HIGH LEVEL MEDIUM CHAIN TRIGLYCERIDE FEEDING, ENERGY EXPENDITURE AND SUBSTRATE OXIDATION IN COLLEGE-AGED, NON-OBESE WOMEN

ELENA ALEXANDROU

NOTE TO USERS

HIGH LEVEL MEDIUM CHAIN TRIGLYCERIDE FEEDING, ENERGY EXPENDITURE AND SUBSTRATE OXIDATION IN COLLEGE-AGED, NON-OBESE WOMEN

by

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Thesis Abstract

This thesis assessed how short term feeding of high levels of dietary medium triglyceride (MCT) affected energy expenditure and postprandial substrate oxidation rates in females. Eight healthy, non-obese (BMI = 21.5 ± 0.7 kg/m2), college aged (22.6 ± 0.5 y) volunteers were fed both a MCT-rich and an isocaloric LCT-rich diet for two 1 week periods separated by 14 d. The energy content in each diet was 45% carbohydrates, 40% fat and 15% protein. The 2 diets had either 60.81% or 1.11% of total fat energy from MCT with the remaining fat energy intake from LCT. On days 1 and 7 of each diet, basal metabolic rate (BMR) and postprandial energy expenditure (PEE) were measured by indirect calorimetry with a ventilated hood. Results on days 1 and 7 showed no significant differences between diets for BMR or mean PEE. Fat oxidation for the MCT-rich diet was significantly greater ($0.0001 \leq p \leq 0.04$) than that for the LCT-rich diet at different time points across the 5.5 h postprandial period both on day 1 and 7 of feeding. It followed that postprandial carbohydrate oxidation rates on days 1 and 7 were significantly lower ($0.0005 \leq p \leq 0.03$) for the MCT-rich vs. the LCT-rich diet. In conclusion, relative to values for a LCT-rich diet, a MCT-rich diet gave a significantly increased postprandial fat oxidation rate but no effect on BMR or mean PEE. The results support that increasing proportions of MCT in the diet may help give a negative fat balance for women.

Acknowledgements

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My whole family, close friends and colleagues deserve much credit for putting up with me throughout my graduate life. I am deeply indebted to my mother Maria, not only financially, but for her constant encouragement and moral support. I dedicate this thesis to her, without her unconditional love and affection, I could not have made it this far.

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List of Definitions and Abbreviations

β (beta)-oxidation: The series of enzymic reactions that oxidizes fatty acyl-CoA esters and shortens them by removal of the C-terminal two carbon atoms as acetyl-CoA. More narrowly, it is the oxidation of a compound, such as a fatty acid, at the β-carbon.

Acyl-enzyme: An intermediate in the hydrolysis of substrates by some peptidases and esterases, e.g. by serine proteinases, in which the acyl moiety of the substrate is transiently attached to a serine hydroxy group of the enzyme.

Acyl transferases: Enzymes of the transferase class that catalyze the transfer of an acyl group from a donor (often the corresponding acyl coenzyme a derivative) to an acceptor compound.

Albumin: Originally, a protein that is soluble in salt-free water and that will coagulate when heated; also the principal protein of plasma or serum.

Basal Metabolic Rate (BMR): The rate of oxygen consumption by a person at rest which represents the energy required for essential functions such as respiration, circulation, maintenance of temperature, after 10-12 hour fast with no activity.

Bile acids: Steroid based acids found in bile; e.g., taurocholic and glycocholic acid's, used when biliary secretion is inadequate and for biliary colic. Their physiological roles include fat emulsification. Their synthesis is reduced in disorders of the peroxisomes.

Bile salts: The salt forms of bile acids; e.g., taurocholate, glycocholate

Carnitine acyltransferase I and II: Acyltransferases in the inner mitochondrial membrane that catalyze the reversible transfer of acyl groups from acyl-CoA to l-carnitine and thereby mediate the transport of activated fatty acids through that membrane.

Doubly labeled water: ($^2H_2{}^{18}O$) is an analytical method used to measure the rate of CO_2 production and an estimate of daily 24h energy expenditure in free-living animals as well as in humans under non-restrained conditions.

Enteral: A method of nutrient delivery where fluid is given directly into the gastrointestinal tract.

Fatty acyl-CoA ligaze (or AcylCoA ligase): the enzyme that hydrolyzes the first reaction during a β-oxidation. This enzyme is located in the outer membrane of the mitochondria. This reaction is the activation of a fatty acid that results in the production of a fatty acyl CoA molecule. A thioester bond is formed between coenzyme A and the fatty acid.

Gravimetric-relating to chemical analysis involving the measurement of the weights of substances used in and produced by a chemical reaction.

Hormone-sensitive lipase: an enzyme whose function of fat hydrolysis is altered depending on particular hormone concentration, i.e. in the presence of insulin, the action of the lipase is decrease for a net outcome of triglycerol synthesis and not lipolysis.

Isomerase: One of a class of enzymes that rearrange the bonds of their substrates, e.g. an epimerase.

Ligase: One of a class of enzymes that join two substrate molecules in energy- (usually ATP-) dependent reaction, e.g. an amino acyl-tRNA synthetase, a carboxylase; in molecular biology, an enzyme that attaches the 3'-end of one polynucleotide to the 5'-end of another.

Lipase: An enzyme that catalyses the hydrolysis of fats (monoglycerides, diglycerides and triglycerides) to glycerol and fatty acids. Calcium ions are usually required.

Medium-chain acyl-CoA synthase: an enzyme which catalyzes the formation of acyl-CoA molecules. Also called Medium-chain acyl-CoA ligase.

Monoacylglycerol lipase: An enzyme that catalyses the hydrolysis of glycerol monoesters of long-chain fatty acids.

Parenteral: Not through the alimentary canal but rather by injection through some other route, as subcutaneous, intramuscular, intraorbital, intracapsular, intraspinal, intrasternal, intravenous, etc.

Parenteral Nutrition: Provision of food other than orally, usually by intravenous infusion. (Origin: Gr. Enteron = intestine).

Postprandial: Occurring after a meal; postcibal.

Protein Kinase A (PKA): An enzyme that uses ATP to phosphorylate a group on a protein, e.g. a serine, threonine or tyrosine hydroxy group. e.g. phosphorylating hormone-sensitive lipase makes the actual protein (enzyme) active, which in turn hydrolyzes lipids.

Tricarboxylic Acid Cycle (TCA): The metabolic pathway in which acetyl-CoA is catalytically oxidized to carbon dioxide, with the concomitant reduction of NAD^+ and FAD via a series of tricarboxylic (citric, cisaconitic and isocitric) and dicarboxylic (succinic, fumaric, malic and oxaloacetic) acids. The actual carbon atoms, that appear as

CO_2 after a single passage through the cycle, are not identical to those that entered as acetyl-CoA. Also known as the citric acid cycle or the Krebs cycle.

Thermic Effect of Feeding: The net difference in energy expenditure between basal levels and post-prandial energy expenditure levels for the duration of the elevation in metabolic rate following a meal.

Chapter 1 Thesis Overview

1.1 Thesis Introduction and Overview

The effects of different types of human dietary fats intake were investigated in this thesis. Specifically, feeding medium chain triglycerides (MCT) or long chain triglycerides (LCT) were tested for their effects on basal metabolic rate, postprandial energy expenditure and substrate oxidation in college-aged women. It was therefore important to provide a biochemical overview as well as a literature review on this topic in Chapter 2 of this thesis. The different metabolic pathways of MCT and LCT are described in the literature review and previous studies reviewed identified a gap in the literature (2, 4, 8, 9). That is that high levels of medium triglycerides have not been examined for their effects on basal metabolic rate, postprandial energy expenditure and substrate oxidation in college-aged or pre-menopausal women (1-9). At the conclusion of Chapter 2 a brief rationale is given for the research hypothesis for this thesis. Following this the testable questions examined in the thesis are given.

Chapter 3 includes the study conducted in this thesis. College-aged females were fed for two separate one week periods with diets rich either MCT or LCT. The two treatments diets were separated by a 2 week wash out period. The two diets were assessed for their effects on basal metabolic rate (BMR) and postprandial energy expenditure (PEE). In addition, substrate oxidation in the postprandial period was assessed between diets. The results from this study add to the evidence in the literature on MCT feeding and energy balance. This is since we assessed effects of higher levels of

medium chain triglycerides feeding on college-aged women's energy expenditure than previously reported (2, 4, 8, 9).

Chapter 4 is an overall summary of the study. It includes the response to the research hypothesis and testable questions listed in Chapter 2, along with some possible real world implications of the results.

1.2 Co-Authorship Statement

i) Design and identification of the research proposal

Elena Alexandrou and Dr. Matthew D. White were responsible for the study design and identification of the research topic. During the initial stages of identification of the research topic. Dr. Gene Herzberg was consulted for his expert advice on the proposed study.

ii) Practical aspects of the research

The data collection for the study in this thesis was primarily by Elena Alexandrou with assistance from Pamela Kirkland who was the dietitian for the study. Gene Herzberg, graduate co-supervisor, kindly donated the equipment use for the GLC analysis to allow assessment of the fatty acid composition in each diet employed in the study. He made a significant contribution to the identification of the proper analytical method for the fatty acid analysis.

iii) Data analysis

Elena Alexandrou and Dr. Matthew White worked together to complete the data and statistical analysis for the study in this thesis.

iv) Manuscript preparation

Elena Alexandrou prepared the first draft of the manuscript and each section of this thesis. Dr. White gave feedback and corrections on the manuscript and each section of the thesis.

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Chapter 2 Literature Review

2.1 Overview

Humans, like any other living organism, need energy to accomplish all of their functions and to stay alive. Food intake is the source of energy, and it can be in the form of carbohydrate, protein and fat. These three energy substrates together form the macronutrients. After their digestion and assimilation, macronutrients are subsequently oxidized to provide energy for cellular work and to allow synthesis of adenosine triphospate (ATP) or other high energy phosphate containing compounds. Energy not chemically retained in the body is either given off as heat, that helps maintain body temperature, or it is used for external work. The energy expended by the human body can be estimated by indirect calorimetry, direct calorimetry (66) or by doubly labeled water (60). For measurement of energy expenditure the method of indirect calorimetry is often used since it allows for estimates of macronutrient oxidation rates. Together with a detailed analysis of food macronutrient composition, the macronutrient balances can be used in the study of weight maintenance and obesity.

In general, as demonstrated by J-P Flatt (21), high-fat diets increase body size and tissue gain. Energy balance can also be influenced by dietary lipid composition (30, 48). This was demonstrated by comparing rats fed diets providing either a low essential fatty acids (EFA) intake of 0.3% of the energy intake or a high EFA energy intake of 10% of the energy intake. Rats fed the high EFA diet developed lower body than in rats fed a low EFA diet. This suggested that the animals fed high amounts of EFA are in a state of decreased metabolic efficiency, i.e. less energy consumed is stored and more was given

off as heat. (44). The same decrease in body weight through increased thermogenesis due to food intake, or thermal effect of feeding (TEF) is also observed by other type of fatty acids, such as medium chain fatty acids (4). As such, fatty acid composition of the diet is one strategy that can be taken to treat obese individuals that display increased risk of developing the metabolic syndrome or other diseases of affluence.

The following brief overview of lipid metabolism aims to provide the needed background to understand Medium Chain Triglyceride (MCT) metabolism, as it relates to energy and macronutrient expenditures. This allowed the development of the rationale of this thesis review and the development of the research hypothesis and the testable questions addressed in the study in this thesis.

2.2 Metabolism of Lipids and Glycerol

Lipids, or fats refer to a member of the group of biological molecules of varying composition that are classified together on the basis of their solubility in nonpolar solvents (3, 16). This classification includes four major groups, being fatty acids, either saturated or unsaturated, glycerides or glycerol containing lipids, non-glyceride lipids, including shingolipids, steroids, waxes, and finally complex lipids or lipoproteins (16). Lipids are found in all cells and perform a variety of functions that are all vital for life. These include that lipids are the predominant energy source and means of energy storage in the human body (62). Oxidation of 1 gram of fat typically releases ~9 kcal of energy, and when the demand for energy is accomplished, excess fat gets mainly stored as

triglycerides in adipose tissue, adipocytes. In addition to triglycerides the other three types of lipids including phosphoglycerides, sphingolipids, and steroids serve mostly as cell membrane structural components. Most often the fat in foods contains long chain triglycerides (LCT) with fatty acid chains that contain greater than 12 carbons. They are comprised of a glycerol backbone, esterified with three fatty acids. Of particular interest for this thesis is a subset of these triglycerides called the saturated MCT, which are 6 to 12 carbons in length. The metabolism of MCT is distinct from other fatty acids and they have been employed both in fat malabsorption (7, 34, 49), energy balance studies (7, 18, 21, 22, 34, 67) and clinically in parallel applications (28, 54, 72). This is because MCT are quickly digested, absorbed and oxidized. This is relevant for individuals with fat malabsorption and this characteristic of MCTs makes them potentially useful in treatment of obesity.

2.1.1 LCT Digestion

The digestion of triglycerides is an aqueous catabolic process, whereby the structurally complex lipids of the diet are converted into smaller absorbable units by pancreatic enzymes, collectively called triacylglycerol lipases, or abbreviated as TAG lipases (9). Due to the lipid insolubility in water, triglycerides must undergo several transformations before being digested and absorbed. Bile salts are cholesterol derivatives (32), secreted by the gall bladder to help emulsify the long chain triglycerides (LCT) (31, 32, 57). Upon ingestion, LCTs interact with bile in the duodenum and are incorporated into mixed micelles (31, 32, 57, 58). This lipid emulsion prevents the fat droplets from

fusion and thereby increases the surface area available for attack by pancreatic lipases. Pancreatic lipase and phospholipase A2 are the enzymes responsible for the breaking down of the LCTs and removal of the fatty acid molecules from the glycerol backbone (31). Pancreatic lipase hydrolyzes the long chain triglycerides into 2-monoglycerides and two free fatty acids, by attacking 3 and 1 positions of the glycerol backbone (32). These almost water-insoluble products are carried into the interior of water-soluble micelles, formed by bile salts. The micelles containing fatty acids and monoglycerides are transported to the luminal surface of the small intestine epithelial cells (32, 58) and then are passively absorbed into the intestinal mucosa, where the free fatty acids are re-esterified with glycerol (31, 32, 57). The intestinal mucosa synthesizes a lipoprotein carrier called a chylomicron to transport the reformed triglyceride (63). Chylomicrons are secreted into the lymph and are released into the venous circulation (Fig. 2-1) via the thoracic duct (8) .

2.1.2 LCT Absorption:

After leaving the micelles and passively diffusing through the luminal membranes, the monoglycerides and free long chain fatty acids are resynthesized into triglycerides in the epithelial cells (57). These triglycerides, containing long chain fatty acids, aggregate and are coated with a layer of lipoprotein to form water-soluble chylomicrons (Fig 2.1), which are pushed out through the basal membrane of the cells by exocytosis (57). Chylomicrons are unable to cross the basement membrane of blood capillaries, so instead they enter the lymphatic vessels. Chylomicrons subsequently

undergo intravascular hydrolysis to yield most of the LCFA to extrahepatic tissues, while the rest are transported to the liver (8). In the bloodstream, lipoprotein lipase again breaks down the triglycerides into two free fatty acids and a monoglyceride. The monoglycerides go to the liver to be further degraded, while many of the circulating free fatty acids (9) are taken up and stored in adipocytes (Fig. 2-2).

2.1.3 MCT Absorption

On the other hand, since MCTs are more hydrophilic than LCTs so they are more easily absorbed, not requiring a prior hydrolysis. MCT are hydrolyzed in the gut or can be absorbed intact and do not require the action of pancreatic lipase or incorporation into chylomicrons. Instead, a lipase within the intestinal mucosal cell degrades the MCT into free fatty acids and glycerol (Fig. 2-1). The released free medium chain fatty acids are bound to hepatic albumin and released into the bloodstream (18), and transported directly for oxidation to the liver by the portal vein (43, 57) (Fig. 2-1). As such, MCTs are digested and absorbed much faster than LCTs and they are immediately available for energy (8, 54, 57). MCT availability as an energy source after consumption follows a similar time frame as glucose (13).

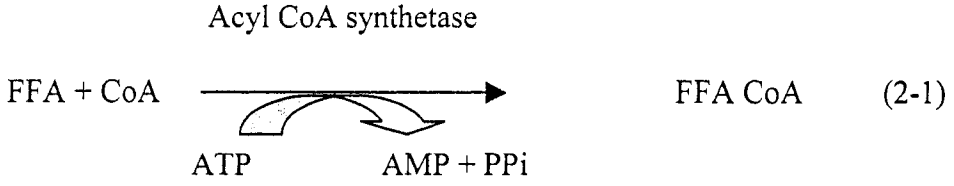
The vast majority of MCFAs are transported directly to the liver and oxidized (8). Fatty acids with short- or medium-length carbon chains can enter the blood via the hepatic portal system (43, 57), but typically very few of these are eaten in the normal diet (57). As such, very little of the MCFAs escape the liver to reach the general circulation

(7). This is evident since only 1-2% of MCTs are incorporated into depot fat in adipocytes (4, 25). It is for these reasons that MCTs are a potential alternative energy source for individuals needing to reduce deposition of lipids in adipose tissue.

2.1.4 Transport and Mobilization of LCT and MCT

Medium and Long chain fatty acids have distinct transport systems. Longer fatty acids are more likely to be found in the lymph (57), circulating as chylomicron-TG; whereas shorter fatty acids are more likely to be found circulating in the portal blood (27), usually bound to albumin (7). Fatty acids from the diet are delivered from the gut to somatic cells via transport in the blood. Fatty acids are stored in the form of triglycerides, primarily within adipocytes of adipose tissue. In response to energy demands, the fatty acids of stored triglycerides can be mobilized for use by peripheral tissues. Mobilization of metabolic energy, in the form of fatty acids, is controlled by a complex series of interrelated cascades that result in the activation of hormone-sensitive lipase (62). The stimulus to activate this cascade, i.e. to mobilize lipids, in adipocytes, can be glucagon, epinephrine or β-corticotropin (62). To initiate these cascades, these hormones bind to cell surface receptors that are coupled to the activation of adenylate cyclase upon ligand binding. The resultant increase in cAMP leads to activation of Protein Kinase A (PKA), which in turn phosphorylates and activates hormone-sensitive lipase (62). This enzyme hydrolyzes fatty acids from carbon atoms 1 or 3 of triacylglycerols. The resulting diacylglycerols are substrates for either hormone-sensitive lipase or for the non-inducible enzyme diacylglycerol lipase. Finally the monoacylglycerols are substrates for monoacylglycerol lipase (62). The net result of the action of these enzymes is three moles

of free fatty acids and one mole of glycerol (62). The free fatty acids diffuse from adipose cells, combine with albumin in the blood, and are transported to other active tissues. Then fatty acids passively diffuse into active tissue cells where they are oxidized. In contrast to the hormonal activation of adenylate cyclase and hormone-sensitive lipase in adipocytes, the mobilization of fat from adipose tissue is inhibited by numerous stimuli (62). The most significant inhibition is that exerted upon adenylate cyclase by insulin (62). When carbohydrates are consumed, insulin is released, which stimulates adipocytes to re-esterify the fatty acids into triglycerides and store them as fat. In general, body fat stores are not mobilized and used for energy to any significant extent in the presence of insulin (62). Therefore, when an individual is in a well-fed state, insulin released from the pancreas prevents the inappropriate mobilization of stored fat. Instead, any excess fat and carbohydrate are incorporated into the triacylglycerol pool within adipose tissue (62). Long chain fatty acids must be activated in the cytoplasm before being oxidized in the mitochondria, or in peroxisomes. Activation is catalyzed by fatty acyl-CoA ligase (also called acyl-CoA synthetase or thiokinase). The net result of this activation process is the consumption of 2 molar equivalents of ATP.



(2-1)

Medium- and long-chain fatty acids are not transported to the mitochondrial matrix in the same manner. The hydrophobic properties of LCFA require specific transport systems for their intracellular delivery. Cytosolic proteins, the fatty acid-binding proteins, transport these fatty acids from the cell membrane to the target organelle (8, 64). The transport of fatty acyl-CoA into the mitochondria is accomplished via an acyl-carnitine intermediate, which itself is generated by the action of carnitine acyltransferase I (7), an enzyme that resides in the cytosolic side of the inner mitochondrial membrane. The acyl-carnitine molecule then is transported into the mitochondria where carnitine acyltransferase II catalyzes the regeneration of the fatty acyl-CoA molecule (62) (Fig. 2-3).

On the other hand, the water soluble MCFA are minimally bound to fatty acid-binding proteins (45) and therefore MCFA oxidation is considered carnitine-independent (7, 14, 70). The activation of the MCFA can occur both in the cytoplasm, and in the mitochondrial matrix, where a medium-chain acyl-CoA synthase is found (1). The extramitochondrial activation of MCFAs is however rare, since MCFA can easily pass across mitochondrial membrane as free fatty acids and be activated to their acyl-CoA esters in the mitochondrial matrix prior to mitochondrial β-oxidation (1). Therefore MCFAs are almost never activated (Fig. 2-3) in the extramitochondrial space (14).

2.1.5 LCFA and MCFA β-Oxidation:

The process of fatty acid oxidation is termed β-oxidation since it occurs through the sequential removal of 2-carbon units by oxidation at the β-carbon position of the fatty acyl-CoA molecule (15). Each round of β-oxidation produces one mole of NADH, one mole of $FADH_2$ and one mole of acetyl-CoA. The acetyl-CoA, the end product of each round of β-oxidation, and then enters the TCA cycle, where it is further oxidized to CO_2 with the concomitant generation of three moles of NADH, one mole of $FADH_2$ and one mole of ATP (19). The NADH and $FADH_2$ generated during the β-oxidation and acetyl-CoA oxidation in the TCA cycle then can enter the respiratory pathway for the production of ATP (Fig. 2-2). It is therefore clear that the lesser the number of carbons in MCFAs provide less energy than LCFAs. The acetyl-CoA molecules can also follow other metabolic pathways, such as ketogenesis (Fig 2-4), elongation of fatty acids in the mitochondria, or could be used in *de novo* synthesis of fatty acids and cholesterol in the cytosol (7). Although the process of β-oxidation is the same for long- and medium-chain fatty acids, oxidation rates and hormonal regulation of the respective oxidations differ. In addition, the effect due to the presence/absence of glucose differs in such a way to increase energy expenditure, as discussed in below in part 2-3.

Ketogenesis, being the metabolic production of ketones or ketone bodies, is more prevalent on an MCT diet (Fig 2.4), due to the MCT faster and more complete oxidation than LCTs (7). The ketogenic effect of MCTs (6, 23, 71) was found not to be reversed by the classic antiketogenic agents, such as glycerol and lactate (41).

The pathway for the formation of ketone bodies starts with the reversal of the last step in fatty acid β-oxidation. The inadequacy of oxaloacetate, the needed intermediate for the catalysis of last step in the Krebs Cycle, shifts the reaction to the fusion of two molecules of acetylCoA to produce one molecule of Acetoacetyl CoA (15), which in turn will be converted to ketone bodies (Fig. 2-4). The formation of ketone bodies occurs in hepatic mitochondria, as a result from over production of AcylCoA formed during β-oxidation of fatty acids. This endogenous formation was found to occur when the acetoacetate/β-hydroxybutyrate ratio is greater than one (19).

2.1.6 Glycerol Oxidation

Glycerol is metabolized to an intermediate (glyceraldehyde-3-phosphate), which can then undergo glycolysis. In glycolysis the glycerol is converted to pyruvate, which can be oxidized to acetylCoA. The later can then enter the citric acid cycle or be used to synthesize more Glucose (Fig. 2-5). Glycerol is very rapidly absorbed via the portal system and the extent of this absorption was found to influence the triglyceride hydrolysis (9). Bergstrom et al. concluded that free glycerol from the hydrolysis of long-chain triglycerides in the intestine is not reutilized for triglyceride synthesis (9).

2.2 Different Metabolic Fates for LCTs and MCTs

MCFAs are more readily oxidized and thought to aid in weight loss or "slimming". The "slimming hypothesis" is based on the following data: a) MCTs

provide lower energy density, 8.3 kcal per gram, compared to LCTs (9 kcal per gram) (7, 8), b) have rapid intra-hepatic delivery (27), and oxidation rates (54), and c) show poor adipose tissue incorporation (Fig. 2-7). Several studies examined whether EE translates into decreased fat mass due to increase energy expenditure (4, 25, 35) relative to LCT feeding. A decrease in the fat depot (4, 25, 35) and a decrease in the adipocyte size (4) reinforce the implication that MCT feeding will help reduce obesity rates (18, 51, 56, 61) (Fig. 2-2).

Natural sources of MCTs include variety of foods but coconut oil is the predominate food source (7). Some MCTs are found in human milk and milk products (7). Since the MCT introduction in the 1950's for the treatment of fat malabsorption problems, these triglycerides have been applied in enteral and parenteral nutrition regimens (7). Before the availability of MCT lipid emulsions suitable for intravenous use, glucose was used as the only nonprotein source of calories (37). Not only did this result in essential fatty acid deficiencies, but it increased hepatic lipogenesis (43) and respiratory work. The result of this was increased respiration to eliminate the excessive carbon dioxide produced during the increased lipid oxidation (37). Increased lipogenesis could lead to hyperlipidemia (7), therefore different emulsions were tested, such as inclusion of LCTs, instead of glucose in intravenous feeding. This approach represented an improvement in regard to the lipogenic effect by glucose feeding, but problems remained, such as the slow clearance of LCTs from the blood circulation (7, 37). Several studies showed by replacing LCTs with MCTs the slow plasma clearance problem was

improved (20, 26, 38). Many studies of human MCT feeding (29, 40) and rat MCT feeding (4, 13) agreed with this finding. In addition, MCT feeding increases energy expenditure compared with other fat feeding (18, 56, 67).

2.3 Studies Using MCT Feeding for Increasing Energy Expenditure.

In agreement with other studies, Hill et al. (29) showed that the MCT/LCT ratio within a diet plays an important role in the thermic effect of feeding. A more recent study (18), conducted in a 24-h whole body respiratory chamber, showed that low to moderate intake of MCT may be a contributing factor in controlling human body weight and enhancing energy expenditure. The study examined a dose-response feeding on 24-h energy expenditure and substrate oxidation in eight healthy young men, on four different occasions. The subjects were fed a total of 30 grams of fat that varied in its proportions of MCT and LCTs. They were randomized between the four different combinations of MCT and LCT supplements in a double-blind method. Their results indicated that 24-h EE of normal-weight men was significantly higher, by about 5% or increased by ~500 kJ, with the diet rich in MCT, relative to a diet rich in LCT. White et al, (67) found a similar level of increase in EE, but focused on basal metabolic rate and thermic effect of feeding. Both studies used diets composed of the same proportion of macronutrients, of 15% protein, 40% of the dietary fat and 45% carbohydrates. White et al. (67) had about one quarter of the 40% of fat in the diet comprised of MCTs. They investigated if fatty acid chain length influences energy expenditure (EE) and substrate oxidation, in twelve, non-obese, premenopausal women. They reported a MCT-diet induced increase in BMR and

postprandial energy expenditure, which was observed at day 7, but had diminished by day 14 of their two week feeding period (67).

Several studies have supported the increase in energy expenditure (13, 18, 46, 67) due to MCT-rich diet intake and focused on different components of EE. Most studies examine basal metabolic rate and thermal effect of feeding during MCT feeding. Increased metabolic clearance for MCTs has been documented (24) and was observed in 28 cirrhotic patients intravenously administered medium chain triglycerides, as a physical mixture of equal portions of 20% MCT and 20% LCT emulsions (20). The patients were compared with 9 healthy individuals as the control group. In this study, intravenous fat tolerance was performed to determine the clearance rate of MCT/LCT mixture. Their results indicated increased serum free fatty acid concentrations, suggesting an increase in metabolic oxidation and therefore basal metabolic rate in both groups. It was therefore concluded that the ability to eliminate (metabolize) the MCT/LCT mixture is seen even in cirrhotic patients.

Baba et al (4) studied the mechanism whereby MCT overfeeding can be correlated with decrease in body weight. Rats were overfed for six weeks with diets with 50% of the total energy expenditure deriving from fat, MCT or LCT. Body weight and fat deposition along with resting metabolic rate were some of the variables the investigators collected. They (4) concluded that metabolic rate was higher for the rats fed

with the MCT diet, where fat deposition along with resting metabolic were higher for the LCT fed rats.

Papamandjaris et al (47) conducted a study comparing the effects on exogenous and endogenous fatty acid oxidation with the use of ^{13}C-labeled long chain fatty acid, following an isotope tracer methodology. Twelve young women were fed with either LCT-rich or MCT-rich diets (~25.6% of total fat) for two weeks, followed by two week washout period. Main results indicate that there is an increase in endogenous long chain fatty acid oxidation in women fed MCT-rich diets. This result implies an increase in metabolic rate, which in turn increases the energy expenditure of those individuals. The same group (46) also investigated the components of total daily energy expenditure on the second week of feeding for the same study using doubly labeled water. Their results suggest that TEE is not affected by the type of diet after day 7 and thus the increase seen in energy expenditure following MCT diets is attenuated by day 14 (46). The study was limited since energy expenditure estimated by doubly labeled water may have been not precise enough to detect the increases in energy expenditure that is reported by studies with indirect calorimetry (18, 30, 67).

White et al (67) studied changes in energy expenditure for women fed MCT for 14 days. On day 7 of the study total energy expenditure (TEE) was increased, but by the day 14 the differences in TEE between MCT and LCT were no longer evident. After a breakfast on day 7 (67), the postprandial energy expenditure was significantly greater

with the MCT than with the LCT diet at different times. Their results included differences in RQ values, which are indicated increased rate of fat oxidation for the MCT diet relative to the LCT. The authors concluded an increase in energy expenditure of ~6% of typical daily intake 2500 kcal, along with increases in BMR, postprandial energy expenditure and fat oxidation in non obese women (67).

Many other studies investigated the positive effects of medium chain triglyceride feeding on postprandial energy expenditure (4, 5, 30, 35, 48). Their findings of increased thermal effect of feeding after the feeding of medium-chain triglyceride diets, has been confirmed by other studies. Hill et al (30) hypothesized that the high rate of energy expenditure produced by MCT overfeeding was due to hepatic *de novo* synthesis of long chain fatty acids (LCFA) from the excess medium chain fatty acids. They tested this hypothesis by overfeeding MCT and LCT and comparing blood lipid profiles in 10 non-obese male patients, who were overfed two formula diets for 6 days. Excess dietary MCT was thought to result in de novo synthesis of hepatic LCFA from MCFA. This process could help account for the higher rate of postprandial energy expenditure with MCT as compared to LCT feeding.

Mascioli et al (39) investigated the thermogenic effects of MCT feeding on eighteen hospitalized patients dependent on total parenteral nutrition (TPN). Patients were compared during intravenous infusions of long-chain triglycerides (LCT) relative to an infusion of 75% MCT and 25% LCT. Resting energy expenditure, as an estimate of fat

oxidation, was shown to increase during MCT infusion but not during LCT administration. They concluded that TPN consisting of MCT causes increased energy expenditure, without an increase in body temperature, most likely through increased fat oxidation. The calculated fat oxidation rose from 10.7±1.5 to 19.3±2.4 kcal/m^2 BSA/hr, reflective of MCT's property as an obligate fuel (39).

The same year Scafi et al (51) concluded that a similar effect is seen in both obese (n=6) and lean (n=6) individuals when LCT diet is replaced with MCT. The researchers examined postprandial thermogenesis (PPT) after the ingestion of mixed meals by thin and obese subjects containing either 38 g long-chain triglycerides (LCTs) or 30 g MCTs plus 8 g LCTs. Postprandial thermogenesis, evaluated as 6-h incremental areas above resting metabolic rate, was significantly greater in both subject groups after meals containing MCTs. The thermic effect of MCTs was found to be especially greater in the obese group, who had a PPT of 144.7± 48.8 kJ/6 h, relative to the lean group with a PPT of 119.7 ±33.9 kJ/6 h.

From the results mentioned in this section, it could be concluded that MCT feeding increases EE (13, 18, 46, 67). This increase is potentially partitioned between an elevated basal metabolic rate (4) and thermic effect of feeding (TEF) (4, 39, 40, 46, 51, 67).

2.4 Tolerance of MCT Feeding.

Borum et al (11) indicated that the fatty acid profiles of formula for preterm neonates are not optimal. Optimization of the fatty acid profile in the diet has also been found to be awaiting an improved understanding of the metabolism of fatty acids of all chain lengths in the preterm neonate (11) as well as in adult human diet (46, 67). Gastric emptying is increased when MCT-rich diets are administered (36, 59) and are suggested for patients with gastric delay (59). Jeukendrup et al. (33) reported that MCT ingestion was associated with increased gastrointestinal complaints i.e. intestinal cramping. On the same topic, Megremis (42) reported that diets containing up to 100 grams daily are easily tolerated and that MCT intakes at 40% of total calories have been reported with no negative effects (42). Several researchers (40, 46, 67) studied female metabolic changes due to introduction of MCTs in the diet, but the optimal level of MCT in diet has not been established. This is since the positive effect of MCT on energy expenditure appears to diminished over two weeks of MCT feeding (40, 46, 67). A possible means to prevent this decrease in energy expenditure is to feed subjects levels of MCT closer to maximal tolerable levels. Matsuo et al (40) compared diets of 20% MCT of total energy intake (~ 9 grams of MCT/day), relative to 100% LCT diets, while White et al (67) investigated the effect of a higher level 10.4% MCTs (26% of total fat energy intake, which was 40% of the total energy intake or ~ 29 grams of MCT/day). It appears that higher levels of MCT feeding would still be tolerable based the comments of Megremis (42) .

The effects of rapid plasma clearance, indicative of rapid availability of MCFAs for oxidative process, was evaluated in a study with postoperative patients receiving intravenous infusions of MCT compared with infusions of LCT (50). The patients received fat emulsions at 1.0 g/kg per day, in the presence of 80% of the basal requirement of non-protein calories. Results of this study (50) suggest that the MCT lipids were not associated with any side effects and were rapidly cleared from the plasma and were oxidized without any significant side effect, such as ketosis.

2.5 Review of Relevant Methods

2.5.1 Assessment of Food Energy Intake and Lipid Composition

Bomb Calorimetry

Energy content of a known mass of food can be measured with a bomb calorimeter by direct calorimetry. The observed value is called the physical caloric value (C_{pc}) for a particular food and its units are calories (17). Within the bomb calorimeter, a known quantity of food is combusted and the energy produced is released to the water which surrounds the chamber. The change in temperature of the water is recorded and is used as a measure for the C_{pc} for that type of food. The complete oxidation of food in the bomb calorimeter is a good representation of catabolism in humans. A difference with bomb calorimetry values and physiological caloric values is that macronutrients are completely oxidized in the bomb calorimeter. As such, although the C_{pc} are reliable, they can be an under or over estimate of the physiological caloric values, depending on the digestibility of the given food. For example some indigestible fibers in the diet are not absorbed but still have a C_{pc} value that does not contribute to the energy intake total by the body.

Food Tables

An alternative method of assessment of food energy intake are food tables that are now available as computer programs. Energy content of foods in these food tables are determined by bomb calorimetry. These tables are employed for their convenience and

their accuracy appears justified (2, 10). The additional information provided by the food tables allows the proportions of macronutrients in the diet to be easily established. A main limitation of the food tables is that they are not detailed enough to assess the composition of lipids in the diet. In the context of this thesis the proportions of different fatty acids are not available in these food tables for given lipids.

Chromatography is a method for separating a sample into its different components that depends on the partitioning of a solute between immiscible solvents. Both a stationary and a mobile phase are included in chromatography Several types of chromatography exist. A few of these include Gas Liquid Chromatography (GLC), High Performance Liquid chromatography (HPLC) and Thin Layer Chromatography (TLC). The GLC is a technique particularly valuable for separating volatile organic compounds such as fatty acids.

With GLC organic compounds are partitioned between a flowing mobile gas phase and a stationary phase. The stationary phase in GLC is a material in a separation column of two kinds, packed or capillary. Capillary tubes provide higher separation efficiency and have been used for separation of fatty acids in lipid fraction of human diets (61, 67). The column is kept at constant temperature, or temperatures, and the mobile phase is an inert gas, such as helium, argon or nitrogen that flows at a fixed rate. The sample is injected/introduced into a port maintained a temperature higher than the boiling point of the least volatile component in the sample. As such the components of the sample enter the stationary phase at the same time but compounds with lower vapor

pressure or higher boiling point remain in the stationary phase (i.e. retention time) longer than do compounds with higher solubility. At the outlet of the column the component separation in GLC is with either flame-ionization (destroys the sample) or thermal conductivity detectors (retains the sample). The choice of the detector depends on its selectivity for the type of molecules of interest in the sample. The electrical output from the detector in the GLC is amplified and the signal is sent to a chart recorder or integrator. An integrator quantifies the peak areas on a chromatogram, that is from the baseline to the peaks. The area under each peak is proportional to the quantity of the compound and identification of different peaks on the chromatogram is made by comparison of the peaks to peaks and locations of standards of known composition that are assessed in the GLC. A given sample is separated into fractions with a large number of boiling points, by increasing the oven temperature from a low to a high temperature.

2.5.2 Assessment of Energy Expenditure

There are several techniques to quantify total daily energy expenditure and its components. The components of energy expenditure include sleeping metabolic rate (SMR), the energy cost of arousal, thermic effect of feeding (TEF) or diet-induced energy expenditure (DEE), and the energy cost of physical activity. For convenience, the first two are taken together making up the basal metabolic rate (BMR), which is the main component of the average daily metabolic rate, making up approximately 66% of total energy expenditure (66). The BMR is estimated at rest and any kind of intensive physical activity prior the testing must be prevented. Ideally, the subjects stay overnight in the

laboratory to make sure they do not take any food and they have no vigorous exercise in the hours preceding the measurement. In practice this means measuring BMR in the early morning after an overnight fast and to exclude DEE or TEF, the measurement should take place at least 12 hours after the last meal. Typically the TEF makes up 5 to 10 % of the total daily calories taken consumed. Measuring the TEF is after the feeding of a standardized meal to the subjects and keeping them in a supine position for approximately 5.5 to 6.0 hours. This is since metabolic rate increases after a meal and does not return to pre-meal levels until at ~ 6 hours. The energy cost of physical activity makes up the remainder of the daily total energy expenditure. Several methods of estimating energy expenditure in humans are available for assessing energy requirements, including doubly labeled water, intake balance and calorimetry (55). Each of these plus its advantages and disadvantages/limitations are given below.

Direct Calorimetry:

This is the process of measuring direct heat production, similar to a bomb calorimeter, where water temperature changes due to body heat are measured in a container of water that surrounds an enclosed air-filled space, where the subject is positioned. These are often large, live-in sealed chambers that that give an estimate of total body energy expenditure by calculating heat lost using the specific heat of water and water temperature changes over a fixed period of time. Although the chambers are a direct measure of energy expenditure, they are large, expensive structures to build and maintain. In addition they give no information about macronutrient oxidation rates.

Indirect Calorimetry:

There are both *closed-system* and *open-system* forms of indirect calorimetry as described below.

Closed-system

A typical *closed-system* involves the animal being confined in sealed box, which contains a chemical CO_2 trap (e.g. calcium carbonate). When the animal respires it takes in oxygen and produces CO_2. The chemical trap takes up the expired CO_2. Consequently, there is a change in the internal pressure. If a manometer is fixed to the chamber, the liquid in the manometer will move upwards as the O_2 is consumed, to maintain a constant pressure within the chamber. The declining volume of the chamber, measured from the rate of movement of the manometer fluid multiplied by the bore of the tube, is a measure of O_2 consumption (66). In more refined systems, the movement of the manometer fluid is detected by a sensor, which also regulates inflow of pure oxygen to the chamber to maintain constant chamber gas volume and pressure. The oxygen consumption is then equivalent to the oxygen delivered from the pure oxygen tank. At the termination of an experiment the accumulated CO_2 in the trap can be assessed by the mass change of the trap (66).

Open-Flow Systems

There are several types of open flow systems. One type of *open-flow system* works on a gravimetric method. Atmospheric air is drawn into the system by a pump , is it is then dried and its CO_2 content removed using a chemical trap. The air passing into

the chamber is therefore dry and CO_2-free. In the chamber the animal consumes oxygen, expires CO_2 and also loses water vapor. Water vapor may also be derived from objects inside the chamber, such as the drinking bowl or the food. Downstream of the chamber the air is dried and the CO_2 content of the stream is then chemically trapped, normally using a series of traps to ensure that all the CO_2 has been removed from the system. Below each CO_2 trap is a water trap that captures any water, which has been formed during the process of trapping CO_2. The two CO_2 and water traps downstream of the drier after the flowmeter are weighed after a period of 24 to 48 hours and after that period, the CO_2 traps are reweighed. The increase in mass represents the mass of trapped CO_2, which can be converted into a gas volume using the ideal gas law (PV=nRT).

In another *open-flow system*, the gas analysis can also be done using gas analyzers. Air is pumped into the system the gas is then is dried and its CO_2 content is removed using chemical traps (e.g. silica gel traps). After the drier, the airflow is divided to an oxygen analyzer or the reference channel, where the ambient oxygen content is measured. The remainder of the flow is passed via a flow regulator, which maintains the flow within 1 to 2%, to a sealed chamber containing the animal. The animal consumes oxygen and expires CO_2 and adds water to the airflow. These mixed gases are pushed out of the chamber and are dried again to remove the water added to the system by the animal. The gases then enter sample lines for the CO_2 analyzer and the oxygen analyzer. The difference between the oxygen content in the reference channel and the sample channel is determined. Because oxygen consumption and carbon dioxide production are

measured simultaneously, the system gives a running estimate of the respiratory quotient (60).

Whole Body Indirect Calorimetry

Similar to *open flow system* that employs gas analyzers, a whole body indirect calorimeter (68, 69) can be employed to estimate energy expenditure. The subject is positioned on a large respiratory chamber of ~ 18,000 liters in volume that is slightly below atmospheric pressure. From the chamber samples are continuously taken by means of two pump heads mounted on a motor drive that and are passed through sensor cells of a oxygen analyzer and carbon dioxide analyzers. These analyzers allow differential readings of O_2 and CO_2 concentrations between outgoing air and ingoing air with a sensitivity better than 0.001% (60, 68, 69). The data of flow rate, differential O_2 and CO_2 concentrations, as well as the room temperature and relative humidity are continuously passed through an on-line computerized data acquisition system, and averaged to provide minute-by-minute readings. From the rates of O_2 consumption and CO_2 production, EE using Weir's (65) equation, as well as the respiratory quotient (RQ) are calculated throughout the measurement periods. (18). This allows estimates of 24 h macronutrient oxidation rates and 24 h EE. One advantage is a 24-h energy expenditure that can be closely controlled if a daily activity routine is set up for the subjects in the chamber. Despite these advantages, like the whole body direct calorimeters, whole body indirect calorimeters are costly to build and expensive to operate and maintain. Disadvantages of whole body indirect calorimetry are is it difficult to measure BMR or

impossible to measure TEF since the precision of the overall system is too low. As well, it can take 4 to 6 hours prior to establishing stable values for CO_2 and O_2 values; this is a prerequisite for expression of valid values for of energy expenditure and macronutrient oxidation rates.

Ventilated Hood:

The ventilated hood is another variant of the *open-system* of indirect calorimetry. The subject rests in a recliner armchair with his or her head enclosed in a transparent plastic canopy, with a plastic sheet draped around the neck and shoulders. Air is pulled from the canopy by a pump through a mixing chamber. A second pump that is connected to both a oxygen analyzer and a carbon dioxide gas analyzer periodically draws small samples gases, typically of 150 ml, from the mixing box. The airflow through the hood can be measured with a dry gasmeter or a pneumotach. The flow through the hood is adjusted to 25 and 50 L/min for healthy adults to keep the differences in oxygen and carbon dioxide concentrations between inlet and outlet air within the range of 0.5-1.0 per cent. The ventilated hoods are most useful for measuring basal metabolic rates, postprandial metabolic rates and the thermic effects of feeding. A disadvantage is that the subject must remain inactive for long period with only short washroom breaks allowed.

Macronutrient Oxidation Rates

As mentioned above, a distinct advantage of indirect calorimetry is it allows an estimation of energy expenditure (EE) or heat production and amounts carbohydrates (C),

protein (P) and fat (F) that are oxidized. Brouwer (12) published equations to allow these estimates that are based on measurements of oxygen consumption (VO_2), carbon dioxide production (VCO_2) and nitrogen loss in the urine. To estimate EE, three equations with three unknowns can be used derive equation 2-5 that gives an energy expenditure.

$$\text{Oxygen consumption} = 0.829\ C + 0.967\ P + 2.019\ F \quad (2\text{-}2)$$

$$\text{Carbon Dioxide Production} = 0.829\ C + 0.775\ P + 1.427F \quad (2\text{-}3)$$

$$\text{Heat production} = 21.1C + 18.7P + 19.6F \quad (2\text{-}4)$$

$$E = 16.20\ VO_2 + 5.0\ VCO_2 - 0.95\ \text{Protein oxidation} \quad (2\text{-}5)$$

In equations 2-2 and 2-3 above, the protein oxidation (P) is calculated is equal to 6.25 times the urine nitrogen content in grams (g). The de Weir equation (65) or the Brouwer (12) equations, as given above, are the basis for estimating macronutrient oxidation rates from expired gases with indirect calorimetry.

Doubly Labeled Water Method

This method involves the isotopic incorporation of oxygen-18 (^{18}O) into carbon dioxide in the tricarboxylic acid cycle. It is based on the difference between the dilution rates of ^{18}O and deuterium (^{2}H) from the total body water pool in order to estimate the carbon dioxide-production rate (55). Doubly labeled water shows an accuracy of 1-3% and precision of ~8% (55). Subjects are orally dosed with ^{18}O and ^{2}H, and the isotope concentrations are measured in the total body water pool from samples of blood and urine

before, during and after dosing (55). The ^{18}O and ^{2}H concentrations are determined by *isotope-ratio mass spectroscopy*, whereas ^{2}H can also be determined by the use of duplicate analysis by *infrared spectrophotometry.* Because of the relative low sensitivity of infrared spectrophotometry, compared with the isotope-ratio spectroscopy, the ^{2}H concentration is increased by a larger dose for accurate measurement. Isotope-pool sizes and disappearance rates are calculated by the use of least-square regression analysis on isotope concentrations in body water as a function of elapsed time from dosing. The CO_2 production rates (r_{CO2}) is calculated from the disappearance rates (K18 and K2).

The clear advantage of the doubly labeled water method is the ability to estimate free living energy expenditure over extended periods of 1 to 2 weeks (52, 53). Another advantage is with detailed food records and EE doubly labeled water from whole body energy balance can be assessed over a period long enough to follow fluctuations in weight. The disadvantages of the doubly labeled water method are the high cost of the isotopes, the equipment for analysis of isotope levels and the inability to estimate macronutrient oxidation rates. Two other disadvantages of doubly labeled water method, like for whole body indirect calorimetry, are that BMR and TEF can not be measured with this technique.

2.6 Summary of Proposed Methods

For the food intake assessment food tables and GLC are appropriate tools to allow adjustment of macronutrient levels in the diet and for assessment of the fatty acid profiles in the lipid component of a given diet.

In controlled experiments looking for whole body measures of either 24 h whole body energy expenditure (direct or indirect calorimetry) or 24 macronutrient oxidation rates (indirect calorimetry) can be assessed. For studies in a free living conditions, doubled labeled water and intake balance (66) are best for investigating EE. In controlled laboratory conditions the individual components of EE can be continuously measured with a ventilated hood (67). This allows measurement of the basal metabolic rate, postprandial energy expenditure and macronutrient oxidation rates. As such, to determine the effects of MCT feeding on basal metabolic rate, postprandial energy expenditure and postprandial macronutrient oxidation rates the ventilated hood system is preferable method for conducting the proposed study in this thesis.

2.7 Research Hypothesis

The effects of feeding high but tolerable amounts of MCTs in diets on basal metabolic rate and on postprandial energy expenditure, as well as on substrate oxidation, remain to be established in women. This research hypothesis is based on the rationale that a higher level of MCT in the diet will give a greater increase in EE and fat oxidation in the postprandial period.

We hypothesize that increasing the proportion of MCTs in the diet to ~25% of total energy intake will increase basal metabolic rate and postprandial energy expenditure by greater than 5%. As well it is hypothesized that with ~25% of the energy intake as MCT that there will be a decrease postprandial carbohydrate oxidation and respiratory quotient with a corresponding increase in postprandial fat oxidation.

2.8 Testable Questions

The testable question for this thesis are:

1. Does feeding of ~25% of total energy intake as MCT for 7 days increase the BMR of young healthy women on days 1 or 7 relative to the BMR of the same women fed a high LCT and low MCT diet?

2. Does feeding of ~25% of total energy intake as MCT for 7 days significantly change the postprandial energy expenditure and postprandial substrate oxidation rates on day 1 or 7 of feeding relative to the postprandial energy expenditure and postprandial substrate oxidation rates of the same women fed to a high LCT and low MCT diet?

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Figure 2-1. Intracellular transport of long chain triglycerides (LCT) vs. the medium chain triglycerides (MCT) and the corresponding fatty acids.

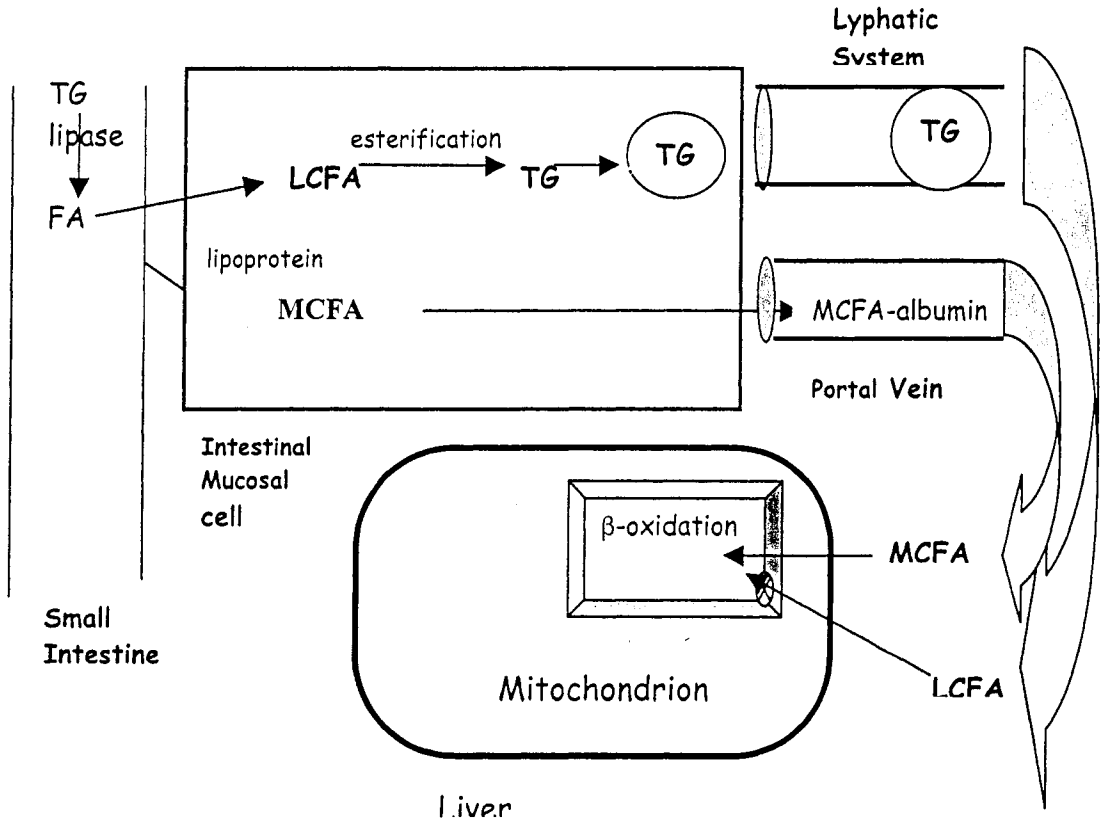


Figure 2-2. Hepatic metabolism of fatty acids. TG. Triglycerides; PL. Phospholipids; CE. Esterified Cholesterol; CPT. Carnitine palmityl transferase. Adopted from Bach and Babayan, 1982.

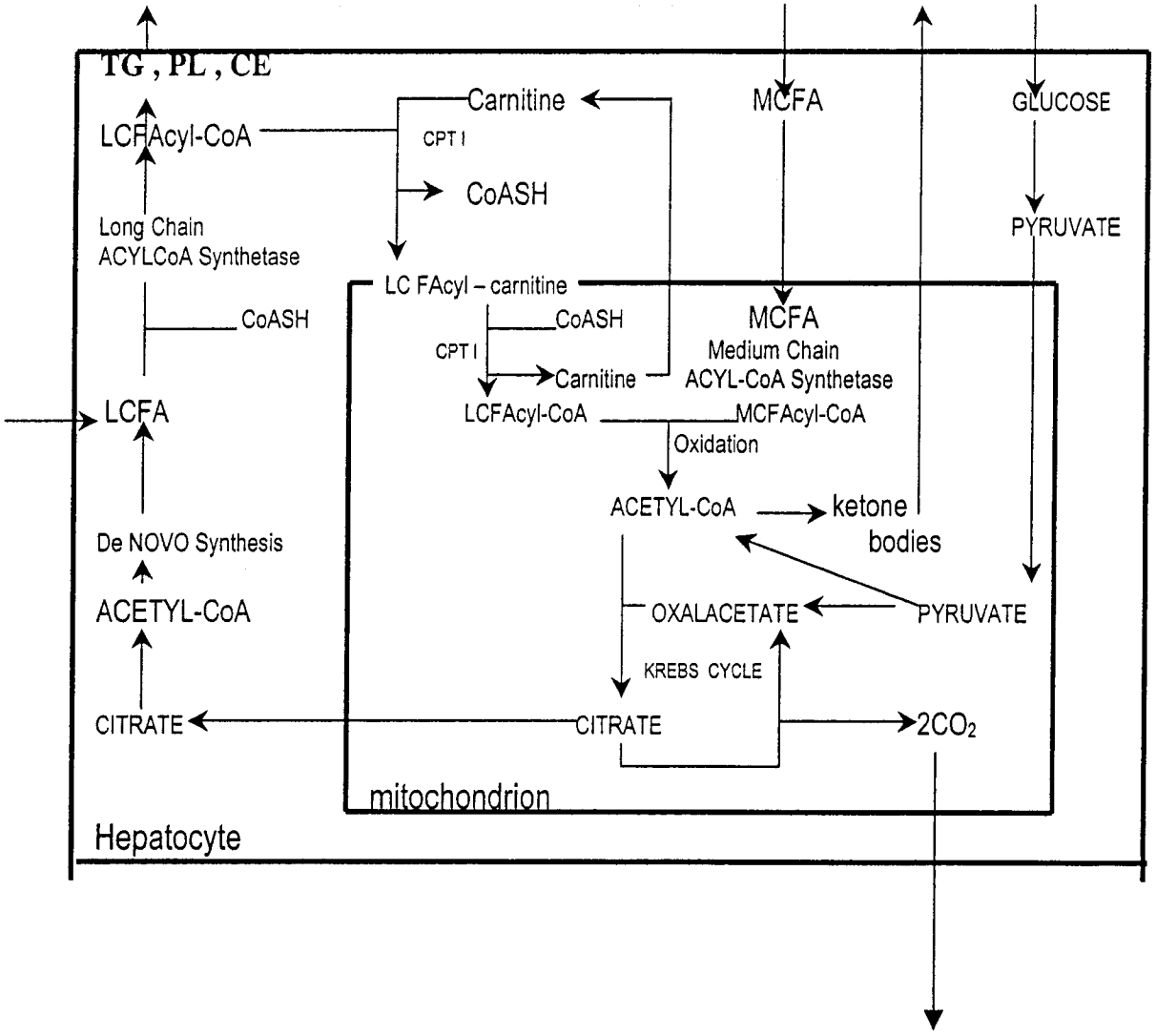


Figure 2-3: Transport, distribution and metabolic fate of exogenous fatty acids. Adopted from Bach, 1996.

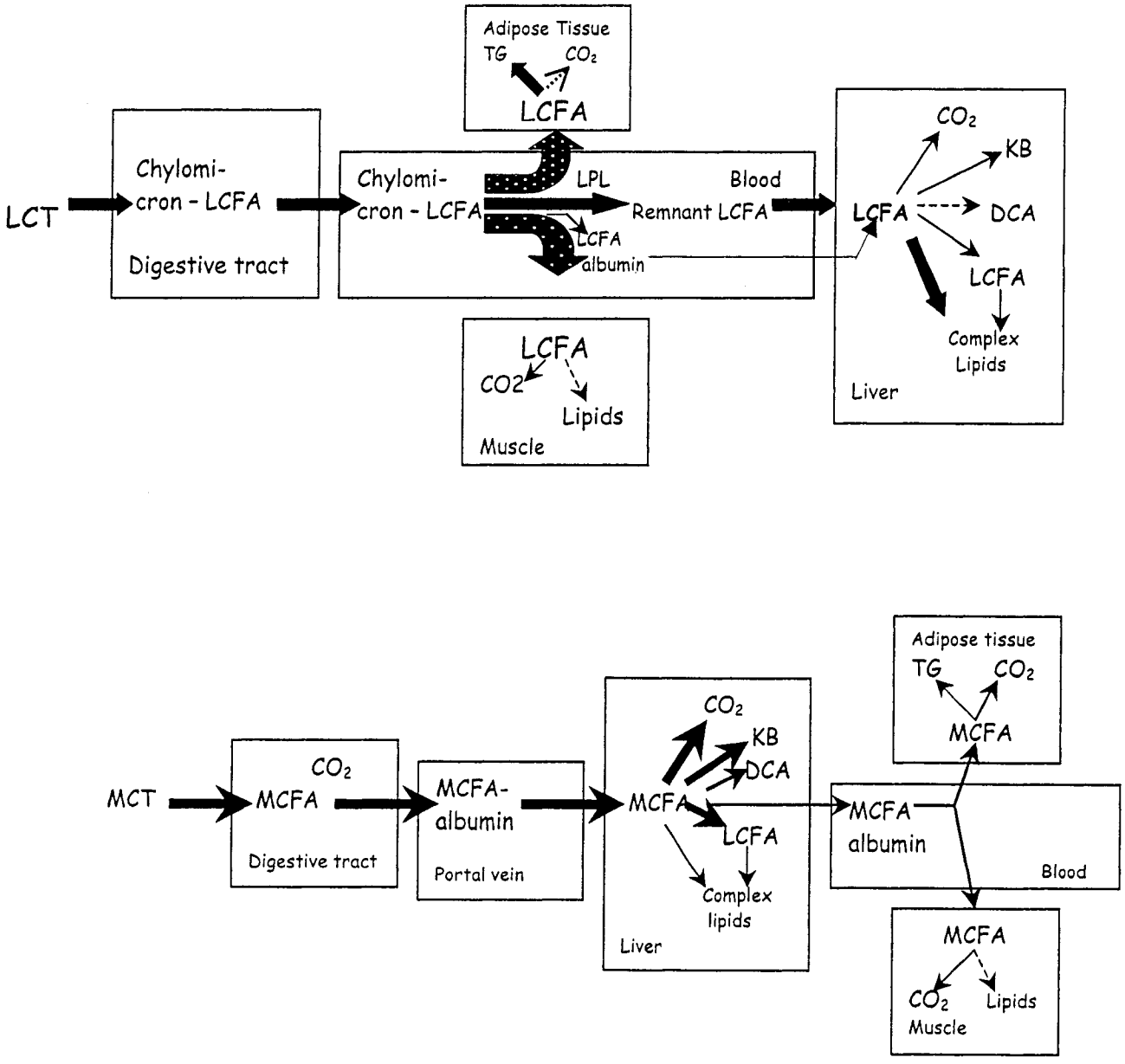


Figure 2-4: The formation of ketone bodies.

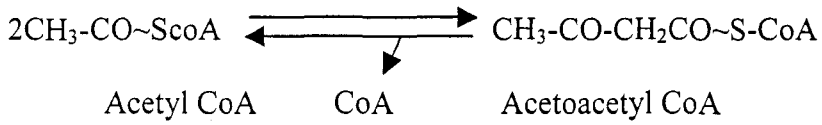


Figure 2-5 Overview of lipid metabolism

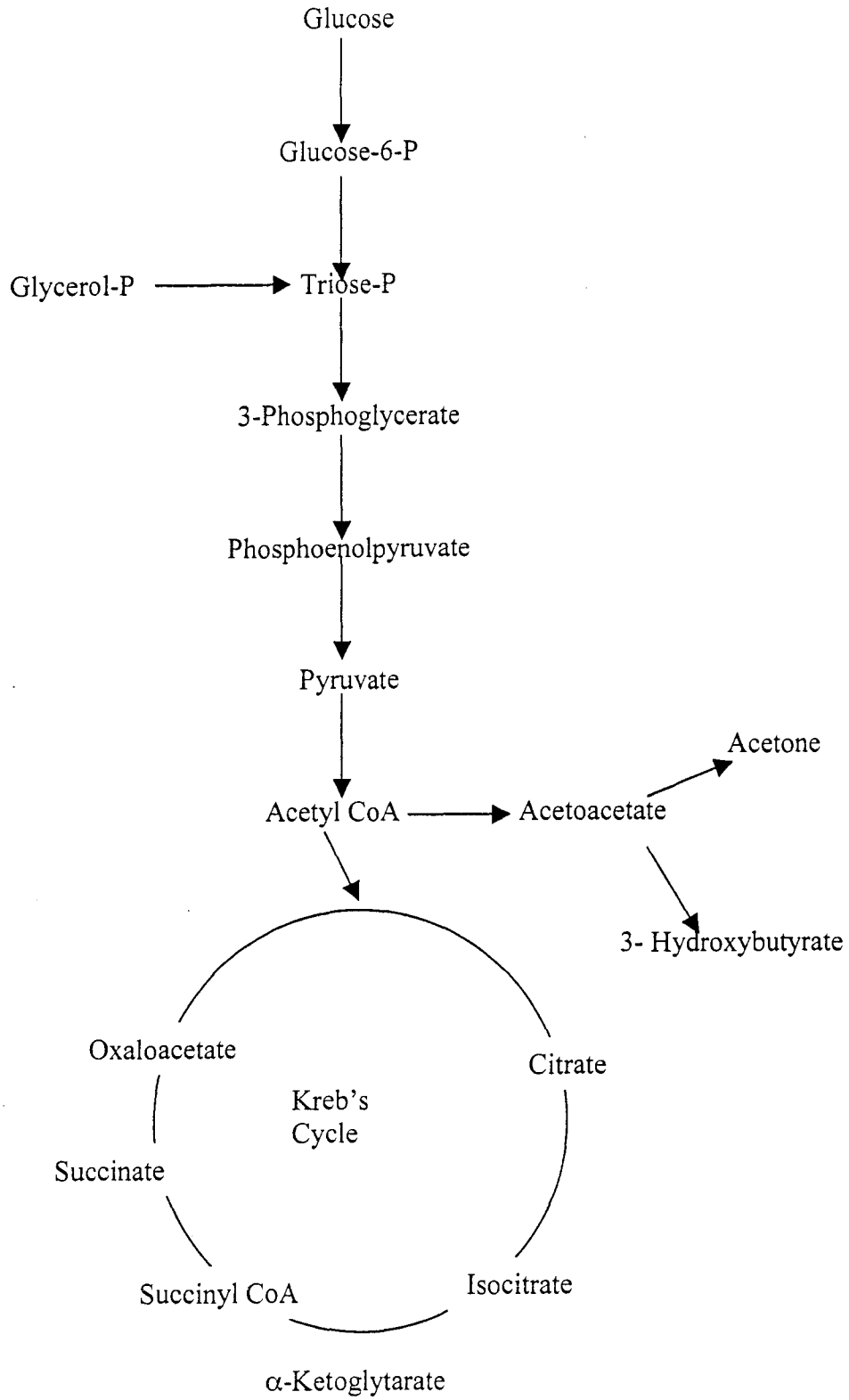


Figure 2-6, Metabolic pathways of exogenous fatty acids in the hepatocyte. Adopted from Back, 1996.

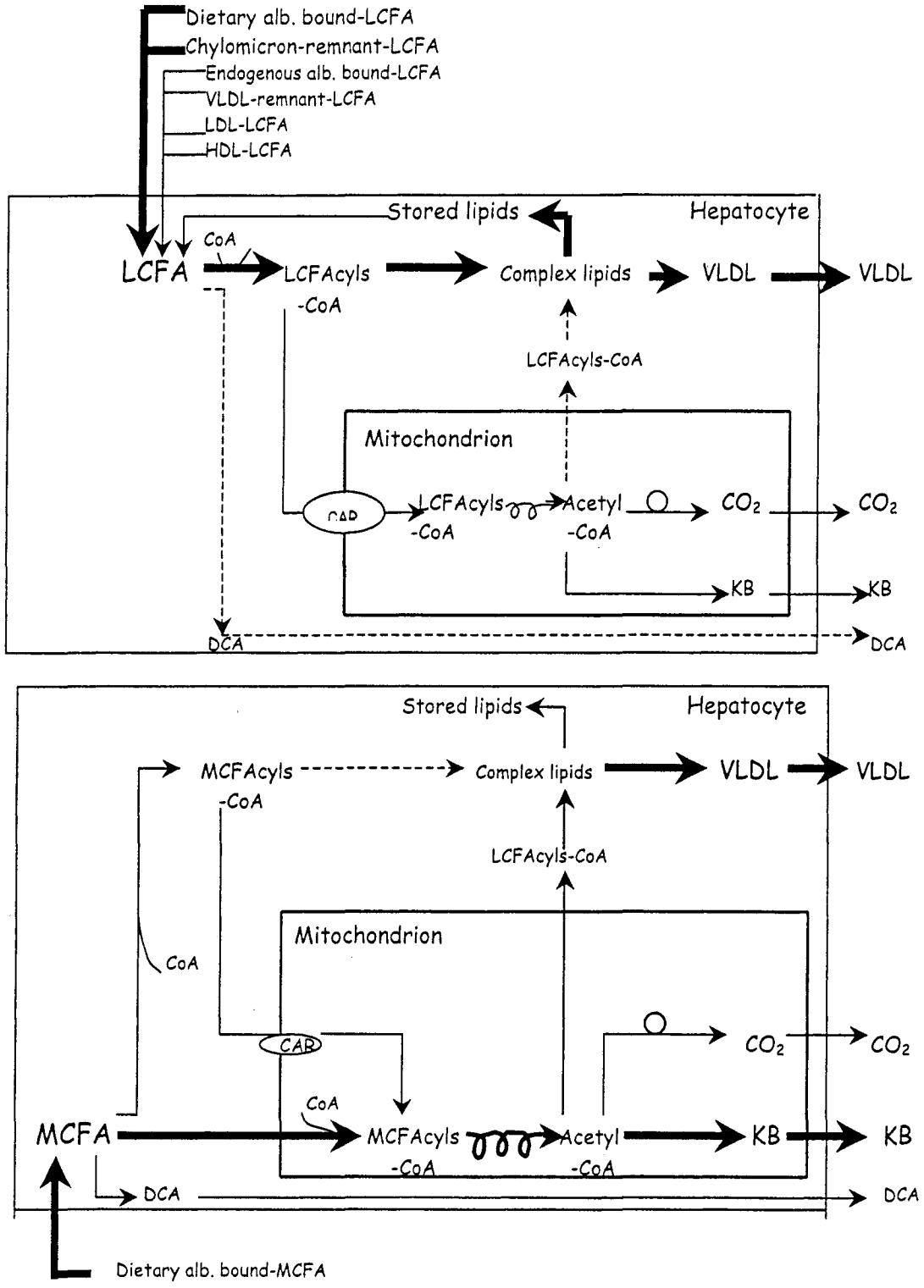
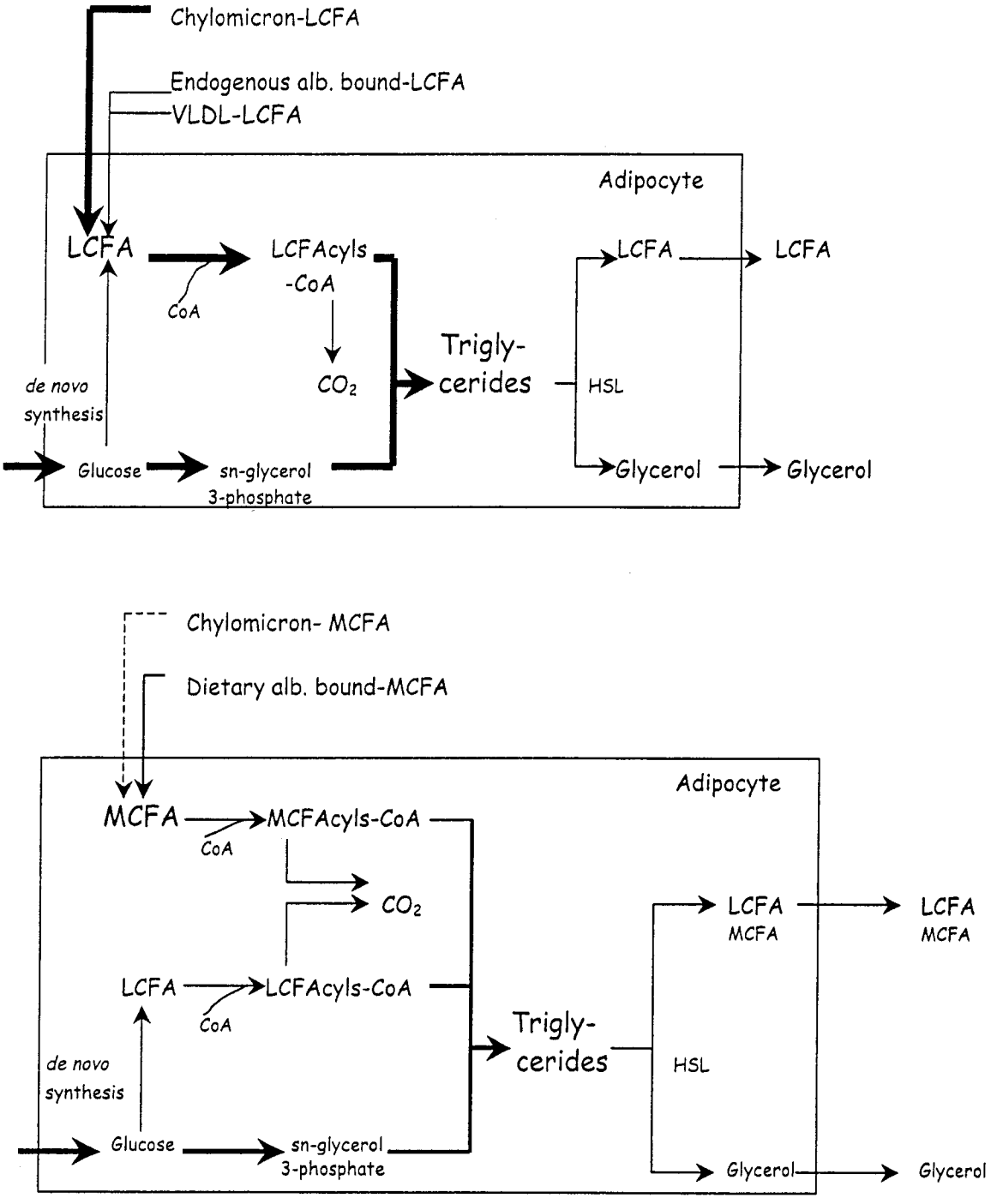


Figure 2-7. Metabolic pathways of fatty acids in the adipocyte. Adopted from Bach, 1996.



Chapter 3 Effects of high levels of medium chain triglycerides feeding on postprandial energy expenditure and substrate oxidation in college aged women

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Keywords: basal metabolic rate, energy expenditure, female, medium and long chain triglycerides, obesity

3.1 Introduction

An increased proportion of dietary medium chain triglycerides (MCT) has been reported to increase preprandial (15, 17, 27) and postprandial energy expenditure (1, 3, 11, 15, 17, 27). In addition, the thermic effect of feeding after MCT feeding was shown to be greater than after LCT feeding (11). Mascioli et al (15) also reported MCT feeding for hospitalized patients with total parenteral nutrition (TPN) and showed an increased thermic effect of feeding relative to an LCT infusion. In agreement with these findings, Scalfi et al (20) saw enhanced postprandial thermogenesis (PPT) with MCT feeding for both obese and non-obese men. Males in a whole body indirect calorimeter were shown to have a significantly increased 24-h energy expenditure of up to ~5% as the ratio of MCT:LCT was increased in the diet (5). Overall there is evidence to suggest a greater proportion of MCTs in the lipid component of the diet increases energy expenditure and some evidence suggests this influences fatty acid oxidation (27).

Recently St-Onge and colleagues illustrated greater fat oxidation and energy expenditure in females (23) and males (24) fed diets high in MCT over approximately a 1 month period. This suggests that long term feeding of MCTs can be an effective dietary strategy to influence weight loss as evidenced for the males in their study. Interestingly, their was a gender disparity in St-Onge and colleagues' studies with males but not females showing weigh adipose tissue losses. Also few MCT feeding studies have been conducted in women and it is not clear how this affects their energy expenditure and substrate oxidation over shorter periods (10, 17, 27, 29). White et al. (27) saw increased

preprandial and postprandial energy expenditure plus fat oxidation for young, non-obese females fed only ~10% of the total energy intake as MCT. This increase of energy expenditure extrapolated to 24 hours was ~6% greater than for the LCT-rich diet (27), but this difference was found to be attenuated by day 14 of feeding of the MCT-enriched diet. Other evidence suggests with long term feeding for women there is an diminished effect of MCT on energy expenditure and weight loss (10, 29). One study for women showed when MCT oil was added to a hypocaloric diet that this had no effect on weight loss(10). The authors did show the MCT feeding slowed the fall in BMR that is often reported in weight loss studies (10). Another study with women showed enhanced insulin action following MCT feeding although this feeding strategy also did not affect the amount of weight loss (29).

The purpose of the present study was to assess in college aged, non obese females if short-term feeding higher amounts of dietary MCT than previously employed (10, 17, 27, 29) would give greater influences on the components of energy expenditure and on postprandial fat oxidation.

3.2 Methods

Subjects

The sample size of eight healthy college-aged women was determined using a power calculation. The difference worth detecting was set at 5%, with an alpha level of 0.05, a beta value of 0.8 and a standard deviation of 7% of the estimated mean scores

(27). Poster advertisements and word of mouth were used to the find the volunteers. The criteria for selection included that subjects a) had regular menstrual periods, b) were non-obese (i.e. BMI < 25 kg/m^2) and c) were in age range of 19-29 years. The participants' characteristics are listed in Table 3-1. All females were informed about possible risks, discomforts and the objectives of the study. They were also informed about their right to withdraw at any time without prejudice. The Memorial University Human Investigation Committee approved the protocol of the study, and all subjects signed their informed consent before starting the study.

Body Composition Assessment

The subject's adiposities were assessed with a bioelectric impedance monitor (Tanita Model TBF521, Tokyo, Japan).

Diets

Subjects were fed isoenergetic MCT and LCT diets of approximately 2700 kcal and each diet was provided in a meal plan which gave a 2-day rotating menu for a total of 6 meals. The total energy intake for each subject was determined after collecting the subject's basal metabolic rate (BMR), which was multiplied with the activity factor according to Passmore and Durnin (19). Diets provided ~45% of the total energy as carbohydrates, ~15% as protein and ~40% as fat. Subjects were instructed to eat nothing but what was provided with the exception of water. All meals were prepared after weighing foods to the nearest 0.1 g and meals were served in the laboratory under

supervision. This was to ensure the intake of the food provided and only on rare occasions were take home meals provided.

The difference between the two diets was the type of treatment fat. For the MCT diet approximately ~78% the total fat by weight was from MCT and this level was achieved by adding commercial MCT oil (Premium Gold MCT) to the MCT-rich diet. For the LCT-rich diet ~65% by weight of the total fat was from beef lard. This was used in the LCT-rich diet as the main source of long chain triglycerides. The remaining LCTs were from naturally occurring LCT in other foods included in 6 meals in the LCT-rich diet.

A gas-liquid chromatography (GLC) analysis was employed to assess the lipid composition of each diet. The 6 meals were homogenized by using a commercial blender. The fat content was extracted from replicate portions of the diet according to the Folch Wash Procedure (8) and then esterified using Boron trifluoride as the reagent. Fatty acid methyl esters were separated by gas-chromatography using a Supelco SP-2330 fused silica capillary column (30m x 0.32 mm ID, 0.20μm film thickness) in a Hewlett Packard 5890 Series II gas chromatograph equipped with a flame ionization detector. Separation was performed isothermally at 200°C, with He as the carrier gas at a flow rate of 1mL/min. The split ratio was 100:1. Table 3-2 showed ~99% of the fatty acids in the MCT oil were MCFA. The fatty acid composition of each type of diet is provided in Table 3-3.

Study Design

Each diet was fed for 7-day period, separated by three week washout period. This gave a total time of 28 days between diets and the length of the washout period was to ensure the testing on a similar day of each menstrual period for each subject. This avoided changes in metabolic rate that are attributed to increases in body temperature in the post ovulation period in these menstruating women (25). During this period, subjects returned to their typical lifestyles. The subjects were not aware of the type of diet consumed. They were randomly assigned to each diet and the cross-over design of the study made every subject her own control. Four subjects started their feeding with the MCT-rich diet and 4 subjects started their feeding with the LCT-rich diet.

On day 1 and 7 of each diet group, the subject rested in a semi recumbent position for at total of 6.0 hours under a transparent ventilated hood. The expired CO_2 and O_2 collection started with a BMR assessment for approximately half an hour and this was following a period of 10 to 12 hours without food or physical activity. After breakfast, which was served at ~7.30 am, the subject was placed again in a semi-recumbent position under the hood for the next 5.5 h. Throughout the postprandial period, subject was relaxing or watching movies.

Gas exchange measurements

A Sensormedics metabolic monitor (Sensometrics, Anaheim, CA) was used to determine oxygen consumption and carbon dioxide production. A ventilated

hood, was connected through Collins tubing to the mixing box of the metabolic monitor. The monitor allowed the continuous gas collections, over 5.5h. The gas analyzers were calibrated with gases of known concentration prior to every testing, after sufficient warm-up time. The first gas was 26% oxygen with the balance from nitrogen, the second gas was 4% CO_2, 16% O_2 and the balance from nitrogen, and the last gas was ambient air. Before all experimental sessions the flow sensor was also calibrated with known syringe volumes and rates. From the VO_2 and the VCO_2 values, the respiratory quotient (RQ) was calculated as the ratio of carbon dioxide production and oxygen consumption. Substrate oxidation rates were estimated according to Weir's formula (26). A constant of 0.707 g protein/kg fat-free mass was assumed because an error in protein oxidation ≤ 30% would have no significant effect on substrate oxidation rates (16).

Statistical Analysis

A repeated measures ANOVA was employed for the statistical analysis of the data. The factors included the kind of DIET (MCT or LCT), the DAY (day 1 or day 7) and TIME (0 to 6 h). Post-hoc comparisons were made by paired orthogonal contrasts. The level of significance was set at $P<0.05$.

3.3 Results

The proportions of fatty acids in the commercial MCT oil used in the MCT diet is given in Table 3-2. The GLC analysis showed that the MCT oil employed is mainly composed of C:8 with medium chain fatty acids making up 99.87% of the total fatty acids in the oil.

The GLC analysis of the fatty acids for the two diets is given is Table 3-3. The MCT diet was found to be composed of 60.81% MCFA (i.e. fatty acids $\leq$ 12 carbons) of the total fatty acid content, with 37.6% of the MCFA as octanoic acid (C8:0). This amount of MCFAs accounted for the 24.32% of the total energy consumed in the MCT diet. The LCT diet was found to be composed of ~98.9% LCFA. This included 34.52% as oleic acid (C18:1n9) and 24.60% as palmitic acid (C16:0). In the LCT diet ~1.1 % of the fatty acids were naturally occurring MCFAs.

The mean rates of EE measured under basal conditions (i.e. BMR) on day 1 and 7 of the study are given in Figure 3-1 at time 0. On day 1 the BMR of 3.55± 0.25 kJ • min^{-1} for the LCT diet was not statistically different than the BMR of 3.46 ± 0.21 kJ • min^{-1} for the MCT diet. As well on day 7 the BMR of 3.44 ± 0.15 kJ • min^{-1} for the MCT diet was not significantly different than the BMR of 3.39 ± 0.15 kJ • min^{-1} for LCT diet. On day 1 a positive, significant effect ($p < 0.05$) of the MCT diet on EE was only observed at 1.5 h and 2.0h in the postprandial period. After the peak in EE at 2h, the difference between

the two diets decreased over time. On day 7 there were no significant differences between diets for postprandial EE.

The difference in mean postprandial EE between MCT and LCT diets (Fig 3-2) over 5.5 postprandial hours on day 1 ($F = 5.1$, $p = 0.06$) and on day 7 ($F = 4.5$, $p = 0.07$) bordered on significance. The overall mean rate of postprandial EE for between the two diets, with data pooled for day 1 and 7, also bordered ($F = 2.4$, $p = 0.06$) on significance.

Fat oxidation for day 1 (Fig. 3-3) was significantly greater for the MCT diet relative to the LCT diet at 0 to 90 min ($F = 26.7$, $p = 0.0001$), at 91 to 180 min ($F = 7.1$, $p = 0.01$). From on day 1 the LCT diet fat oxidation from 271 to 360 min ($F = 8.5$, $p = 0.008$) was significantly greater than fat oxidation for the MCT diet. On day 7 the pattern was similar for MCT diet with significantly greater fat oxidation from 0 to 90 min ($F = 4.9$, $p = 0.04$), and from 91 to 180 min ($F = 10.8$, $p = 0.004$). However, from 181 to 360 min on day 7, there was no significant difference between the two diets for fat oxidation.

The carbohydrate oxidation (Fig. 3-4) was significantly attenuated ($F=16.7$, $p =0.0005$) on day 1 from 0 to 90 min for the MCT diet relative to the LCT diet. From 271 to 360 min on day 1, carbohydrate oxidation was significantly greater ($F = 6.3$, $p = 0.02$) for the MCT diet relative to the LCT diet. On day 7, the MCT diet had a significantly lower carbohydrate oxidation than the LCT diet but only during the 91-180 min period ($F = 2.6$, $p = 0.03$).

The respiratory quotient (RQ) analysis showed similar results when compared for the effects of the two diets over time (Fig. 3-5). On day 1 the RQ was significantly lower for the MCT relative to the LCT diet from 0-90 min ($F = 20.9$, $p = 0.002$) and from 91-180 ($F = 6.58$, $p = 0.02$). From 271 to 360 min on day 1 the RQ was significantly lower ($F = 8.7$, $p = 0.008$) for the LCT relative to the MCT diet. On day 7 the effect of the MCT diet on RQ was bordered on significance from 0 to 90 min ($F = 3.4$, $p = 0.08$) was significantly lower ($F = 10.1$, $p < 0.01$) from 91 to 180 min.

3. 4 Discussion

The energy expenditure with the MCT feeding was only significantly greater relative to the LCT-diet during the first 2 h of the postprandial period on day 1 of feeding (Fig. 3-1). This is despite that the postprandial energy expenditure for MCT-diet showed an overall trend to be higher than those for LCT diet on both days 1 and 7 (Fig. 3-1). The mean postprandial energy expenditure across the two diets was not significantly different (Fig. 3-2). A main result from this study was the increase in fat oxidation and the substrate oxidation profile (Fig 3-4, 3-5) for the MCT diet relative to the LCT. A maintenance of elevated fat oxidation in women for for 7 days (Fig 3-3) suggests that this level of MCT intake, with ~25% of the calories from MCT, is closer to that needed to give longer term influences on postprandial energy expenditure. A previous study of MCT feeding in a group of women showed a diminished affect of MCT feeding on substrate oxidation over time (28). The present study supports that at this level of intake the MCT feeding can increase postprandial fat oxidation for 7 days.

On day 1 in the current study the small but significant increase in postprandial energy expenditure due to MCT feeding compared to LCT feeding is consistent with results from past studies. Regardless of the way the MCTs were administrated, being orally, in normal incorporation in normal diets (21), overfeeding liquid formula (11), or intravenously administration (14), a positive effect on energy expenditure is observed with increased medium and short chain fatty acids in the diet. It appears that there are some discrepancies for the level of energy expenditure response due to differences in the

quantity and source of MCT being administrated, the type of administration and the duration of the study. The diminishing effect of MCT on energy expenditure between day 1 and 7 found in the current study is in agreement with past studies (17, 27, 29). It appears the duration and level of MCT feeding needed to have an affect on women's energy expenditure remains to be established.

On both days 1 and 7, there were greater rates of fat oxidation and lower for carbohydrate oxidation for the MCT feeding than the LCT. Results from some similar studies agree with this finding (14, 18, 27), while some others do not (7, 12). In addition, for both days1 and 7, the RQ levels were higher for the LCT diets at different postprandial time points, which confirms that there was a greater carbohydrate oxidation with LCT feeding Figure 3-5. There does not appear a consensus for the affects of MCT feeding on postprandial fat oxidation. However with ~25% of the total energy intake as MCT in these women it appears to be sufficient to increase fat oxidation for the first three hours following a meal (Fig 3-3). For example, assuming 3 meals per day, this increase in fat oxidation for 3 hours in Fig 3-3 on day 1 could translate to an increased fat oxidation of 0.0165 g • min^{-1} or ~ 335 kJ• day^{-1}. If there are 3500 kcal (14630 kJ) in a pound of fat, this modest increase in fat oxidation could give a loss of ~8.4 pounds of fat in a year.

The reason for the type of dietary triglyceride not affecting the energy expenditure in the same way is due to the different metabolic pathways (3, 22). After digestion, most

MCFAs are bound to albumin in the blood in hepatic tissues (3, 22) in the portal circulation and are preferentially oxidized to acetyl-CoA (4). In contrast, LCFAs get incorporated into chylomicrons and circulated through the lymphatic system prior entry to bloodstream (3, 22) and are taken by peripheral tissues and are incorporated into adipocytes (3).

The inclusion of MCT in a diet can cause ketonemia, as a result of their high rate of oxidation (2, 3). The fast accumulation of acetyl-CoA, as the end product of the fatty acid oxidation could follow the Kreb's cycle, which will eventually generate energy-rich compounds (ATP), be used for fatty acid elongation or follow the ketogenic pathway leading to an increase of the hepatic and plasma ketone bodies and acidosis. The route is determined by the concentrations of various enzymes and key compounds in the Kreb's cycle, such as oxaloacetate. The fast increase of acetyl-CoA will cause the concentration of oxaloacetate not to be sufficient, shifting acetyl-CoA to the ketogenic route (3). The degree of ketonemia and the duration of the effect are found to be proportional to the amount of MCT administrated (2). The production of ketone bodies requires oxygen and if ketogenesis is very high, may cause calculations for energy expenditure and substrate oxidation to be inaccurate (6, 13). The metabolic rate could be overestimated and the respiratory quotient not as accurate. The amount of ketone bodies formed during oxidation can be estimated by adding ketone excretion in the urine and accumulation in blood and other parts of the body (6). However, studies with humans show smaller increase in ketonemia than with animals fed with MCT diets (9). In addition, when the

mixture of the diet includes MCT, LCTs and glucose, the effect of ketogenesis is only minor (9).

3.5 Conclusions

The increase of MCT in the diet for these women to~25% of total energy intake had transient but significant affects on energy expenditure on day 1 of feeding. Over the 7 day feeding period the affect of MCT feeding on the postprandial substrate oxidation was sustained in the initial 2 to 3 postprandial hours. The results support at this level of MCT intake that there is a potential for increased fat oxidation for women in this age group.

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Table 3-1. Subjects' physical characteristics and ages.

Subject (#)	Age (y)	Height (m)	Weight (kg)	BMI (kg/m^2)	Body Fat (%)
1	23	1.71	68.64	23.35	24.50
2	20	1.65	61.70	22.66	36.50
3	22	1.52	48.00	20.64	29.50
4	24	1.62	55.00	20.83	18.00
5	21	1.62	53.50	20.39	29.50
6	24	1.50	42.00	18.67	25.50
7	24	1.75	63.40	20.58	23.50
8	23	1.58	62.60	24.92	35.00
Mean	22.63	1.62	56.86	21.51	27.75
SD	1.41	0.08	8.31	1.86	5.74
SE	0.50	0.03	2.94	0.66	2.03

Table 3-2. Fatty acid profile for the MCT oil employed in the study.

FA Chain Length	MCT oil (%)
8:0	64.05
10:0	35.23
12:0	0.58
14:0	
16:0	
16:1n7	
18:0	
18:1n9	
18:2n5	
18:2n6	
18:3n3	
20:0	
22:0	
22:1	
24:0	
sum	99.87%

Table 3-3. Fatty Acid Profile from the GLC analysis for foods served in either the MCT or LCT diet. Only the main fatty acids identified are given in the table

FA Chain Length	MCT Diet (%)	LCT Diet (%)
8:0	37.60	
10:0	21.80	0.16
12:0	1.41	0.95
14:0	3.44	3.00
16:0	11.98	24.60
16:1n7		2.09
18:0		12.24
18:1n9	4.54	34.52
18:2n5	15.22	2.95
18:2n6		15.33
18:3n3		1.14
20:0		0.46
22:0		0.15
22:1		0.29
24:0		0.29

Figure 3-1. Comparison of mean (± SE) basal metabolic rate at t = 0 h and postprandial energy expenditure on day 1 and day 7 between MCT and LCT diets (* $p < 0.05$).

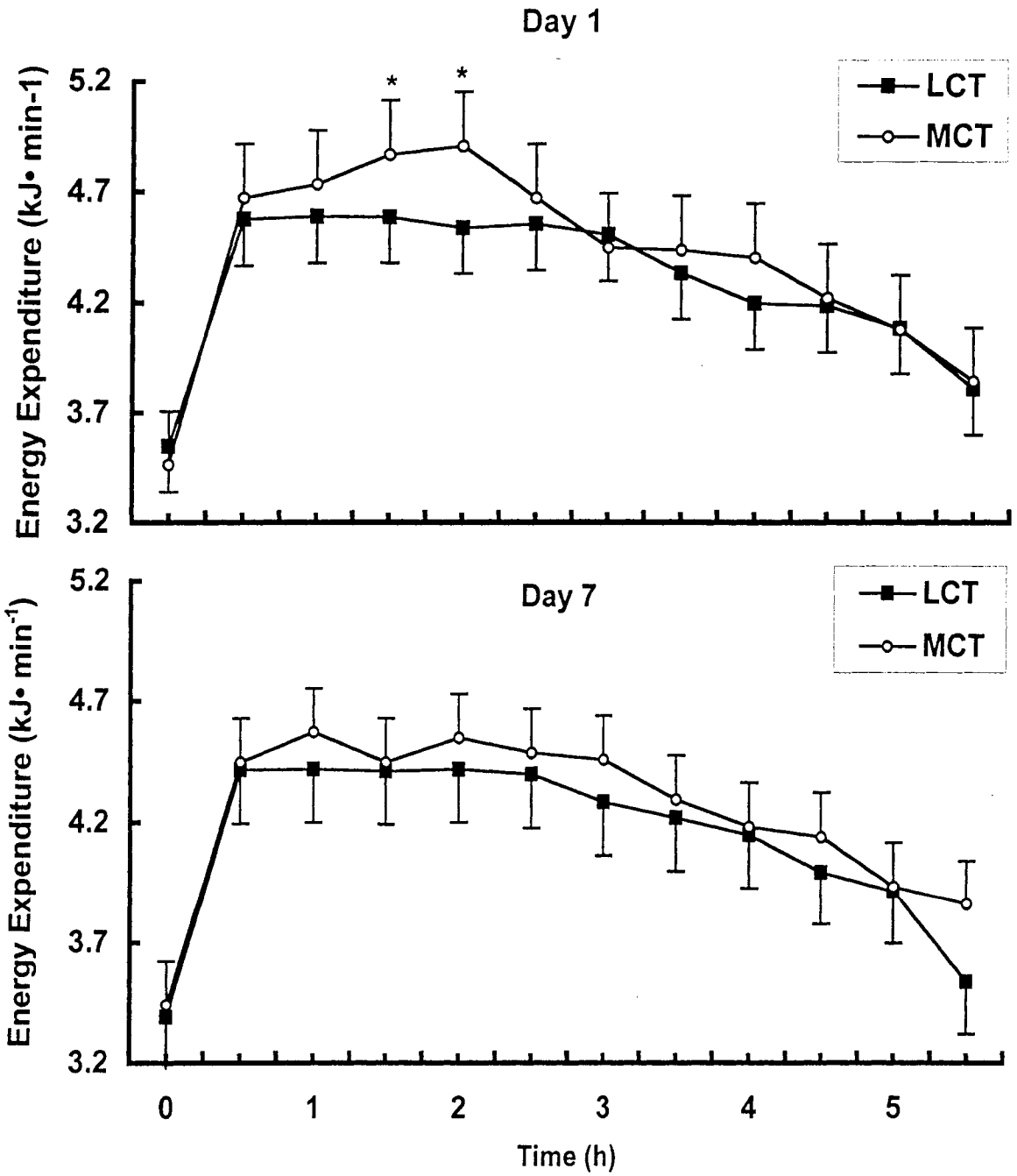


Figure 3-2. Mean (± SE) postprandial energy expenditure across 5.5 h on day 1 and 7 for MCT and LCT diets (NS = non-significant).

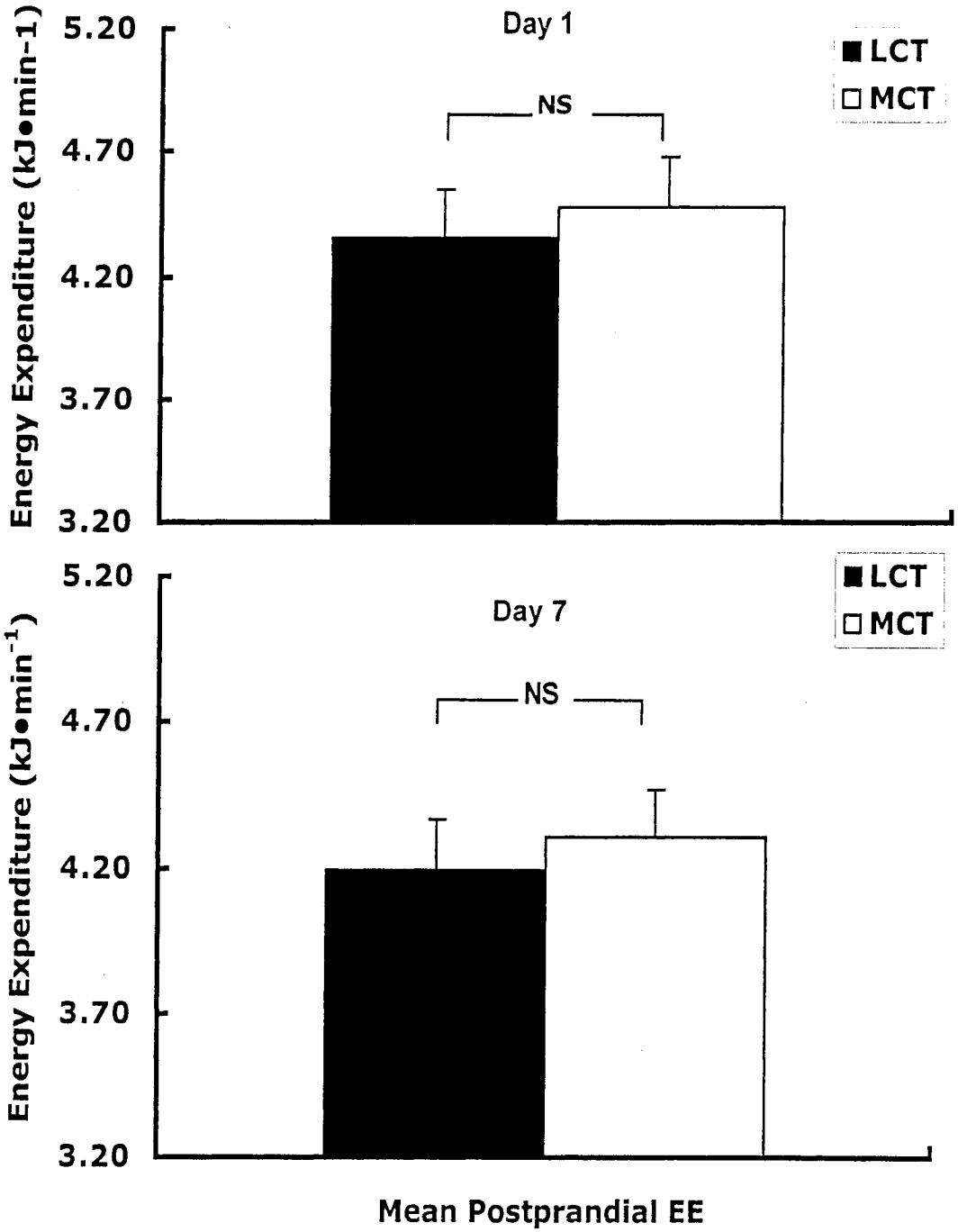


Figure 3-3. Comparison of fat oxidation rates on day 1 and 7 between MCT and LCT diets (* $p < 0.05$, † $p < 0.01$, ‡ $p < 0.001$, NS = non-significant).

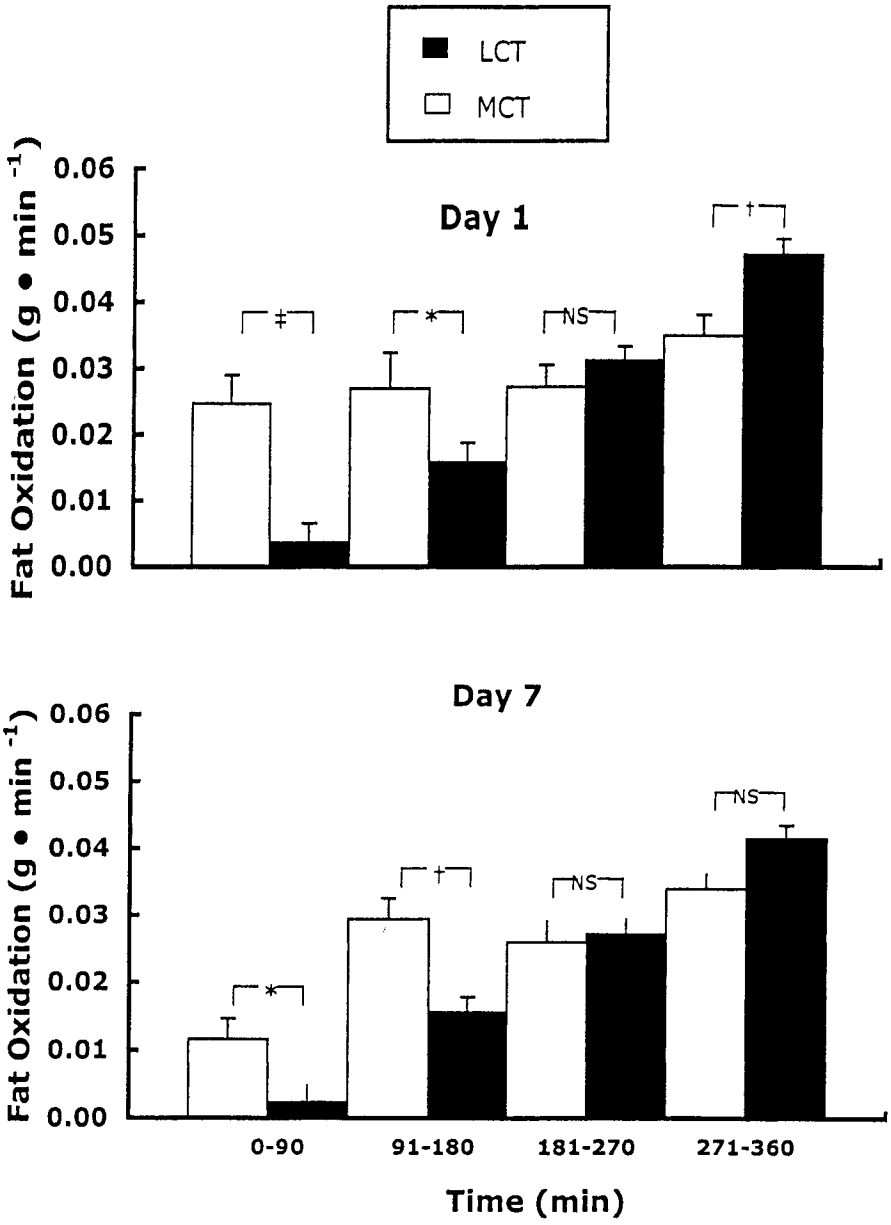


Figure 3-4. Comparison of carbohydrate (CHO) oxidation rates on day 1 and 7 between MCT and LCT diets (* $p < 0.05$, † $p < 0.01$, NS = non-significant).

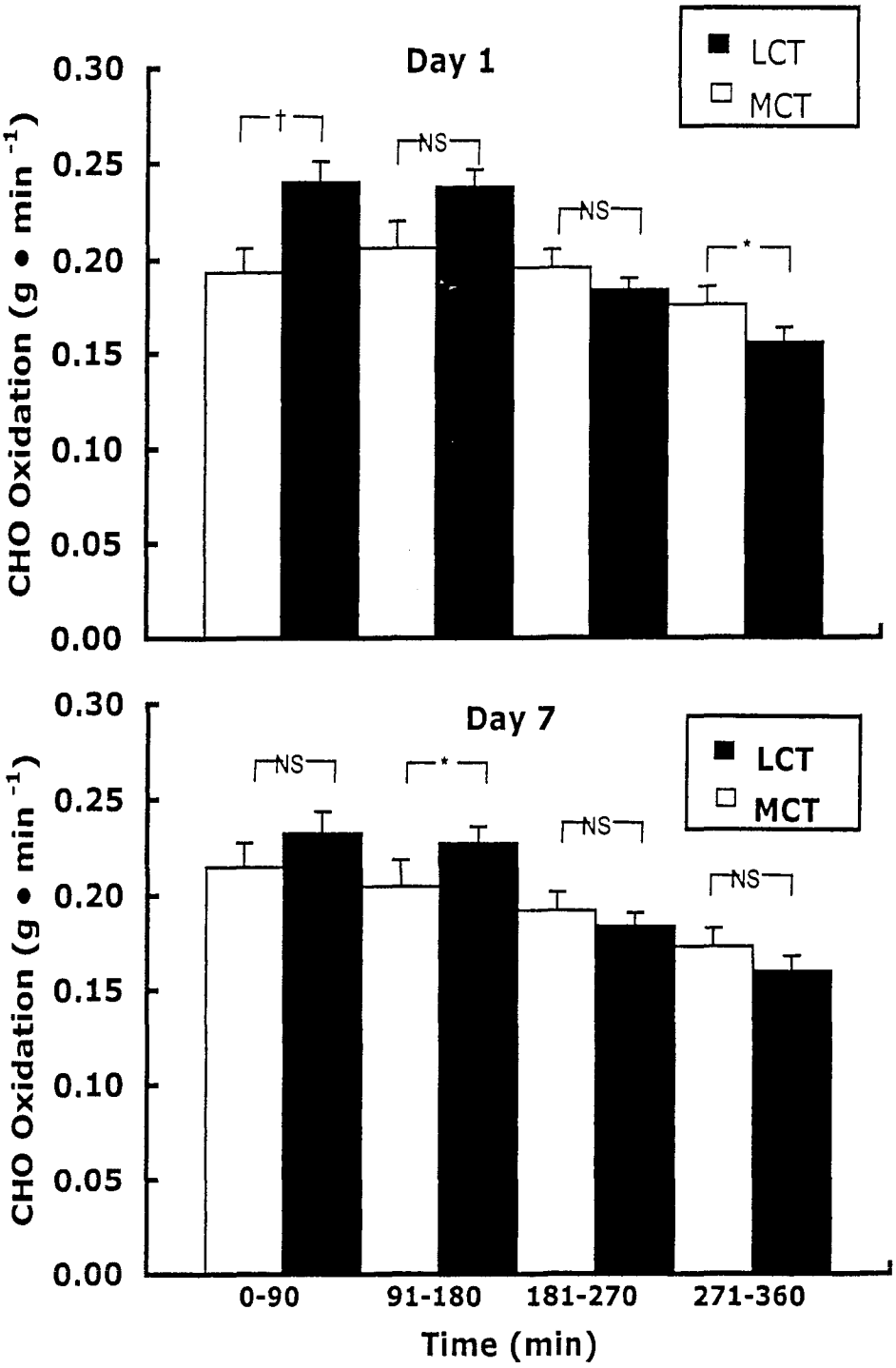
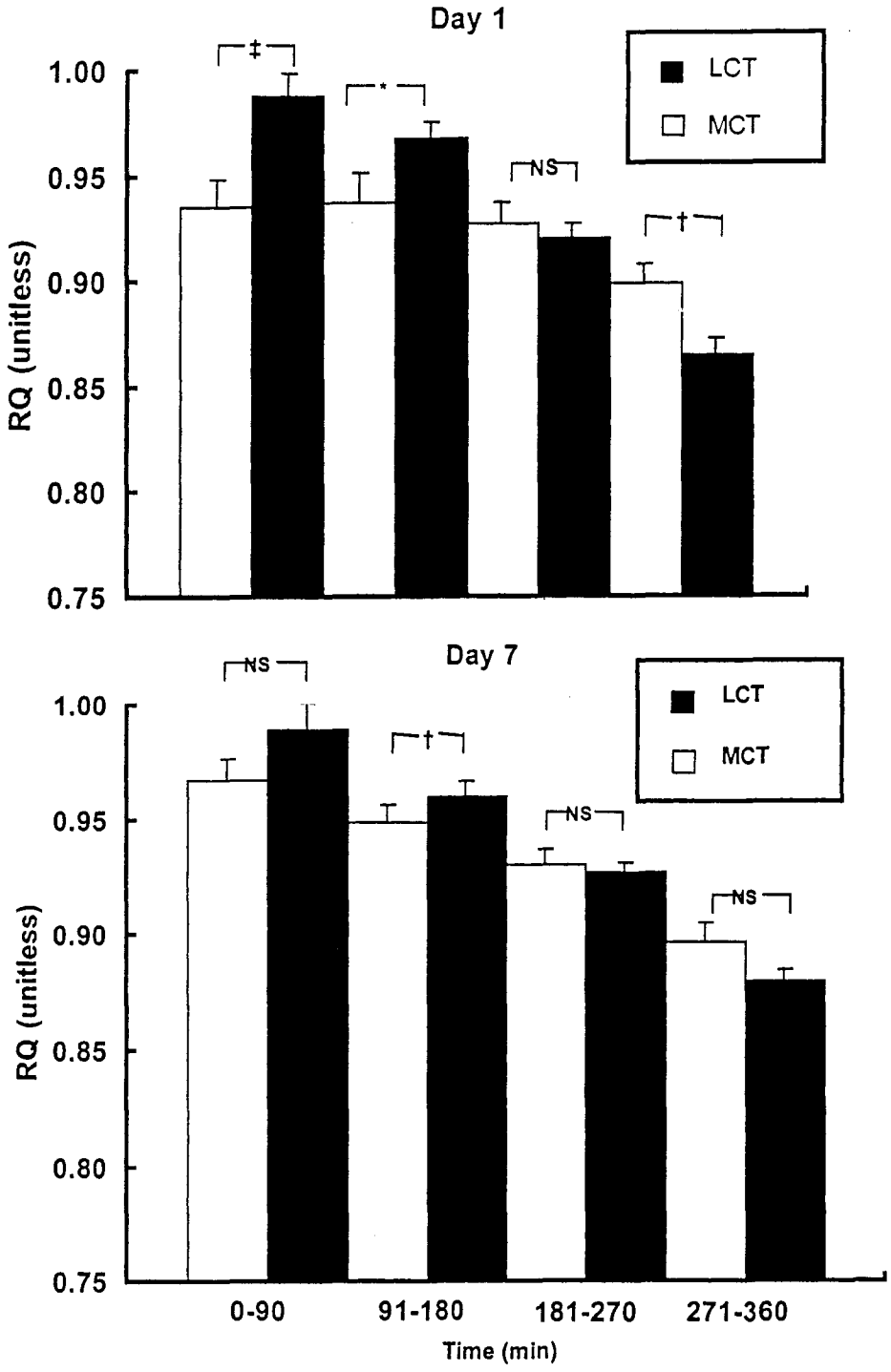


Figure 3-5. Comparison of respiratory quotient (RQ) on day 1 and 7 between MCT and LCT diets (* $p < 0.05$, ‡ $p < 0.001$, NS = non-significant). .



Chapter 4: Thesis Summary and Conclusions

Response to Research Hypotheses

It was hypothesized at the conclusion of the literature review in Chapter 2 that increasing the proportion of MCTs in the diet to ~25% of total energy intake will increase basal metabolic rate and postprandial energy expenditure to greater than 5%. As well it was hypothesized that with ~25% of the total energy intake as MCT that there will be decreased postprandial carbohydrate oxidation and respiratory quotient with an increased postprandial fat oxidation.

The study results partially support the hypothesis that with ~25% of the total energy intake as MCT that energy expenditure will increase. This was evident only within the first 3 postprandial hours on day 1 of the 7 day diet. Although there was a trend for an elevated basal metabolic rate for the MCT diet relative to the LCT-diet, the MCT feeding had no significant effect on basal metabolic rate in these women. There was not a 5% increase in energy expenditure for MCT relative to a LCT feeding.

The study results partially support the hypothesis that with ~25% of the total energy intake as MCT that fat oxidation will increase together with a decrease in RQ and a decrease in carbohydrate oxidation in the postprandial period. This is since in the first three hours of the post prandial period fat oxidation was significantly increased while there were significant decreases in RQ and carbohydrate oxidation for the MCT diet relative to the LCT diet.

Responses to Testable Questions

The responses to the testable questions stated at the end of the literature review in Chapter 2 are given below.

1. Does feeding of ~25% of total energy intake as MCT for 7 days increase the BMR of young healthy women, on days 1 or 7 relative to the BMR of the same women fed a high LCT and low MCT diet?

The results of this study supported that a study with a 7-day feedings of an MCT-rich diet relative to a diet low in MCT and high in LCT had no significant effect on the basal metabolic rate on day 1 nor on day 7.

2. Does feeding of ~25% of total energy intake as MCT for 7 days significantly change the postprandial energy expenditure and postprandial substrate oxidation rates on day 1 or 7 of feeding relative to the postprandial energy expenditure and postprandial substrate oxidation rates of the same women fed to a high LCT and low MCT diet?

The diet with ~25% of total intake energy intake from MCTs relative to the diet low in MCT and high in LCT had a significant effect on the postprandial energy expenditure and substrate oxidation in a time dependent manner. The effect on postprandial energy expenditure was evident during the first 2 hours following feeding on the first day of the study. By day seven of feeding the effect of feeding a high MCT diet on postprandial

energy expenditure was no longer evident. The significant increases in fat oxidation during the postprandial periods for the MCT-rich relative to the LCT-rich diet was evident on day 1 and day 7 of feeding. For carbohydrate and respiratory quotient significant decreases for the MCT-rich relative to the LCT-rich diet in the postprandial period were evident on days 1 and 7 of the feeding.

Applications of Thesis Results

The results suggest that the MCT-rich diet affected the substrate oxidation levels by increasing the fat oxidation and decreasing carbohydrate oxidation. The determination of the optimal level of dietary MCT may help reduce health risks of developing conditions obesity that is associated with conditions such as diabetes, hypertension and cardiovascular disease. In addition, the time frame of consumption may be critical and should be investigated further to determine the most effective way to feed MCT so that eventually they might be applied in the real world challenge of helping to reduce obesity.

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