reserves is associated with fatigue during endurance exercise. The most widely accepted carbo-loading/glycogen-loading regime mainly entails endurance athletes reducing their training volume three to four days prior to a marathon race or endurance event, and ingesting a high carbohydrate (CHO) diet (>7g/kg body mass/day) [2]. This regime typically increases the resting muscle glycogen content from ~100 to >140 mmol/kg wet weight and extends the time to exhaustion during prolonged (>3 hours) submaximal exercise [3]. The following of a glycogen-loading regime, has widely been reported to have ergogenic effects [2,4,5,6,7,8,9,10].

The mechanism whereby carbohydrate consumption benefit the athlete during exercise, has been ascribed to the increased availability of glucose in the blood; glucose utilized by muscles during exercise could therefore be replaced. Another potential mechanism for increased performance observed with CHO feeding during exercise is a change in brain neurotransmitter production [9]. The production of serotonin in the brain is partly related to the amount of the precursor tryptophan available in the blood. Tryptophan and branched-chain amino acids compete for entrance to the brain, and the reduction in branched-chain amino acids that occurs with prolonged exercise can theoretically result in greater

production of serotonin in the brain; the latter would presumably lead to early fatigue [11].

Although ingested CHOs certainly can and do improve endurance performance, their oxidation rate is limited [12]; there is also accumulating evidence that argues against the contention that pre-exercise glycogen concentration is the primary factor that limits endurance performance [9,13,14,15]. Although the increased dietary availability of CHOs, ingested in the form of a carbohydrate-rich beverage during endurance exercise or training, may lessen the perception of fatigue following exercise and may increase time to maximal exhaustion, overall psychological status and physiological responses to incremental maximal exercise seemed not to be affected [16,17,18].

The improved endurance capability observed after aerobic training has also been attributed to increased oxidation of fat relative to carbohydrate; the "carbohydrate sparing" effect presumably delaying the point at which reduced CHO reserves will cause fatigue. Fat is mobilized from adipose tissue in response to stimulation of intracellular lipase by the catecholamines. During endurance exercise lipolysis in fat depots is increased after about 15-20 minutes of exercise by stimulation of hormone sensitive lipase by epinephrine, resulting in the degradation of triglycerides into fatty acids and glycerol. The free fatty acids are transported to muscle in loose combination with plasma albumin where they are released, taken up and oxidized. Glycerol undergoes gluconeogenesis in the liver, and this process helps restock liver glycogen stores which in turn provides glucose as a fuel for the central nervous system and for muscle metabolism. Fat cells increase their sensitivity to hormonal stimulation after training and as a result mobilization of fatty acids more closely matches utilization [1].

When considering lipid metabolism, the important role of carnitine in lipid metabolism and energy production should not be ignored. Carnitine is a cofactor for the transport of long-chain fatty acids across the inner mitochondrial membrane and thus for energy release via ß-oxidation and acetyl CoA production.

The beneficial effects of L-carnitine supplementation on improving endurance capacity, supposedly by means of enhanced lipid oxidation, have been widely reported [19,20,21,22,23]. Carnitine purportedly decreases plasma lactate accumulation during exercise [24]. A possible role for L-carnitine in medium-chain fatty acid oxidation has also been identified [25].

The presumed carbohydrate sparing effect of fat oxidation has led to the hypothesis that a greater availability of fat during exercise can improve endurance performance. Although being a plausible hypothesis, it is not yet supported by a sufficient number of replicated, credible and valid studies. Recently, however, more significant results have been obtained during studies involving medium-chain triglyceride (MCT) supplementation.

Mucio *et al.* [26] studied the effects of dietary manipulations on the VO_2 max and endurance of trained runners, comparing a seven day normal, fat, and carbohydrate diet respectively. The percentage energy contributions from carbohydrate, fat and protein were 61/24/14, 50/38/12, and 73/15/12 for the normal, fat, and carbohydrate diets respectively. They found an increase in running time to exhaustion after the fat diet, concurrent with higher VO_2 max values. They subsequently implied that restriction of dietary fat may be detrimental to endurance performance, suggesting that increased availability of free fatty acids (FFA), consequent to the fat diet, may provide for enhanced oxidative potential (as evidenced by an increase in VO_2 max and running time).

Similar results were obtained in a study where endurance-trained cyclists were accustomized to 14 days of either a high fat or a high carbohydrate diet [27]. In this case the percentage energy contributions from carbohydrate, fat and protein were 7/67/26 for the high fat, and 74/12/14 for the high carbohydrate diet. Cycling time to exhaustion at 60% VO_2 max was significantly longer for the high fat diet. Respiratory exchange ratios (i.e. 0.87 ±0.03 and 0.92 ±0.02 for high fat and high carbohydrate respectively) also supported the contention that dietary manipulations modified fuel utilization. However, relatively low exercise intensities

were used in this study (60-65% VO_2 max) as compared to intensities used by athletes in training and competition for most endurance sports.

The muscle glycogen sparing effect of lipid ingestion was demonstrated by Dyck *et al.* [28]. Their study revealed that intralipid and heparin infusion during 25 minutes of intense aerobic cycling, resulted in elevated plasma FFA, producing a significant sparing of muscle glycogen. The glycogen sparing that occurred was unrelated to changes in muscle citrate and acetyl-CoA contents. Dyck *et al.* [29] also reported a significant decrease in the rate of glycogen degradation (44%) after infusing athletes with a triglyceride emulsion during 15 minutes of aerobic exercise at 85% of VO_2 max. They found that acetyl-CoA concentrations were not affected by the fat infusion, and suggested that the reduced glycogen degradation was mediated by unknown effects of triglycerides on phosphorylase.

The potentialy positive effects of fat utilization during endurance activities thus seems to be certain. Unfortunately, most fat is ingested as long-chain triglycerides (LCT); their ratio of digestion and absorption via the lymphatic system being too slow to be of any advantage to the athlete during exercise. In contrast, ingested MCTs are metabolized similar to glucose [30], and provides a readily available source of energy. Satabin *et al.* [31] reported that only 9% of ~44g of LCTs ingested one hour prior to the start of exercise at 60% VO_2 max were oxidized over a two hour period of exercise as compared to the oxidation of 44% of ~44g exogenous MCTs. However, Massicotte *et al.* [32] compared the oxidation rate of MCTs with that of glucose during prolonged exercise when ingesting 25g of MCTs one hour before exercise, as compared to the ingesting of 57g of glucose dissolved in one litre of water during exercise. They found no significant difference in the contribution of MCTs and glucose to the total energy expenditure over the two hour period of exercise. Neither of the exogenous substrates reduced the endogenous carbohydrate utilization , and ingestion of MCTs as well as glucose contributed mainly to the blood glucose concentration.

Research has shown that the metabolic availability of MCTs during exercise when ingested orally, is greatly enhanced when ingested with carbohydrates. Hawley *et al.* [33] reported a consistent decrease of two minutes in the times of six cyclists during a 40km time trial at 60% of VO_2 max, when adding 4.5% MCTs to a 10% glucose solution. Jenkendrup *et al.* [34] also reported a more rapid increase in the oxidation rate of exogenous MCTs with co-ingestion of carbohydrates. Well-trained athletes cycled at 57-60% of VO_2 max for 180 minutes, ingesting a CHO solution, CHO-MCT suspension, high CHO-MCT suspension, and MCT solution respectively. During the MCT-CHO trials, MCT oxidation showed a sharp rise during the first hour and a plateau thereafter, the amount of MCTs oxidized being 71-76% of the total amount ingested, and representing 6.3-6.8% of total energy expenditure. During the MCT trial, only 33% of MCTs were oxidized, contributing 3.2% to the total energy expenditure. They suggested that the sharp rise in both plasma FFA and ß-hydroxybutyrate concentration in the CHO-MCT and high CHO-MCT trials, but not in the MCT trial, was due to the fact that carbohydrates allowed the medium-chain fatty acids (MCFA) to get into the systemic circulation more rapidly, thereby implying that gastric emptying and intestinal absorption would be the most likely factors responsible for the difference in MCT oxidation. A possible explanation could be that CHO-MCT suspensions emptied faster from the stomach than do MCTs. They ascribed the relatively small contribution of MCTs to energy expenditure (3-7%) to the small amount of MCTs that was provided; the amount of ~30g MCTs in three hours seemed, however, to be the maximal amount that can be tolerated in the gastrointestinal tract without causing distress.

The above mentioned knowledge highlights some topical, and as yet unresolved issues, e.g. the question of "fat adaption" and the efficacy of MCTs as a quick source of rapidly available energy. Does L-carnitine supplementation improve endurance performance, and does carnitine indeed play a role in the transport of MCFAs across the inner mitochondrial membrane?

In a previous study [23], it was demonstrated that daily supplementation with a 2g dose of L-carnitine improved the performance of black male marathon runners, in as much as athletes were able to better their performance (measured by an increase in peak treadmill running speed) at lower levels of oxygen consumption, concurrent with lower heart rates. Oxygen was therefore used more economically. Carnitine supplementation also seemed to potentiate fat oxidation, evident in lower respiratory exchange ratios during exercise (after six weeks of supplementation). An interesting observation was the very low plasma carnitine levels in the athletes. This was ascribed to their diets, which consisted mainly of carbohydrates. These findings prompted a more extensive study.

Hypothesis: A seven week period of combined MCT plus carbohydrate supplementation, will alter the body's metabolism in such a way as to potentiate fat utilization during endurance exercise, thereby improving endurance performance. Repeating the seven weeks supplementation, adding L-carnitine to the exact same MCT plus carbohydrate supplement, will further potentiate fat utilization, thereby proving that carnitine is indeed involved in the transport of MCFAs across the inner mitochondrial membrane.

In order to validate the above hypothesis, the aims of the study were:

- To examine the effects of a seven week period of MCT+CHO supplementation on the performance and metabolic parameters of male marathon athletes. Performance was measured in terms of VO_2 max, peak treadmill running speed and respiratory exchange ratios. Serum organic acid extractions were used to observe individual metabolic variations. Serum lipid profiles, nutritional status evaluation as well as plasma carnitine determinations were included. Dietary intake and individual training programmes were monitored.
- To compare the above effects with those of a seven week MCT+CHO+L-carnitine supplementation period; the same performance and metabolic profiles were compared. The second supplementation period followed after a five week washout period.

- To investigate carnitine's involvement in the transport of MCFAs across the mitochondrial membrane.
- To use serum organic acid extraction profiles (tests developed by Ms Salomé Jooste, Potchefstroom University, 1995) to identify if an individual utilizes mainly carbohydrates (lactic acid response), or fatty acids (fatty acid response) to generate energy during aerobic exercise.
- To investigate if MCT supplementation could potentiate an individual's ability to preferably utilize fatty acids as an energy source during aerobic exercise.
- To validate laboratory results by monitoring athletes during a field test i.e. a standard marathon (42.2km) at the end of each supplementation period.
- To show that continuous supplementation with MCTs (MCTs made up 31% of the total energy content of each supplement) during a marathon event could be well tolerated, and be of benefit.

2. MEDIUM-CHAIN TRIGLYCERIDES

Medium-chain triglycerides (MCTs) were first introduced in 1950 for the treatment of lipid absorption disorders [30]. A great deal has been learned about the metabolism and various clinical uses of MCTs e.g. in infant care, malabsorption syndromes and treatment of the critically ill patient. In the search for alternative non-carbohydrate fuels, MCTs are unique and have established themselves as being a rapidly available, high energy source.

2.1 Properties

Fats of animal, vegetable and marine origin have a fatty acid (FA) spectrum ranging from C2-C24 chain length. The lauric fats (Babassau, Coconut, Cohune, Palm kernel, Tacum) are composed primarily of fatty acids of C14 chain length and shorter. MCTs are composed primarily of saturated C8 and C10 chain length fatty acids, caprylic and capric respectively. Medium-chain triglyceride fatty acid composition is as follows:

C6 1-2%
C8 65-75%
C10 25-35%
C12 2% max [16,35].

The melting point of the medium-chain fatty acids (MCFA) is much lower than that of the long-chain fatty acids (LCFA); C8: 0-16.7°C, C10: 0-31.3°C versus C16: 0-63.1°C. Medium-chain fatty acids, as well as MCTs are thus liquids at room temperature. They possess a low viscosity, 25-31 centi poisies (cp) at 20°C. Medium-chain triglycerides are extremely stable to both high and low temperature extremes; e.g. they remain nonviscous even after prolonged use at frying temperatures. Under these conditions they increase only slightly in viscosity to approach the viscosity of an unheated vegetable oil (when most vegetable oils are heated to high temperatures, they polymerize over time to become thick and viscous). Even at extremely low temperatures of 0°C, MCTs remain a clear and

nonviscous liquid. At these low temperatures no warming is required to use MCTs [35,36]. Due to the saturation of the fatty acids, MCTs are extremely stable to oxidation. They possess a bland taste, are colourless, and have no odour.

Medium-chain triglycerides differ from regular fats and oils in two important ways: firstly, they are absorbed directly into the portal system (independent of pancreatic enzymes), and are rapidly metabolized in the liver. The molecular size of MCTs is smaller than that of long-chain triglycerides (LCT), thus facilitating the action of pancreatic lipase. Consequently MCTs are hydrolyzed faster and more completely than LCTs. The products of MCT hydrolysis are also absorbed faster than those of LCTs, and as rapidly as glucose.

The second important difference is that while MCTs are absorbed as rapidly as glucose, they have more than twice the caloric density of protein and carbohydrate, providing 8.3 calories/g [37]. Since their intraluminal hydrolysis is rapid and relatively complete, MCTs are (unlike LCTs) mainly absorbed as free fatty acids (FFA). They have little tendency to deposit as fat. Being easily oxidized and utilized as fuel and energy, MCTs are an extremely quick, high energy source, favoured in particular by runners and body builders as dense, easily absorbed and rapidly utilized energy.

Research indicates that diets containing up to 50-100g MCTs per day are easily tolerated [36]. Medium-chain triglyceride intakes at 40% of total calories have been reported with no negative effects [35]. Short- to medium-chain fatty acids appear to enhance the absorption of fats in general, whereas LCTs tend to impair the process [38]. It has been shown that in the case of mixed triacylglycerols, the MCFAs are preferentially liberated [38].

Further physicochemical characteristics of MCTs rationalize the use thereof. Medium-chain triglycerides present more interfacial surface for enzyme action per time unit, thereby facilitating the more rapid and complete intraluminal enzymatic hydrolysis of MCTs, as compared to LCTs. Because of the greater water solubility

of MCT hydrolysis products, bile salts are not required for dispersion in water. The greater water solubility of MCFAs is of significance considering the different routes of transport of MCTs: portal transport as MCFAs bound to serum albumin, versus lymphatic transport of LCTs as chylomicrons.

Intramucosal metabolism of MCFAs differs from that of LCFAs because of the small molecular size and lower pKa of fatty acids derived from MCTs; MCFAs display a decreased affinity for esterifying- and activating enzymes, thus minimizing re-esterification of MCFA to MCTs, as well as no chylomicron formation. In the event of diseased mucosal surfaces, more efficient penetration is obtained by the short-chain length fatty acids derived from MCTs.

Although MCTs are fats, they sometimes tend to behave like carbohydrates. Though not hyperglycemic, they stimulate insulin production slightly, through the stimulation of the Islets of Langerhans by either the ketone bodies, or by the MCFAs themselves. Dias *et al.* [37] found a 8%decrease in plasma glucose levels of normal adults from baseline levels after 3 days of high MCT - low carbohydrate intake (MCT: 51% of calories). They ascribed the decrease in serum glucose levels as either a result of decreased gluconeogenesis or increased glucose uptake during the high MCT dietary period.

MCTs are not hyperlipidemic, but they are ketogenic; being donors of hydrogen ions and precursors of acetyl-CoA. MCTs are not drugs and have no pharmacological effect.

2.2 Clinical uses and applications

2.2.1 MCTs have been used successfully in adults, children and newborn babies where the digestion, absorption or transport of natural dietary fats are disturbed: disorders of lipid digestion (e.g. cystic fibrosis), lipid absorption disorders (e.g. Crohn's disease), disorders of lipid transport viz

in deficiency of chylomicron synthesis (e.g. congenital ß-lipoprotein deficiency).

2.2.2 Gallbladder disease: the use of medium-chain monodiglycerides have been investigated in the dissolution of gallstones [36].

2.2.3 The energy-providing and ketogenic properties of MCTs are utilized.

2.2.4 Lipid precursors: the acetyl-CoA produced in the peripheral tissues from MCTs can enter into anabolic pathways. Especially in the brain, synthesis of mainly phospholipids from ketone bodies has been demonstrated.

2.2.5 The anticonvulsive properties of ketone bodies are beneficial in the treatment of epilepsy.

2.2.6 Hyperalimentation: MCTs are a preferable food source for any organism with increased energy needs, such as the critically ill patient, or children during normal or retarded growth.

2.2.7 Hyperlipidemias: decreases in blood and liver cholesterol levels have been reported with a MCT diet.

2.2.8 Deficiency of the carnitine system: deficiencies of carnitine or carnitine palmitoyl transferase (I or II or both) result in diminished capacity to oxidize LCFAs [36].

2.2.9 MCT incorporation into various food applications: MCTs are used as flavor carriers, providing gloss to confections, as substitutes for liquid vegetable oils in reduced calorie foods, and in speciality nutrition and energy dense foods.

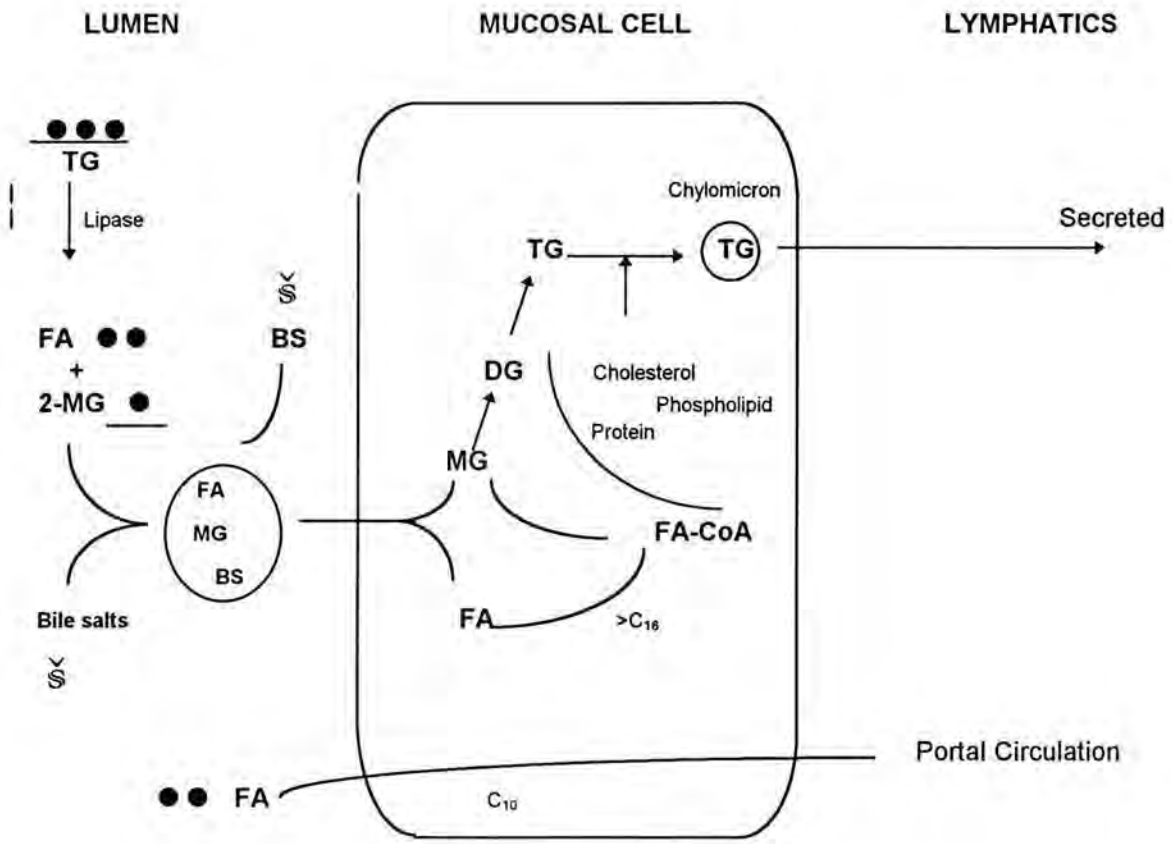
2.2.10 Structured lipids with a MCT backbone and built-in essential fatty acid components (linoleic acid) or other polyunsaturated fatty acids, provide greater flexibility while caring for the critically ill patient [35].

2.3 MEDIUM-CHAIN TRIGLYCERIDE METABOLISM

2.3.1 Absorption and transport

The products of lipid hydrolysis and nonhydrolysable lipids are absorbed by the cells of the intestinal mucosa. The main products of lipid digestion namely long-

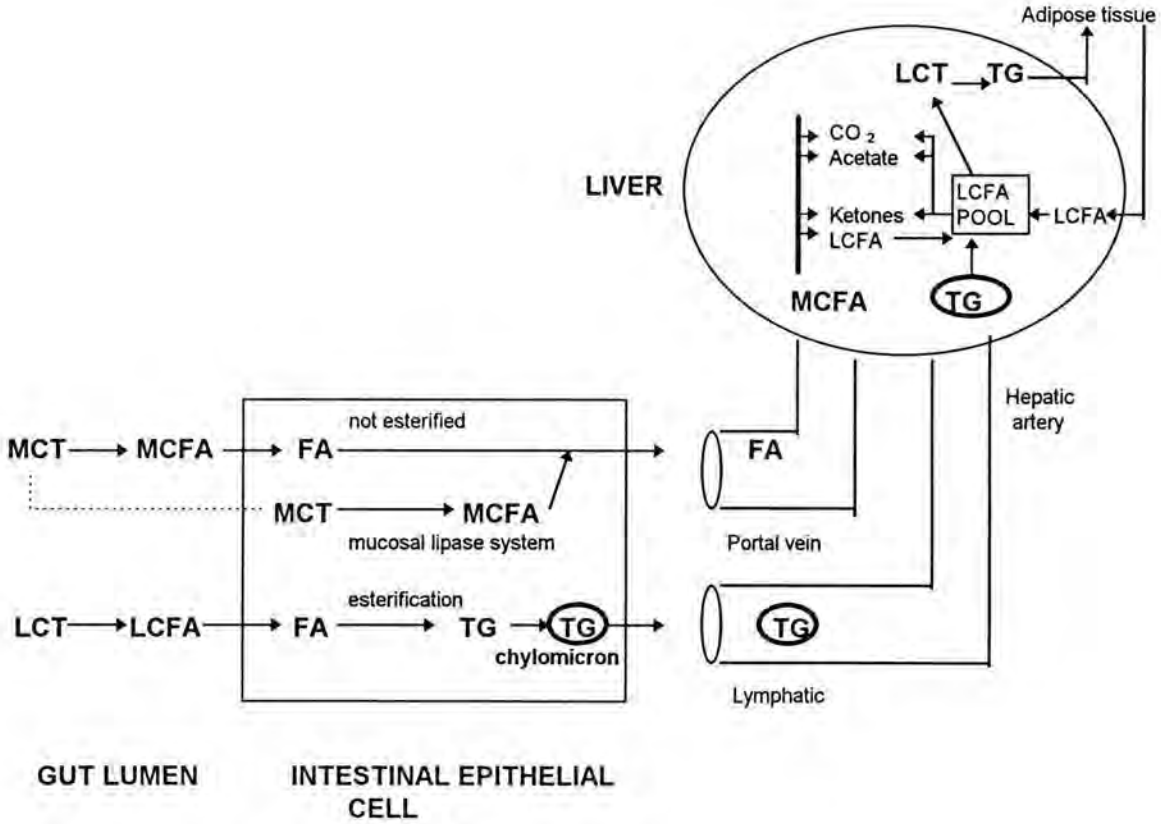
chain triglycerides and 2-monoacylglycerols, which are not water soluble, are made soluble by being incorporated into mixed micelles which contain bile salts (mixed micelles also contain cholesterol and fat-soluble vitamins). These lipids enter the mucosal cells by passive diffusion. Inside the mucosal cells, LCFAs and monoglycerides are resynthesized into triglycerides. Chylomicrons are then assembled within the mucosal cells and secreted into the lymphatics. From the lymphatics, chylomicrons are carried into the bloodstream and transported to peripheral tissues, particularly adipose tissue.



TG - Triacylglycerol; FA - Fatty acid; MG - Monoacylglycerol; DG - Diacylglycerol; BS - Bile salts; C_{10}, C_{16} - 10- and 16-carbon fatty acids ; §- Bile salts [39]

Figure 1: Digestion and absoption of dietary lipid.

In contrast to LCFAs, medium- and short-chain fatty acids (C12 and shorter) are water soluble and are absorbed directly into mucosal cells without the involvement of mixed micelles. MCFAs are not significantly incorporated into chylomicrons, and therefore leave the intestine faster than LCFAs. From the mucosal cells, MCFAs enter the portal venous system and are carried to the liver bound to serum albumin (the bond between MCFAs and albumin is however not as easily formed as that between LCFAs and albumin). Unlike LCFAs, MCTs do not stimulate the flow of lymph. The majority of MCFAs are retained in the liver and only a small amount appears in the peripheral blood for a short period of time [35].



MCT - Medium-chain triglycerides; MCFA - Medium-chain fatty acids; LCT - Long-chain triglycerides; TG - Very low density lipoprotein; LCFA - long-chain fatty acids; (TG) - Chylomicrons [30]

Figure 2: The transport of medium- and long-chain triglycerides

The tendency of fatty acids to be esterified is directly proportional to their ability to bind to fatty acid binding protein. Because MCFAs do not bind easily to this protein, they are not easily esterified, whereas LCFAs are easily bound to this protein and are therefore abundantly incorporated into lipids.

2.3.2 Hepatic metabolism

Fatty acids are oxidized in the mitochondrial matrix through the process of ß-oxidation. Fatty acids arise in the cytosol through triacylglycerol transport from outside the cell, or through biosynthesis. The inner mitochondrial membrane is impermeable to free fatty acids or acyl-CoAs, and therefore a specific transport system, the carnitine transport system, comes into play to move fatty acyl-CoAs into the mitochondrial matrix. The carnitine transport system is discussed in detail in section 3.5.2.

Medium-chain fatty acids however, cross the double mitochondrial membrane very rapidly and, unlike LCFAs, cross the membrane independently of the carnitine transport system. It has however been suggested that carnitine may play a significant role in the metabolism and utilization of MCFAs [24] as is discussed in section 3.5.3.

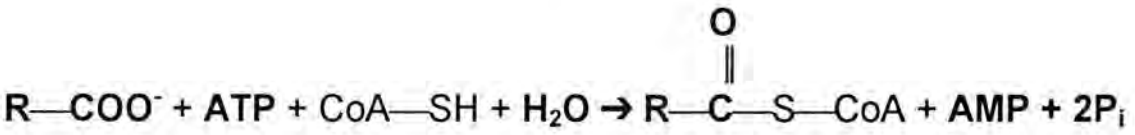
The mentioned transport system operates hand in hand with the metabolic activation needed to initiate the ß-oxidation pathway. A series of fatty acyl-CoA ligases, specific for short-, medium-, or long-chain fatty acids, catalyze the formation of a fatty acyl thioester conjugate with coenzyme A.

Long-chain acyl-CoA ligase was originally found only in the endoplasmic reticulum, but was later discovered in the outer membrane of the mitochondria, as well as outside the permeation barrier to coenzyme A in the inner membrane of the mitochondria. It acts on C10-C20 chain length fatty acids, as well as unsaturated fatty acids (C16-C20). The enzyme functions both in lipid synthesis in the endoplasmic reticulum membrane and in fatty acid oxidation.

Short-chain acetyl-CoA synthetase is found in the mitochondrial matrix of cardiac- and skeletal muscle, kidney and adipose tissue, as well as in the intestines. It is not present in liver mitochondria. Cytosol acetyl-CoA synthetase has been identified in tissues with high lipogenic activity e.g. liver, adipose tissue, intestines and mamma.

The medium-chain fatty acid specific ligase, known as octanoyl-CoA synthetase, is also situated in the mitochondrial matrix of various tissues, activating fatty acids with chain length C3-C7, as well as unsaturated medium-chain carboxylic acids. Thus medium-chain fatty acids are almost never activated extramitochondrial and are consequently not significantly incorporated into the lipids synthesized by the hepatic tissue. The medium-chain ligases act on C4-C12 chain length fatty acids [40].

Fatty acid activation is readily reversible, since each fatty acyl-CoA like ATP itself, is an energy-rich compound. The direction of this reaction is however far to the right because of the active pyrophosphatase present in most cells. The overall reaction can be summarized as follows:



Activated MCFAs are rapidly oxidized through ß-oxidation; the result being an excess of acetyl-CoA which then follows various metabolic pathways, both in the mitochondria - Krebs cycle, ketogenesis, elongation of fatty acids, and in the cytosol - *de novo* synthesis of fatty acids and cholesterol. Many hydrogen atoms are released from this accelerated ß-oxidation.

A slight cholesterol lowering effect of MCTs has been identified [36] that could be accounted for by a decrease in the intestinal absorption of cholesterol and a slowing of it's synthesis from acetyl-CoA in the liver.

Oral MCT ingestion gives rise to the development of a slight hypoglycemia [37], apparently caused by a decrease in hepatic output of glucose and not by an increase in the peripheral utilization of glucose. Interestingly though, the plasma insulin concentration increases at the same time because the islets of Langerhans are stimulated either by ketone bodies produced from MCFA oxidation, or by the MCFAs themselves, or by both. In general it appears that MCTs improve carbohydrate tolerance [36].

2.3.3 Extrahepatic metabolism

Except for the utilization of ketone bodies, the role of the extrahepatic tissues in the metabolism of MCTs is small, given the magnitude of the hepatic uptake of MCFAs. As in the liver, the extrahepatic tissues do not incorporate considerable amounts of MCFAs in the lipids they synthesize. It appears that MCFAs do not need carnitine to cross the mitochondrial membrane of extrahepatic tissues. This has, however, again been questioned by some authors [41]. In extrahepatic tissues, MCFAs are more rapidly oxidized into CO_2 than LCFAs. As in the liver, MCFAs only slightly inhibit the *de novo* synthesis of fatty acids in adipose tissue.

2.4 Energy from medium-chain fatty acids

One of the properties of MCTs is that they are ketogenic [38]; when supplied in the diet, they are rapidly oxidized, rendering many ketone bodies which supply a quick source of energy. The major ketone bodies in humans are acetoacetate and ß-hydroxybutyrate, synthesized from acetyl-CoA [40]. Ketogenesis can be considered an "overflow pathway", stimulated when acetyl-CoA accumulates due to the rapid oxidation of MCFAs in the mitochondria, or due to deficient carbohydrate utilization, so that oxaloacetate levels are low. This reduces flux through citrate synthase and causes acetyl-CoA to accumulate.

Ketogenesis occurs primarily in the liver, because of the high levels of ß-hydroxy-ß-methylglutaryl-CoA (HMG-CoA) synthase in the mentioned tissue. Liver cells

are however unable to metabolize ketone bodies; they are transported from the liver to other tissues where acetoacetate and ß-hydroxybutyrate can be reconverted to acetyl-CoA for energy generation. Energy is thus delivered to the whole body; energy for the brain during extended periods of starvation, and for other tissues especially muscle when available glucose is low. The utilization of ketone bodies for fuel may also spare the oxidation of branched-chain amino acids and reduce skeletal protein catabolism [30].

A modest elevation of the concentration of ketone bodies in the blood is known not to be dangerous, since all extrahepatic tissues can use ketone bodies supplied by the blood. When the blood level of ß-hydroxybuturate and aceotacetate increases, the utilization of ketone bodies is enhanced [36], since extrahepatic tissues are enzymatically equipped to produce acetyl-CoA from ketone bodies.

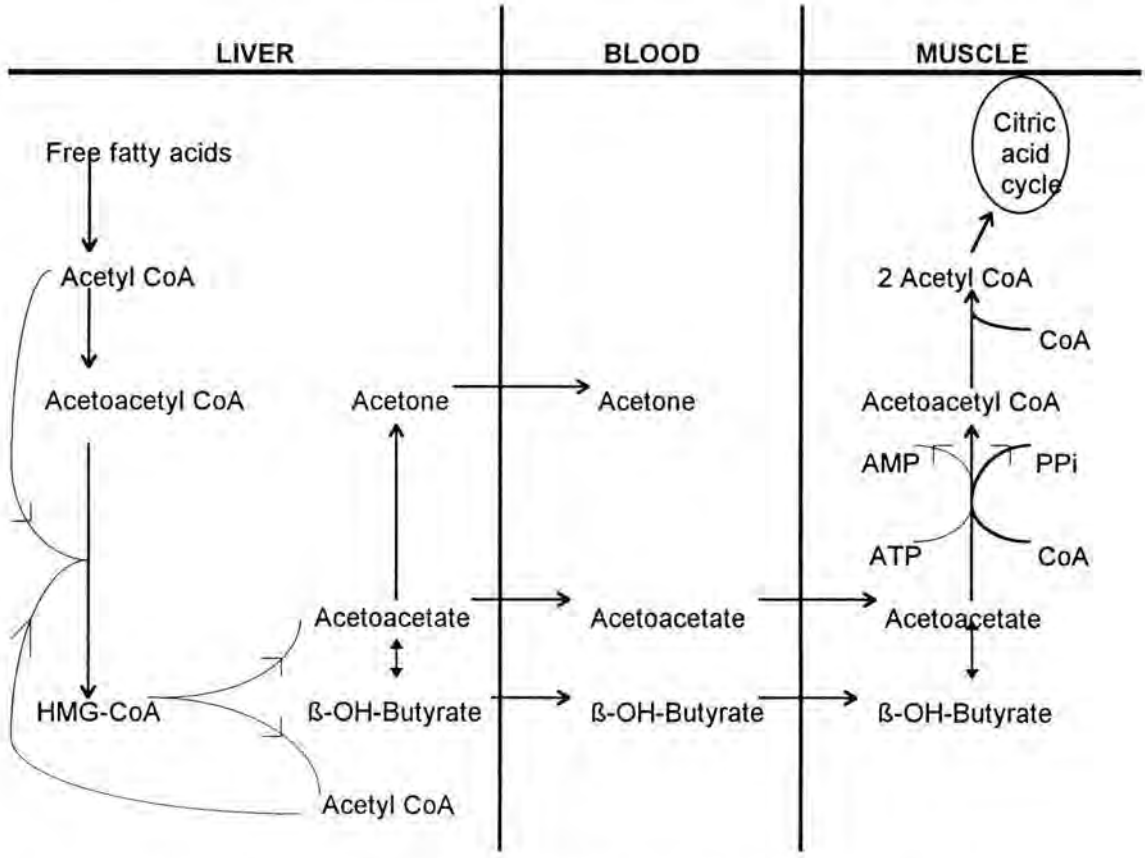


Figure 3: A pathway illustrating the production and fate of ketone bodies [38].

In their study on the effects of a three day high MCT-low CHO intake on urinary organic acid levels, Dias *et al.* [37] found no change in the 24-hour urinary tricarboxylic acid concentration, thereby suggesting that adenosine triphosphate (ATP) production was optimum. Thus, excessive generation of acetyl-CoA from the rapid oxidation of MCFAs resulted in it's conversion to ketone bodies - acetoacetate and ß-hydroxybutyrate. These findings confirmed that diets in which MCTs comprise a high percentage of total caloric intake are highly ketogenic.

3. L-CARNITINE

3.1 Biosynthesis and metabolism

L-carnitine was discovered in muscle tissue in 1905 and at one time was considered to be a vitamin, but today is not considered an essential nutrient because it is known to be synthesized in the body from the amino acids lysine and methionine. The highest carnitine concentrations in the human body, 90% of the body's total carnitine content, is found in skeletal muscle tissue [42] (skeletal muscle 1mg-, cardiac muscle 0.6mg-, kidney tissue 0.3mg/gram dry substance).

The biosynthesis of carnitine in humans is adequate to meet the body's carnitine needs under normal physiologic conditions, evident in the fact that normal plasma carnitine levels are maintained with little or no dietary carnitine [43]. Therapeutic administration of supplemental carnitine is of proven efficacy in patients with renal carnitine wasting and those with increased carnitine requirements for detoxification of accumulating acyl-CoA caused by metabolic defects. Carnitine has also been suggested as a therapeutic agent in a diverse group of disorders including chronic renal failure, hyperlipidemias and peripheral arterial disease [44].

Deficiencies in carnitine biosynthesis as well as dependent enzyme deficiencies have been identified, described by Engel *et al.* [43] as apparently being of genetic origin. Primary muscle carnitine deficiency manifests in mild to severe

muscle weakness and excess of lipids in skeletal muscle fibers. Primary systemic carnitine deficiency is often associated with multiple episodes of metabolic encephalopathy, hypoglycemia, hypothrombinemia, hyperaminonemia and lipid excess in hepatocytes.

3.1.1 Chemical composition

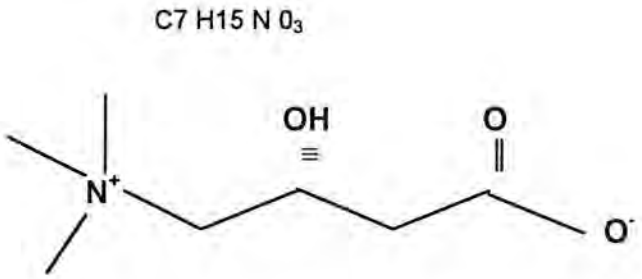
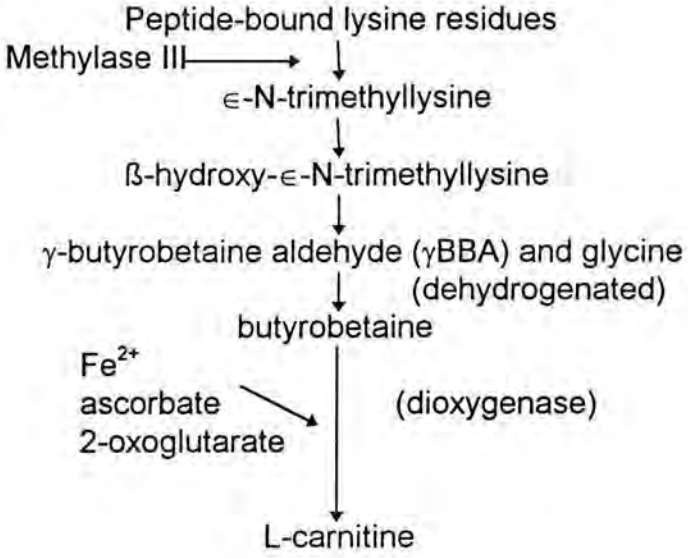


Figure 4: The structural formula of L-carnitine [24].

The pathway of carnitine biosynthesis has been studied extensively in the rat, and virtually all available evidence indicates that the enzymatic pathways in rats and humans are identical [43]. The precursors of carnitine are the amino acids lysine and methionine. The methyl groups for enzymatic trimethylation of peptide-linked lysine are provided by S-Adenosylmethionine. Numerous proteins contain ∈-N-trimethyllysine residues, including histones, cytochrome c, myosin and calmodulin. ∈-N-trimethyllysine residues are released for carnitine biosynthesis by protein turnover, and undergoes the following series of transformations:



Cofactor availability can affect carnitine biosynthesis: Fe^{2+} , ascorbate and 2-oxoglutarate are required [45]. Scorbutic animals were found to have 50% less carnitine in heart and skeletal muscle than do controls [45]. Magnesium is another essential cofactor and will be discussed.

Enzymes for the conversion of ∈-N-trimethyllysine to γ-butyrobetaine were found in all human tissues studied (skeletal muscle, heart, liver, kidney and brain) [43]. However, γ-butyrobetaine hydroxylase activity was not present in skeletal- and heart muscle; thus muscle carnitine is derived from the diet or synthesis by the liver or kidney, and then transported to muscle in plasma entering the muscle via an active transport system [46].

3.1.2 Methylation in the body - the role of magnesium

Magnesium is the fourth most abundant cation in the human body, and plays an important role as a co-enzyme for at least 300 known biochemical reactions, including all reactions involving the generation or use of energy [47]. Some of these activities include methylation, the glycolytic pathway, fat and protein metabolism, adenosine triphosphate (ATP) hydrolysis and the second-messenger system (cyclic AMP), as well as maintaining normal intracellular calcium, potassium and sodium levels. In addition, magnesium acts as a physiological

regulator of membrane stability, and in neuromuscular, cardiovascular, immune, and hormonal function [48]. Of the normal dietary intake of 200-300 mg/day, approximately 30% is absorbed. The absorption of magnesium occurs predominantly from the small intestine. Ninety-five percent of the filtered load of magnesium is reabsorbed in the kidney, thus renal reabsorption is responsible for the maintenance of a significant proportion of total body magnesium stores. Magnesium stores are found either in bone or in cells, with only 1% in the extracellular fluid [47].

The measurement of serum magnesium cannot be considered reliable in the diagnosis of magnesium deficiency, because of independently varying intra- and extracellular magnesium concentrations, i.e. a deficiency in one compartment may not be reflected in another. The main causes of magnesium deficiency are decreased intake, decreased intestinal absorption and excessive urinary losses [47]. Redistribution and increased loss of magnesium from the body has been observed during and immediately after a bout of exercise [48]. A shift in magnesium from the plasma into erythrocytes was found following exercise. Urinary excretion of magnesium was shown to increased by 21% on the day following exercise [49]. Magnesium loss through sweat also increases during exercise [50].

Supplementation of the diets of competitive athletes with magnesium salts (magnesium aspartate), has thus been reported to improve cellular metabolism [51], and has also been implemented in the increase of muscle strength and power [52].

As mentioned, the first step in carnitine biosynthesis is the methylation of lysine residues to trimethyllysine [43]. The gateway to these methylation reactions is the formation of S-adenosylmethionine (SAM) from methionine, catalyzed by methionine adenosyltransferase (MAT). This most important conversion requires magnesium as cofactor [45].

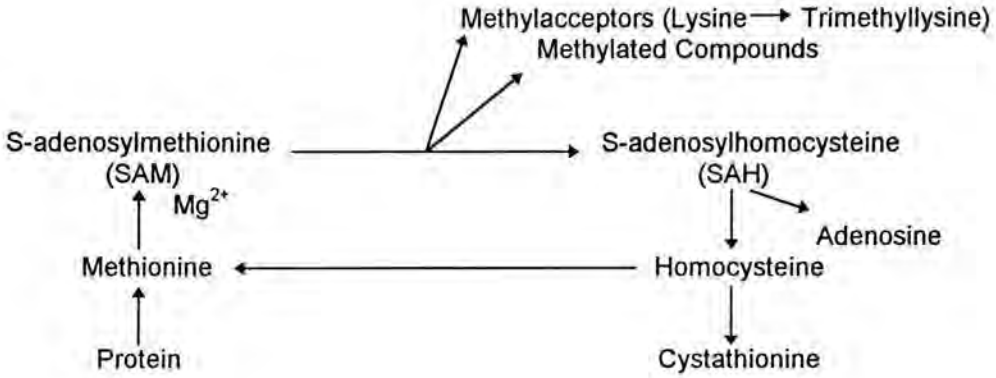


Figure 5: The methylation pathway [53].

S-adenosylmethionine may be regarded as a "high-energy" compound, because of its sulfonium bonds; the substituents of the sulfonium atom being energetically capable of participating in one or more transfer reactions. These transfer reactions produce a common sulfur-containing product, S-adenosylhomocysteine. Many S-adenosylmethionine-dependent methyltransferases are strongly inhibited by S-adenosylhomocysteine; when SAM is present in the ratio of 1:4 with respect to SAM, a variety of methyltransferases will decrease their activities by 10-60%. A hydrolase further metabolizes the S-adenosylhomocysteine thioester, cleaving it to homocysteine and adenosine. Homocysteine lies at an important metabolic branch point; through the transsulfuration pathway it may be converted to cystathionine, or methylated to form methionine, thereby completing the sulfur conservation cycle.

A large percentage of the body's methylation capacity is utilized for the formation of creatine [53], which plays an important role in the supply of energy to muscles (creatine is synthesized in the kidney, liver and pancreas from the amino acids glycine and arginine [54]). Transmethylation reactions occur mainly in the liver; the liver therefore being the most important organ for methionine metabolism [53].

3.2 Carnitine absorption and distribution

L-carnitine is found in various food sources; substantial amounts are found in meat, particularly beef, sheep and lamb. Other animal foods such as milk, cheese and poultry contain somewhat less carnitine, while fruits, vegetables, grains and eggs are minor sources. The typical non-vegetarian diet provides approximately 100-300mg/day [19]. The body may compensate for low dietary intakes by increasing carnitine biosynthesis from lysine and methionine and decreasing renal clearance of carnitine.

Hamilton *et al.* [43] studied carnitine transport across the human proximal small intestinal mucosa, *in vitro*, and concluded that movement across the membrane was via an active process that depended on Na^+ co-transport. A passive diffusional process, which may be important for absorption of large doses of carnitine was also identified. Carnitine is transported rapidly into intestinal mucosa from the lumen, where acetylation of up to 50% of the carnitine accumulated in the tissue occurs. Free and acetylcarnitine are then slowly released into the circulation.

Plasma total carnitine levels in normal adults range from 30-89μM. Mean values tend to be higher in females: 50.3±11.9 μM for males versus 51.5±11.6 μM for females [43]. Carnitine levels in skeletal muscle of normal humans range from 11-52nmol/mg noncollagen protein. Thus the concentration of carnitine in skeletal muscle is approximately 70 times higher than that in plasma. Normal regulatory processes that maintain tissue gradients have not been identified, although hormonal interactions may be important.

Turnover time for carnitine in skeletal and heart muscle is approximately eight days, and for other tissues (thought to be primarily liver and kidney) 11.6 hours. Turnover time for carnitine in extracellular fluid is approximately 1.13 hours, and 66 days for the whole body [43].

3.3 Renal carnitine handling

Carnitine is highly conserved in humans; tubular reabsorption in the kidneys was found to be 96-99%. Normal serum clearance is ~1ml/minute, and the daily excretion is 100-300µmol. Excretion increases after oral administration and after six hours, 10% of a 1g oral dose is recovered in the urine [55]. A true renal plasma threshold for carnitine excretion is difficult to determine as small amounts of carnitine are excreted in the urine even at very low plasma carnitine concentrations. An 'apparent' renal plasma threshold for carnitine excretion was calculated to be 51±7.4µM; this value closely parallels plasma carnitine concentrations in most individuals [43]. Research thus suggests that under normal conditions plasma carnitine concentration is partly regulated by the kinetics of carnitine reabsorption by the kidney.

L-carnitine formed intracellularly in the kidney may be partially secreted into the tubular lumen, either as short-chain acylcarnitine esters or in free form. The significance of renal secretion of carnitine and carnitine esters is unclear. It has been suggested that the excretion of carnitine esters may provide a mechanism for removing excess short- or medium-chain organic acids (occurring in excessive amounts in genetic diseases such as propionic acidemia).

Several factors, including serum thyroxine concentration, affect carnitine excretion in humans. Hyperthyroidism increases urinary carnitine excretion whereas hypothyroidism reduces urinary loss of carnitine. In normal humans fasting for 36 hours decreases renal clearance of free carnitine, but increases clearance of acylcarnitine esters, while total carnitine excretion increases. Under fasting conditions serum free carnitine decreases, but serum acylcarnitine esters and total carnitine concentrations increase. Parallel to the rising plasma carnitine concentrations, the urinary excretion of acylcarnitine esters increases [43].

3.4 Functions of carnitine

- The primary function of carnitine is the transport of long-chain fatty acids from the cytosol, across the mitochondrial membrane, into the mitochondrial matrix. Without carnitine long-chain fatty acids cannot enter the mitochondria to undergo the process of ß-oxidation [43].
- Carnitine is involved in the intracellular process of fat metabolism by way of forming acylcarnitine esters [56,57]. Thereby carnitine lowers the fatty acyl-CoA levels in the mitochondria; the accumulation of fatty acyl-CoA in the mitochondria inhibits ATP transport, and may damage cell membranes. The role of carnitine in the oxidation of long- and medium-chain fatty acids is discussed in detail in section 3.5.2 and 3.5.3 respectively.
- Carnitine participates in modulation of the intramitochondrial acyl-CoA/CoA ratio: carnitine transports acetyl-CoA and acetoacetyl-CoA from within the mitochondria to the cytosol where LCFA synthesis occurs, as well as transporting activated acyl groups from the cytosol into the mitochondrial matrix where oxidation takes place [56].
- Several mitochondrial pathways produce coenzyme-A esters of short- and medium-chain organic acids, which under normal circumstances are further metabolized to regenerate free coenzyme-A. Under conditions of stress, when one or more of these metabolic pathways produces large amounts of these esters, the organic acid may be transesterified to carnitine. Reduced coenzyme A is thereby freed to again participate in other mitochondrial pathways e.g. the tricarboxylic acid cycle.
- Through the formation of acetylcarnitine within the mitochondria, carnitine serves as a reservoir for acetyl groups. Under normal conditions the role of carnitine as mitochondrial buffer for excess organic acids is probably minor. However, this role may be of major importance in the maintenance of mitochondrial function and cell viability under abnormal conditions, such as diabetes, exercise, anorexia, or a defect of mitochondrial ß-oxidation [43].

Carnitine is thus of primary importance in active skeletal muscle tissue, since aerobic endurance conditioning is dependent on fatty acid oxidation.

- The oxidation of branched-chain amino acids has been recognized as a contributing energy source during exercise. Carnitine apparently facilitates the oxidation of these amino acids, thereby incorporating protein catabolism in cellular energy metabolism.
- The actions of carnitine stimulates the metabolism of pyruvate and this lessens the accumulation of lactic acid. The decrease in acetyl-CoA:CoA ratio could stimulate the activity of pyruvate dehydrogenase, a key enzyme in the oxidative metabolism of glucose in the mitochondria, which is normally inhibited by high levels of acetyl-CoA [19]. Theoretically if L-carnitine supplementation could increase the oxidation of glucose while concomitantly decreasing the accumulation of lactic acid, performance might be enhanced in exercise tasks which might be limited by excess lactic acid accumulation.

3.5 The role of carnitine in fatty acid metabolism

3.5.1 Transport and activation of fatty acids

Fatty acids are transported in the circulation as free fatty acids, bound to serum albumin, or as triglycerides in association with lipoproteins. Fatty acids are generated from the hydrolysis of plasma triglycerides and adipose tissue triglycerides by lipoprotein lipase and hormone-sensitive lipase respectively [24].

Evidence exists for both a saturable and nonsaturable uptake of fatty acids into the cell [24]. The saturable uptake predominates in the event of a low concentration of plasma free fatty acids, and a carrier mechanism may be involved. The nonsaturable uptake becomes significant at higher concentrations of plasma free fatty acids, and has been attributed to nonspecific diffusion across the cell membrane. Long-chain fatty acids thus cross the cell membrane and are

either transported to, or diffuse to the outer mitochondrial membrane. The fatty acids are subsequently activated by conversion to their CoA thioesters.

The transport of fatty acids between the plasma and outer mitochondrial membrane via either simple diffusion or a facilitated process, seems to be speculative. A low molecular weight (approximately 14 000) fatty acid binding protein has been identified in the cytosol of various animal tissues. This protein may function as a carrier of fatty acids in the cytosol, or may provide a temporary reservoir for potentially dangerous acyl-CoA thioesters. The best defined fatty acid binding protein is the Z-protein, present in liver, small intestine and adipose tissue [24].

3.5.2 The role of carnitine in long-chain fatty acid metabolism

Enzymes for ß-oxidation of activated fatty acids are situated in the mitochondrial matrix. The inner mitochondrial membrane is however impermeable to coenzyme A and its derivatives. L-carnitine thus carries acyl-CoA thioesters, formed on the outer mitochondrial membrane, across the inner mitochondrial membrane into the matrix.

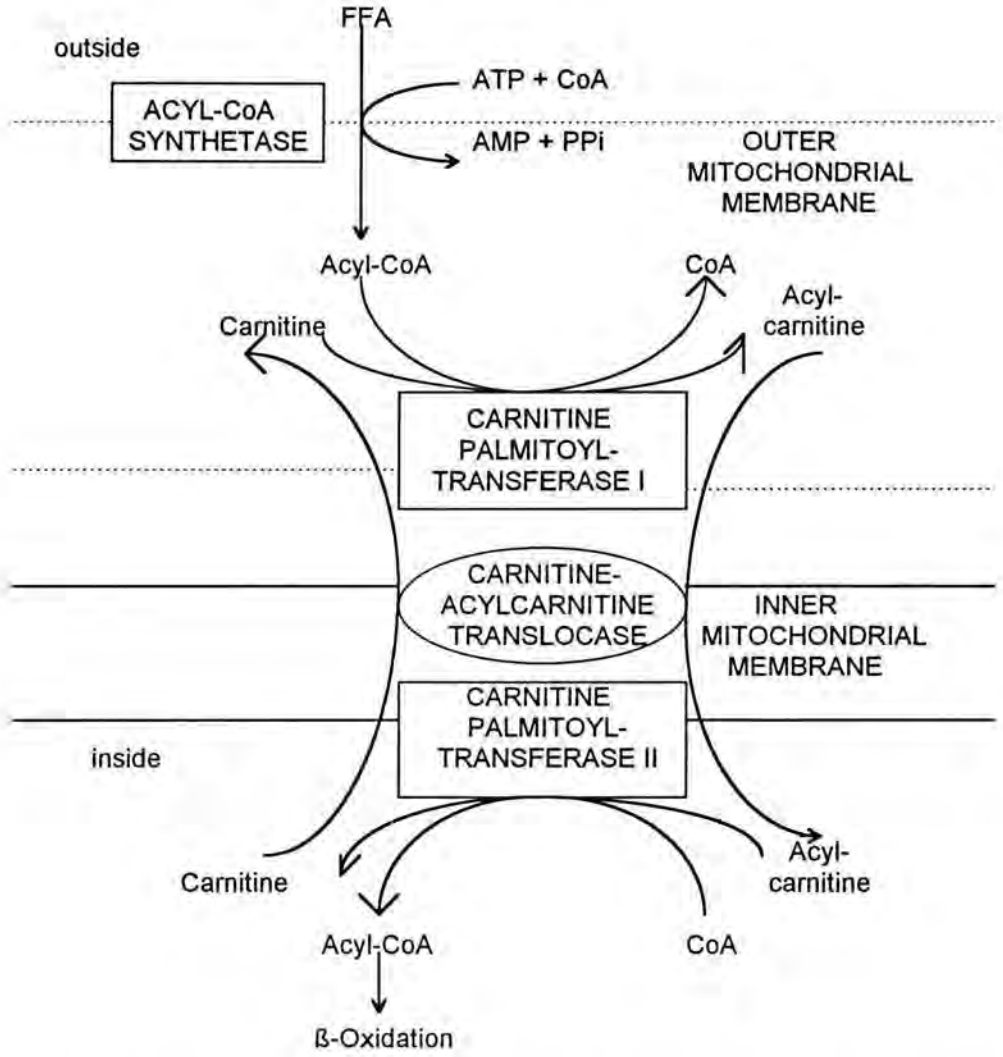


Figure 6: The role of carnitine in the transport of long-chain fatty acids across the inner mitochondrial membrane [24].

- The reversible transfer of fatty acyl residues from CoA to carnitine is catalyzed by carnitine palmitoyltransferase (CPT). Two forms of this enzyme have been identified namely CPT I and CPT II. Both are associated with the inner mitochondrial membrane; CPT I faces the intermembrane-space, whereas CPT II is directed towards the matrix space.
- The transfer of acyl residues from coenzyme A to carnitine during the translocation of fatty acid residues from the cytosol to the mitochondrial matrix is catalyzed by CPT I.
- The carnitine acylcarnitine translocase enzyme then facilitates acylcarnitine across the inner mitochondrial membrane; this enzyme catalyzes the slow

unidirectional diffusion of carnitine both in and out of the matrix, in addition to the much faster mole-to-mole exchange of acylcarnitine for carnitine. The slow unidirectional transfer of carnitine may provide an important mechanism whereby carnitine, after being synthesized in the liver, reaches the mitochondria. The more rapid exchange of mainly acylcarnitine for carnitine, is believed to be essential for the translocation of LCFAs from the cytosol into the mitochondria [24].

- In the mitochondrial matrix, the transfer of acyl residues from carnitine back to CoA to again form acyl-CoA thioesters, is catalyzed by carnitine palmitoyl transferase II. Acyl-CoA thioesters can subsequently be metabolized via ß-oxidation.

The mitochondria also contains a carnitine acetyl transferase, which catalyzes the transfer of acyl groups (C2-C10) to carnitine. The enzyme possibly regenerates free coenzyme A within the mitochondrial matrix through the transfer of acetyl groups and other short- or medium-chain residues from coenzyme A to carnitine. The resulting acylcarnitines leave the mitochondria probably via the carnitine:acylcarnitine translocase and move to other tissues [24].

3.5.3 The role of carnitine in medium-chain fatty acid metabolism

Short- and medium-chain fatty acids (<C10) enter the mitochondria as free fatty acids, crossing the inner mitochondrial membrane independently of the carnitine transport system. These fatty acids are then activated within the mitochondrial matrix where short- and medium-chain acyl-CoA synthetases are situated. Recent research has however suggested that carnitine may be involved in the metabolism and utilization of MCFAs. The enzyme, carnitine octanoyltransferase, is considered to be responsible for the formation of medium-chain length acylcarnitines within the mitochondrial matrix [45].

Rossle *et al.* [58] suggested a possible role for carnitine in MCFA utilization after examining plasma carnitine levels in humans following administration of an

intravenous long-chain triglyceride emulsion, and comparing it with a 50% long-chain, 50% medium-chain triglyceride emulsion. The infusion of the medium-chain triglyceride containing emulsion resulted in:

- a greater decrease in total and free plasma carnitine levels
- an increase in short-chain plasma carnitine levels
- an increase in plasma ß-hydroxybutyrate levels
- an increase in the acylcarnitine:fatty acid ratio

These observations supported the hypothesis that carnitine is indeed involved in medium-chain fatty acid metabolism.

Von Kempen and Odle [25] also suggested a role for carnitine in MCFA oxidation, *in vivo*. Sixteen newborn piglets were infused with MCFAs for 9-12 hours, providing energy equivalent to 50-75% of the animal's metabolic rate. After a five to seven hour carnitine free infusion period, a primed co-infusion of L-carnitine was started, and maintained for four to five hours. It was found that carnitine, independent of the level, increased the fatty acid oxidation rate by as much as 20% if the energy provided as MCFAs exceeded 5% of the metabolic needs of the pig.

A determinant in the fate of MCFAs, that is, oxidation versus lipogenesis, might be the site of activation in the liver; liver mitochondria contain an intramitochondrial medium-chain acyl-CoA synthethase, however a major portion of MCFAs are activated outside the mitochondria. Intramitochondrial activation leads to fatty acid oxidation, while extramitochondrial activation leads to esterification or chain elongation.

The process of lipid oxidation during, especially, endurance exercise, gives rise to the accumulation of long- and short-chain acyl-CoA derivatives within skeletal muscle mitochondria. In an attempt to generate coenzyme A for various metabolic reactions, e.g. further oxidation of fatty acids and substrate for the Krebs cycle, the partially oxidized fatty acids are again transferred to carnitine, thence from the mitochondria to the cytosol and eventually out of the cell [58]. This generation of

coenzyme A can be described as a detoxification mechanism; normal mitochondrial function in the urea cycle and ß-oxidation is inhibited by excess acyl-CoA [25], resulting in increased short- and long-chain plasma carnitine levels. The same increase occurs when medium- and long-chain lipid emulsions are administered intravenously, though due to the increase in the rate of MCFA uptake into the mitochondria, the effect is more dramatic. This again results in an increase in the release of short- and medium-chain carnitine esters into the plasma [58].

This data again supports the hypothesis that carnitine is involved in MCFA oxidation. The postulated role for carnitine does not include the actual transport of MCFAs into the mitochondria. Carnitine may be involved in the export of partially oxidized fatty acids from within the mitochondria in an attempt to generate coenzyme A.

3.6 The effect of exercise on carnitine

Carnitine regulates fatty acid influx to the mitochondria, and thus plays an important role in providing energy to working muscles during exercise.

A complex equilibrium exists between the various carnitine fractions in the body:

- free carnitine versus acylated forms
- muscle carnitine versus plasma carnitine
- the urine carnitine fraction

This equilibrium ultimately determines the size of the free carnitine fraction in the muscle pool, which from an energy standpoint, represents the metabolically active fraction. The amount of free carnitine in muscle can therefore be considered as a limiting factor to its energy supply. During especially prolonged exercise the carnitine fractions of the body compartments change in size, mainly because of the increased esterification of muscle free carnitine with acyl groups from different sources [59]. This phenomenon is chronically activated by physical training, and a

possible consequence thereof is a progressive reduction of the muscle pool free carnitine, which could lead to a potentially deleterious carnitine insufficiency.

In normal human subjects only minimal changes in the muscle carnitine pool are observed during exercise at work loads below the lactate threshold. At work loads above the lactate threshold however, muscle total carnitine is redistributed from carnitine to acetylcarnitine, with the acetylcarnitine content correlating with the muscle acetyl-CoA and lactate contents. According to Brass and Hiatt [46], changes in the muscle carnitine pool during exercise are poorly reflected in the plasma, and that any changes in carnitine metabolism during exercise might be dependent on the exercise work load.

At rest, approximately 80% of the muscle total carnitine pool is present as carnitine, 15% as short-chain acylcarnitine and 5% as long-chain acylcarnitines. During up to 60 minutes of low intensity exercise, i.e. below the lactate threshold, no significant changes are observed in the muscle carnitine pool. In contrast, within 10 minutes of exercise at high intensity work loads, the muscle carnitine pool is redistributed to short-chain acylcarnitines (acetylcarnitine) with only 20-50% of the total pool as carnitine, and 45-75% of the total carnitine as short-chain acylcarnitines. This redistribution of the carnitine pool slowly normalizes after the cessation of high intensity exercise, but has still not returned to the resting distribution 60 minutes after 30 minutes of high intensity exercise. Despite these dramatic changes in the muscle, the plasma carnitine pool is modified to a lesser extent during exercise [46].

Higher basal levels of free and total muscle carnitine were observed in marathon runners when compared to sprinters [59]. This phenomenon was ascribed to the higher concentration of mitochondria found in Type I muscle tissue fibers, predominant in long distance athletes, compared to the lower concentration of mitochondria in Type II muscle fibers, more predominant in skeletal muscle tissue of sprinters.

After exercise, muscle carnitine levels decreased in both marathon runners and sprinters [59], a 20% decrease was observed by Lennon *et al.* [60] after sub-maximal exercise. This reduction in free and total muscle carnitine may lead to reduced availability of carnitine to provide energy substrate to the mitochondria. The reduction seemed to be more pronounced in marathon athletes than in sprinters, seemingly because marathon athletes predominantly rely on the aerobic metabolism of carbohydrates and fatty acids to supply energy [59].

No difference in muscle carnitine levels prior to and after exercise has been observed: Carlin *et al.* [57] found no difference in total muscle carnitine prior to and after 90 minutes of cycling. Decombaz *et al.* [60] described the same results in long distance skiers after a 13 hour 26 minute race. They also found that individual carnitine levels varied, but seemed independent of either training or oral intake of carnitine.

Exercise generally seems to cause a rise in plasma short- and long-chain acylcarnitine levels, whereas plasma free carnitine levels decrease [57,59,61]. The rise in plasma carnitine esters were ascribed to the release of muscle carnitine esters during exercise; during endurance exercise the oxidation of both pyruvate and fatty acids in skeletal muscle increases. Large scale mobilization of fatty acids during prolonged exercise causes rapid accumulation of long-chain acylcarnitine in muscle, and after exercise, any excess not used for oxidative purposes is returned to the blood stream. The increase in plasma carnitine esters may also be due to the exchange of carnitine with hepatic carnitine pools [57].

The combined effect of training and participating in endurance events, seem to cause a wasting of short-chain acylcarnitine in the urine, resulting in reduced availability of muscle carnitine [57].

3.7 The influence of L-carnitine administration on physical exercise

Carnitine's important role in lipid metabolism and energy production has been thoroughly explained. The proposed enhancing effect of carnitine on lipid oxidation, has led to extensive studies, investigating the effect of carnitine supplementation on physical exercise. The utilization of fatty acids as an energy source, progressively decreases when exercise intensity increases above 65% of VO_2 max. At this point, the utilization of carbohydrate as an energy source increases.

The limiting factor in the use of fatty acids as an energy source during high intensity exercise, is as yet still unclear. A decrease in the ability to translocate fatty acids into the mitochondria might be involved [19]. Carnitine administration should therefore theoretically improve mitochondrial fatty acid oxidation, with a subsequent muscle glycogen sparing effect. Endurance performance should be enhanced in events such as marathons, where optimal endogenous carbohydrate stores are considered essential.

However, some discrepancies have arisen concerning the effect of carnitine on physical exericse. After supplementing competitive walkers with 4g of L-carnitine per day, Marconi *et al.* [62] found a significant 6% increase in individual VO_2 max values. They ascribed the increase to a probable activation of substrate flow through the citric acid cycle. They found no change in the contribution of lipid oxidation to energy metabolism during exercise, coinciding with no change in respiratory exchange ratios. Vecchiet *et al.* [22]. also reported higher VO_2 max values, as well as an increase in peak power output during maximal exercise intensity, after 2g L-carnitine were administered orally, one hour prior to exercise, in moderately trained young men. Carbon dioxide production, oxygen uptake, and lactate accumulation were reduced during their study. They did not report on respiratory exchange ratios.

Wyss *et al.* [20], however, found no change in VO_2 max or power output during strenuous exercise, after L-carnitine administration (1.036g L-carnitine 3x/day for seven days prior to exercise testing and during the duration of exercise testing). They did report a significant decrease in respiratory exchange ratios, thereby implicating higher lipid metabolism rates after carnitine administration. Greig *et al.* [63] also reported no effect of carnitine supplementation on VO_2 max, or on maximal heart rate (2g/day for two to four weeks). Intravenous carnitine administration (185µmol/kg) at the start of a cycle ergometer exercise session, was found to have no effect on respiratory exchange ratio, muscle lactate concentration, or muscle glycogen utilization during exercise [44].

From the above it is clear that consensus has not yet been reached on the effect of carnitine administration on exercise performance.

CHAPTER 2: SUBJECTS, MATERIALS AND METHODS

1. Subjects

Nine male marathon athletes, members of the Arcadia Athletics Club, voluntarily participated in the experiment. Athletes varied in age from 18 years to 30 years; mean=25.5 (sd=5.46). None of the athletes exhibited any overt manifestasion of poor health or disease prior to the study. Selection criteria for participation in the experiment included athletes having been actively involved in road running for a minimum of two years, and being able to complete a standard marathon (42.2km) in a maximum time of 3 hours 45 minutes.

The nature and risks of the experimental procedures were explained to the subjects and their written informed consent was obtained (see Appendix A).

2. Preliminary analyses

2.1 Nutritional Analysis

The week prior to the first baseline trial, athletes were instructed to record their food intake for two week days and one weekend day. Energy and nutrient intakes were calculated from the dietary records using a computerized version of Food Fundi Professional (Nutritional Advice Software, Penta Medical Systems, MRC).

2.2 Anthropometric evaluation

The following anthropometric measurements were recorded:

- Stature (height - m) was measured with a calibrated height gauge (Seca Model 220, Germany).
- Mass (weight - kg) was determined with a calibrated medical balance scale

(Seca Model 713, Germany)

- Skin folds (mm) were measured using a Harpenden skinfold caliper, and included the Triceps, Subscapular, Supra illiac, Biceps, and Medial calf folds.
- Circumferences (girths - cm) were measured using a Rabone-Chesterman calibrated steel tape and included flexed arm, and calf.
- Diameters (cm) - humerus and femur - were measured with a spreading caliper.
- Anthropometric somatotype (endomorphic, mesomorphic and ectomorphic component) was determined according to the method of Heath and Carter [64].
- The following formula was used to calculate the % bodyfat [64]:

$$\% \text{ Bodyfat} = \left[\frac{4.95 - 4.5}{D}\right] \times 100$$

male: $D = 1.1610 - 0.0632\ X$

where X = sum of triceps, subscapular, supra-iliac and biceps skin folds (mm).

- Basal metabolic index (BMI) was calculated according to the formula

$$BMI = \frac{\text{weight (kg)}}{\text{height}^2\text{ (m)}} \qquad [65]$$

- The formula of Katch, Behuke and Katch [67] was used to calculate basal surface area (BSA)

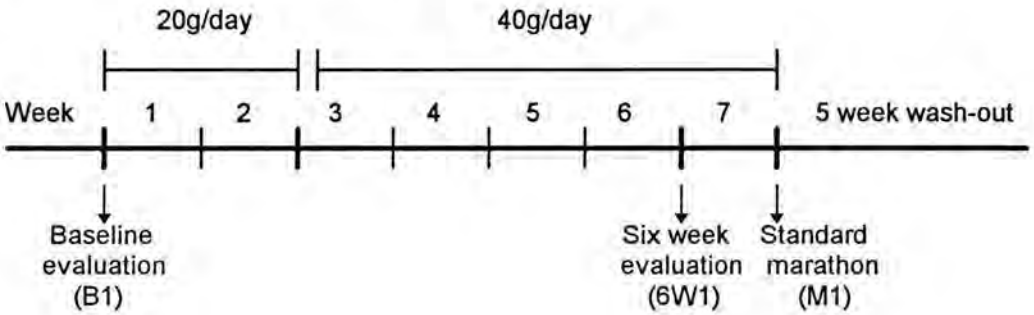
$$\text{Surface area } (m^2) = \sqrt{\frac{\text{weight (kg)}}{\text{height (cm)}}} \times 3 \times \text{height (cm)} \times 0.01762$$

3. Experimental design

During the experiment each athlete served as his own control. The experiment consisted of two seven week (49 days) trial periods, separated by a five week wash-out period. Athletes continued with their individual training programmes as

well as their normal diets throughout the experiment. During trial period one and trial period two, the same test protocol and supplementation regime were followed, the only difference being the form of supplementation - Supplement I (SI) and Supplement II (SII) respectively.

Trial period 1 - Supplement I (SI)



Trial period 2 - Supplement II (SII)

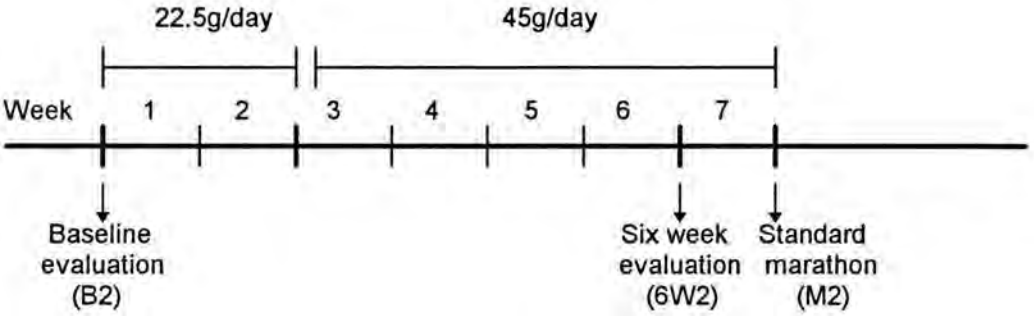


Figure 7: Schematic presentation of experimental design

Each trial period commenced with athletes reporting to the Sport Sciences Laboratory of Technikon Pretoria for a series of laboratory tests and blood sampling, following an overnight fast. Data obtained from the first test series served as baseline reference for every supplementation period. Each athlete followed the following regime:

- an anticubital venous blood sample was collected by a medical doctor

- athlete was instrumented with ECG electrodes and a mouthpiece assembly for measuring respiratory gasses by open circuit spirometry (Med Graphics Spirometer)
- athlete performed a multiphase progressive treadmill test to exhaustion. Criteria for exhaustion included respiratory exchange ratio exceeding 1.1, stabilization of VO_2, and athlete indicating exhaustion. VO_2 max and peak treadmill running velocity were determined. Expiratory gasses were analysed, respiratory exchange ratio determined and heart rate monitored
- directly after completing the treadmill test an anticubital venous blood sample was collected
- athlete rested for 30 minutes whereafter an anticubital venous blood sample was collected.

During the following two weeks, athletes daily ingested orally 20g of Supplement I (SI) (in powder form), dissolved in 250ml of water. Athletes were instructed to ingest the supplement 30-45 minutes prior to their main training session of the day. After two weeks, (which served to accustomize athletes to the supplement and monitor them for any adverse effects), the dose was increased to 40g/day for the remaining five weeks. Athletes were subsequently instructed to dissolve 40g of SI in 500ml of water and to drink 250ml, 30-45 minutes prior to their main training session, and 250ml, 30-45 minutes into their training session. Athletes were provided with an appropriate water bottle, and weekly received a seven day supply of supplement prepared in powder form, sealed in sachets containing 20g of powder, and measured on a Mettler PE 3000 (Germany) electronic balance.

Athletes were instructed to continue with their normal diet and normal training programme throughout the duration of the experiment. Together with their weekly supply of supplement, athletes were also provided with a 7-day training log (see Appendix B) in which they indicated taking the supplement, recorded their daily training programme, as well as commenting on how they felt during training. Athletes regularly competed in league road running events throughout the year,

forcing them to maintain a certain level of fitness. The two baseline evaluations served to monitor the consistency of their fitness.

During weeks three and seven, athletes again recorded their food intake for two week days and one weekend day, in order to monitor the consistency of their food intake throughout the experiment. Energy and nutrient intakes were calculated and compared with preliminary dietary analyses. Dietary intakes during week seven were specifically recorded on the three days prior to the marathon event to identify whether the athletes significantly increased their carbohydrate intake prior to a marathon event.

At the end of week six athletes again reported to the Sport Sciences Laboratory following an overnight fast. The weight (kg) of each athlete was recorded and thereafter each athlete followed the following test protocol:

- an anticubital venous blood sample was collected by a medical doctor
- athlete ingested a single 20g dose of SI, dissolved in 250ml of water
- athlete rested for 30 minutes
- athlete was instrumented with ECG electrodes and a mouthpiece assembly for measuring respiratory gasses by open circuit spirometry (Med Graphics CardiO_2 VO_2/ECG exercise system)
- athlete performed a multiphase progressive treadmill test to exhaustion during which VO_2 max and peak treadmill running velocity were determined. Expiratory gasses were analysed, respiratory exchange ratio determined, and heart rate monitored
- directly after completion of the treadmill test an anticubital venous blood sample was collected
- athlete rested for 30 minutes whereafter an anticubital venous blood sample was collected.

During week seven athletes continued on the supplementation regime as described. After achieving accustomization (seven week supplementation period), at the end of week seven, athletes participated in a competitive standard

marathon event (42.2km) during which they were monitored and provided with the appropriate supplement (SI, SII respectively). The objective of supplementation during the marathon event was to induce each athlete to consume 500ml of fluid containing 40g of supplement per hour. The supplement was prepared in advance (40g supplement powder/500ml water) and provided to the athletes throughout the race in sachets containing 125ml fluid.

Prior to the marathon event an anticubital venous blood sample was collected from each athlete. Starting time of the marathon events was at 6h00 and due to practical reasons pre-event blood samples were collected two hours prior to the start of the race, whilst athletes were rested and calm (no forced dietary adjustments were made prior to the marathon events; athletes followed their normal pre-event eating patterns). Athletes were provided with two 125ml sachets before the race, to drink 60 minutes and again 30 minutes prior to start.

Directly after completion of the marathon an anticubital venous blood sample was collected from each athlete. Their respective times were recorded and athletes reported back on how they experienced the race, their drinking patterns during the race, and if any adverse affects (e.g. gastro-intestinal discomfort) occurred during the race.

4. Laboratory tests

4.1 VO_2 max exercise test

Each athlete performed a multiphase progressive treadmill test on four occasions, twice during each supplementation period (SI: baseline and after six weeks, SII: baseline and after six weeks). The treadmill test was performed on a Quinton Q-65 treadmill (Quinton Instrument Co., Seattle, WA), during which cardio-respiratory measurements were recorded by means of the Med Graphics Cardio$_2$ combined VO_2/ECG exercise system (Medical Graphics Corporation, St. Paul MN, USA).

Athletes expired through a bi-directional differential pressure preVent Pneumotach; accuracy ±3%, resolution 8.64 ml/sec, dead space <20ml. Expired air was continuously monitored and mixed expired CO_2 and O_2 concentrations determined through infrared absorption and polarographic analyser respectively. Zircoma-type O_2 analyses - response of <80 msec and accuracy ±0.1% was utilized. CO_2 analyses were recorded by NDIR type analyser - response <90msec and accuracy ±0.1%. Expiratory gas volumes collected by Med Graphics Spirometer were analysed and data for VO_2, VCO_2 and respiratory exchange ratio (RER) were calculated by computer (Med Graphics System). VO_2 max was determined as the highest rate of O_2 consumption measured during any 60 seconds of the progressive treadmill test [69]. The gas analyser was calibrated manually and electronically using Analar grade standard bottled gas concentrations. The gas sample was drawn using a patented gas drying sample circuit with a side-stream sample flow rate of 80-100ml/min with a warm-up time of 30 minutes from cold start.

Throughout the exercise tests, an electro-cardiogram (ECG) was recorded to determine heart rate (HR). Athletes were instrumented with ECG electrodes. The 12 lead ECG was monitored throughout and graphically depicted on an electro-cardiogram as well as visually displayed on the computer monitor (Med Graphics CardiO_2 ECG mode). Heart rate and ST curve deviations (if any) were established from the ECG. The digital sampling rate of simultaneous input from all 12 leads was 500 s/sec/channel, with frequency response of 0.05-160 Hz. The on-screen display included three 5.0 sec rhythm leads displayed with one average complex for each of the 12 leads, four times standard gain complex and gas exchange trend graph showing real time and breath-by-breath data.

The treadmill protocol for each VO_2 max exercise test was as follows: Athlete started running at 8km/h at a 0° gradient [70]. The gradient was kept constant (0°) throughout the exercise test. Treadmill speed was increased by 2km/h every three minutes up to 16km/h. Thereafter the speed was increased by 1km/h until

exhaustion (criteria for exhaustion has been explained), whereupon the test was terminated. Peak treadmill running velocity was determined as the highest running speed (km/h) the athlete was able to maintain during any 60 seconds of the treadmill test [70]. Tests were conducted in a closed laboratory space with a constant temperature of 21°C (±2°), and humidity of approximately 55%, whilst barometric pressure was recorded every day (640-659mmHg). The temperature, humidity and barometric pressure were interpolated separately with each data recording.

4.2 Blood analyses

Before, directly after, and 30 minutes after each treadmill test (of which each athlete performed four), anticubital venous blood samples were obtained from the athletes by a medical doctor. The pre-exercise sample included a 5ml sample collected in an EDTA tube (Disodium ethylenediamine-tetraacetic acid), plus two 5ml samples collected in 7ml clotting tubes (Vacutainer system with hemoguard closure). Directly post- and 30 minutes post-exercise a 5ml sample was again collected in clotting tubes, as well as before and directly after each marathon event. One pre-exercise 5ml sample, the directly after, and 30 minute after exercise samples, as well as the marathon event samples were on each occasion allowed an adequate coagulation period. Samples were centrifuged and serum frozen at -4°C. At every sampling occasion a drop of blood was collected on filter paper (Whatman 3, Whatman Laboratory Division. Springfield MU, Maidstone, Kent.), allowed to dry, sealed in plastic sachets and cooled. The frozen serum samples and filter papers were subsequently transported to the Biochemistry Department of the Potchefstroom University for serum organic acid extractions, and carnitine determinations respectively.

The pre-exercise EDTA and the remaining clotting tubes were placed on ice and brought to the Institute of Pathology - University of Pretoria, where the following chemical pathological analyses were performed using standard methods -

Technicon DAX System SM4-1141L93. A brief outline of each individual method follows.

Serum analyses included:

- Total serum cholesterol - Cholesterol esters in serum are completely hydrolyzed to free cholesterol by cholesterol esterase. Free cholesterol in the presence of oxygen and cholesterol oxidase generates hydrogen peroxide, which in turn is combined with 4-aminoantipyrine and a phenol derivative to form a quinoneimine dye. A red colour is produced which is directly proportional to the cholesterol concentration in the sample and is quantitated by an endpoint measurement at 524nm.
- Serum triglycerides - The triglycerides (GPO) method is based on the enzymatic determination of glycerol with glycerol phosphate-oxidase (GPO) after hydrolysis with lipoprotein lipase.

Plasma analyses included:

- Sodium - The Technicon DAX system sodium method is based on an indirect potentiometric procedure using an ion-selective electrode. The sodium ion-selective electrode responds selectively to sodium ions according to the Nernst equation.
- Potassium - The potassium method is based on an indirect potentiometric procedure using an ion-selective electrode. The potassium ion-selective electrode responds selectively to potassium ions according to the Nernst equation.
- Magnesium - Magnesium ions form a red chelate with xylidyl blue in an alkaline medium which results in a spectral shift. The change of absorbance at 660nm is directly proportional to the magnesium concentration and can be quantified by an endpoint measurement.
- Calcium - Cresolphthalein complexone forms a colored complex with calcium ions. Absorbance is measured at 572nm.
- Chloride - The serum sample is mixed with DCL chloride reagent. This reagent, an equilibrium solution of ferric, mercuric, and thiocyanate ions, when

combined with the chloride ions in the serum, undergoes a double displacement reaction, forming a red-brown chromophore, ferric thiocyanate. The endpoint measurement is read at 476nm.

- Carbon dioxide - The enzymatic carbon dioxide method is based on the phosphoenolpyruvate carboxylase (PEPC) catalyzed reaction of HCO_3^- with phosphoenolpyruvate to give oxaloacetate. Malate dehydrogenase (MDH) is used to catalyze the indicator reaction in which the amount of NADH oxidized is followed spectrophotometrically and is proportional to the amount of CO_2 in the sample.
- Glucose - D-glucose is phosphorylated by adenosine-5'-triphosphate (ATP) in the presence of hexokinase and magnesium ions. The product of this reaction, glucose-6-phosphate, is converted to 6-phosphoglucono-δ-lactone in a reaction catalyzed by D-glucose-6-phosphate dehydrogenase (G6PDH). The reaction is coupled with a reduction of the coenzyme, nicotinamide adenine dinucleotide (NAD). The amount of NADH produced is directly proportional to the concentration of glucose present in the sample and is measured by its absorbance at 340nm.
- Albumin - BCG (bromcresol green) preferentially binds serum albumin at pH 4.2 causing a shift in the absorption spectrum. The increase in absorbance measured at 604nm after thirty (30) seconds is directly proportional to the concentration of complexed albumin.
- Total protein - The peptide bond of proteins forms a colored complex with Cu^{2+} ions in an alkaline solution. The shift of spectral absorption is measured photometrically, and is directly proportional to the protein concentration in the sample.
- Globulin - Is a calculated value: globulin = total protein - albumin.
- Creatinine - Creatinine reacts with picric acid in an alkaline medium to produce a red colored complex. The absorbance of the analytical mixture is measured at 500nm on a first-order reaction curve.
- Urea nitrogen - Urea is hydroyzed by urease to form ammonia, which in turn acts as substrate in a NADH dependent reaction with glutamic dehydrogenase.

The decrease in absorbance at 340nm due to the consumption of NADH is directly proportional to the concentration of urea in the sample.

- Uric acid - Enzymatic methods are based on the specific uricase-catalysed oxidation of uric acid to allantoin. This reaction can be monitored directly at 293nm or indirectly by coupling to indicator reactions and thus measuring hydrogen peroxide.
- Lactate dehydrogenase (LDH) - Lactate dehydrogenase catalyzes the conversion of L-lactate to pyruvate in the presence of NAD. The enzymatic activity of LDH is proportional to the rate of production of NADH. The reaction is monitored at 340nm as a zero-order kinetic assay.
- Alkaline phosphatase (ALP) - The reaction is initiated by the addition of p-nitrophenyl phosphate (PNPP) as substrate to the sample. During the reaction, the alkaline phosphatase hydrolyzes the PNPP to form p-nitrophenol. The kinetic analysis is followed by colorimetric measurement at 404nm; the rate of formation of p-nitrophenol is proportional to the alkaline phosphatase activity.
- Alanine aminotransferase (ALT) - The reaction is initiated by the addition of reagent (Technicon Omnipak reagents - ALT Reagent 1 & ALT Reagent 2) to the patient sample. The rate of decrease in the concentration of NADH is directly proportional to the ALT activity in the sample. The reaction is monitored at 340nm as a zero-order kinetic assay.
- Aspartate aminotransferase (AST) - The reaction is initiated by the addition of the reagent (Technicon Omnipak reagents - AST Reagent 1 & AST Reagent 2) to the patient sample. The rate of decrease in the concentration of NADH is directly proportional to the AST activity in the sample. The reaction is monitored at 340nm as a zero-kinetic assay.
- Gamma-glutamyltransferase (GGT) - Synthetic substrate glycylglycine acts as acceptor for the gamma-glutamyl residue and p-nitroaniline is liberated. The liberated product has an absorption maximum near 400nm; the rate of formation is measured photometrically at 404nm as a zero-order kinetic assay.
- Aspartate Aminotransferase (AST) - The reaction is initiated by the addition of the reagent to the patient sample. The rate of decrease in the conentration of

NADH is directly proportional to the AST activity in the sample. The reaction is monitored at 340nm as a zero-order kinetic assay.

- Full blood count - Full blood and hemoglobin counts were determined using standard methods. The Coulter Counter Model T890 (Coulter Electronics, Inc., Hialeah) was used for the procedure.

Serum LDL- and HDL cholesterol:

Were determined using the CHOD-Iodine method, which is a highly sensitive enzymatic UV test being suitable for both routine and special determinations. Low density lipoproteins (LDL) are precipitated by heparin at their isoelectric point (pH 5.12). After centrifugation the high density lipoproteins (HDL) and very low density lipoprotein (VLDL) remain in the supernatant and can then be determined by enzymatic methods. LDL cholesterol = total cholesterol minus cholesterol in supernatant.

4.3 Serum organic acid extractions

A brief outline of the method used to obtain organic acid extractions (non-esterified fatty acids and L-lactate) follows. One hundred millilitres internal standard (3-φ-butyric acid; 26.25mg/50ml) was used for 1ml serum. The above was acidified with six drops 5N HCl. Six millilitres ethyl acetate was added to the serum and mixed for 30 minutes, then centrifuged for 10 minutes. The organic phase was collected and placed in a clean kimax 15ml tube. Three millilitres diethyl ether was added to the serum, mixed for 15 minutes and centrifuged for 10 minutes. The organic phase was collected and added to the first phase, then dried with anhydrous sodium sulphate and centrifuged for 10 minutes. The organic solutions were transferred to smaller kimax 10ml tubes and dried under nitrogen. It was then derivatised with bis(trimethylsilyl) trifluoroacetamide (BSTFA) 50ml, and trimethyl-chlorosilane (TMCS) 10ml at 70°C for 60 minutes, and left to cool. Gas chromatographic analysis was performed [66].

4.4 Plasma carnitine determinations

Plasma free and acetylcarnitine concentrations were determined on a VG Quattro II mass spectrometer.

The Whatman 3 filter paper was perforated, and the sample, equivalent to seven µl blood, was placed in an eppendorf tube. Ten µl internal standard (Minstoel carnitine - 10µM) and 100µl ethanol were added, and left at room temperature for 30 minutes, to allow for extraction of the relevant compounds. The liquid was then transferred to a clean kimax tube, and dried under N_2. The dried liquid was then butylated with 50µl butanolic/HCl for 15 minutes at 65°C, and dried under nitrogen. It was then resuspended with 250µl acetonitrile:H_2O (1:1) and analysed. The starting temperature was 70°C for two minutes; thereafter the temperature was increased with 5°C/minute, to reach 280°C.

5. Supplementation

5.1 Supplement I (SI) - Medium-chain triglycerides + carbohydrates

The supplement was based on a commercially available product, ENERGO (Registered Trademark, PVM Products (Pty) Ltd, Pretoria, SA.), designed as a mid- and post race energy replacement. The product is fully isotonic and free of preservatives. It consists mainly of carbohydrates of various chain lengths to maintain a constant blood glucose level, and is supplemented with protein, vitamins and minerals to provide nutrient levels as indicated in Table 1.

Table 1: Nutritional information ENERGO (100g)

NUTRITIONAL INFORMATION		ENERGO (100g)
Energy	kJ	1406.60
Carbohydrate	g	86.60
Protein	g	5.30
Fat	g	0.06
Vitamins		
Thiamine B1	mg	2.60
Riboflavin B2	mg	0.80
Nicotinic acid B3	mg	10.10
Folic acid B9	µg	133.30
Vit B6	mg	1.06
Vit B12	µg	1.06
Pantothenic acid B5	mg	5.30
Biotin	µg	160.00
Vit C	mg	46.60
Vit A	µg	533.30
Vit E	mg	8.00
Minerals		
Sodium	mg	506.60
Potassium	mg	220.00
Calcium	mg	78.60
Magnesium	mg	333.30
Zinc	mg	5.30

For the purposes of the experiment, the above described product was used, but modified to include medium-chain triglycerides to the value of 31% of the total energy value (protein 3%- and carbohydrates 66% of the total energy value). The nutritional details of a 20g dose (ingested during the first two weeks of supplementation), as well as a 40g dose (ingested during the remaining five weeks of supplementation) are shown in Table 2.

Table 2: Nutritional information Supplement I (SI) (medium-chain triglyceride + carbohydrate) per 20- and 40g dose

NUTRITIONAL INFORMATION		SUPPLEMENT I	
		20g	**40g**
Moisture %	g	0.40	0.80
Energy	kJ	368.00	736.00
Protein	g	0.74	1.48
Total fat	g	2.97	5.94
Total carbohydrate	g	14.54	29.08
Calcium	mg	11.40	22.80
Iron	mg	0.13	0.26
Magnesium	mg	61.20	122.40
Potassium	mg	64.00	128.00
Sodium	mg	106.40	212.80
Zinc	mg	0.87	1.74
Vit A	IU	684.00	1368.00
Thiamine B1	mg	0.28	0.56
Riboflavin B2	mg	0.36	0.72
Nicotinic acid B3	mg	4.20	8.40
Vit B6	µg	0.34	0.68
Folic acid B9	µg	41.60	83.20
Pantothenic acid B5	mg	0.96	1.92
Vit C	mg	12.60	25.20
Vit E	mg	3.80	7.60

Bergabest MCT-oil (Berg + Schmidt, GmbH & Co., Hamburg, Germany) was used in the supplement. The product details and composition of the MCT-oil is shown in the following table:

Table 3: Bergabest MCT-oil composition

	BERGABEST MCT-OIL	
Typical Data	Saponification value	325-345
	Acid value	<0.1
	Iodine value	<1
	Water (Karl Fisher)	<0.2%
	Peroxide value meq/kg	<1
	Viscosity (20%) mPa.s	25-33
	Density (20%) g/ml	0.930-0.960
	Hydroxyl value	max. 5
Fatty acid composition (%)		
	C-6	<2
	C-8	50-56
	C-10	30-45
	C-12	<3

The quantity of MCT-oil included in the supplement was at the level of 15% m/m to be able to present the product in powder form, and to be prepared as a beverage by the athletes. This incorporation was achieved by using an emulsifier, Dimodan PV (Grindsted Products A/S, Denmark), initially dissolved in the oil phase, followed by rapid high speed mixing of the ingredients such that a soft granular powder was obtained.

Dimodan PV is a distilled monoglyceride made from edible, refined, hydrogenated soya bean oil. Product data is shown in Table 4.

Table 4: Chemical and physical data - Dimodan PV

DIMODAN PV Chemical and physical data	
Monoester content	min. 90%
Fatty acid composition, typical	
Palmitic acid	11%
Stearic acid	88%
Iodine value	max. 2%
Free glycerol	max. 1%
FFA	max. 1.5%
Melting point	approx. 70°C

In addition, the normal product (ENERGO) contains L-carnitine, but for the purpose of this experiment the L-carnitine was omitted from Supplement I. L-carnitine was however included in Supplement II as described below.

5.2 Supplement II (SII) - Medium-chain triglycerides + carbohydrates + L-carnitine

Supplement II was formulated to contain exactly the same ingredients as Supplement I. In addition L-carnitine was incorporated in the form of a commercially available product, Carnesium* (Omeara (Pty) Ltd, Pretoria SA. Patent Application no: 94/3003). Each 2.5ml Carnesium* contains:

Magnesium chloride	730mg
L-carnitine	200mg

in the presence of a complex of L-arginine monohydrochloride and glycine.

To maintain the same nutrient value, the dose of Supplement II was 22.5g. Each 22.5g of supplement contained 2.5ml Carnesium* (20g MCT + CHO + 2.5ml Carnesium*, with 2.5ml ≡ 2.5g).

Carnesium* was incorporated in the first stage of blending (oil and emulsifier), followed by high speed mixing of the powder ingredients to again obtain a soft granular powder.

The nutritional information of a 22.5g dose (ingested during the first two weeks of supplementation), as well as a 45g dose (ingested during the following five weeks of supplementation) is shown in Table 5.

Table 5: Nutritional information of Supplement II (medium-chain triglycerides + carbohydrates + L-carnitine) per 22.5- and 45g dose.

NUTRITIONAL INFORMATION		SUPPLEMENT II	
		22.5g	45g
Moisture %	g	1.61	3.22
Energy	kJ	360.00	720.00
Protein	g	0.73	1.46
Total fat	g	2.90	5.80
Total carbohydrate	g	14.22	28.44
L-carnitine	mg	200.00	400.00
Calcium	mg	11.22	22.44
Iron	mg	0.13	0.26
Magnesium	mg	793.32	1586.64
Potassium	mg	62.48	124.96
Sodium	mg	104.06	208.12
Zinc	mg	0.85	1.70
Vit A	IU	669.00	1338.00
Thiamine B1	mg	0.28	0.56
Riboflavin B2	mg	0.35	0.70
Nicotinic acid B3	mg	4.10	8.20
Vit B6	µg	0.33	0.66
Folic acid B9	mg	40.70	81.40
Pantothenic B5	mg	0.94	1.88
Vit C	mg	12.30	24.60
Vit E	mg	3.71	7.42

6. Statistical analysis

Computorised statistical analyses were performed using Statistix Version 4.1 ((c) 1985, Analytical Software). The Paired T-test, and the Wilcoxon signed rank test were used to analyse the data.