EFFECT OF MEDIUM VERSUS LONG CHAIN TRIGLYCERIDE CONSUMPTION ON ENERGY EXPENDITURE, SUBSTRATE OXIDATION AND BODY COMPOSITION IN OVERWEIGHT MEN AND WOMEN

Marie-Pierre St-Onge

School of Dietetics and Human Nutrition

McGill University, Montreal

Québec, Canada

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements of the degree of Doctor of Philosophy.

Your file Votre référence
ISBN: 0-612-88581-X
Our file Notre référence
ISBN: 0-612-88581-X

ABSTRACT

Medium chain triglycerides (MCT) have long been advocated as potential weight-lowering agents or potential tools in the treatment and prevention of human obesity. These statements have been made after findings from human and animal trials that consumption of MCT increases energy expenditure and fat oxidation compared to long chain triglycerides (LCT). In addition, animal studies have resulted in lower body weight gain and smaller fat depots when animals were fed MCT compared to those fed LCT. However, long-term controlled trials studying the effects of consumption of MCT in humans have not been conducted and the longest trial to date, 14 d of duration, has shown that the effect of MCT on energy expenditure may be transient. Therefore, we aimed to determine whether, in controlled feeding conditions, consumption of MCT for 4 wk would lead to differences in energy expenditure and substrate oxidation versus consumption of an isocaloric diet rich in LCT. Furthermore, our aim was to establish whether consumption of MCT for 4 wk would lead to greater changes in body composition than would LCT consumption. We conducted two randomized, controlled, crossover feeding trials involving overweight women and men to test our objectives. A secondary objective was to examine the potential satiating effect of MCT, and this was tested in men. Finally, a third objective was to determine whether, when combined with phytosterols and flaxseed oil, MCT consumption would result in different blood lipid profile compared to LCT. Nineteen healthy overweight women and 24 healthy overweight men participated in two separate randomized controlled trials to test these objectives. Energy expenditure and body composition were assessed at the beginning and end of each experimental phases, which differed only in the type of fat included in

the controlled diets. Blood samples were also taken at baseline and endpoint of each phase to determine plasma lipid concentrations. Results showed that energy expenditure and fat oxidation were increased with MCT relative to LCT consumption in both women and men. In women, this rise in energy expenditure did not result in significant differences in changes in body composition, whereas in men, upper body adiposity was diminished to a greater extent with consumption of the diet rich in MCT compared to the one rich in LCT. The combination of MCT, phytosterols and flaxseed oil resulted in a significant lowering of plasma total and low-density lipoprotein concentrations in both women and men and did not impact triglyceride concentrations. It can thus be concluded that consumption of an oil rich in MCT, phytosterols, and flaxseed oil can be considered a functional food in weight maintenance and prevention of weight gain while promoting cardiovascular health.

RÉSUMÉ

Les acides gras à chaînes moyennes (AGCM) ont longtemps été proposés comme agents pouvant aider à la perte de poids ou pour le traitement ou la prévention de l'obésité humaine. Ces conclusions sont basés sur des résultats d'études chez les humains et les animaux qui démontraient qu'une consommation accrue d'ACGM augmente la dépense énergétique et l'oxydation des gras comparativement à une consommation égale d'acides gras à chaînes longues (AGCL). De plus, les études chez les animaux ont démontrés que les rats consommant les AGCM avaient un gain de poids plus faible et de plus petits dépôts adipeux que les rats consommant les AGCL. Par contre, des études à long terme vérifiant les effets d'une consommation d'AGCM n'ont pas été produites et la plus longue étude à date, durant 14 jours, a démontré que les effets des AGCM sur la dépense énergétique pouvaient être transitoires. Nous avons donc voulu déterminer si, dans des conditions contrôlées, une consommation élevée d'AGCM pour 4 semaines produirait une différence de la dépense énergétique et de l'oxydation des substrats comparativement à une consommation d'AGCL. De plus, notre objectif était d'établir si une consommation, sur une période de 4 semaines, d'AGCM mènerait à de plus grands changements dans la composition corporelle qu'une consommation d'AGCL. Nous avons produits deux études contrôlées, randomisées, en chassé croisées impliquant des femmes et des hommes ayant un surplus de poids pour vérifier nos objectifs. Un deuxième objectif était d'examiner l'effet des AGCM sur la satiété, et ceci a été testé chez les hommes. Finalement, un troisième objectif était de déterminer si, lorsque combiné à des phytostérols et de l'huile de lin, les AGCM produisent un profile lipidique différent des AGCL. Dix-neuf femmes en santé et ayant un surplus de poids et 24

hommes en santé ayant un surplus de poids ont participé à 2 études différentes, contrôlées et randomisées, pour tester ces objectifs. La dépense énergétique et la composition corporelle des sujets ont été analysées au début et à la fin de chaque phase expérimentale, qui différaient seulement dans le type de gras inclus dans la diète. Des échantillons sanguins ont été prélevés au début et à la fin de chaque phase pour déterminer les concentrations de lipides plasmatiques. Les résultats ont démontré que la dépense énergétique était plus élevée avec la consommation d'AGCM comparativement aux AGCL chez les femmes et les hommes. Chez les femmes, cette augmentation de la dépense énergétique ne s'est pas traduite en différences significatives du changement de composition corporelle, tandis que chez les hommes, l'adiposité du haut du corps était réduite de plus grande façon avec la consommation de la diète riche en AGCM qu'avec la diète riche en AGCL. La combinaison d'AGCM, de phytostérols, et d'huile de lin a causé une diminution significative des taux de cholestérol total et de cholestérol LDL chez les femmes et les hommes tout en n'ayant aucun impact sur le taux de triglycérides. Il peut donc être conclu que la consommation d'une huile riche en ACGM, phytostérols, et huile de lin peut être considérée comme aliment fonctionnel dans le maintien du poids corporel ou la prévention d'une prise pondérale tout en promouvant la santé cardiaque.

PREFACE

This thesis examines the effects of an oil combining medium chain triglycerides (MCT), phytosterols, and flaxseed oil, on energy expenditure, substrate oxidation, and body composition compared to oils containing long chain triglycerides (LCT). As secondary and tertiary objectives, we examined the effects of an oil rich in MCT on satiety and on blood lipid profiles compared to an oil rich in LCT.

The results of the trials conducted to test our objectives are presented in manuscript format. Each chapter of this thesis contains a review of the literature pertinent to each topic. Chapter 1 is a broad literature review spanning the effects of MCT consumption on energy expenditure, body composition, and satiety as a means of preventing weight gain. Chapter 2 reports the results of a randomized crossover controlled trial examining the effects of a 4 wk consumption of a diet rich in MCT versus LCT on energy expenditure and body composition in overweight women. Chapter 3 reports the results of a randomized crossover controlled trial examining the effects of a 4 wk consumption of a diet rich in MCT versus LCT on energy expenditure, body composition, and satiety in overweight men. Chapter 4 examines the effects of a 4 wk consumption period of an oil rich in MCT, phytosterols and flaxseed oil on blood lipid and aminothiol concentrations in overweight women and chapter 5 examines the effects of this oil on blood lipids as well as low-density lipoprotein particle size in overweight men.

This thesis ends with a general summary and conclusion as well as a description of the limitations of the results reported. This thesis is based on 2 published papers, 2 manuscripts in press, and 2 manuscripts submitted to peer reviewed journals.

ADVANCE OF SCHOLARLY KNOWLEDGE

1. Original contribution to knowledge:

- Extends current knowledge of the effects of acute consumption of medium chain triglycerides versus long chain triglycerides on energy expenditure and substrate oxidation rates;
- Improves knowledge of the effects of chronic medium chain triglyceride consumption versus long chain triglycerides on energy expenditure and substrate oxidation rates;
- Shows the effects of MCT consumption, as part of a diet targeting weight-maintenance on body composition;
- Shows that the thermogenic effects of MCT may be more pronounced in individuals of lower initial body weights;
- Adds to the current knowledge concerning effects of medium chain triglyceride consumption on ad libitum food intakes;
- Shows that addition of phytosterols and flaxseed oil to medium chain triglycerides prevents the predicted expected rise in total and LDL-cholesterol concentrations, as well as triglyceride levels.
- Shows, for the first time, that MCT consumption, in combination with phytosterols, does not affect low density lipoprotein particle size.

2. Research publications in refereed scientific journals:

- St-Onge M-P, Jones PJH. A review of the physiological effects of medium chain triglycerides as potential agents in the prevention of obesity. *J Nutr* 2002;132:329-32.

- St-Onge M-P, Bourque C, Jones PJH, Ross R, Parsons WD. Consumption of medium- versus long-chain triglycerides for 27 days increases fat oxidation and energy expenditure without resulting in changing body composition in overweight women. *Int J Obes* 2003;27:95-102.
- St-Onge M-P, Ross R, Parsons WD, Jones PJH. Consumption of a functional oil containing medium chain triglycerides by overweight men increases energy expenditure and decreases body adiposity compared to a diet rich in olive oil. *Obes Res* (in press).
- Bourque C, St-Onge M-P, Papamandjaris AA, Cohn JS, Jones PJH. Consumption of a functional oil composed of medium chain triacylglycerols, phytosterols and n-3 fatty acids improves the overall cardiovascular risk profile of overweight women. *Metabolism* (in press).

3. Research manuscripts submitted or to be submitted to refereed journals:

- St-Onge M-P, Mauger J-F, Lamarche B, Jones PJH. Consumption of a functional oil containing phytosterols and medium chain triglyceride oil for 28 days decreases plasma lipid concentrations relative to olive oil and leads to increased LDL particle size. (Submitted to *J Nutr*).
- St-Onge M-P, Jones PJH. Greater rise in fat oxidation with medium chain triglyceride consumption relative to long chain triglyceride is associated with lower initial body weight and greater loss of adipose tissue. (Submitted to *Int J Obes*).

CONTRIBUTION OF CO-AUTHORS TO MANUSCRIPTS

The original idea for the review article came from the candidate, who contributed to the written work by collecting articles, preparing the figure and writing the text. The study design and protocol for trial I in women was elaborated by Peter Jones, Marie-Pierre St-Onge, and Andrea Papamandjaris. The candidate conducted and coordinated the research trial in women with Christine Bourque. All energy expenditure measurements and analyses were performed by the candidate. The candidate also analyzed all magnetic resonance images. All statistical analyses performed on energy expenditure, body composition and fecal fat excretion were executed by the candidate. The protocol for trial II in men was elaborated by the candidate with some help from Peter Jones. The candidate recruited all subjects and coordinated the research trial. The candidate also performed all work during the research trial, with some hired help for cooking and food portioning. All energy expenditure measurements and statistical analyses were performed by the candidate. The candidate also analyzed most magnetic resonance with some help from Lindsay MacKinnon, who analyzed the images for 2 subjects, and performed all statistical analyses on body composition data. Blood lipid concentrations were analyzed by the candidate with some help from Sarah Howe.

Dr. Peter JH Jones, the candidate's supervisor, edited all of the manuscripts presented in this thesis. Dr. Jones developed the protocol for the initial trial in women with some help from the candidate and assisted the candidate in the development of the trial in men. Furthermore, Dr. Jones provided regular meetings to assess progress with the work.

Dr. Robert Ross provided training for analysis of body composition using magnetic resonance imaging. Dr. Ross provided valuable input in all manuscripts in which he was a co-author.

Christine Bourque helped with recruitment of subjects and coordination of the research trial in women. Ms. Bourque also provided input for the manuscript in which she was a co-author and conducted all lipid analyses for the women's trial as well as write the manuscript in which she was first author.

Dr. William D Parsons was the study clinician. He was available to meet subjects should they have any medical issues related to the trials and to provide consent for magnetic resonance imaging scanning at the Montreal Neurological Institute. Dr. Parsons also provided valuable comments during the editing of the manuscripts in which he was a co-author.

Dr. Jeffrey Cohn provided the expertise for the analysis of aminothiols. He provided valuable input for lipid methodologies and was also involved in editing the manuscript in which he was a co-author.

Dr. Andrea Papamandjaris helped obtain the grant for this research and helped design the original protocol for the trial in women. She also helped with editing of the manuscript in which she was a co-author.

Dr. Benoît Lamarche provided the analyses of low-density lipoprotein particle size and helped with the editing of the manuscript in which he was a co-author.

Jean-François Mauger helped with the analyses of LDL particle size.

Dr. Stan Kubow, the candidate's committee member, edited the final draft of this thesis.

This research was supported by Dairy Farmers of Canada and Forbes Medi-Tech Inc.

The candidate was supported in part by the Fonds de recherche en santé du Québec (FRSQ-FCAR-Santé).

ACKNOWLEDGEMENTS

I would not have been able to go through this adventure with a smile without the help of several people. My parents and sister have always been very supportive and I would like to thank them for going through the range of emotions that come with graduate studies with me. You have always encouraged me to pursue me dreams and go to the maximum of my abilities. I can never be thankful enough for your support.

I would like to thank my fellow lab mates and friends, Yan-Wen Wang, Mélanie Journoud, Makiko Yoshika, Catherine Vanstone, Chris Vanstone, Stephanie Wollin, Geoff Hynes, Vivian Lau, and Mahmoud Raeini-Sarjaz for their support throughout this experience. They were of great assistance in helping me get through with sanity. I would also like to thank my friends in the School of Dietetics and Human Nutrition, especially Patrick Owen, Uma Palanappian, Lindi Sibeko, and Tanya Verrell for their encouragements.

Last, but certainly not least, a most special thank you to my supervisor, Dr. Peter Jones, who has seen me through it all! Your positive outlook and enthusiasm has made the world of difference for me. Thank you for always being available and supportive during my endeavours. I will certainly miss your open-mindedness and intellectual stimuli. I would like to thank you especially for making my graduate life at McGill the most wonderful and fulfilling experience.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Triglycerides differ in their modes and rates of digestion and absorption depending on the chain lengths of their fatty acids. It is well understood that triglycerides are digested to their respective long or medium chain fatty acids in the gastrointestinal tract. However, the metabolic fate of these fatty acids differs. Long chain fatty acids (LCFA) must be repackaged as triglycerides into chylomicrons for transportation into the peripheral circulation, where they travel to extrahepatic tissues (Bach and Babayan, 1982). These long chain triglycerides (LCT) thus have a great potential for deposition into adipose tissue, where lipoprotein lipase breaks them down to LCFA for storage into adipose tissue. Medium chain fatty acids (MCFA) owing to their smaller size of 6 to 12 carbons, are more easily solubilized (Bach and Babayan, 1982) and do not require chylomicron formation for transportation across the intestinal mucosa (Babayan, 1987). Medium chain fatty acids are thus not transported throughout the lymphatic system and peripheral circulation but travel through the portal circulation directly to the liver. This mode of transportation renders MCFA less susceptible to deposition into adipose tissue and makes them highly and rapidly available for oxidation and energy production. In addition, medium chain triglycerides (MCT) do not require carnitine for entry into the mitochondria, also leading to faster oxidation and a decreased likelihood of undergoing elongation to LCT and deposition into adipose tissue (Babayan, 1987).

These differences in modes of absorption and metabolism of LCT and MCT have prompted researchers to examine whether differences exist between LCT and MCT consumption on thermic effect of food and substrate oxidation in humans. Animal studies have also been

conducted to determine whether the different transport systems for LCFA and MCFA result in differences in adiposity, weight gain, and fat cell size. Most human studies have established that MCT consumption leads to increased energy expenditure and fat oxidation relative to LCT consumption (Dulloo et al., 1996; Hill et al., 1989; Scalfi et al., 1991; Seaton et al., 1986; White et al., 1999) while animal trials have shown that animals fed MCT gain less weight, have smaller fat depots, and fewer fat cells than those fed LCT (Baba et al., 1982; Crozier et al., 1987; Geliebter et al., 1983; Lavau and Hashim, 1978). Although it has been speculated that MCT could be considered functional foods in the prevention of obesity, no controlled clinical trial has been of sufficient duration to determine whether the rise in energy expenditure with MCT consumption results in significant changes in body composition. This thesis therefore focuses on the effects of MCT versus LCT consumption for 4 wk on energy expenditure, substrate oxidation, and body composition in overweight women and men.

There have been some concerns regarding the effects of MCT consumption on plasma lipid concentrations and hence, cardiovascular disease risk. Some trials have observed increased triglyceride concentrations (Hill et al., 1989; Swift et al., 1992) while others have noted increased total (TC) and low-density lipoprotein (LDL-C) cholesterol concentrations (Cater et al., 1997) with MCT compared to LCT consumption. In order to circumvent these adverse effects of MCT consumption, we have added phytosterols and flaxseed oil to our diets rich in MCT. Phytosterols have been known to produce reductions in TC and LDL-C of approximately 10 and 13%, respectively (Moghadasian and Frohlich, 1999). Flaxseed oil, a rich source of n-3 fatty acids, may help prevent any rise that may occur in triglyceride

concentrations with MCT consumption. Therefore, a secondary objective of this thesis was to examine plasma lipid concentrations with MCT versus LCT consumption in overweight men and women.

CHAPTER 1. Manuscript 1. ***Published in J Nutr 2002:132:329-32, American Society for Nutritional Sciences***

PHYSIOLOGICAL EFFECTS OF MEDIUM CHAIN TRIGLYCERIDES AS POTENTIAL AGENTS IN THE PREVENTION OF OBESITY

Marie-Pierre St-Onge and Peter JH Jones

School of Dietetics and Human Nutrition, McGill University, Ste-Anne-de-Bellevue, Quebec, Canada, H9X 3V9.

Correspondence to: Dr. Peter J.H. Jones

School of Dietetics and Human Nutrition

21 111 Lakeshore Rd.

Ste-Anne-de-Bellevue, Québec, Canada

H9X 3V9

Tel.: (514) 398-7547 Fax: (514) 398-7739

jonesp@macdonald.mcgill.ca

Running head: Use of medium chain triglycerides in weight maintenance

1.1 ABSTRACT

Medium chain fatty acids (MCFA) have long been known to be readily oxidized in the liver. Animal and human studies have also shown that the fast rate of oxidation of MCFA leads to greater energy expenditure (EE). Most animal studies have also demonstrated that the greater EE with MCFA relative to long chain fatty acids (LCFA) results in less body weight gain and decreased size of fat depots after several months of consumption. Furthermore, both animal and human trials suggest a greater satiating effect of medium chain triglycerides (MCT) compared to long chain triglycerides (LCT). The aim of this review is to evaluate existing data describing effects of MCT on EE and satiety and determine their potential efficacy as agents in the treatment of human obesity. Animal studies are summarized and human trials more systematically evaluated since the primary focus of this article is to examine the effects of MCT on human energy metabolism and satiety. Hormones including cholescytokinin, peptide YY, gastric inhibitory peptide, neurotensin, and pancreatic polypeptide have been proposed as involved in the mechanism by which MCT may induce satiety; however, the exact mechanisms have not been established. From the literature reviewed, we conclude that MCT increase energy expenditure, may result in faster satiety and facilitate weight control when included in the diet as a replacement for fats containing LCT.

Key words: medium chain triglycerides, satiety, energy expenditure, obesity

1.2 INTRODUCTION

Differences in the metabolism of fats varying in fatty acid chain lengths have been well established (Babayan, 1987; Bach and Babayan, 1982; Dulloo et al., 1996; Flatt et al., 1985; Hill et al., 1989; Scalfi et al., 1991; Seaton et al., 1986; White et al., 1999). Medium chain triglycerides (MCT), containing 6 to 12 carbon fatty acids, differ from long chain triglycerides (LCT), which have fatty acids of more than 12 carbons, in that they are absorbed directly into the portal circulation and transported to the liver, for rapid oxidation (Babayan, 1987). LCT, however, are transported via chylomicrons into the lymphatic system allowing for much uptake into adipose tissue. Therefore, it has been hypothesized that the rapid metabolism of MCT may increase energy expenditure (EE), decrease their deposition into adipose tissue, and result in faster satiety. The objective of the present article is to review literature concerning the effects of MCT on EE, fat deposition and food intake as a means to establish the potential efficacy of MCT in the prevention of obesity in humans.

1.3 EFFECT OF MEDIUM CHAIN TRIGLYCERIDES ON ENERGY EXPENDITURE

Animal trials studying the effects of MCT versus LCT consumption on lipid and energy metabolism have shown that body weight (BW) is reduced with MCT consumption compared to LCT consumption and that feed efficiency is thus reduced (Lasekan et al., 1992; Mabayo et al., 1994; Rothwell and Stock, 1987). In a study in which animals infused with MCT gained one third of the weight gained by those infused with LCT, Lasekan et al. (1992) concluded that replacing LCT with MCT over long periods could produce weight loss without decreasing energy intakes.

Human studies have mostly compared the effects of MCT versus LCT in single meal or single day experiments. Scalfi et al. (1991) evaluated the effects of a single mixed meal containing MCT on post-prandial thermogenesis and examined possible differences in the thermic response between lean and obese male subjects. Subjects consumed a meal containing 15% of energy from protein, 55% from carbohydrate, and 30% from fat, in the form of corn oil (CO) and animal fat or MCT oil (56% octanoate, 40% decanoate) in random order. Energy expenditure measurements were conducted prior to and for 6 h after consumption of the meal. Total EE was 48% and 65% greater in lean and obese individuals, respectively, after MCT compared with LCT consumption. Similar results were obtained by Seaton et al. (1986) comparing the effects of MCT or CO on EE after a single meal. Energy expenditure peaked at 16% above baseline after MCT consumption compared to 5% for CO.

Dulloo et al. (1996) investigated the thermogenic effects of low to moderate amounts of MCT consumption in healthy adult males. Subjects were required to spend 24 h in a respiratory chamber on 4 separate occasions during which time diets differed in the ratio of MCT: LCT (0:30, 5:25, 15:15, 30:0) provided as added fat. The diet was given at a level 1.4 times energy requirements and the 30 g of added fat was distributed evenly across all meals. The authors found that EE between 8 am and 11 pm increased by 45, 135, and 265 kJ with 5, 15, and 30 g of MCT in the diet, respectively. Mean 24 h EE also increased by 162 and 475 kJ with 15 and 30 g of MCT in added fat. These results showed that the greater effects of MCT than LCT on EE are not only evident in the few hours following the meal but are longer-lasting.

Most results (Dulloo et al., 1996; Scalfi et al., 1991; Seaton et al., 1986) from single day experiments conclude that replacing LCT for MCT in the diet could produce weight loss after prolonged consumption. However, when Flatt et al. (1985) compared diets rich in MCT, LCT, and low in fat, they concluded that a low fat diet was more prudent when aiming for weight loss. However, MCT consumption resulted in greater EE at several time points compared to the low fat diet.

Few trials have been conducted over longer periods. One of those studies examined energy balance during overfeeding liquid formula diets containing MCT (61% octanoate, 32% decanoate) or LCT (32% oleate, 51% linoleate) for 7 d (Hill et al., 1989). EE was measured on d 1 and 6 for 10-15 min every 30 min for 6 h following meal consumption. The thermic effect of food (TEF) was identified as 8% of ingested energy after MCT consumption compared to 5.8% after LCT consumption on d 1. After 6 d, TEF was 12% and 6.6% of ingested energy with MCT and LCT consumption, respectively. This study showed that the difference in EE between MCT and LCT persists, even after a week of overfeeding.

The longest published study to date (White et al., 1999) aimed at determining whether fatty acid chain length influences EE and substrate oxidation in women over 14 d. Subjects consumed a controlled, weight maintenance diet containing 40% of energy as fat, either in the form of butter and coconut oil (MCT; 38.9% of fatty acids contained chains with less than 16 carbons) or beef tallow. Energy expenditure was measured over a 30 min period prior to and for 5.5 h following breakfast. Post-prandial total EE following MCT

consumption was greater than after LCT consumption on d 7 but not d 14. It was concluded that the effects of MCT consumption on EE may be transient.

All animal studies (Lasekan et al., 1992; Mabayo et al., 1994; Rothwell and Stock, 1987) and most human studies (Dullloo et al., 1996; Hill et al., 1989; Scalfi et al., 1991; Seaton et al., 1986; White et al., 1999) have shown that MCT consumption increases EE when compared to a meal containing LCT. Studies that have found greatest differences in EE with MCT compared to LCT consumption also concluded that MCT could be used in the treatment or prevention of human obesity (Dullloo et al., 1996; Scalfi et al., 1991; Seaton et al., 1986). However, the studies conducted to date have been of short duration, ranging from a single meal (Dullloo et al., 1996; Flatt et al., 1985; Scalfi et al., 1991; Seaton et al., 1986) to several days (Hill et al., 1989; White et al., 1999). Whether effects of MCT on EE and RQ are long-lasting and result in actual measurable and sustainable changes in body composition of humans remain to be established.

1.4 EFFECT OF MEDIUM CHAIN TRIGLYCERIDES ON FAT DEPOSITION

Given that feed efficiency studies in animals and energetic studies in humans indicate enhanced EE following MCT consumption (Dullloo et al., 1996; Flatt et al., 1985; Hill et al., 1989; Lasekan et al., 1992; Mabayo et al., 1994; Rothwell and Stock, 1987; Scalfi et al., 1991; Seaton et al., 1986; White et al., 1999), additional work has examined whether increased EE translates into decreased fat mass. Results from these studies showed that in animals consuming MCT, BW were lower, fat depots smaller (Baba et al., 1982; Crozier et al., 1987; Geliebter et al., 1983; Lavau and Hashim, 1978) and adipocyte size decreased

(Baba et al., 1982; Crozier et al., 1987) with MCT compared to LCT consumption. These results led authors to conclude that MCT could potentially prevent (Crozier et al., 1987) or control (Lavau and Hashim, 1978) obesity in humans. However, MCT consumption has not been observed by Hill et al. (1989) to cause greater weight loss than lard, CO, or fish oil (FO). Body adipose tissue during the first 3 mo was not different between groups but after 6 mo, the group fed FO had lower body fat than all other groups. Also, MCT storage tended to decrease with duration of feeding. From these results it was concluded that although both FO and MCT feeding resulted in small fat cells, only FO feeding was associated with inhibition of cell proliferation.

Only one study evaluated the ability of MCT to facilitate weight reduction in humans (Yost and Eckel, 1989). Sixteen obese women consumed MCT (58% octanoate, 22% decanoate) or LCT (soy oil) in random order for either 4 wk if they were inpatients or 12 wk if they were outpatients, at a level of 191 kJ/d. There was no difference in weight loss or rate of weight loss between diets. It was thus concluded that a liquid diet containing 24% of energy as MCT failed to increase the rate of weight loss when compared to LCT. This lack of agreement with animal trials and EE experiments may have been due to the low fat content of the diets, which contained 1.5 g of total fat/d, of which 1.2 g was treatment fat or to gender differences in the effects of MCT. Differences detected in EE with MCT and LCT consumption are considerably greater in males than females. When data are extrapolated from trials conducted on men (Dullloo et al., 1996; Hill et al., 1989; Scalfi et al., 1991; Seaton et al., 1986), average EE was approximately 460 kJ/d greater with MCT than with LCT consumption, with a peak difference between treatments at of 669 kJ/d (Hill et al.,

1989). In contrast, data from White et al, who studied female subjects, found differences in EE of 138 kJ/d between MCT and LCT consumption. Our own work with overweight female subjects also reveals a difference in EE of approximately 188 kJ/d (St-Onge et al., 2001, abstract). From these preliminary data, it appears that women respond less readily to treatment with MCT than men.

1.5 EFFECT OF MCT ON FOOD INTAKE AND SATIETY: Animal studies

Lower weight gain and decreased fat depot size with MCT feeding compared to LCT feeding in animals have been attributed to two different effects of MCT, increased EE and decreased food intake. Satiety may also be affected by fatty acid chain length of dietary fat. Bray et al. (1980) observed greater feed intake when LCT were included in the diets of the rats compared to diets containing MCT. After 80 d of being fed diets containing 60% of energy from CO, MCT, or a mixture of the two, rats fed the CO and the CO-MCT diets had greater BW than those fed the MCT diet alone. It was concluded that rats fed the MCT diet ate fewer calories and that ß-hydroxybutyrate may play a role in the difference in food intake between MCT and CO fed rats.

Following from these results, Maggio and Koopmans (1982), in 1982, conducted a study to clarify the origin and the nature of the signals that terminate short-term food intake of mixed meals containing triglycerides (TG) with fatty acids of different chain lengths. Sprague-Dawley rats were intragastrically intubated and given free access to a liquid diet containing 21% of energy as fat. The TG infusions consisted of 70% TG (tributyrin, tricaprylin, or triolein in different concentrations) and 30% carbohydrate. The authors found that shifting

chain length from medium to long did not differentially affect food intake when the infusions were equicaloric. Therefore, it was concluded that satiety may be related to the number of calories ingested rather than to the physical characteristics of the specific nutrients. This contrasts results obtained by Denbow et al. (1992). Here white leghorn cockerels were infused intrahepatically or intubated intragastrically with isocaloric quantities tributyrate, tridecanoate, or trioleate and their feed consumption measured. Feed consumption with SCT and MCT infusion was suppressed within 1 h after intrahepatic infusion until 180 min. However, when infusions were given intragastrically, only SCT was effective in decreasing feed intake. The authors concluded that these results reflect the relatively rapid rate of digestion and absorption of short chain fatty acids (SCFA) from the gut along with oxidation of SCFA by the liver.

Furuse et al. (1992) also investigated the effects of two different levels of MCT on feed intake in rats. They further examined endogenous cholecystokinin's (CCK) capacity to modulate feed intake with MCT. Feed intake of male Wistar rats fed diets containing CO, MCT, or a 1:1 mixture of CO and MCT was determined every hour for 12 h and then at 2 h intervals for the following 12 h. In a separate trial, Devazepide (DVZ), a CCK-A receptor antagonist, was intraperitoneally injected 40 min before feeding and feed intake was measured at 1, 2, 3, and 6 h post-injection. Feed intake decreased in a dose-dependent manner with increased concentration of MCT in the diet and was enhanced 2 h after DVZ injection. After 3 h, feed intake with the MCT diet was less than after the CO diet. It was thus concluded that satiety is affected by carbon chain length in dietary TG sources.

1.6 EFFECT OF MCT ON FOOD INTAKE AND SATIETY: Human studies

If MCT consumption enhances satiety and decrease food intake satiety in animals, an equivalent response might be expected in humans. Stubbs and Harbron (1996) have examined whether the effects of ingesting MCT can limit the hyperphagia associated with high-fat, energy-dense diets in humans. Six men participated in a three-phase inpatient trial in which they had ad libitum access to experimental high fat foods (61.5% of energy as fat) for 14 d. Each experimental phase differed in the amount of MCT included in the diet; either low, medium or high MCT content with 20%, 31% and 40%, respectively, of total energy as MCT. Subjects consumed 15.1 MJ and 17.6 MJ less on the diet containing the most MCT compared to the diets containing the low and medium amounts of MCT, respectively, over the 14 d period. Body weights on low and medium MCT diets increased by 0.45 and 0.41 kg, respectively, and decreased by 0.03 kg with the high MCT content diet. Food and energy intakes were thus suppressed when two thirds of the fat content of a high-fat diet was derived from MCT, but BW were not affected.

Another clinical trial (Van Wymelbeke et al., 1998) aimed to establish the influence of chain length and degree of saturation on food intake in normal weight men. Breakfasts differing in the nature of the fat; olive oil, lard, MCT, or a fat substitute served and food intake at lunch and dinner was measured. Energy intake at lunch was lower after the MCT-containing breakfast than after all other breakfasts (3100 kJ vs 3715 kJ with the fat substitute, 3278 kJ with olive oil, and 3798 kJ with lard) but there was no difference in food consumption at dinner.

1.7 HORMONES INVOLVED IN THE SATIATING EFFECT OF MEDIUM AND LONG CHAIN TRIGLYCERIDES

Clinical trials (Stubbs and Harbron, 1996; Van Wymelbeke et al., 1998) have thus shown that MCT consumption can lead to lower energy intakes but failed to explore the underlying mechanism. More recently, research has focused on specific hormones that may be involved in the satiating effect of MCT. McLaughlin et al. (1999) examined the relationship between fatty acid chain length, CCK secretion, and proximal and distal gastric motor function. Fifteen healthy volunteers were studied for their response to a control meal and orogastric infusion of 250 mL of a 0.05 mol/L fatty acid emulsion. It was found that fatty acid emulsions containing fatty acids of 11 carbon chains and less did not increase plasma CCK concentrations compared to the vehicle whereas LCFA did. This study showed that the human proximal gut differentiates between fatty acid molecules, however, does not support the role of CCK in mediating the satiating effect of MCT.

Several other studies have also reported that MCT do not stimulate CCK secretion in humans (Barbera et al., 2000; Maas et al., 1996; Maas et al., 1998) and trials have attempted to establish which hormone is responsible for the observed effects of MCT on food intake. Barbera et al. (2000) compared effects of MCT and LCT on sensations of satiety, gastric tone, gastric inhibitory peptide (GIP), pancreatic polypeptide, and CCK. Nine subjects were infused with either saline, LCFA (mainly oleate and linoleate), and MCFA (octanoate and decanoate) on three separate occasions in random order. LCFA infusion resulted in greater rise in satiation than MCFA but there was no difference between the two fats on perception of fullness and bloating. The rise in gastric volume was also greater with LCFA infusion than MCFA infusion. Similarly, LCFA increased baseline levels of plasma CCK, GIP,

neurotensin, and pancreatic polypeptide compared to saline and MCFA, whereas MCFA infusion did not. It was thus concluded that MCFA induces gastric relaxation without increasing satiation or plasma levels of gut hormones. However, since Stubbs and Harbron (1996) and Van Wymelbeke et al. (1998) have shown lower food intakes with diets rich in MCT, it is likely that other factors play a role in regulating energy balance with MCT consumption.

Maas et al. (1998) examined effects of MCFA and LCFA on peptide YY (PYY) release to determine whether PYY, which inhibits gastric acid secretion in humans, is involved in the enterogastrone effect of MCFA. These investigators had previously observed that infusions of MCFA suppressed gastrin-stimulated gastric acid secretion without the involvement of CCK (Maas et al., 1996). Fourteen men were intraduodenally infused for 2.5 h with MCFA (56% octanoate, 43% decanoate), LCFA (CO), or saline in random order. The caloric loads differed between MCFA and LCFA infusions, the former providing a load of 11.6 kJ/min and the latter providing a load of 22.7 kJ/min. Both infusions increased plasma levels of PYY, however, LCFA resulted in a greater increase than MCFA infusion (10.3 vs 2.8 pmol/L). LCFA inhibited gastrin-stimulated gastric acid secretion by 4.1 mmol/15 min compared to 2.7 mmol/15 min for MCFA. PYY is therefore involved in the enterogastrone effect of MCFA, however, MCFA are less potent at inducing PYY release than LCFA. Greater induction of PYY release by LCFA may be due to CCK discharge by LCFA, since CCK has been shown to stimulate PYY secretion. Other hormones may thus be involved in the mechanism by which MCFA inhibit gastric acid secretion yet, except for GIP, which is not released in response to MCFA, these have not been studied.

Recently, Feinle et al. (2001) have investigated the ability of TG with fatty acids of varying chain lengths to induce gastrointestinal sensations and symptoms. Five different infusions were studied; LCT (soybean oil), MCT, soy lecithin, Orlistat^{tm} and sucrose polyester. LCT and MCT both increased gastric volume, with LCT causing the greater rise. All infusions resulted in increased feelings of fullness, bloating and nausea, and decreased hunger but were most pronounced with LCT infusion. It was concluded that the mechanism of action of fat in the generation of gastrointestinal symptoms required digestion of TG. Furthermore, since MCT do not release CCK yet affect sensations of fullness, bloating and nausea, CCK-dependent and CCK-independent mechanisms must be involved.

It is thus well established that, in humans, MCFA do not stimulate CCK secretion. Therefore, CCK must not be the hormone responsible for their satiating effect (McLaughlin et al., 1999; Barbera et al., 2000; Maas et al., 1996; Maas et al., 1998; Feinle et al., 2001). Although MCT have been shown to induce satiety and to stimulate hormone secretion, no one particular hormone has been found to be strongly secreted due to MCT digestion. PYY has been found to be secreted in response to MCFA yet it is still more potently secreted in response to LCT (Maas et al., 1998).

1.8 POTENTIAL BENEFITS TO CONSUMPTION OF MEDIUM CHAIN TRIGLYCERIDES ON BODY WEIGHT

There is evidence to suggest that short-term consumption of MCT increase EE in humans (Scalfi et al., 1991; Seaton et al., 1986; Dulloo et al., 1996; Hill et al., 1989; White et al., 1999) and results in decreased fat cell size and body weight accretion in animals (Baba et al.,

1982; Crozier et al., 1987; Geliebter et al., 1983; Lavau and Hashim, 1978; Hill et al., 1993; Bray et al., 1980). Human studies have shown that replacing dietary LCT with MCT results in an increase in daily energy expenditure of 100 kJ (Flatt et al, 1985) to 669 kJ (Hill et al., 1989) in men and 138 kJ/d (White et al, 1999) in women. Studies examining the satiating effect of fats of different chain lengths found that energy intake was approximately 1070 kJ lower when meals contained MCT than when they contained LCT as fat source (Stubbs and Harbron, 1996). Van Wymelbeke et al. (1998) found that intakes were 175 to 698 kJ lower, depending on the chain saturation of the LCT, at the subsequent meal when MCT substituted LCT. Therefore, in the most optimistic scenario where EE would be increased by 669 kJ/d (Hill et al., 1989) and intakes decreased by 698 kJ/d (Stubbs and Harbron, 1996), then a weight gain of 1.35 kg/mo could be avoided by replacing LCT with MCT in the diet. On the other hand, the least optimistic scenario would give an increase in daily EE of 100 kJ (Flatt et al, 1985) and decreased daily food intake of 350 kJ/d (2 subsequent meals at 175 kJ less each, Van Wymelbeke et al., 1998). In this case, a weight gain of 0.45 kg/mo would be avoided (**Figure 1-1**). If we project these data to long-term weight balance, a negative weight balance of 5.4 to 16.2 kg/y would be produced. However, more work is needed to establish whether prolonged consumption of MCT results in a decrease in BW or smaller weight gain compared to LCT.

1.9 SUMMARY

In summary, research conducted to date in animals show that replacing dietary LCT by MCT causes a rise in EE, depression of food intake and lower body fat mass. Similarly in humans, MCT increase EE relative to LCT consumption. Fewer studies have examined the effects of

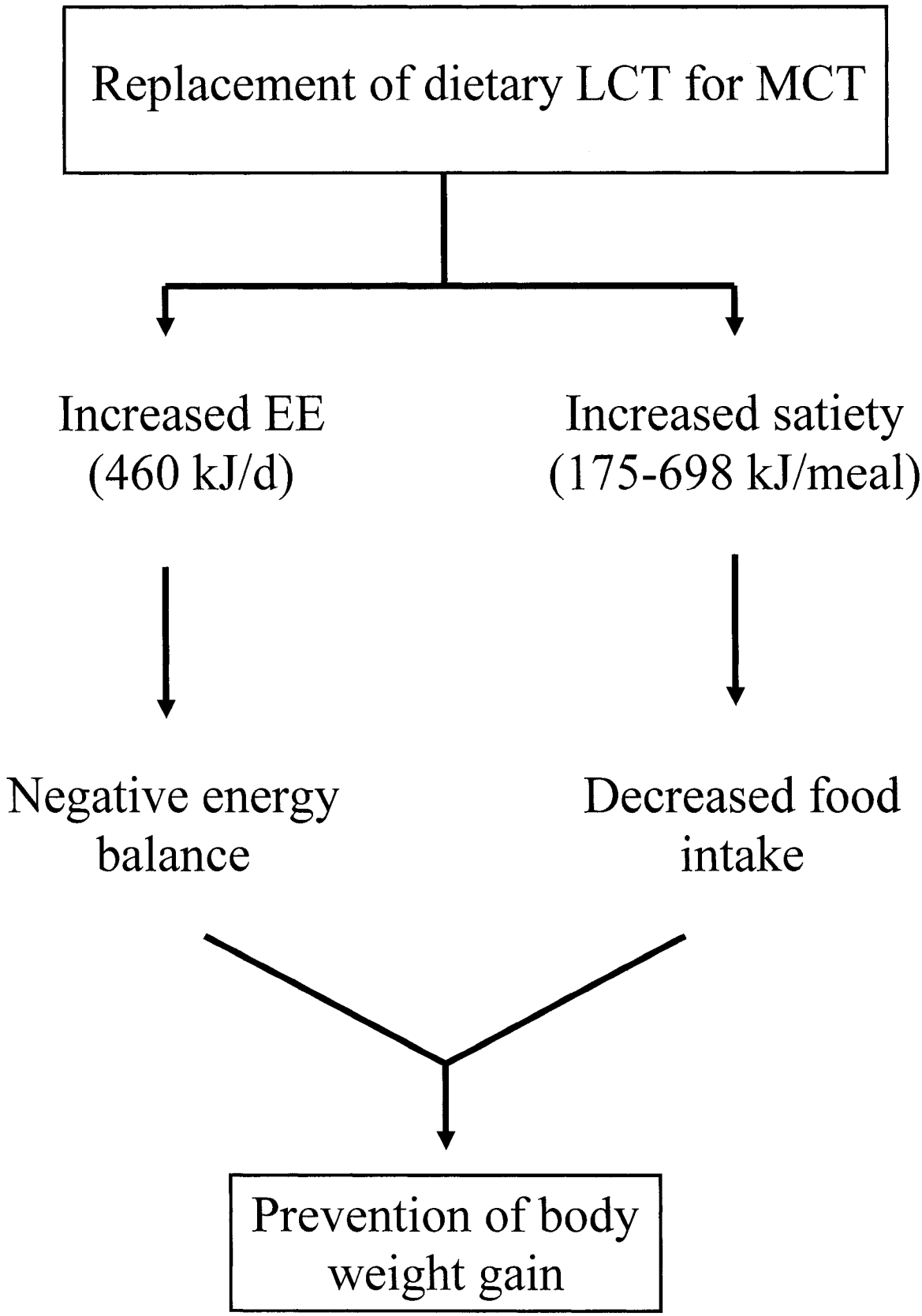
MCT on satiety but, although results vary, these also suggest decreased food intake when LCT are replaced with MCT in the diet. Therefore, greater EE and lower food intake with MCT compared to LCT suggest that replacing dietary LCT with MCT could lead to better weight maintenance in humans.

1.10 FIGURE LEGEND

Figure 1-1

Replacement of dietary long chain for medium chain triglycerides can lead to increases in energy expenditure and satiety level. Energy expenditure can be increased by up to 460 kJ/d and decreased food intake by 175 to 698 kJ/d. The combination of increased energy expenditure and satiety can lead to prevention of body weight gain.

Figure 1-1.


Replacement of dietary LCT for MCT
Increased EE
(460 kJ/d)
Increased satiety
(175-698 kJ/meal)
Negative energy
balance
Decreased food
intake
Prevention of body
weight gain

BRIDGE 1.

Most research trials examining the effects of medium chain triglyceride versus long chain triglyceride consumption have shown that intakes of a meal rich in medium chain triglycerides increase energy expenditure and fat oxidation compared to a meal rich in long chain triglycerides. However, the longest trial conducted to date, spanning over 14 d, showed that there may be an attenuation of the effects of medium chain triglyceride consumption on energy expenditure after 14 d of intake. Moreover, methodological limitations have prevented direct observation of the effect of the rise in energy expenditure on body weight and adiposity. Since medium chain triglycerides have only been studied for short durations, ranging from one meal to 14 d, and since their effect on energy expenditure is reportedly small, it has not been possible to identify actions of medium chain triglycerides on body weight. Accordingly, it was proposed to conduct a trial of longer duration, 27 d, to observe whether the increased energy expenditure observed with medium chain triglycerides would occur during both acute and chronic intakes. Furthermore, it was attempted to determine whether elevated energy expenditure with medium chain triglycerides relative to long chain triglycerides would translate into actual body weight changes as well as specific changes in regional adiposity. The use of magnetic resonance imaging to examine changes in body composition allows measurement of small changes in adiposity and is therefore the most precise and appropriate method to assess the effects of medium chain triglycerides versus long chain triglycerides on body composition. The first trial reported was conducted in overweight women (n=17). Feeding periods extended over 27 d and compared beef tallow as a source of saturated long chain triglycerides to a functional oil combining medium chain triglyceride oil, coconut oil, butter, flaxseed oil, and phytosterols. Both diets were identical

except for the quality of the treatment fat and were designed to provide all nutrients and essential fatty acids in amounts that met the Canadian recommendations for nutrient intakes. Phytosterols were included in the medium chain triglyceride-containing diet in order to prevent a potential rise in plasma total and low-density lipoprotein cholesterol concentrations that has been observed in previous feeding studies involving medium chain triglycerides.

CHAPTER 2. Manuscript 2. ***Published in Int J Obes 2003;27:95-102, International Association for the Study of Obesity.***

MEDIUM- VERSUS LONG-CHAIN TRIGLYCERIDES FOR 27 DAYS INCREASES FAT OXIDATION AND ENERGY EXPENDITURE WITHOUT RESULTING IN CHANGES IN BODY COMPOSITION IN OVERWEIGHT WOMEN

Marie-Pierre St-Onge[1], Christine Bourque[1], Peter JH Jones[1], Robert Ross[2], William D Parsons[1]

[1]School of Dietetics and Human Nutrition, McGill University, Ste-Anne-de-Bellevue, Québec H9X 3V9

[2]School of Physical and Health Education, Queen's University, Kingston, Ontario K7L 3N6

Corresponding author: Peter JH Jones

School of Dietetics and Human Nutrition

21 111 Lakeshore Rd.

Ste-Anne-de-Bellevue, Québec

H9X 3V9

Phone: (514) 398-7547

e-mail: jonesp@macdonald.mcgill.ca

Supported by Dairy Farmers of Canada and Forbes Medi-Tech Inc.

Running head: MCT and energy expenditure

2.1 ABSTRACT

The objective of this research is to determine the effects of long-term consumption of medium chain (MCT) versus long chain triglycerides (LCT) on energy expenditure (EE), substrate oxidation and body composition. Our hypothesis is that MCT consumption will not result in greater EE, substrate oxidation, and body weight loss compared with LCT consumption. Seventeen healthy obese women participated in this randomized, crossover inpatient trial. Meals were prepared and consumed on site for two periods of 27 d. Diets, containing 40% of energy as fat with treatment fat comprising 75% of the total fat, were designed to supply each subject with their individual weight-maintaining energy needs. The functional oil (FctO) diet contained 67% of treatment fat as MCT oil (49% octanoate, 50% decanoate) whereas the control diet contained exclusively beef tallow (BT) as treatment fat. Body composition was assessed by magnetic resonance imaging (MRI) on d 1 and 28 of each phase while energy expenditure was measured on d 2 and 27. Changes in total and subcutaneous adipose tissue volumes following consumption of FctO and BT were not different (-0.61 ± 0.38 L vs -0.54 ± 0.48 L and -0.58 ± 0.35 L vs -0.48 ± 0.40 L, respectively). Average EE and fat oxidation were greater ($p<0.05$) during FctO than BT consumption (0.95 ± 0.019 versus 0.90 ± 0.024 kcal/min, respectively, for EE and 0.080 ± 0.0026 versus 0.075 ± 0.0022 g/min, respectively, for fat oxidation). These results show that long-term consumption of FctO enhances EE and fat oxidation in obese women, when compared to BT consumption. The difference in body composition change between FctO and BT consumption, although not statistically different, was consistent with differences predicted by the shifts in EE. It can be concluded that substitution of FctO for BT, rich in LCT, in a targeted energy balance diet may prevent long-term weight gain via increased EE.

Key words: medium chain triglycerides, body composition, magnetic resonance imaging, energy expenditure

2.2 INTRODUCTION

The contribution of dietary fat to daily caloric intake has long been recognized. Diet fat is efficiently stored, being an important factor in the growing problem of obesity in North America. However, all fats may not be partitioned for storage with similar efficiency. Animal studies have shown decreased weight gain and smaller fat depots with medium chain triglyceride (MCT) consumption compared to long chain triglyceride (LCT; Crozier et al., 1987; Geliebter et al., 1983; Hashim and Tantibhedyangkul, 1987), thus, suggesting a lower feed efficiency of MCT. The more rapid oxidation of MCT also results in greater energy expenditure (EE) when compared to LCT in both animals (Baba et al., 1982; Geliebter et al., 1983; Lasekan et al., 1992; Rothwell and Stock, 1987) and humans (Dulloo et al., 1996; Seaton et al., 1986; Scalfi et al., 1991; White et al., 1999). Greater EE and lower respiratory quotients (RQ) in humans consuming single or several meals containing MCT compared to LCT have been observed by various groups (Dulloo et al., 1996; Seaton et al., 1986; Scalfi et al., 1991). MCT consumption resulted in thermic effect of food (TEF) close to 50% greater compared to LCT consumption in men consuming MCT or LCT containing diets for up to 7 d (Hill et al., 1989). The results of these short-term studies on both animals and humans have prompted researchers to conclude that consumption of MCT, found in butter and coconut oil, may be useful in the treatment or prevention of weight gain associated with the development of obesity. However, studies have been of limited duration; with few experiments exceeding 7 d. One controlled study (White et al., 1999) showed that the difference in EE between LCT and MCT consumption is reduced after 14 d of consumption compared to 7 d. The same researchers, using doubly-labelled water methodology to assess total energy expenditure (Papamandjaris et al., 1999), found no difference between MCT and LCT consumption on

total EE (2246 vs 2186 kcal/d, respectively) after one week of intake. However, close to 60% of the fatty acids in the high MCT diet contained 16 or more carbons, while only 8% were octanoic and decanoic acids (White et al., 1999). The similar fatty acid composition of the diets may explain this lack of agreement with previous research.

In light of the findings on EE and fat oxidation with short-term MCT consumption, we expect that long-term consumption of MCT would result in negative energy balance. This would ultimately lead to different body weight trajectories with MCT consumption showing negative energy balance and decreased body weight and LCT consumption showing equilibrium and no body weight change. Assuming that the EE with consumption of triglycerides containing octanoate and decanoate remains elevated relative to LCT and that little or no adaptation occurs, then a weight loss in the order of 0.67 kg/mo of consumption can be predicted by replacing LCT with MCT in the diet (Dulloo et al., 1996). Tsuji et al. (2001) recently reported a difference in body weight of 0.80 kg after 4 wk of consumption of a diet containing MCT compared to one with LCT. However, this study was not crossover and diets were not precisely controlled.

The present objective was therefore to determine whether MCT, when compared to LCT, consumption influences EE and substrate oxidation in overweight women consuming a controlled diet, targeted to meet energy balance, rich in MCT or LCT for 27 d. The specific aim was to establish whether a difference exists between MCT and LCT on EE and substrate oxidation in obese women and, if such a difference exists, whether it results in changes in body composition.

2.3 SUBJECTS AND METHODS

2.3.1 Subjects

Seventeen healthy obese women (mean age ± SEM = 44.3 ± 3.8 yrs, mean body mass index = 31.8 ± 0.9 kg/m^2) were recruited by advertisement (**Table 2-1**). Subjects accepted into the study had plasma total cholesterol and triglyceride concentrations below 7.0 mmol/L and 3.0 mmol/L respectively, were not taking cholesterol-lowering drugs, had no history of cardiovascular disease, and did not report having diabetes, gastrointestinal, or thyroid problems. Subjects were required to have been weight stable in the past 3 mo and not perform more than 5 sessions of physical activity per week. The study protocol was approved by the Human Ethical Review Committee of the Faculty of Agriculture and Environmental Sciences of McGill University and all subjects signed informed consent forms prior to entrance into the protocol.

2.3.2 Study design

This study was a randomized, crossover trial in which subjects became inpatients at the Mary Emily Clinical Nutrition Research Unit (CNRU) of McGill University (Ste-Anne-de-Bellevue, Canada) for two phases of 27 d each. During each experimental phase, subjects were required to reside in and consume all meals provided by the CNRU, but were not confined to the unit. Subjects were permitted to leave the research unit for work or to attend classes, although they were required to remain at the CNRU after the evening meal. Subjects were instructed that any physical activity performed in one experimental phase had to be repeated at the same intensity level and on the same day of the next experimental phase.

Activity outside of the unit was not controlled, however, subjects were instructed not to deviate from habitual levels.

Meals were provided as a 3 d cycle menu. Subjects consumed an amount of energy required to maintain weight as calculated using the Mifflin equation (Mifflin et al., 1990) with an activity factor of 1.7. The activity factor was chosen since our group has shown that a factor of 1.7 was appropriate for weight maintenance (Bell et al., 1985). Energy intake was adjusted for weight gain or loss during the first 7 d of the first phase of the research and remained constant for both phases thereafter. As a result, subjects consumed the same number of calories during both phases. Diets contained 40% of energy as fat, 15% as protein and 45% as carbohydrate. Of the total amount of fat, 75% was derived from either beef tallow (BT) or a blend of saturated and unsaturated vegetable oils (FctO). In the FctO diet, 50% of the total fat was provided by MCT oil, rich in octanoate and decanoate (49 and 50% of total fatty acids, respectively, Neobee 1053, Stepan Company, Northfield, USA), 10% by olive oil, and 5% by each butter, coconut oil and flaxseed oil. Flaxseed oil was added as source of n-3 fatty acids and olive oil was incorporated into the FctO diet to increase the level of monounsaturated fatty acids. The remaining 25% of total dietary fat was intrinsic to foods common to both diets. **Table 2-2** shows the fatty acid composition of each experimental diet as determined using gas chromatography. An unesterified plant sterol/stanol mixture (Forbes Medi-Tech, Vancouver, Canada), at a level of 22 mg/kg body weight/d, was added to the MCT diet to maintain normal levels of cholesterol concentrations. Results of plasma lipid concentrations with consumption of FctO and BT are reported separately (Bourque et al., 2002, submitted). Subjects were randomly assigned to the FctO or

BT diet for the first experimental phase and consumed the alternate dietary fat during the second phase. Both phases were separated by a 4 or 8 wk washout period during which subjects resumed their habitual lifestyles. This resulted in all measurements being taken in the same phase of the menstrual cycle for each subject.

Body weights were measured daily before breakfast. Body composition measurements were performed using magnetic resonance imaging (MRI) on d 1 and 28 of each experimental phase. For all women the MRI images were acquired using a Siemens 1.5 Tesla MRI scanner (Siemens, Mississauga, Canada) using a T-1 weighted spin-echo sequence with a 210 ms repetition time and a 17 ms echo time. The MRI protocol is described in detail elsewhere (Ross et al., 1996). Briefly, subjects lay in the magnet in a prone position with their arms straight overhead. Using the intervertebral space between the fourth and fifth lumbar vertebrae (L4-L5) as the point of origin, transverse images (10 mm slice thickness) were obtained every 40 mm from hand to foot, resulting in a total of approximately 41 images for each subject. The total time required to acquire all the MRI data for each subject was approximately 45 min. All MRI data were analyzed using specially designed image analysis software (Tomovision Inc, Montreal, Canada).

The model used to segment the various tissues has been fully described and illustrated elsewhere (Ross et al., 1996). A multiple step procedure was used to identify tissue area (cm^2) for a given MRI image. In the first step a threshold was selected for adipose tissue (AT) and lean tissue (LT) based on the analysis of a sample of typical images and their respective grey level-histograms. Each image was then reviewed by an interactive slice

editor program which allowed for verification, and where necessary, correction of the segmented results. The original grey level image was superimposed on the binary segmented image using a transparency mode to facilitate the corrections. In the final step, the observer labeled the different tissues by assigning them different codes. The areas (cm^2) of the respective tissues in each image were computed automatically by summing the given tissues' pixels and multiplying by the individual pixel surface area. The volume (cm^3) of the different tissues in each slice was calculated by multiplying the tissue area (cm^2) by the slice thickness (10 mm). The volume of the tissues for the space between two consecutive slices was calculated by using a mathematical algorithm given elsewhere (Ross et al., 1996). The intra-observer differences for total, subcutaneous and visceral adipose tissue was calculated by comparing two analyses of 5 MRI data sets by a single observer. The intra-observer difference was 2.1 ± 1.2% for total, 1.8 ± 1.1% for subcutaneous and 8.1 ± 3.9% for visceral AT.

Energy expenditure was measured using a metabolic monitor (Delta-Trac, Sensor Medics, Anaheim, USA) for 30 min before breakfast (baseline) and 30 min during each hour for 6 h after breakfast on days 2 and 27 of each experimental phase. A transparent ventilated hood was placed on subjects' heads with Collins tubing connecting the hood to the monitor, as previously described (White et al., 1999). The metabolic cart was calibrated daily, after an overnight warm-up period, with calibration gas containing 96% O_2 and 4% CO_2 and local atmospheric pressure. In addition, accuracy and precision of the metabolic cart were verified using the weighed methanol burning test and respiratory quotient test, respectively, at the beginning of the trial. During EE tests expired gases were collected and analyzed against

ambient air and readings from the monitor were collected every minute. Fat and carbohydrate oxidation rates were calculated minute-by-minute using the equations derived by Lusk (1928). Total daily EE was calculated as the difference between energy intake and the sum of the changes in body AT, LT, and fecal energy excretion. Fecal energy excretion was calculated by measuring fecal fat excretion and multiplying by 9 kcal/g of fat.

$$\text{Total EE} = \text{E intake} - (\Delta \text{ total AT} + \Delta \text{ LT} + \text{E excreted}) \qquad \text{(eqn 1)}$$

Change in total AT was converted to its change in AT energy store by multiplying the change in AT volume by 0.9 (Katch and McArdle, 1993) to convert from L to kg, further multiplied by 7700 kcal/kg of fat and divided by 27 d of intake. Similarly for the conversion of change in lean tissue stores to change in energy content of lean tissues, the change in lean tissue was multiplied by 1.1 (Katch and McArdle, 1993) to convert from L to kg, further multiplied by 2920 kcal/kg, taking into account approximately 73% hydration (Groff and Gropper, 2000), and divided by 27 d of intake.

Total fecal samples were collected for 3 d at mid-point through each experimental phase for determination of fecal fat excretion. Samples were diluted by 50% with water, aliquoted and dried. Fecal lipids were extracted from approximately 3 g of the combined 3 d dried samples with each day of sampling being proportionately represented. Lipid extraction was carried out in duplicate using the method of Folch et al. (1957). Dried lipid extracts were saponified then methylated using boron trifluoride methanol and heated at 80°C for 55 min. Methylated samples were analyzed using gas chromatography (Hewlett Packard, GC 5890, Series II)

equipped with 30 m fused silica capillary column (0.25 mm ID, 0.20 μm film, Supelco, Bellefonte, USA). Total fatty acid recovery was calculated by summing individual fatty acids and comparing their quantities with C 17:0 as internal standard.

2.3.3 Statistical analyses

Analysis of variance of results was carried out using a mixed model procedure with diet (FctO or BT), day (2 or 27), hour (each half hour period between 0 and 6.5 h), and sequence as factors in the model. Interactions between diet and day and between diet, day and hour were also examined. Paired Student's t-test was then used to determine differences between diets at each hour on each individual day. Paired Student's t-test was also used to establish differences between FctO and BT consumption on fecal fat excretion and changes in body composition. All statistical analyses were conducted using SAS statistical software (SAS/STAT version 6.12, SAS Institute, Cary, USA). A p-value of 0.05 was taken as statistically significant. Data are reported as means ± SEM.

2.3.4 Sample size determination

Dulloo et al. (1996) found that subjects consuming MCT had an increase in total daily energy expenditure of 5%, which was equivalent to 113 kcal/d. Results from White et al. (1999) suggest that the difference in total energy expenditure between LCT and MCT diets could be as high as 160 kcal/d. Assuming that subjects expand 160 kcal/d over total energy expenditure for the entire 28 d period, a total of 4,500 kcal will be expanded. The result of this increase in EE will be loss of approximately 600 g of body fat mass. A sample size of 18

subjects was found to be required to detect a change in adipose tissue stores of 600 g (Ross, 1996) over the total experimental phase with 95% confidence.

2.4 RESULTS

Twenty-two subjects were recruited into the study and five did not complete. Reasons for failure to complete the study included intolerance to the control diet (n=2) and personal reasons (n=3). Compliance with the study protocol and feeding regimen within subjects completing the trial was considered high as only 2% of the meals were not consumed at the research unit due to exceptional circumstances. We assume that consumption of non-study foods was low since study foods were consumed under supervision and subjects did not gain weight. Although an exercise room was available for use by the subjects, only 2 subjects used it sporadically. These individuals followed instructions to repeat similar exercise level at approximately the same day during each experimental phase.

Eight of the women were post-menopausal. For the remaining 9 women, 4 were in the follicular phase of their menstrual cycle during the first EE measurement period, 3 were in the luteal phase, and the remaining 2 were in their menstrual period during this initial measurement period.

There was a decrease ($p<0.01$) in body weight, as measured using a standard scale, within each dietary phase but no difference in weight loss between the 2 phases (-0.87 ± 0.16 kg versus -0.84 ± 0.22 kg during FctO and BT consumption, respectively). **Figures 2-1** and **2-2** show individual changes in total and subcutaneous body adipose tissue compartments,

respectively, between d 1 and d 28 of each dietary phase. There was no effect of diet or day on any of the body compartment volumes. Total AT varied from 37.7 ± 2.4 L on d 1 of FctO consumption to 37.1 ± 2.5 L on d 28. During BT phase, total AT volume was 37.9 ± 2.6 L on d 1 and 37.3 ± 2.6 L on d 28. Average subcutaneous AT volume at the onset of the FctO phase was 33.5 ± 2.2 L and 32.9 ± 2.3 L after 28 d. At the start of the BT phase, average subcutaneous AT volume was 33.6 ± 2.3 L versus 33.1 ± 2.4 L at the end of the phase. Mean muscle volume was 19.6 ± 0.6 L on both d 1 and 28 during the FctO phase and, similarly, for the BT phase, average muscle volume was 19.5 ± 0.6 L on d 1 and 19.4 ± 0.6 L on d 28. Inter-individual coefficients of variation for total body volume, total AT and subcutaneous AT volumes were 28.7%, 25.2%, and 23.0%, respectively.

Resting metabolic rate (RMR) was not different between FctO and BT consumption. On d 2 of the FctO phase, RMR was 0.84 ± 0.02 kcal/min versus 0.82 ± 0.03 kcal/min during the BT phase. On d 27, RMR was 0.81 ± 0.03 kcal/min and 0.79 ± 0.02 kcal/min for the FctO and BT phases, respectively.

Thermic effect of food was calculated as the difference between post-prandial (PP) EE and RMR at each time point after breakfast. Average TEF was 0.15 ± 0.01 kcal/min on d 2 and 0.17 ± 0.01 kcal/min on d 27 of FctO consumption whereas it was 0.14 ± 0.02 kcal/min for both d 2 and 27 of BT consumption. Average PP EE was 0.99 ± 0.02 kcal/min on d 2 and 0.97 ± 0.02 kcal/min on d 27 of FctO consumption versus 0.96 ± 0.03 kcal/min and 0.93 ± 0.03 kcal/min on d 2 and 27 of BT consumption.

Mean EE during d 2 and 27 on each dietary treatment are shown in **Figure 2-3**. Inter-individual coefficient of variation was calculated to be 2.3% for EE. The mean rates of EE measured in the 30 min interval immediately after breakfast are shown as time 1 hr on both days. Average EE on d 2 of the FctO phase was 0.97 ± 0.023 kcal/min compared to 0.94 ± 0.028 kcal/min for the BT phase. On d 27 of the FctO phase, average EE was 0.95 ± 0.024 kcal/min versus 0.90 ± 0.019 kcal/min for the BT phase. There was a main effect of diet ($p<0.01$), day ($p<0.01$) and hour ($p<0.01$) on EE but there was no diet by day interaction. Area under the curve (AUC) was greater ($p<0.01$) with FctO compared to BT intake. FctO consumption resulted in greater EE than BT consumption and EE was lower on d 27 than d 1 for both dietary phases.

Figure 2-4 shows basal and PP fat oxidation on d 2 and 27 with each diet. Average fat oxidation on d 2 of the FctO phase was 0.081 ± 0.0035 g/min compared to 0.077 ± 0.0033 g/min for the BT phase. On d 27, average values were 0.080 ± 0.0026 g/min and 0.075 ± 0.0022 g/min for the FctO and BT phases, respectively. Area under the curve was greater ($p<0.05$) with FctO consumption compared to BT consumption. There was a main effect of diet ($p<0.05$) and hour ($p<0.01$) and a treatment by day by hour interaction ($p<0.01$) on fat oxidation. The inter-individual coefficient of variation for fat oxidation was calculated to be 0.3%.

Fecal lipid analyses showed a trend ($p=0.10$) towards greater fat excretion with BT consumption compared to FctO consumption. The fat content of the fecal collection on the FctO diet was 0.47 ± 0.09 g/d compared to 0.61 ± 0.08 g/d on the BT phase. This represents

approximately 99.6% and 99.4% fat absorption during periods of FctO and BT consumption, respectively.

The individual and mean calculated total EE during FctO and BT consumption periods are shown in **Figure 2-5**. Total EE was 2640 ± 138 kcal after 27 d of FctO consumption and 2628 ± 177 kcal/d after BT consumption (NS).

2.5 DISCUSSION

The results of this 27 d inpatient trial show that controlled consumption of FctO increases EE and fat oxidation in obese women compared to a situation where energy intakes from BT were equal. However, although EE was greater with FctO consumption throughout the 27 d period, this did not result in a measurable enhancement of weight loss during FctO consumption compared to the period of BT consumption. Accordingly, no changes in total or regional AT distribution were observed with MRI consequent to these perturbations.

This study is unique since the inpatient and crossover controlled feeding design utilized precise monitoring of food intake. The use of MRI to assess body composition also allows very small differences in tissue volume to be observed. Magnetic resonance imaging is a well-established tool for measuring total and sub-components of total body fat and has been shown to accurately measure total body and sub-components of total AT when repeated contiguous slices are acquired (Thomas et al., 1998; Ross et al., 1992). The reliability of MRI as a tool in the assessment of body fat compartment volumes has been demonstrated by Ross et al. (1992) who reported that the mean difference for repeat measurements of whole

body AT and LT was < 3% and < 2%, respectively. These same researchers reported that the mean difference for subcutaneous and visceral AT for repeat measurements at the L4-L5 vertebrae was 1.1% and 5.5% respectively (Ross et al., 1993). Magnetic resonance imaging thus measures the different AT compartments with an error of estimate of 2 to 10% (Ross, 1996). More recently, Mitsiopoulos et al. (1998) studied the reproducibility of MRI subcutaneous AT volume measurements by comparing the intra- and inter-observer estimates for MRI measurements. The inter-observer and intra-observer differences were -2.9 ± 1.2% and 1.5 ± 1.5%, respectively, for subcutaneous AT (Mitsiopoulos et al., 1998). It was reported that changes in subcutaneous and visceral AT compartments must be greater than 5 and 10%, respectively, to reach statistical significance (Thomas et al., 1998). The degree of weight loss observed in this trial was therefore below the threshold needed for MRI to identify the changes in AT volumes.

Our research trial is the longest study to date to examine the effects of high intakes of MCT on EE and the first to look at corresponding changes in total body composition in obese women. Quantities of MCT provided in the FctO diet were much higher than those normally consumed in the general population. Sevenhuysen et al. (1993) reported that middle-aged women consume on average 15 g/d of butter as added fat on bread and potatoes. This would represent a MCT intake of approximately 0.52 g/d, considering that 3.5% of total fatty acids in butter are found as octanoic and decanoic acids (Hyvonen et al., 1993). Furthermore, the inpatient study design employed for this trial is the first of its kind in this area of nutrition research. With such a rigorous study design and analytical methods, it was thus possible to not only carefully study the effects of MCT on EE, but also the resulting effects on body

composition that have been proposed previously (Dulloo et al., 1996; Scalfi et al., 1991; Seaton et al., 1986).

Our findings strengthen and extend previous observations wherein greater EE and fat oxidation were obtained with consumption of MCT compared to LCT (Scalfi et al., 1991; Seaton et al., 1986; White et al., 1999; Hill et al., 1989). However, the magnitude of the difference in EE between the FctO and the BT diet appears to be lower than that observed in studies conducted on male subjects (Dulloo et al., 1996; Scalfi et al., 1991; Seaton et al., 1986; Hill et al., 1989). Binnert et al. (1998) have also found that diet-induced thermogenesis after consumption of a bolus of a 50-50 mixture of MCT and olive oil, compared to olive oil alone, was lower in their female subjects than observed by Seaton et al. (1986). In fact, Binnert et al. (1998) failed to observe any difference in fat oxidation between MCT and olive oil intake in obese women. This may be due to the small sample size (n=8) and the quality and quantity of the experimental oil, which was a 30 g bolus of a 1:1 mixture of MCT and olive oil. However, the extent of the difference in fat oxidation is similar to that observed in the present trial. It was suggested that obese women had greater deposition of fatty acid due to their larger AT stores and that they also had increased uptake of fatty acids per unit of fat (Thomas et al., 1998). This may partly explain the discrepancy between our results and those obtained with male subjects (Dulloo et al., 1996; Scalfi et al., 1991; Seaton et al., 1986) in terms of differences in EE and fat oxidation with MCT and LCT consumption. Indirect calorimetry is a valid method for measurement of EE, with an intra-individual coefficient of variation of 3.6%, however, variability for fat oxidation is much higher (17.4%) (Gasic et al., 1997).

Body weight variations observed in this trial are much smaller than those observed by Tsuji et al. (2001), however, their trial was not as well controlled and included more men than women. Foods were not consumed under strict supervision and authors relied on food diaries to observe compliance with protocol. Subjects tend to underestimate food consumption and therefore a negative energy balance in both groups could have caused the weight loss observed. Body weight change in the present experiment is known to have been the result of FctO consumption since energy intake in both phases was identical.

Our analyses of fecal fat excretion show almost complete fat absorption (99.4-99.6%). The total fat excretion observed was indeed similar to that observed by Lia et al. (1997) who found that ileostomy patients consuming oat bran or wheat bran excreted 465 mg or 194 mg of fat/d, respectively. These values correspond to 99.1 and 99.7% fat absorption for oat bran and wheat bran consumption, respectively. Earlier studies of the absorbability of fats in rats showed that coconut oil is 99.7% absorbed compared to 98% for lard (Calloway et al., 1956).

If we consider that RMR accounts for approximately 8 h daily and that the remaining time is spent in the post-meal period, then the average difference in EE with FctO consumption compared to BT, as measured by indirect calorimetry, can be extrapolated to be 40.2 kcal on d 2 and 50.2 kcal on d 27. This would translate into a 0.14 to 0.18 kg weight loss over a 27 d period. Using the energy balance equation with precisely known energy intake, changes in body composition and fecal energy excretion, the daily energy difference between FctO and BT consumption is 10 kcal (NS). This difference in EE over a 27 d period would result in a weight loss of 0.03 kg. Although not statistically significant, the magnitude of the difference

in adipose tissue loss between FctO and BT consumption in this study is similar to the weight loss that would be expected following the differences in EE between the two dietary periods. When consuming an equal number of calories, subjects lost an average of 0.076 L of adipose tissue more with the FctO diet than the BT diet, which is equivalent to 0.07 kg. Therefore, although the difference in adipose tissue loss between the two diets is not statistically significant, the trends in body weight changes can be explained by the extrapolated difference in EE.

In conclusion, present results show that EE and fat oxidation are increased with FctO consumption compared to BT consumption in healthy overweight women. Furthermore, raised levels of EE and fat oxidation remained consistently elevated during 27 d of consumption of a diet rich in MCT but were not associated with a detectable difference in effect on body fat depot size. Although it cannot be concluded that prolonged FctO consumption results in greater weight loss compared to BT consumption, FctO intake resulted in increased EE and fat oxidation. This may promote long-term weight maintenance in obese women.

2.6 ACKNOWLEDGEMENTS

We would like to thank Dr. Andrea Papamandjaris, Chris Vanstone, Andrea D'Souza, Erika Motoie and Sarah Miller for their technical assistance and all subjects for participating in this trial.

2.7 FIGURE LEGENDS

Figure 2-1

Individual changes in volume of total adipose tissue with consumption of FctO and BT diets.

Figure 2-2

Individual changes in volume of subcutaneous adipose tissue with consumption of FctO and BT diets.

Figure 2-3

Energy expenditure after consumption of a breakfast containing FctO or BT on day 2 (A) and day 27 (B). Closed squares = FctO phase; open squares = BT phase. Values are means ± SEM, n=17. * FctO significantly different from BT, $p < 0.05$.

Figure 2-4

Fat oxidation after consumption of a breakfast containing FctO or BT on day 2 (A) and day 27 (B). Closed squares = FctO phase; open squares = BT phase. Values are means ± SEM, n=17. * FctO significantly different from BT, $p < 0.05$.

Figure 2-5

Calculated energy expenditure with consumption of diets containing FctO or BT for 27 days.

Table 2-1. Subject characteristics.

Characteristic	Average (SEM)
Age, yrs	44.3 (3.8)
Weight, kg	82.2 (2.7
Height, cm	160.6 (1.5)
Body mass index, kg/m^2	31.8 (0.9)
Energy intake, kcal	2458 (73)

Table 2-2. Fatty acid content of the experimental diets determined by gas chromatography.

Fatty acid (%)	Functional oil	Beef Tallow
C6:0	Trace	Trace
C8:0	19.4 ± 2.0	Trace
C10:0	23.6 ± 2.3	0.2 ± 0.1
C12:0	3.9 ± 0.6	0.3 ± 0.1
C14:0	2.6 ± 0.5	3.4 ± 0.4
C14:1	0.2 ± 0.1	0.6 ± 0.1
C15:0	10.1 ± 1.1	26.1 ± 0.9
C16:0	0.6 ± 0.2	2.7 ± 0.3
C16:1n7	3.8 ± 0.6	20.3 ± 1.1
C18:0	23.6 ± 3.5	38.5 ± 1.6
C18:1n9	7.1 ± 1.6	6.4 ± 1.6
C18:2n6	4.6 ± 1.3	0.8 ± 0.1
C18:3n3	0.3 ± 0.1	0.3 ± 0.1
C22:6n3	Trace	Trace

Figure 2-1.

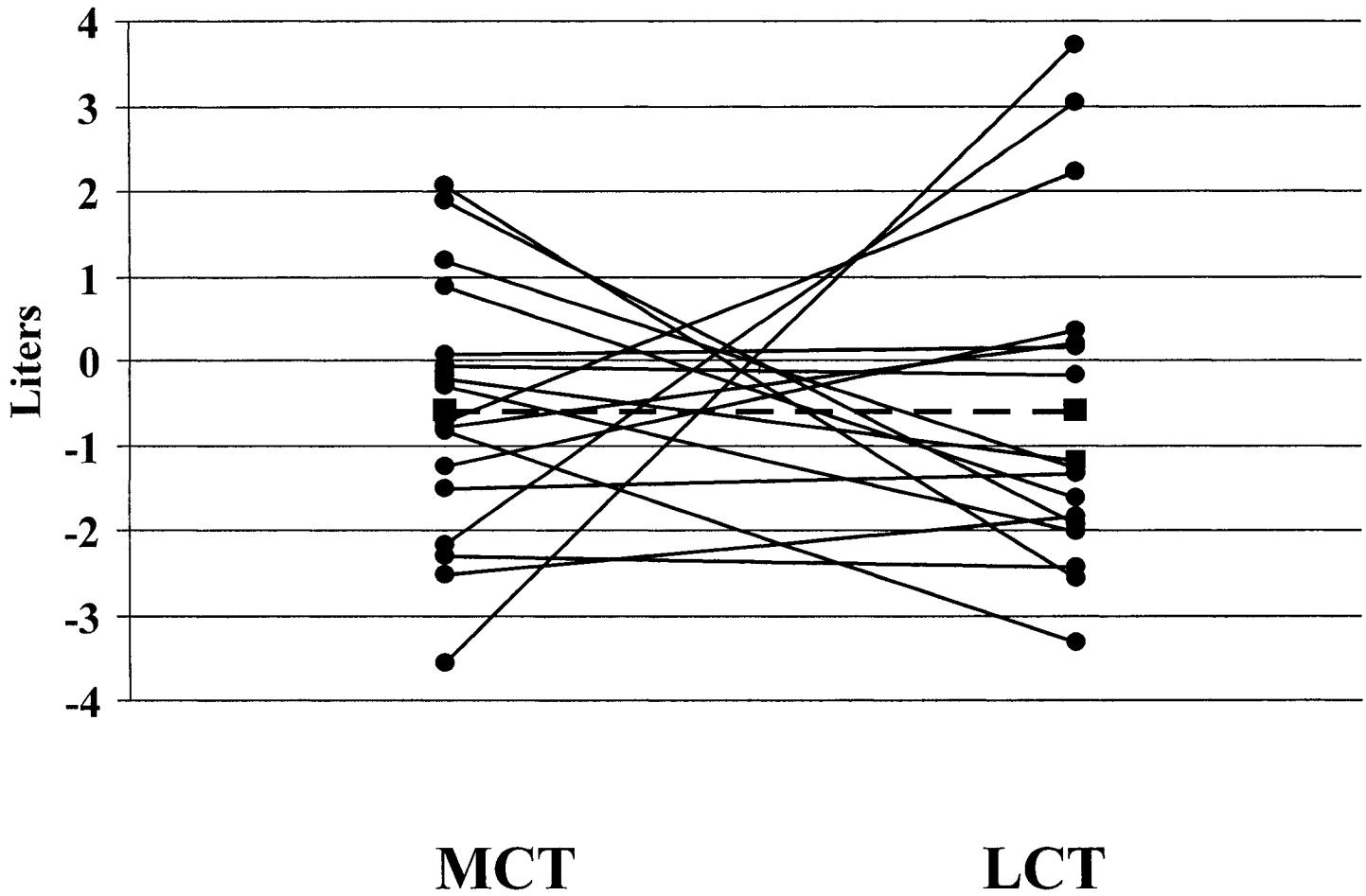


Figure 2-2.

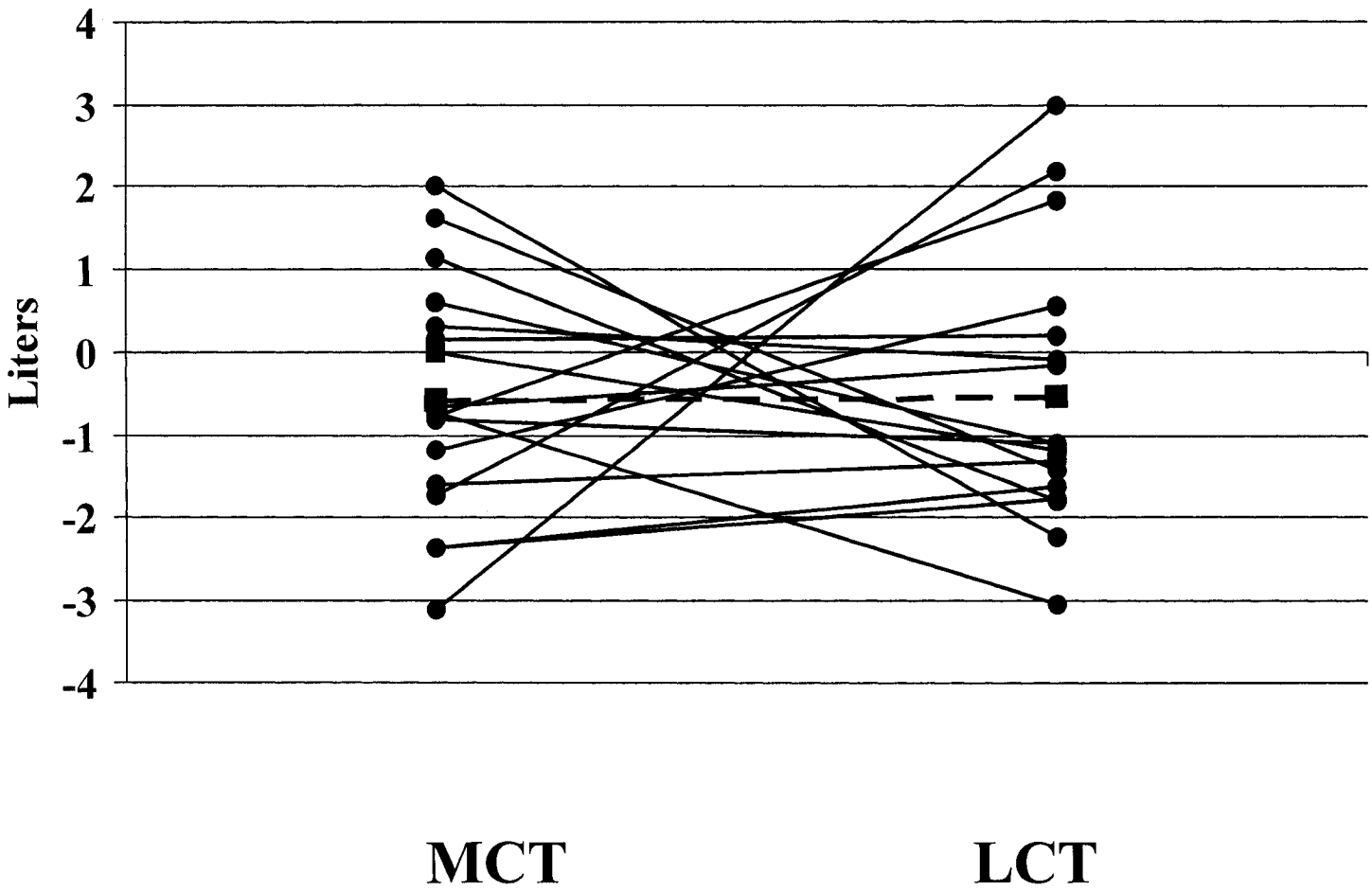


Figure 2-3.

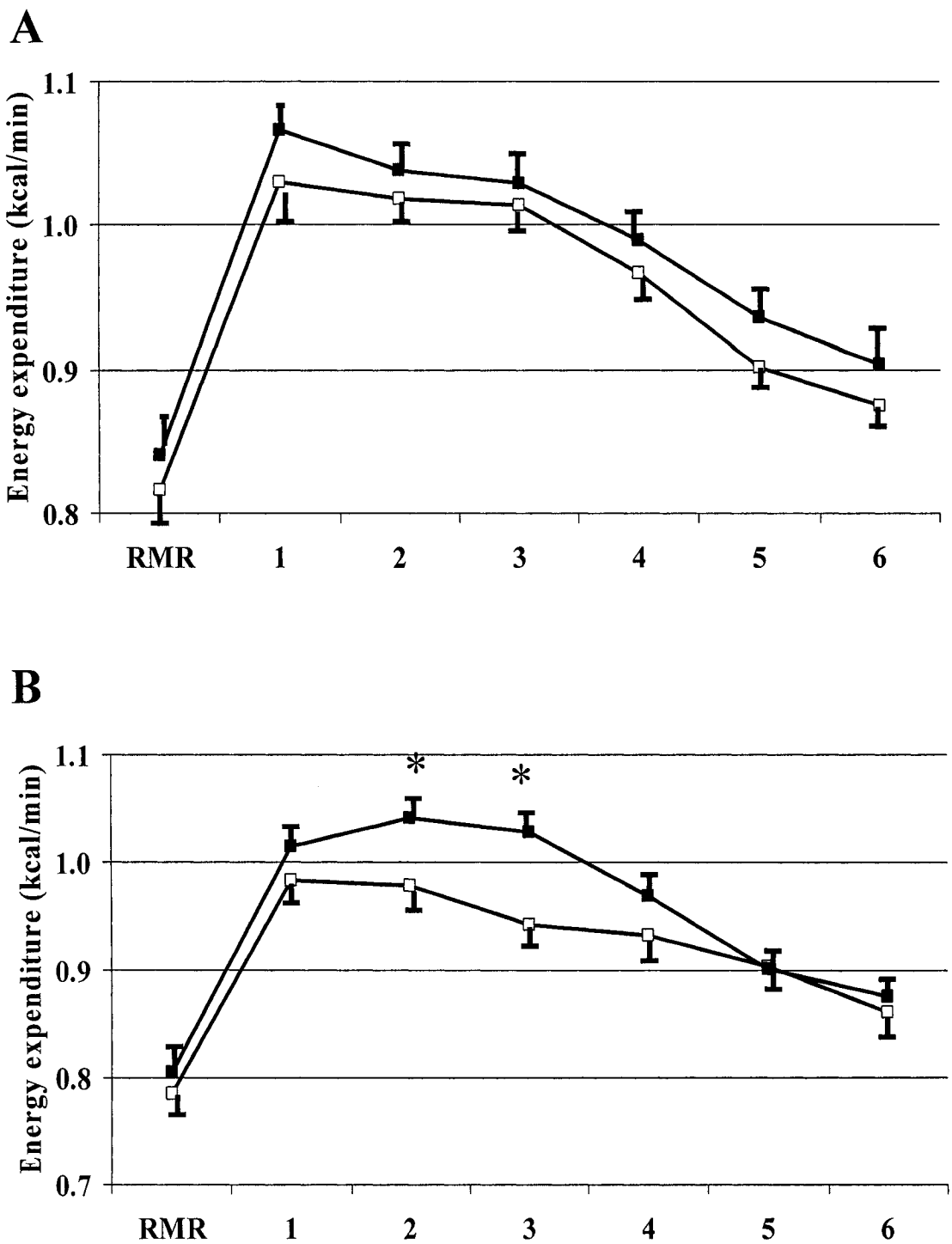

A
Energy expenditure (kcal/min)
1.1
1.0
0.9
0.8
RMR
1
2
3
4
5
6
B
Energy expenditure (kcal/min)
1.1
1.0
0.9
0.8
0.7
*
*
RMR
1
2
3
4
5
6

Figure 2-4.

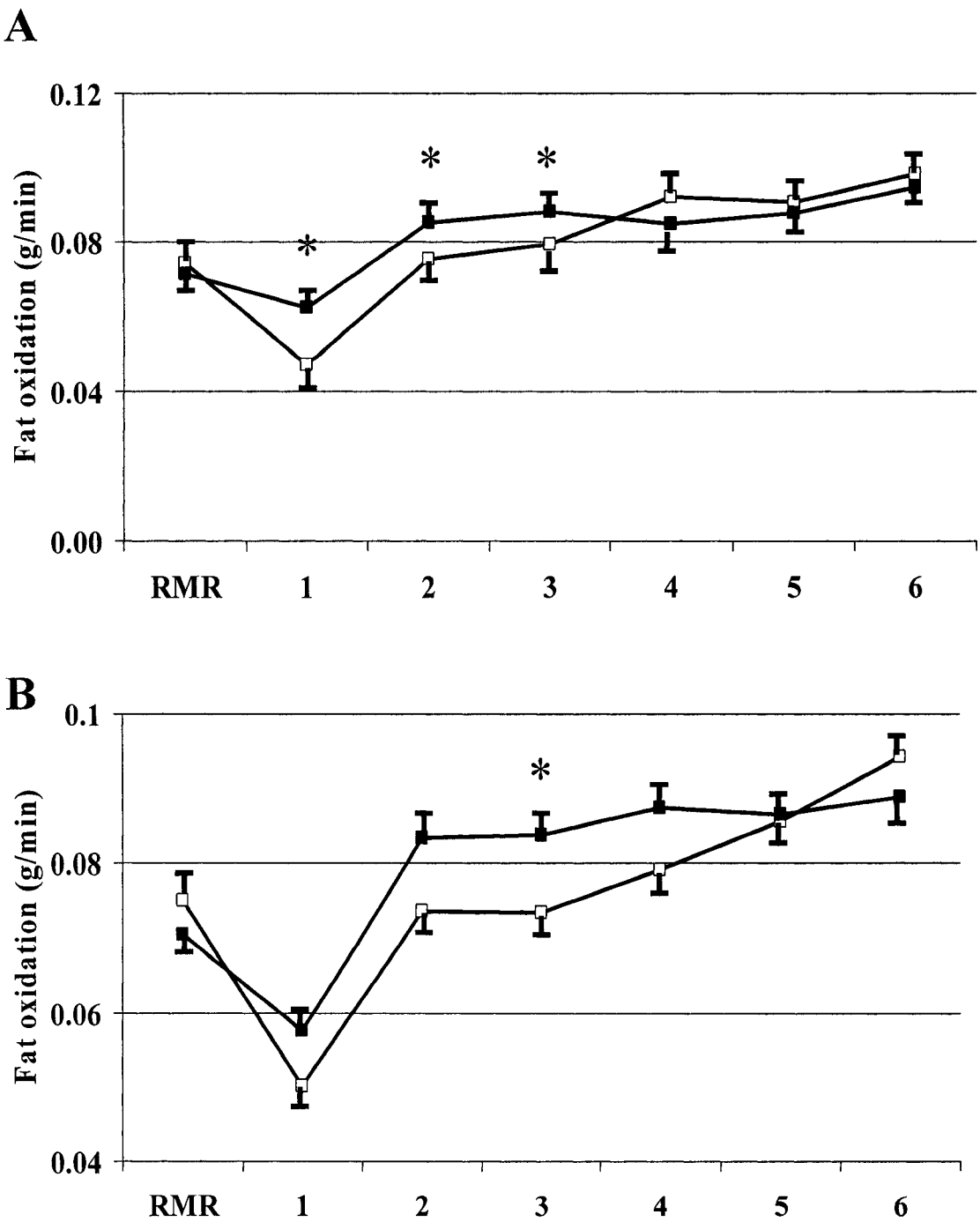
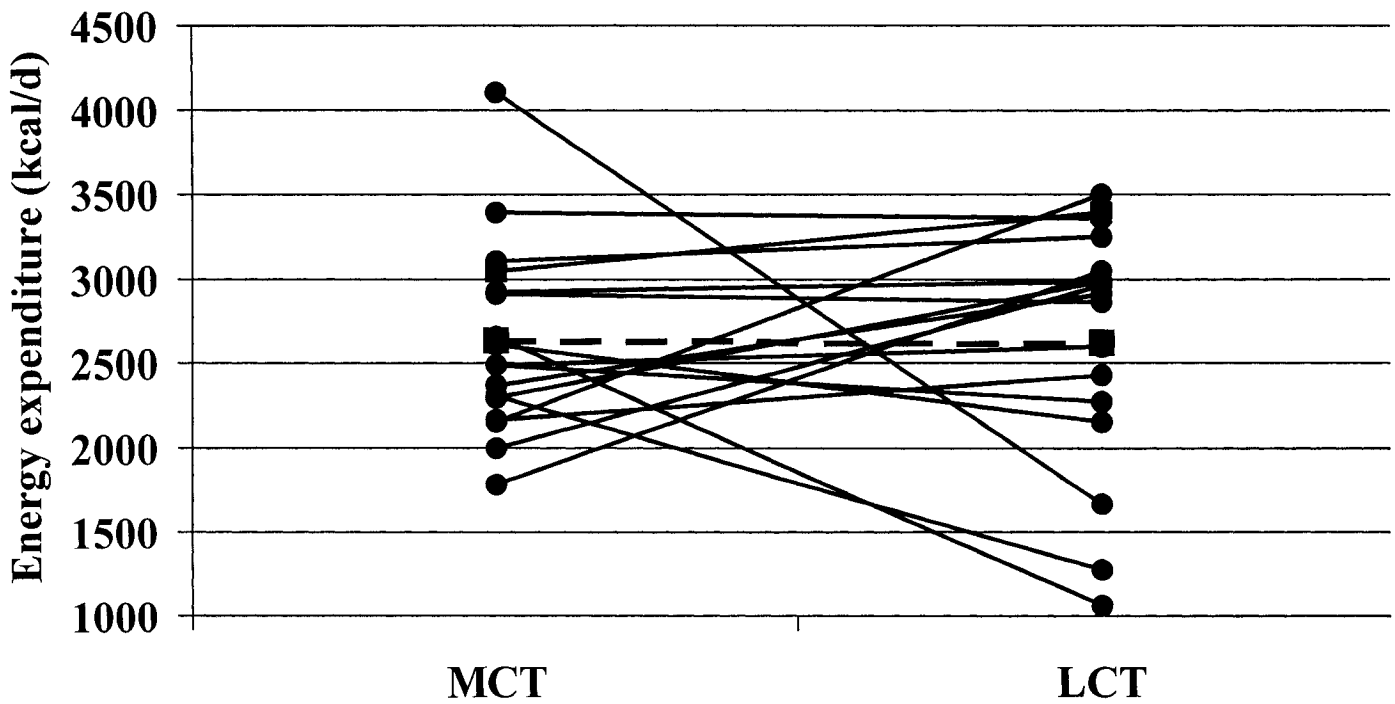

A
0.12
0.08
0.04
0.00
Fat oxidation (g/min)
RMR
1
2
3
4
5
6
*
*
*
B
0.1
0.08
0.06
0.04
Fat oxidation (g/min)
RMR
1
2
3
4
5
6
*

Figure 2-5.


Energy expenditure (kcal/d)
4500
4000
3500
3000
2500
2000
1500
1000
MCT
LCT

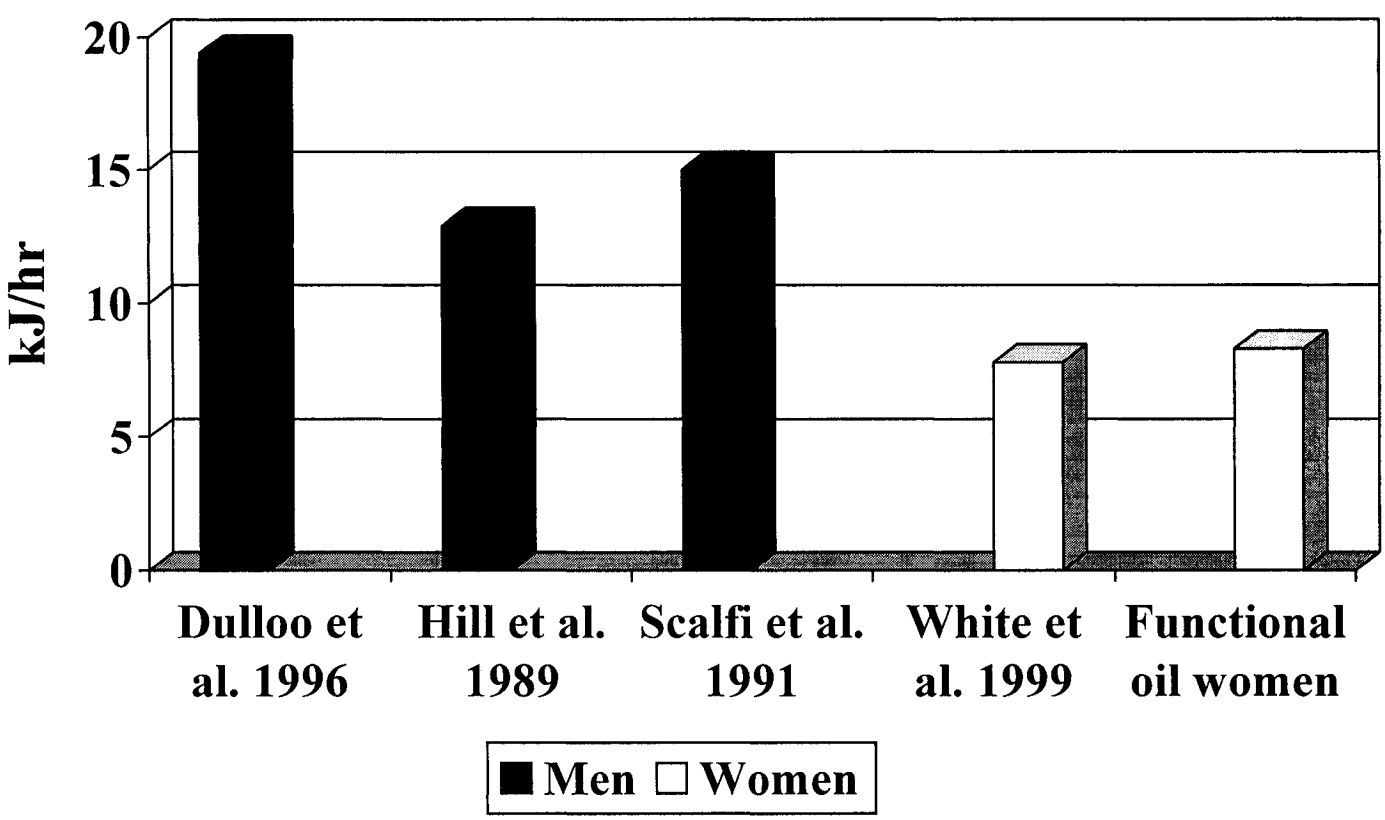
BRIDGE 2.

Results obtained in the trial in women led to the hypothesis that women may not respond as well to the effects of medium chain triglycerides on energy expenditure as men (**Figure Bridge 2**). The difference in body weight loss between medium and long chain triglyceride consumption in women matched the difference in energy expenditure observed with consumption of the medium and long chain triglyceride diets. This small difference in energy expenditure was not sufficient to result in significant changes in body composition over a 4 wk period.

From previous studies conducted on men and women, it was observed that the difference in energy expenditure between medium and long chain triglyceride consumption was greater in men than in women. Therefore, since long-term consumption of medium chain triglycerides as part of a controlled diet has not been studied in men, our second trial aimed to establish whether medium chain triglyceride consumption by men would raise energy expenditure and lead to decreased body weight and adipose tissue stores over 28 d. Subjects consumed our experimental diets, containing medium chain triglycerides or long chain triglycerides for a period of 28 d each. The control, long chain triglyceride-containing diet, consisted of olive oil and the experimental diet consisted of a functional oil containing medium chain triglyceride oil, olive oil, canola oil, coconut oil, and phytosterols. The composition of the control diet was modified since it was found, from our previous work in women, that subjects did not tolerate beef tallow very well and blinding could not be guaranteed due to taste and behavioral differences between beef tallow and functional oil. Beef tallow would solidify on the plate when cooling occurred, whereas the functional oil remained liquid. Another

difference between the two trials is the composition of the functional oil diet. In the first trial, all sources of fat in the functional oil diet were added to the basal diet and weighed separately. In the second trial, the functional oil was made by blending the different oils and phytosterols and this blended oil was weighed and added to the other ingredients of the diet. Length was increased by one day to obtain a full 4-wk feeding period.

Figure Bridge 2.



CHAPTER 3. Manuscript 3. ***In press in Obes Res.***

CONSUMPTION OF A FUNCTIONAL OIL CONTAINING MEDIUM CHAIN TRIGLYCERIDES BY OVERWEIGHT MEN INCREASES ENERGY EXPENDITURE AND DECREASES BODY ADIPOSITY COMPARED TO A DIET RICH IN OLIVE OIL

Marie-Pierre St-Onge[1], Robert Ross[2], William D Parsons[1], Peter JH Jones[1]

[1]School of Dietetics and Human Nutrition, McGill University, Ste-Anne-de-Bellevue, Québec H9X 3V9

[2]School of Physical and Health Education, Queen's University, Kingston, Ontario K7L 3N6

Corresponding author: Peter JH Jones

School of Dietetics and Human Nutrition

21 111 Lakeshore Rd.

Ste-Anne-de-Bellevue, Québec

H9X 3V9

Phone: (514) 398-7547

Fax: (514) 398-7739

e-mail: jonesp@macdonald.mcgill.ca

Supported by Dairy Farmers of Canada and Forbes Medi-Tech Inc.

Running head: MCT, energy expenditure and body composition

3.1 ABSTRACT

The objectives of this study were to compare the effects of diets rich in medium chain triglycerides (MCT; FctO) or long chain triglycerides (LCT; OL) on body composition, energy expenditure, substrate oxidation, as well as subjective appetite and ad libitum energy intake. Twenty-four healthy, overweight men with body mass index between 25 and 31 kg/m^2 consumed 2 diets, FctO and OL, for 28 d each in a crossover randomized controlled trial. Energy expenditure was measured using indirect calorimetry and body composition was analyzed using magnetic resonance imaging (MRI) at baseline and after 4 wk of both dietary interventions. Upper body adipose tissue decreased to a greater extent ($p < 0.05$) with functional oil (FctO) compared to olive oil (OL) consumption (-0.67 ± 0.26 kg and –0.02 ± 0.19 kg, respectively). There was a trend towards greater loss of whole body subcutaneous adipose tissue volume ($p = 0.087$) with FctO compared to OL consumption. Average energy expenditure (EE) was 0.04 ± 0.02 kcal/min greater ($p < 0.05$) on d 2 and 0.03 ± 0.02 kcal/min (NS) on d 28 with FctO compared to OL consumption. Similarly, average fat oxidation was greater ($p = 0.052$) with FctO compared to OL intake on d 2 but not d 28. Consumption of a diet rich in MCT results in greater loss of adipose tissue compared to LCT perhaps due to increased EE and fat oxidation observed with MCT intake. Medium chain triglycerides may thus be considered as agents that aid in the prevention of obesity or potentially stimulate weight loss.

Key words: body composition, magnetic resonance imaging, energy expenditure, medium chain triglycerides, weight loss

3.2 INTRODUCTION

The prevalence of overweight and obesity has been increasing worldwide. In the US, the prevalence of overweight has increased from 25.4 to 33.3% in the adult population from NHANES II to phase I of NHANES III (Kuczmarski et al., 1994; Galuska et al., 1996). It is apparent, therefore, that strategies designed to prevent or treat obesity are of paramount importance. Medium chain triglycerides (MCT) have been previously proposed as a tool in the prevention of human obesity (Geliebter et al., 1983; Hashim and Tantibhedyangkul, 1987) due to their effects on fat deposition in animals. Medium chain triglycerides are known to increase energy expenditure in humans (Seaton et al., 1986; Hill et al., 1989; Scalfi et al., 1991; Dulloo et al., 1996; White et al., 1999; Bendixen et al., 2002; St-Onge et al., 2003) and are associated with lower body weight gain and fat depot size (Baba et al., 1982; Geliebter et al., 1983; Crozier et al., 1987; Hashim and Tantibhedyangkul, 1987) in growing animals when compared to long chain triglyceride (LCT) consumption. Recent findings (Tsuji et al., 2001; St-Onge et al., in press a), however, have failed to demonstrate convincing results regarding the long-term benefits of MCT consumption on body weight in humans.

From our previous research in women (White et al., 1999; St-Onge et al., 2003), there is evidence to support the hypothesis that women do not respond to MCT intake as strongly as men. In fact, studies previously published that have been conducted in men (Seaton et al., 1986; Scalfi et al., 1991; Dulloo et al., 1996) report greater differences in EE between MCT and LCT consumption than those observed by our group when studying women (White et al., 1999; St-Onge et al., 2003). Since long term controlled feeding studies examining the effects of MCT on EE and body composition have not been conducted in men, the present objective

was to assess whether feeding men diets rich in MCT or olive oil (OL), as a source of LCT, would result in greater EE and weight loss after 4 wk. We hypothesized that MCT consumption would result in greater EE and weight loss compared to OL over a 28 d feeding period. In addition, it is suggested that MCT consumption may lead to increased levels of satiety and thus lower caloric ingestion than with LCT consumption (Stubbs and Harbron, 1996; Van Wymelbeke et al., 1998; Van Wymelbeke et al., 2001; St-Onge and Jones, 2002). Therefore, a secondary objective was to determine if appetite ratings on a visual analog scale (VAS) and food intake at an ad libitum intake lunch session would be altered by the type of fat contained in the diet.

3.3 RESEARCH METHODS AND PROCEDURES

3.3.1 Subjects

Subjects were recruited by advertisement in local newspapers and were required to have a body mass index between 25 and 31 kg/m^2, fasting plasma total cholesterol and triglycerides concentrations below 7.0 mmol/L and 3.0 mmol/L, respectively. Exclusion criteria were reported history of cardiovascular disease, diabetes, hypertension, gastrointestinal disorders, and unusual eating patterns. The study protocol was reviewed and accepted by the Human Ethical Review Committee of the Faculty of Agriculture and Environmental Sciences of McGill University and all subjects signed informed consent forms prior to entrance into the protocol.

3.3.2 Study design

The study employed a randomized crossover controlled feeding design with periods of 28 d each, separated by a 4 wk washout period. The research was conducted at the Mary Emily Clinical Nutrition Research Unit (CNRU) of McGill University. Subjects were required to come to the CNRU every morning for breakfast and to return to consume one other meal under supervision at the CNRU every day. The third meal was prepared and packed for the subjects to consume outside of the unit. Diets were designed to resemble a typical North American diet and contained 40% of energy as fat, 15% as protein, and 45% as carbohydrates. The 2 different diets were consumed in random order for a period of 4 wk each, separated by a 4-wk washout period. These diets were identical except for the quality of the fat. The MCT-containing diet contained a functional oil (FctO) composed of 64.7% MCT oil (Neobee 1053, Stepan Company, Northfield, USA), 12.6% olive oil, 6.8% each of canola and flaxseed oil, and 5.8% coconut oil as the main source of fat (75% of total fat) and the control diet (OL) contained 75% of total fat as olive oil. The rest of the fat came from the other foods in the meals that were identical in both diets. The fatty acid composition of the FctO is provided in **Table 3-1**. The FctO also contained 3.4% of unesterified stanol/sterol mixture (Forbes Medi-Tech, Vancouver, Canada) since previous studies have shown increased plasma lipid concentrations with MCT consumption (Hill et al., 1989; Swift et al., 1992). Lipid level data were obtained and form the basis for a complementary manuscript currently under consideration for publication.

Energy intake was calculated on an individual basis using the Mifflin equation (Mifflin et al., 1990) and an activity factor of 1.7. This activity factor was shown to be appropriate for

weight maintenance (Bell et al., 1985) and has been used by our group previously (White et al., 1999; St-Onge et al., 2003). During the first wk of the first experimental phase, energy intake was adjusted to compensate for any change in body weight that may have occurred. However, after this initial 1 wk period, energy intake was kept constant throughout both experimental phases. Meals were isoenergetic and were provided in a 3 d rotating cycle menu. Subjects were instructed to consume all foods provided to them and nothing else for the duration of the trial. They were also advised to maintain a regular exercise pattern throughout the trial in accordance with habitual levels.

3.3.3 Methods

Body weight was measured every morning before breakfast using a standard scale. Body composition was assessed using magnetic resonance imaging (MRI) on d 1 and 29 of each experimental phase. The MRI protocol is described in detail elsewhere (St-Onge et al., 2003). Briefly, images were acquired using a Siemens 1.5 Tesla MRI scanner (Siemens, Mississauga, Canada) using a T-1 weighted, spin-echo sequence with a 210 ms repetition time and a 17 ms echo time. Subjects lay in the magnet in a prone position with their arms straight above their head. Using the intervertebral space between the fourth and fifth lumbar vertebrae (L4-L5) as the point of origin, transverse images with 10 mm slice thickness were obtained every 40 mm from hand to foot, resulting in a total of approximately 45 images for each subject. Magnetic resonance imaging data was analyzed using specially designed image analysis software (Tomovision Inc, Montreal, Canada). Details of the data analysis procedure have been previously published (St-Onge et al., 2003).

It is reported that the mean difference for repeat measurements of whole body adipose tissue (AT) and lean tissue was < 3% and < 2%, respectively (Ross et al., 1992) and of subcutaneous and visceral AT at the L4-L5 vertebrae was 1.1% and 5.5%, respectively (Ross et al., 1993). Magnetic resonance imaging thus measures the different AT compartments with an error of estimate of 2 to 10% (Ross, 1996). More recently, Mitsiopoulos et al. (1998) determined the reproducibility of MRI subcutaneous AT volume measurements by comparing the intra- and inter-observer estimates for MRI measurements and found that these were 2.9 ± 1.2% and 1.5 ± 1.5% for intra- and inter-observer variability of subcutaneous AT, respectively. Results from our group showed intra-observer differences of 2.1 ± 1.2% for total, 1.8 ± 1.1% for subcutaneous and 8.1 ± 3.9% for visceral AT when comparing two analyses of 5 MRI data sets by a single observer (St-Onge et al., 2003).

Energy expenditure was measured using a metabolic monitor (Delta Trac, Sensor Medics, Anaheim, USA) on d 2 or 3 and 27 or 28 for 19 of the subjects. The metabolic monitor was calibrated daily after an overnight warm-up period using gas containing 96% O_2 and 4% CO_2 at ambient pressure. Expired gases were analyzed against ambient air. Subjects were required to arrive at the CNRU one hour prior to the start of the measurement period to allow for their metabolism to return to a state that approximated basal state. Energy expenditure was then measured for 30 min prior to consumption of a standard breakfast. Subjects were required to consume the breakfast within a 30 min period after which EE measurements resumed for 5.5 h. This length of monitoring was previously recommended to capture most of the thermic effect of food (Reed and Hill, 1996). Energy expenditure was measured for 30

min of each hour after breakfast. Fat and carbohydrate oxidation rates were calculated every minute using the equations derived by Lusk (1928).

Total fecal samples were collected over 3 d midway through each experimental phase for 19 of the subjects for determination of fecal fat excretion. Samples were weighed and diluted by 50% with water, aliquoted and lyophilized. Fecal lipids were extracted from approximately 3 g of the combined 3 d dried samples with each day of sampling being proportionately represented. Lipid extraction was carried out using method of Folch et al. (1957) in duplicate. The lipid fraction was then weighed and used to calculate total fecal fat excretion over 3 d.

Total daily EE was calculated using the following equation:

$$\text{Total EE} = \text{E intake} - (\Delta \text{ total AT} + \Delta \text{ LT} + \text{E excreted}) \qquad \text{(eqn 1)}$$

where AT represents total energy stored in adipose tissue mass and LT represents total energy stored in lean tissue mass. Energy stored in AT was determined by multiplying the change in AT volume by 0.92 g/cm^3 (Snyder et al., 1975) and further multiplying by 7650 kcal/kg, assuming that 85% of adipose tissue is fat (Garrow, 1978). To determine energy stored in LT, the change in LT volume was multiplied by 1.04 g/cm^3 (Snyder et al., 1975) and again by 2920 kcal/kg, assuming 73% hydration of lean tissue (Groff and Gropper, 2000). Both values were then divided by 28 to obtain daily values. Energy excreted was determined as the product of daily fecal fat excretion multiplied by 9 kcal/g of fat.

For a subgroup of 5 subjects, for whom we did not measure EE and fecal fat excretion, we tested the effects of FctO and OL on satiety and food intake. Subjects were required to rank their level of satiety by answering 6 questions on a validated visual analog scale (VAS) (Hill and Blundell, 1982) immediately after (hr 0), at hr 2 and hr 4, after consuming a standard breakfast containing either FctO or OL. Questions included in the VAS were: 1) how hungry do you feel; 2) how full do you feel; 3) how strong is your desire to eat; 4) how much do you think about food; 5) urge to eat; 6) preoccupations with thoughts on food. Subjects were asked to place a mark on a continuous scale from 0 to 10, where 0 meant "not at all" and 10, "very much". After answering the last questionnaire (hr 4), subjects were given foods, which did not contain the test fats, in excess of their expected food intake and were instructed to consume as much of these foods as they wanted until they felt satiated. The amount of food consumed at this ad libitum lunch session was measured and energy and macronutrient intakes analyzed using Food Processor Nutrition Analysis Software (version 7.81, ESHA Research, Salem, USA).

3.3.4 Statistical analyses

The effect of each diet on energy expenditure and substrate oxidation was analyzed using analysis of variance with a mixed model procedure. Diet, day, and hour were tested as variables in the model. Interactions between diet and day and between diet, day and hour were also examined. Paired Student's t-test was used to determine differences in EE between FctO and OL at each time point. Paired Student's t-test was used to assess differences between FctO and OL on average post-prandial (PP) EE, average thermic effect of food (TEF), as well as average PP fat oxidation, calculated total daily EE, changes in body

composition, fecal fat excretion, and food intake during the satiety test. Analysis of variance was used to assess differences between treatments in response to questions on the VAS. Diet, hour and diet-by-hour interactions were used as variables in the model. All statistical analyses were conducted using SAS statistical software (SAS/STAT version 8.0, SAS Institute, Cary, USA). A p-value of 0.05 was taken as statistically significant. Data are reported as means ± SEM.

3.4 RESULTS

Twenty-five of the thirty men enrolled into the study successfully completed the protocol. Three subjects were asked to withdraw from the study due to poor compliance, one withdrew due to medical reasons unrelated to the trial, and one withdrew for work-related reasons. Data from one subject was not analyzed due to difficulties with the acquisition of images during the last MRI scan. Subject characteristics at recruitment are shown in **Table 3-2**.

Body weights at endpoint were lower than at baseline for both FctO ($p < 0.001$) and OL ($p < 0.05$) feeding periods. Body weights were 87.4 ± 2.0 kg at baseline and 86.3 ± 1.9 kg at the end of the FctO feeding period and were 86.6 ± 2.0 kg and 85.9 ± 1.8 kg at baseline and at the end of the OL feeding period, respectively. **Table 3-3** shows changes in BW and body composition values with FctO and OL consumption. Using MRI to assess body composition changes, there was a significant decrease ($p < 0.01$) in total body mass from 70.2 ± 1.6 kg on d 1 to 69.2 ± 1.5 kg on d 29 with FctO consumption whereas the change from 69.5 ± 1.5 kg to 69.0 ± 1.5 kg with OL consumption was not statistically significant. Total adipose tissue masses were 24.7 ± 1.0 kg and 23.9 ± 1.1 kg on d 1 and 29, respectively, with FctO

consumption ($p < 0.01$, within diet difference) and 24.3 ± 1.0 kg and 24.0 ± 1.0 kg on d 1 and 29, respectively, with OL consumption. There was a trend ($p = 0.087$) for greater loss of total subcutaneous adipose tissue with FctO compared to OL consumption. Functional oil consumption resulted in a significant decrease ($p < 0.01$) from 18.1 ± 0.9 kg to 17.6 ± 0.9 kg in subcutaneous adipose tissue. With OL consumption, subcutaneous adipose tissue mass varied from 17.8 ± 0.9 kg on d 1 to 17.7 ± 0.9 L on d 29. When regional adiposity was analyzed, there was greater ($p < 0.05$) loss of upper body adipose tissue with FctO consumption compared to OL. Functional oil consumption resulted in a decrease ($p < 0.05$) in upper body adipose tissue from 12.5 ± 0.6 kg on d 1 to 11.8 ± 0.6 kg on d 29. Upper body adipose tissue masses were 12.1 ± 0.6 L on d 1 and d 29 during OL feeding. Abdominal and lower body adipose tissue volumes were not altered by FctO or OL consumption.

Resting metabolic rate (RMR) was not different between periods of FctO and OL consumption. Average RMR with FctO consumption was 0.82 ± 0.02 kcal/min on d 2 and 0.80 ± 0.03 kcal/min on d 28 and with OL consumption, RMR was 0.81 ± 0.02 kcal/min on d 2 and 0.83 ± 0.02 kcal/min on d 28.

Figure 3-1 shows basal and PP EE on d 2 and 28. There was a significant effect of diet ($p < 0.01$) and hour ($p < 0.01$) on EE. Average PP EE tended to be greater ($p = 0.055$) with FctO consumption compared to OL for both d 2 and 28. On d 2, average PP EE was 1.04 ± 0.02 kcal/min and 0.99 ± 0.03 kcal/min for FctO and OL consumption, respectively. On d 28, average PP EE was 1.01 ± 0.02 kcal/min after consumption of the breakfast containing FctO, compared to 0.98 ± 0.03 kcal/min for OL. Average TEF was calculated as the difference

between average PP EE and RMR. On d 2, TEF with FctO consumption was 0.21 ± 0.02 kcal/min compared to 0.19 ± 0.01 kcal/min with OL consumption. For d 28, TEF after consumption of the breakfast containing FctO was greater ($p < 0.01$) than that observed after the breakfast containing OL. After consumption of the FctO-containing breakfast, TEF was 0.21 ± 0.01 kcal/min versus 0.15 ± 0.02 kcal/min for the OL-containing breakfast.

Average EE over the entire measurement period, from RMR until 6.5 hr after breakfast, was greater ($p < 0.05$) with consumption of the breakfast containing FctO compared to OL on d 2 although this was no longer significant for d 28. Average EE was 1.00 ± 0.02 kcal/min with FctO consumption and 0.96 ± 0.03 kcal/min with OL consumption, on d 2 and 0.98 ± 0.02 kcal/min with FctO intake and 0.95 ± 0.03 kcal/min with OL intake on d 28. Using equation 1 to calculated total daily EE, we found that EE during FctO consumption was 3169.7 ± 125.8 kcal/d and 3050.9 ± 114.6 kcal/d during OL consumption.

Figure 3-2 shows basal and PP fat oxidation on d 2 and 28. There was a significant effect of diet ($p < 0.01$), hour ($p < 0.01$), and diet by hour interaction ($p < 0.01$) on fat oxidation. Basal fat oxidation was not different between phases of FctO and OL consumption. On d 2, basal fat oxidation was 0.054 ± 0.004 g/min and 0.055 ± 0.003 g/min with FctO and OL consumption, respectively. Similarly, on d 28, basal fat oxidation was 0.055 ± 0.003 g/min with FctO and 0.056 ± 0.004 g/min with OL consumption. Average PP fat oxidation was greater ($p = 0.052$) after consumption of the breakfast containing FctO compared the breakfast containing OL, but this difference was not present on d 28 ($p = 0.32$). Fat oxidation after the FctO-containing breakfast was 0.052 ± 0.003 g/min versus 0.044 ± 0.003 g/min after

the OL-containing breakfast. On d 28, average fat oxidation was 0.049 ± 0.003 g/min and 0.047 ± 0.003 g/min after the FctO- and the OL-containing breakfasts, respectively.

Fecal fat excretion was similar between FctO consumption and OL. Average fecal fat recovery was 0.481 ± 0.05 g/d with FctO intake and 0.334 ± 0.04 g/d with OL intake. This represents approximately 99.6 and 99.7% fat absorption for periods of FctO and OL consumption, respectively.

There was no effect of diet, but a significant effect of hour, on hunger and satiety perceptions using the VAS. Also, there was no significant interaction between diet and hour on responses to the questions on the VAS. However, there was a trend ($p = 0.062$) towards lower energy intake at the ad libitum lunch session following the breakfast containing FctO compared to the session following OL breakfast consumption. This was mostly due to lower ($p < 0.05$) fat consumption at the ad libitum lunch session following FctO breakfast compared the one following OL breakfast.

3.5 DISCUSSION

This study shows, for the first time, that when consumed as part of a strictly controlled targeted weight maintenance diet, a FctO rich in MCT leads to greater loss of adipose tissue stores compared to a diet rich in LCT. This change in total adiposity may be due to a rise in EE and fat oxidation with FctO consumption relative to a diet rich in LCT in the form of olive oil.

Results obtained in this trial on body composition are in contrast with those obtained previously in women, which showed no significant effect of MCT consumption compared to LCT on total adiposity (St-Onge et al., 2003). Differences in MCT and LCT consumption on EE between men and women may be due to hormonal differences or, more likely, to differences in intakes. Men generally consume more calories than women and would therefore have a greater absolute intake of MCT.

Although the magnitude of the difference observed in total adipose tissue reduction between diets contrast, our results agree with those of Tsuji et al. (2001) who found greater body fat loss with MCT compared to LCT supplementation in their overweight subgroup. Reasons for discrepancies in results likely include differences in study design. Since the study by Tsuji et al. (14) was a supplementation trial, it is possible that subjects consuming the MCT supplement altered their diet or spontaneously consumed fewer calories than those supplemented with LCT. In addition, the dose of MCT given was low (10 g) to produce such an effect on body composition (Tsuji et al., 2001). Also, our results are similar to those of Matsuo et al. (2001), who found that subjects supplemented with structured MCT gained less body fat than subjects supplemented with LCT over a 12 wk period. However, in this trial, intakes were not strictly controlled and, as in the trial by Tsuji et al. (2001), the dose of MCT supplied was low.

The use of MRI in this study allowed determination of small variations in tissue volumes and is a well-established method for measuring total and regional adiposity since contiguous slices are acquired (Ross et al., 1992; Thomas et al., 1998). The accuracy of MRI in

assessing body fat compartments has been previously demonstrated (Ross et al., 1992; Ross et al., 1993). Therefore, we are confident that the changes observed are biological and not due to methodological error.

When extrapolating average measured EE to total daily EE, the difference in energy expenditure between FctO and OL feeding periods represents approximately 63 kcal/d on d 2 and 43 kcal/d on d 28. This is slightly lower than observed by Scalfi et al. (1991) and Dulloo et al. (1996) who have reported differences in daily EE between MCT and LCT consumption of approximately 86 and 120 kcal/d, respectively. However, when we calculated total EE based on the difference between energy intake and energy output, we found a difference of 119 kcal/d (NS) between FctO and OL consumption. Differences between previous results (Scalfi et al., 1991; Dulloo et al., 1996) and those obtained in the present trial may be due to differences in the quantities of MCT provided in the diet. In this trial, subjects consumed an average of 21.5 g of MCT per meal compared to 30 g for the previous trials (Scalfi et al., 1991; Dulloo et al., 1996). Furthermore, it is possible that MCT exert more profound increments in EE when given in a single acute ingestion than during chronic ingestion. Our results and those of White et al. (1999) support the idea that the initial increase in EE with MCT consumption compared to LCT is lessened when measured again after 14 d (White et al., 1999) and 28 d, as observed with diminished statistical power on d 28 in this trial. Nevertheless, the extent of increase in EE observed in this trial can explain the differences in BW change between FctO and OL phases. When daily EE is extrapolated over a 28 d period, the total difference in EE would lead to differences in BW change between the 2 diets of 0.36 to 0.51 kg, when using EE values measured on d 28 and d 2, respectively.

Fat absorption was 99.6% with FctO consumption which is similar to that observed in an animal trial comparing the digestibility of different types of fat (Kaplan and Greenwood, 1998). Also, earlier studies of the absorbability of fats in rats showed that coconut oil, which is rich in MCT, is 99.7% absorbed (Calloway et al., 1956). With OL consumption, fat absorption was measured to be 99.7%, which is greater than the 97.4% absorption rate reported by Jones et al. (1985) for oleic acid. However, OL also contains approximately 11.4% of fatty acids as linoleic acid (Jones et al., 1989) which was found to be 99.4% absorbed (Jones et al., 1985).

Our data on subjective satiety and ad libitum intake at lunch, although collected on only a small number of subjects, extend and support existing literature. Data obtained using VAS to assess perceived satiety showed no difference between FctO and OL phases, as was observed by Van Wymelbeke et al. (1998) when comparing olive oil, lard, MCT oil, and a fat substitute and by Bendixen et al. (2002) when comparing the effects of modified fats containing medium chain fatty acids to rapeseed oil. The use of the VAS to assess satiety sensations has been shown to be reproducible under controlled conditions and with the use of within subject designs (Stubbs, 2000).

Bendixen et al. (2002) also found no difference in ad libitum food intake with consumption of modified fats compared to the rapeseed oil. This is in contrast with what was observed in this trial and that of others (Stubbs and Harbron, 1996; Van Wymelbeke et al., 1998; Van Wymelbeke et al., 2001). We found a strong trend towards lower energy intake of 221 kcal at the lunch following the breakfast containing FctO compared to the breakfast containing

OL. Van Wymelbeke et al. (1998) found differences in energy intakes between MCT and olive oil diets of 43 kcal whereas in a subsequent study, the same group (Van Wymelbeke et al., 2001) found that subjects consumed 129 kcal less at a meal following MCT consumption compared LCT. Similarly, Stubbs and Harbron (1996) found differences in daily energy intakes of 258 kcal between a diet containing large amounts of MCT compared to that containing the least amount of MCT, when food intakes were precisely recorded.

In conclusion, we have shown that consumption of a diet rich in MCT for 28 d improves adiposity, particularly upper body adiposity in overweight men. This may be due to enhanced EE and fat oxidation compared to olive oil consumption and to greater fecal fat excretion. In addition, there was a strong trend towards lower spontaneous energy intake at the free lunch session following a standard breakfast rich in MCT, compared to one rich in olive oil. Therefore, it is possible that, under free-living conditions, subjects would consume less energy and fat when their diet contained MCT and thus would obtain this added benefit to increased EE, resulting in better weight maintenance and possibly weight loss. Future studies should therefore be conducted on a free-living population replacing the major source of added fat in the diet with medium chain triglycerides for a period of 6 mo and comparing to a control group consuming an oil rich in long chain triglycerides. This design would allow all aspects of medium chain triglyceride consumption, increased EE and satiety, to exert their effect and possibly produce beneficial changes in body composition. Results from the present trial suggest that medium chain triglycerides may be considered as a potential tool in the prevention of weight gain and obesity.

3.6 ACKNOWLEDGEMENTS

We would like to acknowledge the help of Chris Vanstone for the processing of fecal samples, the staff of the Mary Emily Clinical Nutrition Research Unit for assistance in meal preparation, Cathy Tucci for help in recruitment of subjects, and Lindsay MacKinnon for help with MRI analyses.

3.7 FIGURE LEGENDS

Figure 3-1

Energy expenditure after consumption of a breakfast containing OL or FctO on day 2 (A) and day 28 (B). Closed squares = FctO phase; open squares = OL phase. Values are means ± SEM, n=19. * FctO significantly different from OL, $p < 0.05$. ** Trend for diet difference, $p < 0.1$.

Figure 3-2

Fat oxidation after consumption of a breakfast containing OL or FctO on day 2 (A) and day 28 (B). Closed squares = FctO phase; open squares = OL phase. Values are means ± SEM, n=19. * FctO significantly different from OL, $p < 0.05$. ** Trend for diet difference, $p < 0.1$.

Table 3-1. Fatty acid composition of the functional oil.

Fatty acid	Functional oil (%)
6:0	0.17
8:0	36.95
10:0	30.35
12:0	3.61
14:0	1.06
16:0	3.52
16:1	0.23
18:0	0.65
18:1	13.81
18:2n-6	4.62
18:3n-3	4.94
20:0	0.05

Table 3-2. Subject characteristics at screening.

Characteristic	Average (SEM)
Age, y	43.1 (2.3)
Weight, kg	87.2 (1.9)
Height, m	1.76 (0.01)
Body mass index, kg/m^2	28.2 (0.4)
Total cholesterol, mmol/L	5.62 (0.18)
Triglyceride, mmol/L	1.86 (0.15)

Table 3-3. Change in body weight and body compartment volumes with functional oil and olive oil consumption.

Body compartment	Functional oil (SEM)	Olive oil (SEM)
Body weight, kg	-1.03 (0.25)	-0.62 (0.29)
Total adipose tissue, kg	-0.83 (0.25)[1]	-0.31 (0.30)
Subcutaneous adipose tissue, kg	-0.54 (0.16)[1]	-0.17 (0.19)
Upper body adipose tissue, kg	-0.67 (0.26)[1 2]	-0.02 (0.19)
Abdominal adipose tissue, kg	-0.17 (0.13)	-0.07 (0.08)
Lower body adipose tissue, kg	-0.24 (0.21)	-0.27 (0.16)

[1] Significant within diet change, $p < 0.05$, using paired t-test.

[2] Significantly different from change with olive oil consumption, $p < 0.05$, using paired t-test.

Figure 3-1.

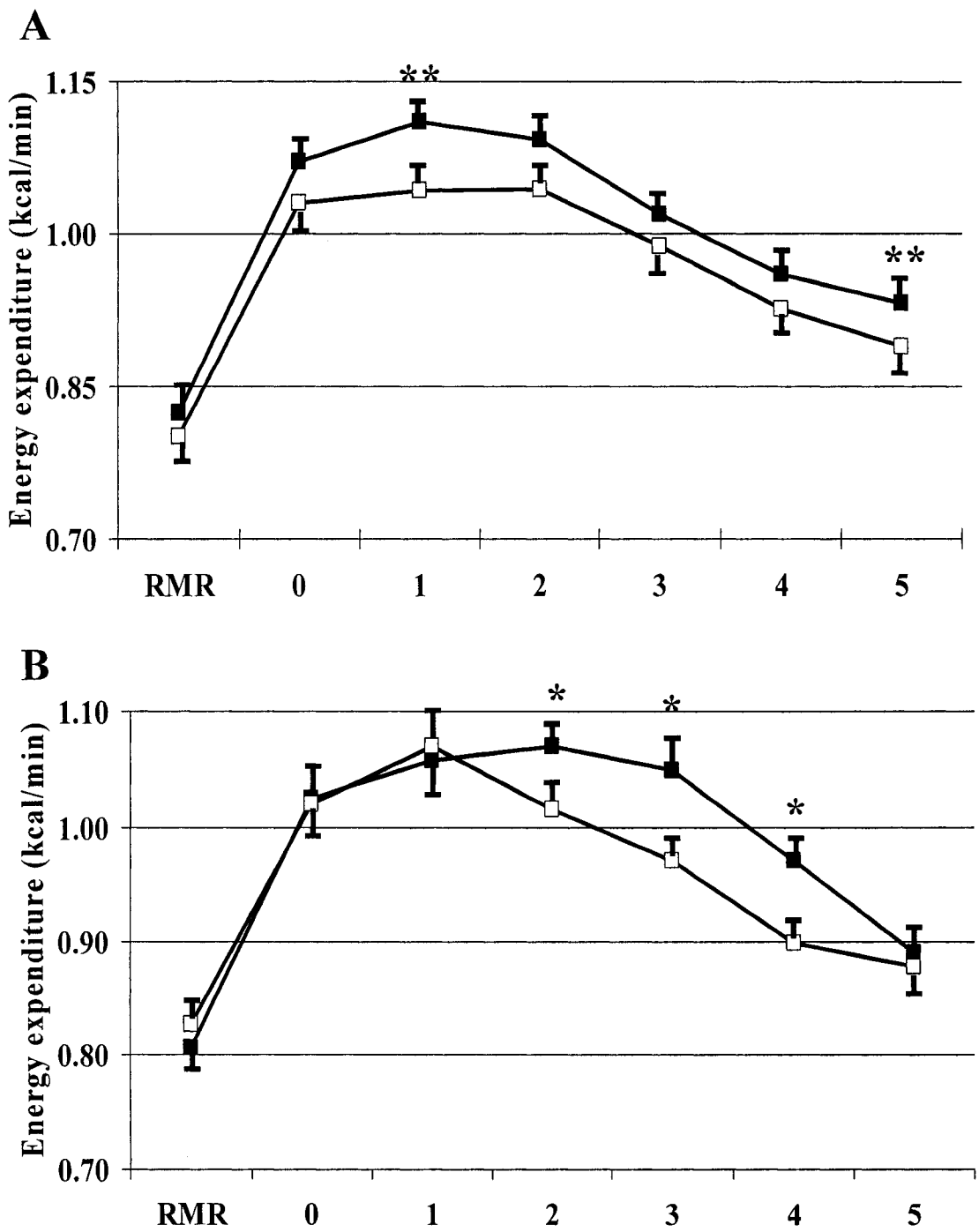
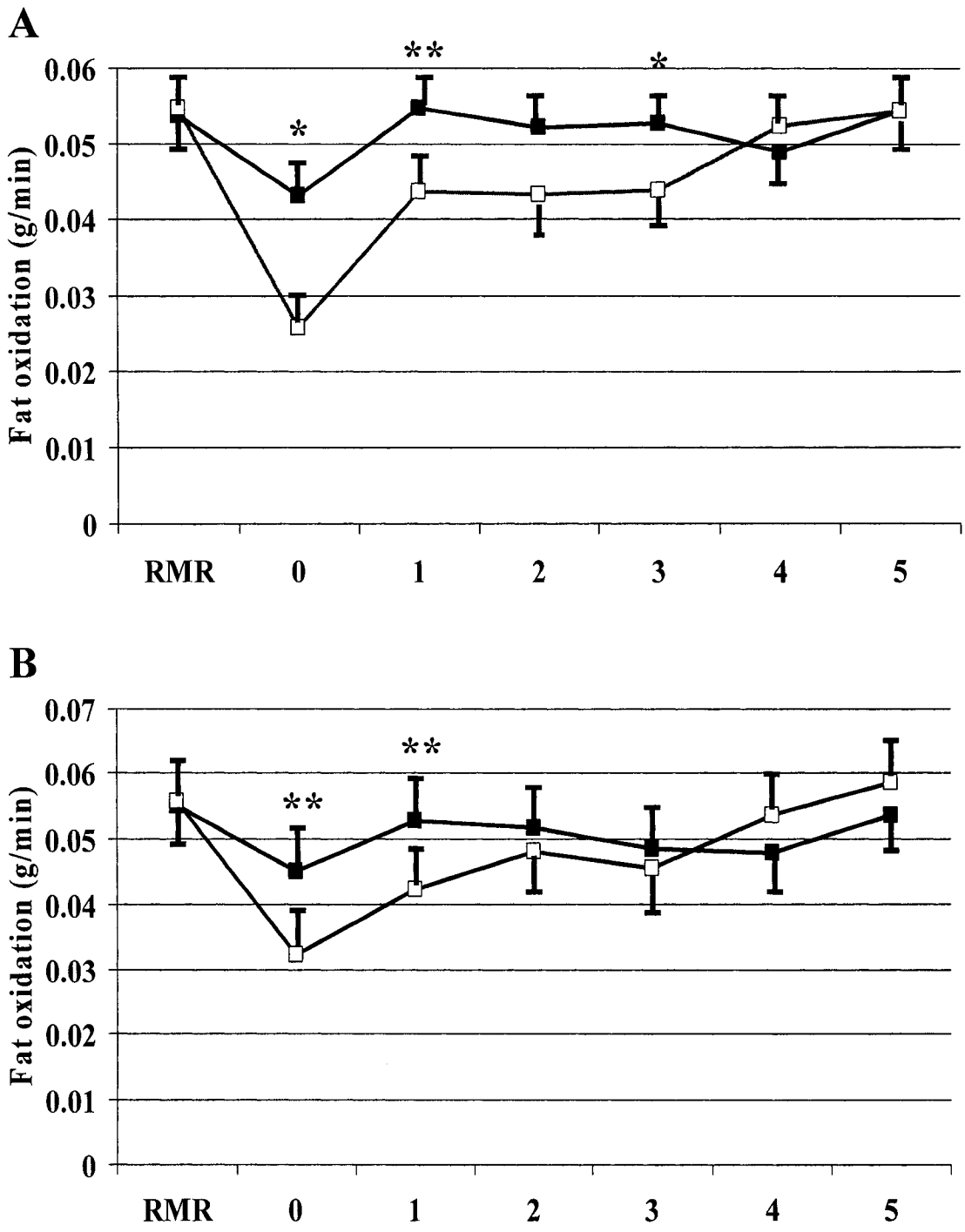

A
1.15
1.00
0.85
0.70
Energy expenditure (kcal/min)
**
**
RMR
0
1
2
3
4
5
B
1.10
1.00
0.90
0.80
0.70
Energy expenditure (kcal/min)
*
*
*
RMR
0
1
2
3
4
5

Figure 3-2.


A
Fat oxidation (g/min)
0.06
0.05
0.04
0.03
0.02
0.01
0
RMR 0 1 2 3 4 5
B
Fat oxidation (g/min)
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
RMR 0 1 2 3 4 5

BRIDGE 3.

Results from the previous two trials showed that men and women had increased energy expenditure and fat oxidation with medium chain triglyceride consumption. This increased thermogenesis, however, did not lead to weight loss in all subjects. In addition, not all subjects responded to medium chain triglyceride consumption with increased energy expenditure. This prompted us to examine the data more closely to determine the characteristics of subjects that were linked to responsiveness to a thermogenic fat, medium chain triglyceride oil. Also, data from Binnert et al. (1998) seemed to indicate that women of greater body weight would benefit most from medium chain triglyceride consumption. In addition, medium chain triglyceride oil has been proposed as agents useful in the treatment of obesity (Scalfi et al., 1991) or prevention of body weight gain (St-Onge et al., 2003; St-Onge et al., in press). It was thus important to determine whether subjects with greater initial body weight had greater response to the thermogenic effects of medium chain triglycerides. Data from the trial conducted in men was chosen since they responded to the shift from high long chain triglyceride diet to a high medium chain triglyceride diet with greater body weight loss.

Chapter 4. Manuscript 4. ***Submitted to Int J Obes.***

GREATER RISE IN FAT OXIDATION WITH MEDIUM CHAIN TRIGLYCERIDE CONSUMPTION RELATIVE TO LONG CHAIN TRIGLYCERIDE IS ASSOCIATED WITH LOWER INITIAL BODY WEIGHT AND GREATER LOSS OF ADIPOSE TISSUE

Marie-Pierre St-Onge[1, 2], and Peter JH Jones[2]

[1] School of Dietetics and Human Nutrition, McGill University, Montreal, Quebec H9X 3V9

[2] Obesity Research Center, Institute of Human Nutrition, Columbia University, New York, NY 10025

Address correspondence to: Marie-Pierre St-Onge

St-Luke's-Roosevelt Hospital

Obesity Research Center

1090 Amsterdam Avenue, suite 14A

New York, NY 10025

Telephone: 212-523-3615

Fax: 212-523-3571

E-mail: marie-pierre.st-onge@mail.mcgill.ca

Running title: MCT, body weight, and energy expenditure

4.1 ABSTRACT

Medium chain triglyceride (MCT) consumption has been shown to increase energy expenditure (EE) and lead to greater losses of adipose tissue (AT) in animals and humans. The objective of this research was to examine the relationship between body composition and thermogenic responsiveness to MCT treatment. Randomized, crossover controlled feeding trial, with diets rich in either MCT or LCT (as olive oil) for periods of 4 wk each. Nineteen healthy overweight men aged (x ± SEM) 44.5 ± 2.5 y with body mass index (BMI) of 27.8 ± 0.5 kg/m^2. Energy expenditure and body composition were measured using indirect calorimetry and magnetic resonance imaging, respectively, at baseline and endpoint of each feeding period. Energy expenditure was measured for 30 min before consumption of a standard meal and for 5.5 h following the meal. The difference in average EE between MCT and LCT consumption was related to initial body weight (BW), such that men with lower initial BW had greater rise in EE with MCT consumption relative to LCT on d 28 ($r = -0.472$, $p = 0.04$) but not d 2 ($r = -0.368$, $p = 0.12$). Similar results were obtained with fat oxidation on d 28. Energy expenditure during each of the treatment phases was not associated with any change in BW or adiposity but greater fat oxidation on d 2 was predictive of greater loss of BW for both MCT ($r = -0.6065$, $p = 0.0059$) and LCT ($r = -0.5824$, $p = 0.0089$) consumption and was also predictive of greater loss of subcutaneous adipose tissue (SAT) during MCT consumption only ($r = -0.4781$, $p = 0.04$). These data suggest that shunting of dietary fat towards oxidation results in diminished fat storage, as reflected by loss of BW and SAT. Furthermore, MCT consumption may stimulate EE and fat oxidation to a lower extent in men of greater BW compared to leaner men, indicative of lower responsiveness to a rapidly oxidized fat by overweight men.

Key words: medium chain triglycerides, body weight, adipose tissue, energy expenditure, magnetic resonance imaging

4.2 INTRODUCTION

Obesity is a worldwide problem with prevalence rates reaching over 30% in the American adult population (Flegal et al., 2002) and is linked to a plethora of disorders, ranging from type II diabetes to cardiovascular disease, hypertension, and certain cancers. Dietary treatments to prevent and reverse obesity have largely been unsuccessful, with the majority of dieters regaining lost weight (Brownell and Jeffrey, 1987; Hensrud, 2001). In a recent meta-analysis, subjects regain, on average, 77% of the weight lost within 5 y of the weight loss program (Anderson et al., 2001). Another approach to weight loss involves tipping the energy balance scale towards greater energy output. This can be achieved through increased physical activity but has also been observed with dietary modifications. Medium chain triglycerides (MCT), which do not require chylomicron formation for absorption into the circulation, travel directly to the liver where they have been shown to be rapidly and completely oxidized (Babayan, 1987; Bach and Babayan, 1982). Human studies have shown that replacing the more common dietary long chain triglycerides (LCT) for MCT leads to increased energy expenditure (EE) and fat oxidation (Bendixen et al., 2002; Dulloo et al., 1996; Hill et al., 1989; Scalfi et al., 1991; Seaton et al., 1987; St-Onge et al., 2003; St-Onge et al., in press; White et al., 1999) resulting in body adipose tissue loss (St-Onge et al., in press; Tsuji et al., 2001). However, studies linking increased EE with MCT consumption to body composition changes are scarce (St-Onge et al., 2003; St-Onge et al., in press) and the underlying mechanism relating these factors are not well developed.

The aim of the current trial was to verify whether replacement of LCT by MCT in a typical North American diet would lead to changes in EE and body composition in overweight men.

The objectives of this trial were therefore to examine whether differences in EE and substrate oxidation between MCT and LCT consumption would be related to initial body weight (BW) and composition as well as to the different degree of body composition change after consumption of isocaloric diets for 4 wk each.

4.3 METHODS

4.3.1 Subjects

Nineteen healthy, overweight men, aged (x ± SEM) 44.5 ± 2.5 y with a body mass index (BMI) of 27.8 ± 0.5 kg/m^2 participated in this trial. Subjects were recruited from newspaper advertisements. Inclusion criteria were BMI between 25 and 31 kg/m^2 and total cholesterol and triglyceride concentrations below 7.0 mmol/L and 3.0 mmol/L, respectively. Subjects were excluded if they had previously been diagnosed with cardiovascular disease, diabetes, hypertension, gastrointestinal disorders, and if they were taking cholesterol-lowering medications or had unusual eating patterns. The study protocol was reviewed and accepted by the Human Ethical Review Committee of the Faculty of Agriculture and Environmental Sciences of McGill University. Eligible subjects signed informed consent forms in the presence of the study coordinator prior to entry into the study.

4.3.2 Study design

This randomized, crossover, controlled feeding trial involved subjects consuming 2 different diets differing only in the type of added fat for 2 periods of 28 d each. Experimental phases were separated by a 4-wk washout period to allow for measured parameters to return to basal values. Diets were designed to maintain BW and each subject's energy requirement was

calculated using the Mifflin equation (Mifflin et al., 1990) multiplied by an activity factor of 1.7. This factor was previously shown to adequately estimate energy requirements for weight maintenance (Bell et al., 1985). Both diets contained 40% of energy as fat, 55% as carbohydrates, and 15% as protein. Of the total amount of fat provided by the diets, 25% was intrinsic to foods common to both experimental diets and the remaining 75% was added fat. For the MCT diet, two thirds of the added fat was MCT oil (Neobee 1053, Stepan Company, Northfield, USA) and the rest was provided by olive oil, coconut oil, flaxseed oil, and canola oil. For the LCT diet, all of the added fat was olive oil. Energy intakes were adjusted in the first week of the first phase to account for any BW variations that may occur when one adapts to a new diet. After this initial 1 wk period, energy intakes remained constant throughout the rest of the first phase and this caloric load was used for the second phase.

Subjects were required to eat 2 meals/d under supervision at the Mary Emily Clinical Nutrition Research Unit (CNRU) of McGill University and could eat the third meal away from the CNRU. Subjects were repeatedly instructed to consume all foods provided and nothing else during the 2 experimental phases. In addition, subjects were asked not to deviate from habitual physical activity level and to maintain a constant pattern of physical activity during the 2 experimental phases.

4.3.3 Body composition measurements

Body weights were measured every day before breakfast using a standard scale. On d 1 and 29 of each experimental phase, body composition was assessed using magnetic resonance

imaging (MRI). The MRI protocol is described in detail elsewhere (St-Onge et al., 2003). Briefly, images were acquired using a Siemens 1.5 Tesla MRI scanner (Siemens, Mississauga, Canada) using a T-1 weighted, spin-echo sequence with a 210 ms repetition time and a 17 ms echo time. Subjects were required to lie in the magnet in a prone position with their arms above their head while images were being acquired. Using the inter-vertebral space between the fourth and fifth lumbar vertebrae (L4-L5) as the point of origin, transverse images with 10 mm slice thickness were obtained every 40 mm from hand to foot, resulting in a total of approximately 45 images for each subject. The entire MRI protocol took approximately 45 min. Data were analyzed using specially designed MRI analysis software (Tomovision Inc, Montreal, Canada). Details of the data analysis procedure have been previously published (St-Onge et al., 2003).

4.3.4 Energy expenditure measurements

Energy expenditure measurements were taken on d 2 and 28, after a 12 h overnight fast. On each testing day, subjects were required to arrive at the CNRU approximately 1 h prior to the start of the measurement period for a resting period designed to allow the subject's metabolic rate to return to near-basal state. After this initial resting period, resting metabolic rate (RMR) was assessed using indirect calorimetry with ventilated hood methodology (Delta Trac, Sensor Medics, Anaheim, USA). The metabolic cart was calibrated daily, after an overnight warm-up period, at ambient pressure with gas containing 96% O_2 and 4% CO_2. Following RMR measurement, subjects were provided with a standard breakfast to be consumed in a 30 min period, after which EE measurements were resumed. Energy expenditure was measured 30 min every hour for 5.5 h following breakfast consumption.

This measurement period was determined to be appropriate to capture most of the thermic effect of a meal (Reed and Hill, 1996). Energy expenditure and fat and carbohydrate oxidation rates were calculated for every minute using standardized equations (Lusk, 1928).

4.3.5 Statistical analyses

Energy expenditure values for d 2 and 28 for each correlation analysis used the average EE values over each 6.5 h EE measurement period. Change in BW was calculated as the change between the average last 3 d and first 3 d of each experimental period. Body composition values used for correlation analyses include total adipose tissue (TAT), subcutaneous adipose tissue (SAT), upper body adipose tissue (AT), visceral adipose tissue (VAT), and lean tissue (LT). Upper body AT was computed by summing all AT compartment volumes from images starting at L4-L5 and above. Initial LT and VAT volumes were calculated by averaging volumes at baseline of both dietary phases. All correlations were performed using SAS system for windows version 8.2 (SAS Institute, Cary, USA). Data are reported as means ± SEM. Statistical significance was set at a p-value of 0.05.

4.4 RESULTS

Body weights decreased with both MCT and LCT consumption. Body weight, body composition, and EE data with MCT and LCT consumption are reported elsewhere (St-Onge et al, in press). In short, MCT consumption resulted in increased EE and fat oxidation relative to LCT consumption. Furthermore, MCT consumption reduced TAT, SAT, and upper body AT but only the change in upper body AT was significantly different from the change that occurred with LCT consumption.

Figure 4-1 shows relationships between differences in EE between MCT and LCT consumption and initial BW. Correlation analyses show that the difference in EE between MCT and LCT on d 2 tended to be related to initial BW ($r = -0.368$, $p = 0.12$). This inverse relationship became significant on d 28 ($r = -0.472$, $p = 0.04$). These effects were largely driven by relationships between EE and LT volume. The differences in EE between MCT and LCT on d 2 and 28 were inversely related to LT volume ($r = -0.478$, $p = 0.038$ for d 2; $r = -0.590$, $p = 0.0079$ for d 28). Correlations for differences in fat oxidation between MCT and LCT consumption and initial BW are shown in **Figure 4-2**. Similar results as those seen with EE were obtained when correlating differences in fat oxidation and initial BW for d 28 ($r = -0.553$, $p = 0.01$) but the reverse trend was found for d 2 ($r = 0.367$, $p = 0.12$). In addition, initial VAT and LT volumes were inversely correlated with difference in fat oxidation between MCT and LCT ($r = -0.496$, $p = 0.031$ and $r = -0.613$, $p = 0.0053$, for VAT and LT, respectively) on d 28.

Responsiveness to MCT feeding by increased EE relative to LCT on both d 2 and 28 was not related to differences in BW and body composition changes between MCT and LCT feeding periods. There was a trend on d 2 for the increase in fat oxidation with MCT consumption relative to LCT to be inversely related to changes in BW between MCT and LCT feeding periods ($r = -0.4075$, $p = 0.08$). This trend was no longer apparent when the same correlation was computed using fat oxidation data from d 28.

Data from both treatment phases were merged to assess whether BW and body composition were correlated with EE and fat oxidation. This analytical method is considered appropriate

since it is not expected that diet treatment would modify the effect of BW and body composition on EE or fat oxidation. Results from these analyses showed that EE on d 2 and 28 were positively correlated with initial BW ($r = 0.5919$, $p < 0.0001$ and $r = 0.5451$, $p = 0.0004$, for d 2 and 28, respectively). This correlation was largely drive by LT volume since AT compartments, either total, subcutaneous or visceral, were not related with EE or fat oxidation rates. As a result, EE was positively correlated with LT volume on d 2 ($r = 0.654$, $p < 0.001$) and 28 ($r = 0.439$, $p = 0.0058$). When examining the relationship between fat oxidation and LT, correlation coefficients were smaller, 0.376 ($p = 0.020$) for d 2 and 0.23 ($p = 0.165$) for measurements taken on d 28. Rate of fat oxidation was not correlated with initial BW or AT volumes.

Again with data from both treatment phases combined, EE on d 2, but not d 28, was significantly inversely related to change in BW ($r = -0.3511$, $p = 0.03$). Energy expenditure was not significantly correlated with change in any of the AT compartments. Fat oxidation, however, was significantly and inversely correlated with change in BW for d 2 ($r = -0.3423$, $p = 0.04$) and 28 ($r = -0.3158$, $p = 0.054$) yet, as for EE, was not related to changes in body AT compartments.

When each treatment phase was examined separately, EE with MCT consumption was not related to body composition changes for any of the parameters measured. However, fat oxidation with MCT consumption on d 2 was related to change in BW ($r = 0.6065$, $p = 0.006$) and SAT ($r = -0.4782$, $p = 0.04$). Correlations when d 28 values for fat oxidation were used were weaker and no longer statistically significant. For LCT consumption, only EE and

fat oxidation on d 2 were related to change in BW ($r = -0.5824$, $p = 0.009$ and $r = -0.5003$, $p = 0.029$, for EE and fat oxidation respectively). Changes in AT compartment volumes with LCT consumption were not related to EE or fat oxidation on either d 2 and 28.

We then examined whether subjects with greater volumes of LT had more pronounced changes in body composition. With data from both treatment phases grouped, LT volume on d 2 was not associated with any change in AT compartment. When correlations were done for each treatment separately, there was a correlation between LT volume on d 2 and change in intramuscular AT, but only during LCT consumption ($r = 0.524$, $p = 0.0082$).

4.5 DISCUSSION

This trial is the first to link responsiveness to a thermogenic oil to initial BW as well as to changes in BW and body compartment volumes. Results from this study show that, in overweight men, those with lower BW had the greatest rise in EE and fat oxidation when replacing dietary LCT with MCT. It was previously suggested that MCT could be used in the treatment of obesity (Scalfi et al., 1991), however, the results reported here seem to indicate that MCT may be a better tool in the prevention of weight gain when BW is not yet highly elevated.

Few trials have compared the effects of LCT substitution by MCT in overweight and lean subjects (Scalfi et al., 1991; Binnert et al., 1998). In the study by Scalfi et al. (1991), lean and morbidly obese men did not differ in their postprandial response to MCT compared to LCT; both groups had similar increases in postprandial EE over RMR values. However, as

observed in the present analyses, postprandial respiratory quotient was reduced with MCT consumption relative to LCT, indicative of greater fat oxidation, in the lean but not in the obese subjects. In a similar experiment in women (Binnert et al., 1998), cumulative exogenous lipid oxidation over 6 h increased from 3.2 to 8.1 g in obese and from 6.0 to 9.2 g in lean subjects. When total lipid oxidation was compared between lean and obese subjects, it was found that in the lean, but not obese women, total lipid oxidation was greater after the MCT-containing challenge than after the LCT challenge. Again, these results are in agreement with those observed in this experiment, where leaner subjects had a greater rise in fat oxidation with replacement of LCT by MCT in the diet compared to those with higher initial BW. This may be due to enhanced uptake of dietary fatty acids by the greater adipose tissue mass of overweight subjects, in whom lipoprotein lipase activity is increased (Ferraro et al., 1993). Release of exogenous fatty acids into plasma has previously been shown to be defective in obese women compared to lean women (Binnert et al., 1998), possibly due to greater uptake by elevated AT stores. The results from this study show that fat oxidation and EE were enhanced to a greater degree with MCT consumption compared to LCT in leaner men. This may be related to the quantity of VAT stores, which would favor greater fat uptake for storage rather than oxidation.

It is unknown, at this time, why a trend towards greater rise in fat oxidation with MCT consumption relative to LCT was linked to greater initial BW early in the trial whereas a significant correlation in the opposite direction was seen after 4 wk of dietary treatment. It may be that MCT help restore rates of fat oxidation early on but that an adaptation occurs whereby differences in fat oxidation between MCT and LCT are diminished. In the study by

Binnert et al. (1998), it was reported that MCT oxidation is similar in obese as in lean women, whereas LCT oxidation seems to be defective in obese compared to lean women. Since their observations only extended over a single meal, it is not known whether long-term feeding would have resulted in maintenance of the improved fat oxidation noted with MCT consumption.

Although not unexpected, data showing that subjects with the greater rise in fat oxidation had the greatest loss of BW and SAT support the purported mechanism of action of MCT on BW. It has been hypothesized that the direct transport of MCT to the liver by the portal circulation diminishes their potential for deposition into AT (Babayan, 1987). MCT are rapidly and almost completely oxidized by the liver, further reducing the probability that they will be elongated and returned to the circulation, where deposition into AT is more likely (Babayan, 1987; Bach et al., 1996). The results reported here show that in fact, greater fat oxidation with MCT was correlated with smaller SAT depots. Moreover, subjects with the greatest difference in fat oxidation between MCT and LCT consumption also tended to have the greatest loss of BW.

Our research demonstrates that an oil rich in MCT increases EE and fat oxidation and leads to beneficial body composition changes. Previous studies from our group have shown that MCT, when combined with flaxseed oil and phytosterols, also improve plasma lipid profiles in men (St-Onge et al, 2002b abstract) and women (Bourque et al., in press). These effects make this oil combination a suitable candidate for classification as a functional food. A functional food is a food that is similar in appearance to a conventional food but that provides

demonstrated health benefits or reduces the risk of chronic disease above and beyond its basic nutritional functions. Our MCT-containing oil meets this definition since it increases EE and fat oxidation to a greater extent than a conventional LCT-containing oil, helps reduce body fatness, and decreases plasma lipid concentrations. These effects can lead to lower risk of developing obesity and cardiovascular disease, 2 prevalent debilitating conditions in Western societies. However, MCT are not readily available in the American diet but are found in small quantities in coconut oil and dairy fats. Sevenhuysen et al. (1993) reported that middle-aged women consume on average 15 g/d of butter as added fat on bread and potatoes. If we consider that 3.5% of fatty acids in butter are the medium chain fatty acids octanoic and decanoic acids (Hyvonen et al., 1993), this would represent an intake of MCT of approximately 0.52 g/d. However, the versatility of MCT oil, and its tasteless and odorless properties (Bach et al., 1996) make it suitable for incorporation into a variety of different food items.

Finally, the replacement dietary LCT with MCT in a typical North American diet for a period of 28 d leads to significant increases in EE and fat oxidation. In addition, BW and AT compartment volumes were diminished when MCT were consumed in place of LCT. Furthermore, correlation analyses show that the rise in fat oxidation with MCT consumption relative to LCT consumption was responsible for the greater loss of BW. Also, subjects with the greatest EE and fat oxidation rates also had the greatest loss of BW. These beneficial effects of LCT-replacement by MCT on EE and fat oxidation were correlated with initial BW. Therefore, these results show that substitution of dietary LCT for MCT can be

beneficial in the prevention of weight gain in subjects with lower BW but may not be as useful to those with greater BW.

4.6 ACKNOWLEDGEMENTS

We would like to acknowledge the excellent work from the staff of the Mary Emily Clinical Nutrition Research Unit for assistance in meal preparation. Funding was provided by Dairy Farmers of Canada and Forbes Medi-Tech Inc.

4.7 FIGURE LEGENDS

Figure 4-1

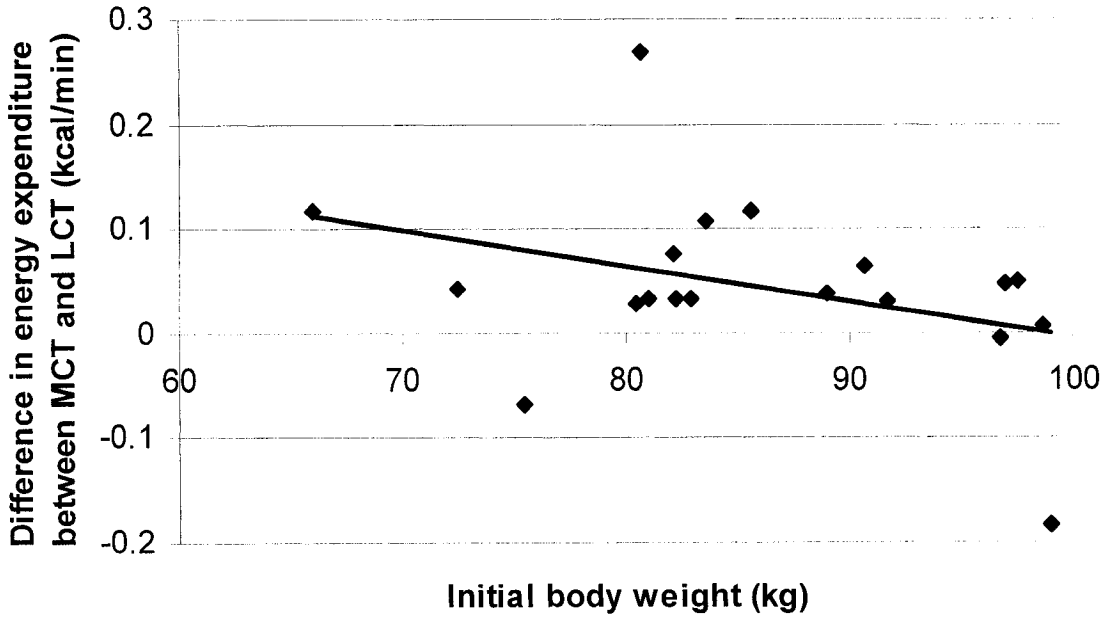
Correlation between difference in EE with MCT and LCT consumption on d 2 (a; r = -0.3679, p = 0.12) and d 28 (b; r = -0.4718, p = 0.04) and initial BW, n = 19.

Figure 4-2

Correlation between difference in fat oxidation with MCT and LCT consumption on d 2 (a; r = 0.3666, p = 0.12) and d 28 (b; r = -0.5526, p = 0.01) and initial BW, n = 19.

FIGURE 4-1.

A


Difference in energy expenditure between MCT and LCT (kcal/min)
0.3
0.2
0.1
0
-0.1
-0.2
60
70
80
90
100
Initial body weight (kg)

B

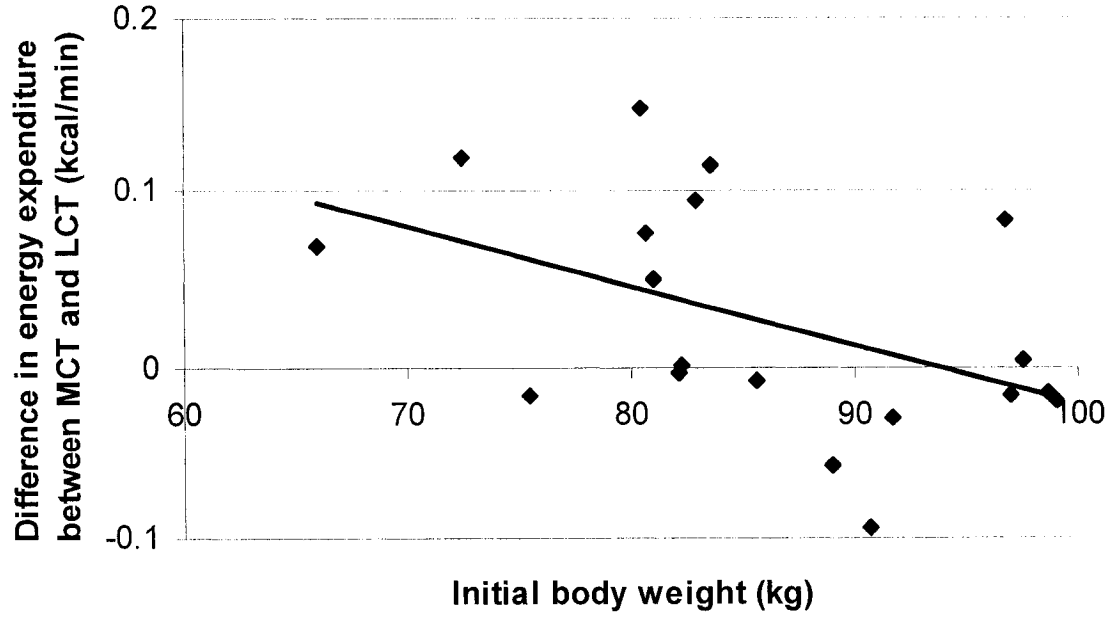
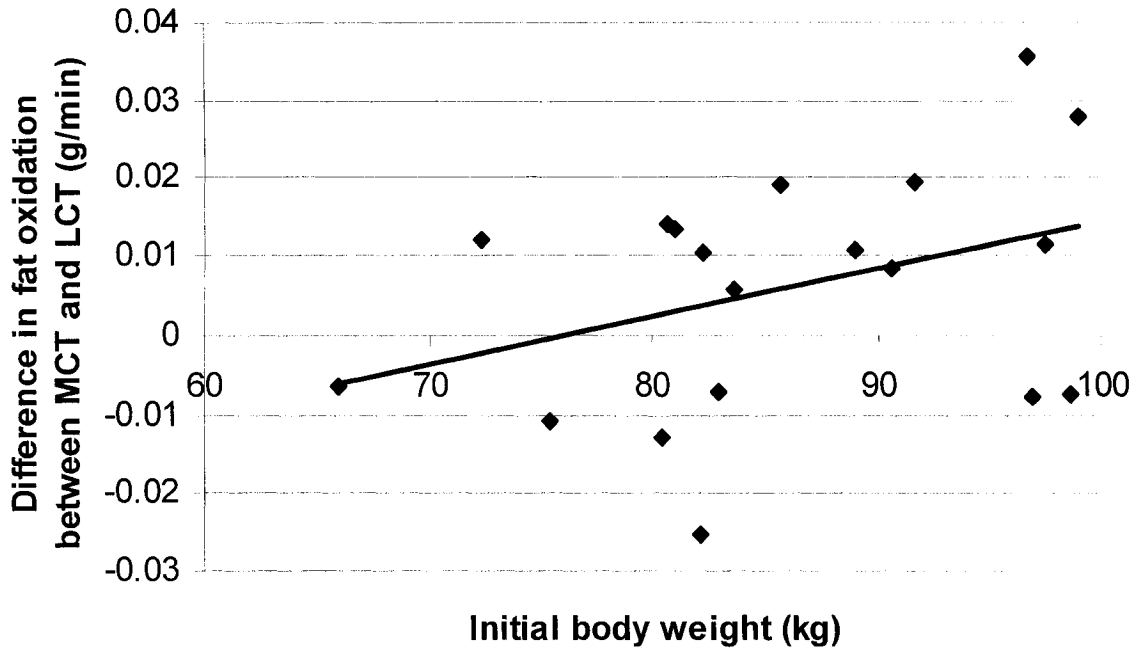
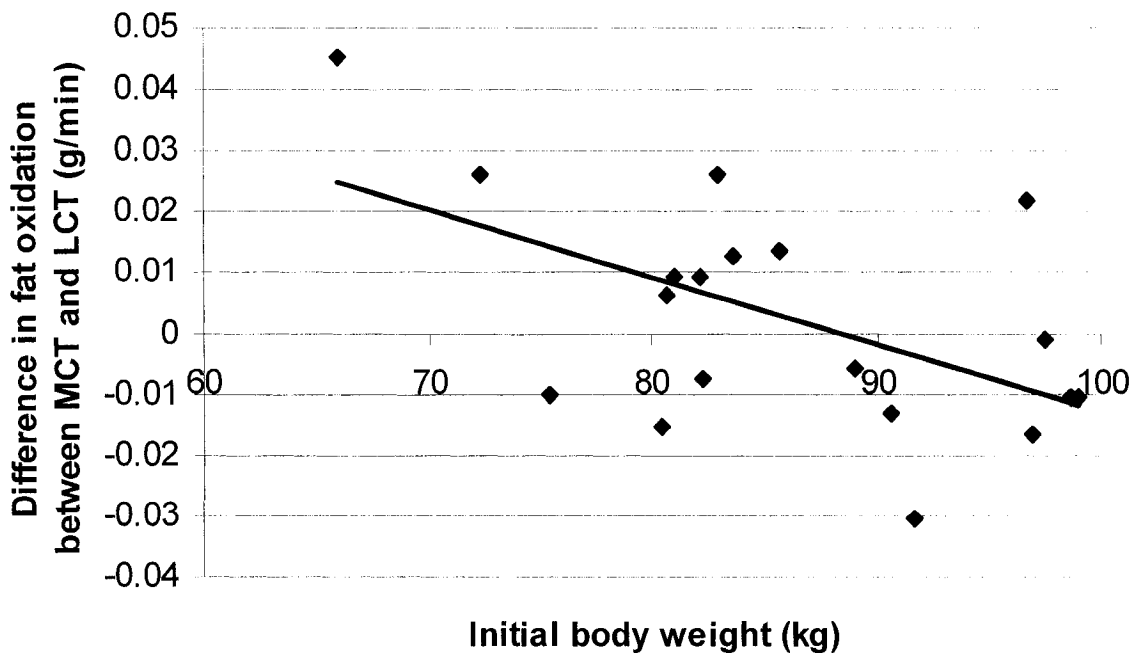

Difference in energy expenditure between MCT and LCT (kcal/min)
0.2
0.1
0
-0.1
60
70
80
90
100
Initial body weight (kg)

FIGURE 4-2.

A


0.04
0.03
0.02
0.01
0
-0.01
-0.02
-0.03
60
70
80
90
100
Difference in fat oxidation between MCT and LCT (g/min)
Initial body weight (kg)

B


0.05
0.04
0.03
0.02
0.01
0
-0.01
-0.02
-0.03
-0.04
60
70
80
90
100
Difference in fat oxidation between MCT and LCT (g/min)
Initial body weight (kg)

BRIDGE 4.

Results from the studies in women and men showed that medium chain triglyceride consumption increases energy expenditure and fat oxidation relative to long chain triglyceride consumption. In men, augmented energy expenditure with medium chain triglyceride consumption resulted in significant changes in body composition. However, several studies have shown that medium chain triglyceride consumption increases triglyceride concentrations and some also report increases in total and low density lipoprotein cholesterol concentrations. These effects of medium chain triglyceride consumption would render its use in weight management unfavorable from a cardiovascular standpoint. In order to circumvent these effects of medium chain triglycerides on plasma lipid concentrations, we have added phytosterols and flaxseed oil, the latter providing a source of n-3 fatty acids, to the medium chain triglyceride-containing diets in both trials in females and males. Phytosterols have been known to decrease circulating cholesterol concentration by preventing cholesterol absorption, and n-3 fatty acids have been shown to lower triglyceride concentrations. Therefore, adding phytosterols and flaxseed oil to medium chain triglyceride oil was anticipated to prevent any undesirable changes in plasma lipid concentrations observed previously. In addition, since medium chain triglycerides increase oxygen consumption and fat oxidation, there may be an augmented generation of oxidative by-products associated with their consumption. In the following chapter, plasma homocysteine and glutathione concentrations were measured to assess oxidative status. Homocysteine is an established risk factor for cardiovascular disease and has been shown to be modulated by fat and cholesterol intakes. Glutathione is an antioxidant and thus measurement of its circulating

levels in plasma is an index of oxidative stress. These measurements add to the information concerning the atherogenicity of the functional oil.

Chapter 5. Manuscript 5. ***In press in Metabolism.***

CONSUMPTION OF AN OIL COMPOSED OF MEDIUM CHAIN TRIACYGLYCEROLS, PHYTOSTEROLS AND N-3 FATTY ACIDS IMPROVES CARDIOVASCULAR RISK PROFILE IN OVERWEIGHT WOMEN

Christine Bourque[1], Marie-Pierre St-Onge[1], Andrea A. Papamandjaris[1], Jeffrey S. Cohn[2], Peter J.H. Jones[1]

[1] School of Dietetics and Human Nutrition, McGill University, Ste-Anne-de-Bellevue, Quebec, Canada

[2] Hyperlipidemia and Atherosclerosis Research Laboratory, Clinical Research Institute of Montreal, Montreal, Quebec, Canada

Address correspondence to: Peter J.H. Jones, Ph.D.
School of Dietetics and Human Nutrition
21,111 Lakeshore Road
Ste-Anne-de-Bellevue, Quebec, Canada, H9X 3V9
Phone: (514) 398-7841
Fax: (514) 398-7739
E-mail: jonesp@macdonald.mcgill.ca

Funding was provided by Dairy Farmers of Canada and Forbes Medi-Tech Inc.

Running head: MCT, phytosterols, n-3 fats: lipid metabolism

5.1 ABSTRACT

Medium chain triacylglycerols (MCT) have been suggested as efficacious in weight management, since they possess greater thermogenic qualities relative to long chain triacylglycerols; however, MCT may also increase circulating lipid concentrations and risk of cardiovascular disease. The present objective was to examine the effect of a diet supplemented with a functional oil (FctO) composed of energy expenditure-enhancing MCT (50% of fat), cholesterol-lowering phytosterols (22 mg/kg body weight) and triacylglycerol-suppressing n-3 fatty acids (5% of fat), vs a beef tallow based diet (BT), on plasma lipid and aminothiol concentrations. In a randomized, single-blind, crossover design, partially-inpatient trial, 17 overweight women consumed each oil as part of a controlled, supervised, targeted energy balance diet for 27 d, with 4 or 8 wk of washout between phases. Mean plasma total cholesterol concentration was lower ($p < 0.0001$), by 9.1%, on FctO (4.37 ± 0.20 mmol/L) vs BT (4.80 ± 0.20 mmol/L). Mean plasma LDL cholesterol was also lower ($p < 0.0001$) following FctO (2.39 ± 0.15 mmol/L) vs BT (2.86 ± 0.16 mmol/L), representing a 16.0% difference between diets. HDL cholesterol and circulating triacylglycerol concentrations remained unaffected by treatment. Ratios of HDL:LDL and HDL:total cholesterol were higher ($p < 0.01$), by 22.0% and 11.0% respectively, on FctO vs BT. Plasma total homocysteine remained unchanged with FctO but decreased ($p < 0.05$) with control, hence higher ($p < 0.05$) endpoints were observed with FctO (6.95 ± 0.33 umol/L) vs BT (6.27 ± 0.28 umol/L). Plasma glutathione increased ($p < 0.05$) by 0.44 umol/L with FctO supplementation. In conclusion, consumption of a functional oil composed of MCT, phytosterols and n-3 fatty acids for 27 d improves the overall cardiovascular risk profile of overweight women.

KEY WORDS: Medium chain triacylglycerols, phytosterols, n-3 polyunsaturated fatty acids, cholesterol, homocysteine

5.2 INTRODUCTION

Obesity is an independent risk factor for cardiovascular disease (CVD), the most common cause of mortality and morbidity in North America (Heart and Stroke Foundation, 1997; National Institute of Health, 1998). Substitution of medium chain triacylglycerols (MCT), containing fatty acids with 6 to 12 carbon chains, for conventional dietary fats has been suggested as beneficial to weight management, since MCT increase energy expenditure and fat oxidation relative to long chain triacylglycerols (LCT) (Scalfi et al., 1991; Hill et al., 1989; Dulloo et al., 1996; White et al., 1999). Medium chain triacylglycerols are rapidly absorbed through the portal circulation (Swift et al., 1990) and undergo oxidation to carbon dioxide (Johnson et al., 1990; DeLany et al., 2000) or conversion to long chain fatty acids in the liver (Carnielli et al., 1994), thus avoiding deposition in peripheral tissues. Animal studies have indeed shown substantial weight loss following MCT consumption (Lavau et al., 1978; Geliebter et al., 1983; Simon et al., 2000), and this has been shown in a supplementation trial (Tsuji et al., 2001), but not in controlled feeding situations in humans (St-Onge et al., 2003).

Possible benefits of MCT consumption on energy balance may be offset by undesirable effects on circulating cholesterol and triacylglycerol (TAG) concentrations, both of which are important risk factors for CVD. Long-term feeding of caprylic (8:0) and capric (10:0) acids, has resulted in plasma cholesterol concentrations higher than those of polyunsaturated fatty acids (PUFA) (Cater et al., 1997; Tsai et al., 1999; Asakura et al., 2000), lower than those of lauric acid (Tsai et al., 1999), and intermediate between those of myristic and oleic acids (Temme et al., 1997). Conversely, some researchers have reported 8:0 and 10:0 to be

similarly cholesterol-raising as palm oil (Cater et al., 1997; Wardlaw et al., 1995) and butter (Wardlaw et al., 1995). With respect to effects on TAG, short term MCT supplementation has demonstrated a 3-fold increase following overfeeding (Hill et al., 1990), and a 42% increase following weight-maintenance feeding (Swift et al., 1992), in comparison to soybean oil. However, long-term MCT feeding has resulted in unchanged fasting TAG, as compared to LCT feeding (Cater et al., 1997; Tsai et al., 1999; Asakura et al., 2000; Temme et al., 1997; Wardlaw et al., 1995).

Phytosterols have been shown to block the absorption of dietary and endogenously-derived cholesterol from the gut, while being only minimally absorbed themselves (Jones et al., 1997). Daily consumption of moderate quantities of phytosterols, has been shown to consistently reduce plasma total cholesterol by 5-13%, and LDL cholesterol by 7-16%, in both hyper- (Miettinen et al., 1995; Jones et al., 1999; Jones et al., 2000; Hendriks et al., 1999) and normocholesterolemic (Hendriks et al., 1999; Pelletier et al., 1995; Tammi et al., 2000) individuals, without affecting HDL cholesterol or TAG concentrations.

Alpha-linolenic acid (ALA), an n-3 fatty acid found in flaxseed oil, has been shown to undergo conversion (Mantzioris et al., 1994; Layne et al., 1996; Cunnane et al., 1993) to the potent hypotriacylglycerolemic (Harris et al., 1997) eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in tissue in vivo, and may thus have anti-atherogenic properties, making it a healthy addition to a blended oil product.

We thus hypothesized that consumption of MCT along with cholesterol-lowering phytosterols and TAG-suppressing n-3 PUFA would prevent undesirable increases in blood lipid concentrations, thus allowing MCT use in prevention of weight gain. The objective of the study was to evaluate the effect of this functional oil (FctO), vs beef tallow as a control fat, on circulating lipids, as well as fatty acid metabolism. Since there is some discrepancy in the literature regarding the effects of fish oil on Hcy (Grundt et al., 1999; Olszewski and McCully, 1993), a secondary objective was to measure plasma total homocysteine (Hcy) and other aminothiols following dietary fat modification.

5.3 SUBJECTS AND METHODS

5.3.1 Subjects

Twenty-two healthy overweight women were recruited from the surrounding community through newspaper advertising. Enrolled subjects had BMI > 25kg/m^2, plasma total cholesterol concentration $\leq$ 7.0 mmol/L and total circulating TAG concentration $\leq$ 3.0 mmol/L, as measured at screening after 12 h of fasting and 24 h of alcohol abstinence. Subject body weights were required to have been stable (± 5%) for at least 3mo prior to study entrance. All subjects were screened through interview for reported absence of existing chronic illnesses including diabetes, hypertension, cardiac, hepatic, renal, and gastrointestinal dysfunction. Other exclusion criteria included use of lipid-lowering drugs, beta-blockers or diuretics, and personal history of CVD. Those reporting exercise at a frequency of $\geq$ 5 times per wk, or ongoing pregnancy or lactation were excluded. Prior to study onset, subjects received a complete description of the protocol before signing a consent form in presence of the study investigators. The experimental protocol was approved by the Human Ethical

Review Committee of the Faculty of Agriculture and Environmental Sciences for the School of Dietetics and Human Nutrition at McGill University.

5.3.2 Study Protocol

The study was a randomized, single-blind, controlled, partially-inpatient clinical trial. A crossover design was used, with two 27-d dietary feeding cycles separated by 8 wk of washout (4 wk for one subject), during which subjects resumed their habitual diets. Subjects were randomly allocated to receive one of two treatment sequences, with a balanced number of subjects assigned to each dietary treatment per phase. Subjects were partially inpatients at the Mary Emily Clinical Nutrition Research Unit (CNRU) of McGill University during both feeding periods. Participants were allowed to leave the facility between meals for work or other approved purposes but were expected to remain at the unit after the evening meal and overnight. Meals were consumed under supervision at the CNRU, however, under unusual circumstances, study coordinators permitted meals to be packed and consumed outside of the CNRU; this occurred for < 2% of meals. Although physical activity was not encouraged, an exercise facility was available at the unit. Subjects were instructed to report any physical activities in a journal during the first phase of the study, and to reproduce the same intensity and duration of activity on corresponding days of the second phase. A physician familiar with the study protocol and diets was available throughout the trial in case subjects experienced discomfort. Fasting blood samples were collected on d 1, 26 and 28 of each dietary phase. Measurements on d 26 and 28 were taken to obtain a better endpoint estimate of circulating lipid concentrations and to quantify the day-to-day variation in these variables. Since the same menu was assigned to corresponding days across phases, foods consumed

preceding d 26 blood draws were identical, except for treatment fat, and similarly for d 28 blood draws. In order to measure fatty acid excretion, total fecal samples were collected for 3 d at midpoint of each phase, a period chosen to allow for sufficient adaptation to the experimental diets. Using the present protocol, each subject was tested during the same phase of her menstrual cycle for corresponding time points across study periods.

5.3.3 Diets

Experimental diets consisted of prepared North American solid foods, precisely weighed, and based on a 3-d rotating cycle menu. Diets were served as 3 isoenergetic meals per day, and provided 45% of energy as carbohydrate, 15% as protein, and 40% as fat, of which 75% was delivered as treatment fat. The remaining 25% of total fat was found in the basal diet food items identical to both diets. Treatment fat, either FctO or control fat (BT), was directly incorporated into food items during meal preparation and cooking to effect blinding. The FctO consisted of 3 major lipid components: MCT, phytosterols and n-3 PUFA. A combination of MCT oil (50% of fat) (Neobee 1053, Stepan Company, Northfield, IL, US), butter (5% of fat) and coconut oil (5% of fat) comprised the MCT portion of the FctO. Tall oil phytosterols, with major components sitosterol, campesterol and sitostanol, in the unesterified form (Forbes phytosterols; Forbes Medi-Tech Inc, Vancouver, BC, Canada), were administered at a concentration of 22 mg/kg body weight per day (average daily intake = 1.81 g). Since plant sterols were dispersed in fat before being incorporated to foods, sufficient solubilization and intestinal availability were ensured. The n-3 PUFA portion of the oil was provided by flaxseed oil (5% of fat). Olive oil (10% of fat) was also present in the FctO to approach the proportion of monounsaturated fatty acids found in the BT diet.

The intake of each fat component was equally distributed over the three daily meals. Treatment fat for the control diet was composed exclusively of beef tallow. Forty-seven percent of total fatty acids in the FctO diet had ≤ 12 carbons, while 66% of the fatty acids in the BT diet had ≥ 18 carbons (**Table 5-1**). Average daily cholesterol intakes on FctO and BT diets were 349 mg and 418 mg, respectively. Non-fat and non-sterol constituents were identical across diets.

In order to provide a targeted energy balance diet, the nutrient intake was adjusted to individual subject energy requirements using the Mifflin equation (Mifflin et al., 1990), to which an activity factor of 1.7 was multiplied to compensate for additional energy needs of active adults (Shils et al., 1999). The different energetic contribution of MCT and LCT, 34 and 38 kJ/g respectively, were accounted for in the calculation of energy intake, hence FctO and BT diets were isoenergetic. During the first week of phase 1, energy intake was readjusted in order to re-establish energy balance. Energy intake was fixed thereafter and was identical during both dietary treatment phases. Body weight was monitored daily before breakfast during feeding periods. No extra food was allowed between meals, except for decaffeinated, energy-free carbonated beverages and herbal teas, which were obtained from kitchen staff. One black coffee was allowed per day at breakfast. Health Canada recommendations (Health Canada, 1990) were met for all vitamins, minerals, fibre, carbohydrate subcomponents and essential fatty acids. The nutrient content of the diets, other than fatty acids, was determined with Food Processor (Esha Research, Salem, OR, US), a computerized dietary analysis program equipped with a Canadian database. A weight

maintenance protocol was chosen to specifically determine the effects of the treatment oil, not weight loss, on the different parameters measured.

5.3.4 Analyses

5.3.4.1 Blood lipid measurement

Blood samples were drawn after a 12 h overnight fast, and at least 24 h of alcohol abstinence (for d 1), and collected in EDTA-containing Vacutainer® (BD, Franklin Lakes, NJ, US) tubes. Samples were immediately centrifuged at room temperature using a table top centrifuge for 15 min at 250 X g, and resulting plasma and red blood cell subfractions were refrigerated, separated within 1 h of collection, and stored at –80°C until analysis. All tubes were coded by an external party to blind investigators for analysis and data compiling procedures. Plasma total and HDL cholesterol, and TAG concentrations, were analyzed in quadruplicate with standardized reagents using a VP Autoanalyser (Abbott Laboratories, North Chicago, IL, US). Calibration of the machine prior to each run was performed as per the standardization protocol of the Canadian Reference Laboratory (1996, Vancouver, BC, Canada), which involves direct comparison with fresh specimen samples. Certification for traceability using this method was maintained through the National Reference System. Measurement of HDL cholesterol in plasma was done after precipitation of apolipoprotein B with dextran sulphate and magnesium chloride (Warnick et al., 1982). LDL cholesterol concentrations were calculated using the Friedewald equation (Friedewald et al., 1972). Coefficients of variation for replicate analyses of total, HDL cholesterol and TAG concentrations were 1.4%, 2.3%, and 3.1%, respectively.

5.3.4.2 Homocysteine and other aminothiols measurements

Total Hcy, cysteine, cysteinylglycine and glutathione were measured in fasting plasma samples using a modified isocratic high-performance liquid chromatography method with fluorescence detection of derivatized aminothiols, as previously described (Durand et al., 1996). Aminothiols were reduced and released from proteins by incubation with tri-n-buthylphosphine (10%) in dimethylformamide for 30 min at 4°C. Proteins were then precipitated with 0.6 mol/L cold perchloric acid containing EDTA. Derivatization was done with ammonium 7-fluorobenzo-2-oxa-1,3-diazole-4-sulphonate (SBD-F) in sodium hydroxide and potassium borate at 60°C for 60 min. After cooling on ice, 10uL of samples were injected into a high-performance liquid chromatograph (System Gold; Beckman, Fullerton, CA, US) equipped with an analytical reverse phase column (C18ODS, 250X4.6mm, Beckman). Isocratic conditions were used with a 0.45:2 acetate:acetic acid buffer, pH 4.0, and a flow rate of 1.3 mL/min for 25 min. Fluorescence detection was carried with a 305-395nm excitation filter and a 430-470 nm emission filter. Acetyl-cysteine was used as internal standard to allow quantification. The day-to-day CVs were 2.5%, 6.5%, 3.2%, and 6.6% for Hcy, cysteine, cysteinylglycine, and glutathione, respectively.

5.3.4.3 Fatty acid composition determination

Individual meals in each 3 d cycle diet, red blood cells on d 1 and d 28, and 3 d total fecal samples were analysed in duplicate for fatty acid composition by gas-liquid chromatography after lipid extraction (Folch et al., 1957), sodium hydroxide saponification (Al Makdessi et al., 1985) and boron-trifluoride methylation (Al Makdessi et al., 1985). Briefly, 3 g of homogenized food or freeze-dried feces, or 2 g of red blood cells, were agitated with

methanol at 55°C for 15min. Following choloroform:hexane (1:4) extraction, non-polar phases were evaporated under nitrogen gas. The residue obtained was saponified for 15 min at 80°C with 0.5 mol/L sodium hydroxide in methanol. After acidification with sulphuric acid, hexane extraction was performed to recover lipids. Fatty acids were methylated with boron trifluoride methanol:hexane:methanol (7:6:7, v/v/v) for 1h at 100°C, and the resulting fatty acyl methyl esters (FAME) were extracted with hexane and sealed in crimp vials. Prior to lipid extraction of red blood cell samples, 0.1% butylated hydroxytoluene (BHT) was added to minimize peroxidation of long chain PUFA.

Derivatized samples were injected via an autoinjector into a gas chromatograph (HP 5890 Series II; Hewlett Packard, Palo Alto, CA, US) equipped with a flame ionization detector, and a 30 m capillary column, with 0.2 mm internal diameter and 0.25 um film thickness (SP2330; Supelco, Bellefonte, PA, US). The injector and detector temperatures were 200°C and 210°C respectively, and the split ratio was 100:1. A multiple ramp temperature program was used to separate individual FAME, and identification was done using relative retention times of authentic standards (Supelco, Bellefonte, PA, US). Fatty acid composition of each diet is reported in Table 4-1. The MCT oil was determined to be 0.1 g 6:0, 49.1 g 8:0, 49.8 g 10:0 and 1.0 g 12:0, per 100 g of total fatty acids. Coefficients of variation for replicate food total fatty acid analyses, fatty acid content of different meals, and menu cycle days were 12.3%, 6.6%, and 2.6%, respectively.

5.3.5 Statistical analyses

Data are reported as mean ± SEM. Dietary fatty acid composition is presented descriptively but was not analyzed statistically. ANOVA was carried out using a mixed model (Littell et al., 1998) for repeated measures, appropriate for crossover models (Ratkowsky et al., 1993), with factors of phase, sequence, diet, time, time-by-phase interaction and time-by-diet interaction. Age and initial body weight were also tested as covariates. Paired Student's t test was then applied to the model to compare time points within dietary phases. Scheffe's adjustment was performed to identify significant differences between control and FctO diets at corresponding times. Separate comparisons between endpoints (mean of d 26 and 28) were also carried out with the mixed model. Pearson's product-moment correlations were carried out on residuals computed from the model, in order to assess specific relationships between outcome measures. The level of significance for rejection of the null hypothesis was set at $p < 0.05$. Version 8.0 of SAS software (SAS Institute, Cary, NC, US) was used for all statistical analyses.

5.4 RESULTS

Twenty-two subjects enrolled, and 17 subjects completed both phases of the trial. Women who completed the trial were 44 ± 4 y of age and had initial BMI of 32 ± 1 kg/m^2. Mean fasting total cholesterol and TAG concentrations at screening were 5.12 ± 0.17 mmol/L and 1.57 ± 0.14 mmol/L, respectively. Mean energy and fat intakes were 10.28 ± 0.31MJ/d (2458 ± 73 kcal/d) and 109.25 ± 3.25 g/d. Four subjects were smokers, and 8 were post-menopausal. For 16 of the subjects who completed the protocol, the washout period was 8-wk; for logistical reasons, the washout period lasted only 4 wk for one subjects.

The FctO diet was generally well tolerated, except for minor gastrointestinal discomfort and occasional nausea during the first few days of consumption. Some subjects reported that the BT diet had an unpleasant smell, taste, aftertaste and/or mouth-feel. The FctO diet was generally preferred by subjects and was not reported to have any particular smell, taste or texture.

Baseline values of all variables were not statistically different between dietary phases. Mean body weight decreased ($p < 0.01$) during both FctO (-0.87 ± 0.16 kg) and BT (-0.84 ± 0.22 kg) phases, but no differential weight loss was discerned between dietary cycles.

Mean plasma lipid concentration on d 1, 26 and 28 of each feeding period, and percent change over time are presented in **Table 5-2.** Mean plasma total cholesterol concentration at endpoint was lower ($p < 0.0001$) on the FctO vs BT diet (4.37 ± 0.20 mmol/L vs 4.80 ± 0.20 mmol/L), corresponding to a 9.1% difference between diets. Relative to baseline, mean total cholesterol concentration declined by 0.24 mmol/L on d 26 of the FctO diet, while there was no change on the BT diet. The mean decrease in total cholesterol after 27 d of FctO consumption was 4.8%.

Mean plasma LDL cholesterol concentration at endpoint was lower ($p < 0.0001$), by 16.0%, with FctO (2.39 ± 0.15 mmol/L) compared to BT (2.86 ± 0.16 mmol/L) consumption (**Figure 5-1**). Relative to baseline, LDL cholesterol concentrations decreased by 0.25 mmol/L on d 26 and 0.28 mmol/L on d 28 of the FctO diet, while no significant change was observed on the control diet. A mean 10.4% decline in LDL cholesterol was found on the FctO phase.

HDL cholesterol concentrations remained unchanged during both feeding periods. Endpoint HDL:LDL and HDL:total cholesterol ratios were higher ($p < 0.01$), by 22.0% and 11.0% respectively, with FctO vs BT (0.574 ± 0.035 vs 0.478 ± 0.029 for HDL:LDL cholesterol and 0.304 ± 0.12 vs 0.276 ± 0.010 for HDL:total cholesterol) feeding. Although no significant difference between diets was identified from the analysis of separate day measurements, a main effect of diet was noted for both ratios. Relative to baseline, HDL:LDL and HDL:total cholesterol ratios increased by 19.5% and 9.4% respectively on the FctO diet.

Mean circulating plasma TAG concentrations were not different between diets. A marginally significant decrease from baseline was noted on d 26 of the BT diet. Percent changes in body weight and BMI were correlated with changes in TAG concentrations ($r = 0.554$, $p < 0.001$, for weight, and $r = 0.544$, $p < 0.01$ for BMI). Separate dietary correlation analysis revealed that the association between changes in TAG and body weight was only present in the BT diet ($r = 0.705$, $p < 0.01$) but nonexistent in the FctO diet, and likewise for the association between TAG and BMI ($r = 0.697$, $p < 0.01$).

Plasma aminothiol concentrations on d 1, 26 and 28, and percent change over time is presented in **Table 5-3**. Mean endpoint plasma total Hcy concentration was higher ($p < 0.0001$) on the FctO vs BT diet (6.95 ± 0.33 μmol/L vs 6.27 ± 0.28 μmol/L), representing a mean 10.9% difference across diets. Relative to baseline, total Hcy concentration decreased on the BT diet by 5.4%, while a marginal increase was seen on d 26 of the FctO diet. Cysteine concentrations remained unchanged, although percent variations were marginally different between diets. Cysteinylglycine remained unchanged with BT, but decreased by

1.77 μmol/L on d 26 of FctO consumption. Glutathione increased from baseline on d 28 of the FctO diet by 0.44 μmol/L and did not vary with BT.

Relative fatty acid composition of RBCs on d 1 and 28 of each dietary phase are presented in **Table 5-4**. Fatty acids with chain lengths of 6, 8, 10 and 12 carbons were not detected in this tissue. Proportion of 14:0, 16:0, 18:3n-3, EPA (20:5n-3) and the sum of n-3 fatty acids was higher, and n-6:n-3 ratio was lower, on FctO compared to the BT diet. Beef tallow diet consumption decreased the proportion of 14:0, 16:0, 18:3n-3 and EPA, and increased the proportion of 18:0, 20:4n-6 and 22:4n-6 in RBCs, compared to baseline. The sum of n-6 fatty acids increased by 1.2%, while the n-6:n-3 ratio increased by 7.9% after 27 d of BT feeding. FctO diet consumption increased tissue 18:3n-3, EPA and the sum of n-3 fatty acids, and decreased 18:1n-9, the sum of monounsaturated fatty acids and the ratio of n-6:n-3 fatty acids by 12.2%. DHA (22:6n-3), 22:5n-3 and the sum of saturated fatty acids remained unchanged on either diets. Changes in RBC fatty acids were not associated with those of plasma cholesterol or lipoproteins. There was a trend towards greater fecal FA excretion with FctO consumption compared to BT (0.47 ± 0.09 g/d versus 0.61 ± 0.08 g/d, respectively, $p = 0.1$). Changes in plasma cholesterol were not correlated with fecal fatty acid excretion.

5.5 DISCUSSION

The present results demonstrate that consumption of a combination of MCT, phytosterols and n-3 PUFA in a controlled diet for 27 d substantially lowers plasma total and LDL cholesterol concentrations, but does not affect circulating TAG or HDL cholesterol in healthy

overweight women. This research thus shows that dietary incorporation of MCT as a means of preventing weight gain or treating obesity, as previously suggested (Geliebter et al., 1983; Hashim and Tantibhedyangkul, 1987), is also advisable in the context of CVD risk management. This FctO can therefore be of dual benefit. Although it is not possible from the present research to attribute each aspect of the lipid modulations to an individual component of the FctO, we have shown that, from a holistic approach, when combined with oils known for their health benefits, MCT can be consumed without adverse effects on CVD risk.

A rigorously controlled partially-inpatient setting and crossover design were used for this study. Subjects slept and resided exclusively at the CNRU and consumed precisely controlled diets under supervision, thereby assuring compliance to the dietary regimen. The results obtained were therefore due to the differences in treatment oils.

Beef tallow was selected as control fat to parallel the high degree of saturation of the FctO, while being free of MCT. Beef tallow consumption did not alter any plasma lipid parameters; thus being an appropriate control. However, BT was associated with poor palatability for some subjects, and provided an average additional 69 mg/d of cholesterol to the control diet compared to the FctO diet. This is unlikely to have confounded plasma cholesterol concentrations since dietary cholesterol is now recognized to have a minor impact on circulating cholesterol values, as opposed to dietary fatty acid type or endogenous cholesterol (Jones and Papamandjaris, 2001). Although the BT diet was less palatable for some subjects, this would not have affected the parameters under study since subjects

remained healthy and compliant with food intake. Comparison of the fatty acid profile of the beef tallow diet with results from a recent nutritional survey show that when compared to recent data on the fat intake of Americans, our beef tallow diet contained more saturated fat (50.9% versus 33.3% for the typical American diet) and less polyunsaturated fat (7.2% versus 20.5% for the typical American diet; Popkin et al., 2001).

The extent of cholesterol-lowering relative to control observed in the present trial, 9.1 and 16.0% for total and LDL cholesterol, respectively, correlates well with those seen in other plant sterol supplementation trials (Hendriks et al., 1999; Jones et al., 1999; Jones et al., 2000; Miettinen et al., 1995; Pelletier et al., 1995; Tammi et al., 2000). When hypercholesterolemic subjects were fed tall oil phytosterols as part of a controlled diet for 30 d, total and LDL cholesterol were lower by 9.1% and 15.5%, compared to placebo (Jones et al., 1999). Therefore, it is appropriate to attribute all of the cholesterol-lowering of the FctO diet to phytosterols. In fact, the degree of LDL cholesterol reduction relative to control with FctO consumption would produce a reduction of up to 31% in CVD risk, considering that each 1% decrease in LDL cholesterol concentrations corresponds to a 2% drop in coronary heart disease risk (The Lipid Research Clinics Coronary Primary Prevention Trial, 1984).

A major concern with MCT feeding in an overweight population is the anticipated rise in plasma TAG concentrations. To offset this possible negative effect of MCT consumption, n-3 PUFA, obtained from flaxseed oil were added in the FctO, which provided approximately 5.0g of ALA. In contrast to marine sources of n-3 PUFA, flaxseed oil is tasteless, odourless, and does not require encapsulation for stability, thus, it can be acceptably incorporated to a

great variety of foods. The lack of effect of FctO consumption on TAG concentrations may be due to a TAG-suppressing action of ALA, or alternatively, to a lack of TAG-raising effect of MCT. Unchanged plasma TAG concentrations have indeed been reported following MCT (Cater et al., 1997; Tsai et al., 1999; Asakura et al., 2000; Temme et al., 1997; Wardlaw et al., 1995), and flaxseed oil supplementation (Layne et al., 1996; Cunnane et al., 1993; Pang et al., 1998; Goh et al., 1997). However, TAG were reduced at the high flaxseed oil intake of 60 mL per day for 2 wk (Singer et al., 1986). With background diets low in total and saturated fat, lower ALA intakes were needed to effect TAG lowering (Indu and Ghafoorunissa, 1992). The 2-fold increase in tissue EPA on the FctO diet confirms the conversion of ALA to this long chain n-3 PUFA. Flaxseed oil feeding has been reported to increase EPA only (Mantzioris et al., 1994; Singer et al., 1994; Goh et al., 1997), although small changes in DHA have also been observed (Layne et al., 1996). Nevertheless, EPA and DHA should have similar TAG-suppressing abilities (Grimsgaard et al., 1997).

Reduced dietary fat excretion, perhaps reflecting greater intestinal absorption, was observed on the FctO as compared to the BT diet. The easier and faster digestion and absorption of MCT vs LCT has been documented and is confirmed by MCT use in malabsorption disorders (Indu and Ghafoorunissa, 1992). Improved absorption of MCT relative to LCT, combined with equal fat intake, may have compensated for the negative effect of MCT on energy balance, thus explaining the lack of differential weight loss between diets. Differences between MCT and LCT absorption should be corrected for in future energy balance studies.

A reduction in plasma total Hcy was anticipated since the experimental diets were high in folate, vitamin B_{12}, and vitamin B_6 (respective mean daily intakes: 409 μg, 4.29 μg and 2.67 mg). Therefore, the observed higher Hcy endpoints following FctO vs BT consumption were unexpected. Total Hcy concentrations remained within normal ranges in these subjects. Thus, this effect should be of little clinical significance, especially in comparison to the substantial improvement in CVD risk profile imparted by the reductions in circulating cholesterol.

In conclusion, consumption of MCT, when administered together with phytosterols and ALA, results in an overall positive influence on lipid profiles in healthy overweight women. This finding supports the concept of use of such a combination of dietary ingredients in the optimization of health risk reduction from the cardiovascular disease perspective.

5.6 ACKNOWLEDGEMENTS

We acknowledge the excellent work of the staff of the Mary Emily Clinical Nutrition Research Unit for help in meal preparation and the care of subjects. We are thankful to all study participants for their time and compliance with the study protocol.

5.7 FIGURE LEGEND

Figure 5-1.

Effect of the BT diet and the FctO diet on mean and individual end-point (d 26/28) plasma LDL cholesterol concentrations in overweight women subjects (n=17). * FctO < BT, $p < 0.0001$.

TABLE 5-1. Fatty acid composition of experimental diets [1]

	Beef tallow diet	Functional oil diet
	g/100g of total fatty acids	
8:0	Trace	19.4 ± 2.0
10:0	0.2 ± 0.1	23.6 ± 2.3
12:0	0.3 ± 0.1	3.9 ± 0.6
14:0	3.4 ± 0.4	2.6 ± 0.5
16:0	26.1 ± 0.9	10.1 ± 1.1
18:0	20.3 ± 1.1	3.8 ± 0.6
18:1n-9	38.5 ± 1.6	23.6 ± 3.5
18:2n-6	6.4 ± 1.6	7.1 ± 1.6
18:3n-3	0.8 ± 0.1	4.6 ± 1.3
Σ SFA	50.9 ± 0.5	63.8 ± 1.0
Σ MUFA	41.9 ± 0.4	24.4 ± 0.8
P:S ratio	0.14 ± 0.01	0.19 ± 0.01
n-6:n-3 ratio	7.2 ± 0.3	1.5 ± 0.1

[1] Mean±SEM composition of 9 meals from the 3-day menu, analyzed in duplicates, thus representing 18 measurements for each diet.

TABLE 5-2. Effect of experimental diets on plasma lipid concentrations [1]

Plasma lipid parameter	Beef tallow diet	Functional oil diet
Total cholesterol [2] (mmol/L)		
Baseline	4.77 ± 0.17	4.58 ± 0.21
Endpoint	4.80 ± 0.20	4.37 ± 0.20 [3, 4]
Change (%)	0.6	-4.6 [5]
LDL cholesterol [2] (mmol/L)		
Baseline	2.76 ± 0.12	2.66 ± 0.15
Endpoint	2.86 ± 0.16	2.39 ± 0.15 [3, 4]
Change (%)	3.6	-10.2 [4]
HDL cholesterol (mmol/L)		
Baseline	1.33 ± 0.07	1.30 ± 0.08
Endpoint	1.32 ± 0.07	1.32 ± 0.08
Change (%)	-0.8	1.5
HDL:LDL cholesterol ratio [6]		
Baseline	0.490 ± 0.029	0.495 ± 0.026
Endpoint	0.481 ± 0.031	0.576 ± 0.036 [3]
Change (%)	-1.8	16.4
HDL:total cholesterol ratio [7]		
Baseline	0.279 ± 0.012	0.281 ± 0.010
Endpoint	0.276 ± 0.010	0.304 ± 0.012 [3]
Change (%)	-1.0	8.2
Total triacylglycerols (mmol/L)		

Baseline	1.48 ± 0.12	1.36 ± 0.15
Endpoint	1.37 ± 0.13 [8]	1.42 ± 0.13
Change (%)	-7.4	4.4

[1] Mean±SEM; n=17 women for each period, except for day 1 and percent change on the functional oil diet (n=16).

[2, 6, 7] Significant main effect of diet, [2] $P<0.0001$, [6] $P<0.01$, [7] $P<0.05$.

[3] Significantly different from day 1 within dietary phase, $P<0.05$.

[4] Significantly different from the control diet, $P<0.05$.

[5] Trend toward significant difference from the control diet, $P<0.1$.

[8] Trend toward significant difference from day 1 within dietary phase, $P<0.1$.

TABLE 5-3. Effect of experimental diets on plasma aminothiol concentrations [1]

		Beef tallow diet	Functional oil diet
Homocysteine [2]	(umol/L)		
Day 1		6.68 ± 0.33	6.55 ± 0.33
Day 26		6.27 ± 0.29 [3]	6.95 ± 0.34 [4, 5]
Change (%)		-6.1	6.1 [6]
Cysteine	(umol/L)		
Day 1		225 ± 7	217 ± 9
Day 26		221 ± 7	228 ± 9
Change (%)		-1.7 ± 1.8	5.1 [7]
Cysteinylglycine	(umol/L)		
Day 1		24.9 ± 1.4	24.9 ± 1.3
Day 26		24.2 ± 1.3	23.5 ± 0.9 [3]
Change (%)		2.8	-5.6
Glutathione	(umol/L)		
Day 1		3.10 ± 0.32	2.93 ± 0.36
Day 26		3.23 ± 0.27	3.34 ± 0.25
Change (%)		4.2	14

[1] Mean±SEM; n=17 women for each period, except for day 1 and percent change on the functional oil diet (n=16).

[2] Significant main effect of diet, $P<0.0001$.

[3] Significantly different from day 1 within dietary phase: $P<0.05$.

[4, 6] Significantly different from the control diet, [4] $P<0.05$, [6] $P<0.01$.

[5] Trend toward significant difference from day 1 within dietary phase, $P<0.1$.

[7] Trend toward significant difference from the control diet, $P<0.1$.

TABLE 5-4. Fatty acid composition of red blood cells at beginning and end of experimental diet supplementations [1]

	Beef tallow diet		Functional oil diet	
	Day 1	Day 28	Day 1	Day 28
	G /100g of identified fatty acids			
14:0 [2]	0.34 ± 0.03	0.26 ± 0.02 [3]	0.34 ± 0.03	0.38 ± 0.03 [4]
16:0 [5]	21.57 ± 0.37	19.93 ± 0.30 [6]	21.15 ± 0.41	21.19 ± 0.44 [4]
18:0	11.21 ± 0.40	12.41 ± 0.33 [3]	11.13 ± 0.45	11.48 ± 0.43
18:1n-9	21.93 ± 0.40	21.68 ± 0.38	22.19 ± 0.62	21.05 ± 0.37 [3]
18:2n-6	12.20 ± 0.54	11.87 ± 0.45	12.17 ± 0.62	11.46 ± 0.65
18:3n-3 [7]	0.08 ± 0.01	0.05 ± 0.01 [3]	0.08 ± 0.01	0.18 ± 0.02 [6, 8]
20:4n-6 [9]	16.75 ± 0.30	17.72 ± 0.32 [3]	16.16 ± 0.41	16.60 ± 0.37
20:5n-3 [7]	0.84 ± 0.04	0.66 ± 0.06 [10]	0.80 ± 0.09	1.13 ± 0.08 [6, 8]
22:4n-6 [2]	3.51 ± 0.17	4.18 ± 0.24 [3]	4.81 ± 0.45	4.64 ± 0.42
22:5n-3	3.84 ± 0.15	3.76 ± 0.19	3.72 ± 0.17	4.00 ± 0.19
22:6n-3	4.49 ± 0.20	4.47 ± 0.16	4.30 ± 0.27	4.56 ± 0.18
Σ SFA	33.11 ± 0.37	32.60 ± 0.47	32.62 ± 0.55	33.05 ± 0.67
Σ MUFA	22.81 ± 0.41	22.41 ± 0.41	22.94 ± 0.60	22.04 ± 0.40 [3]

[1] Mean±SEM; n=17 women for each period, except for day 1 of the functional oil diet (n=16).

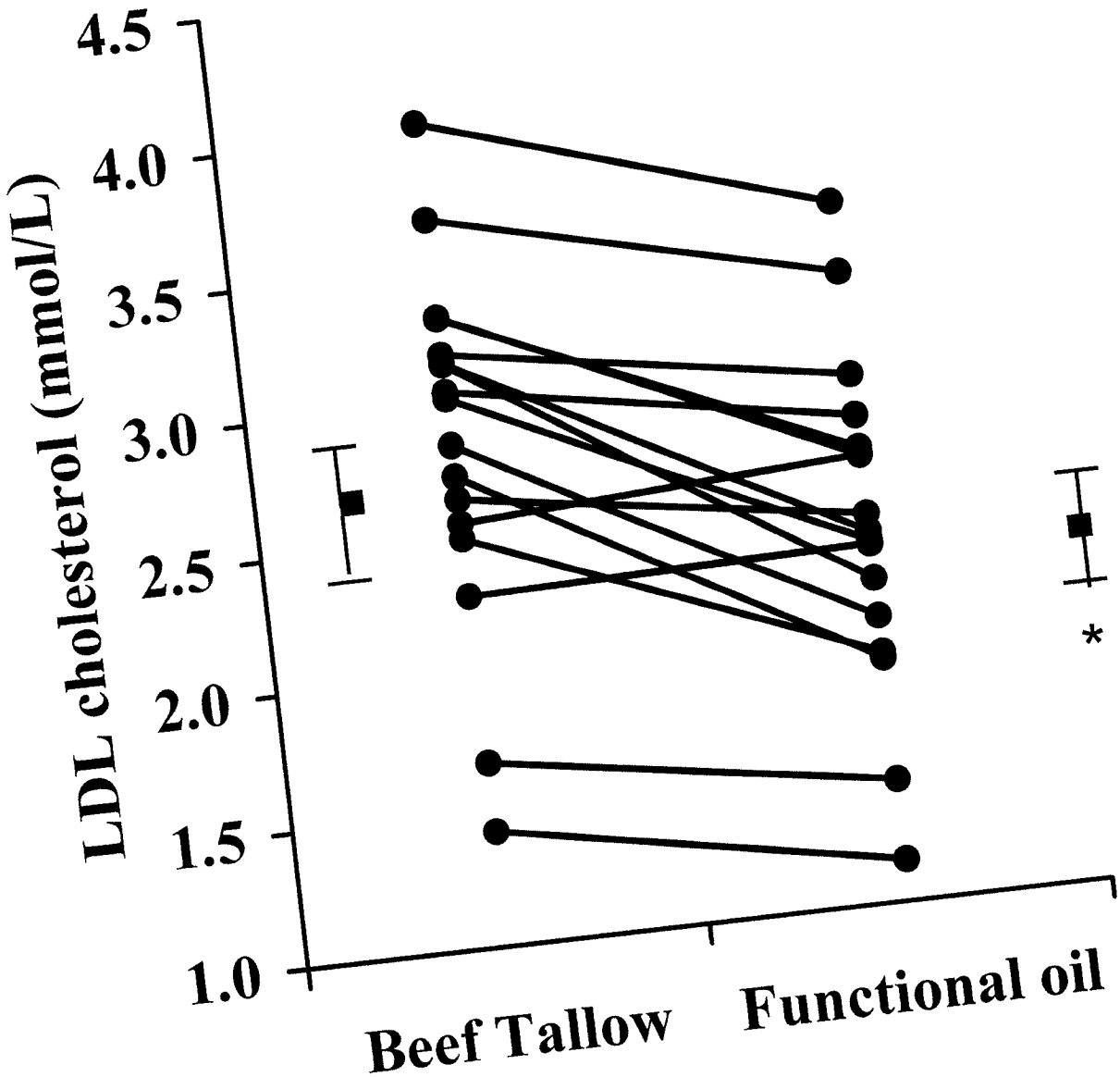
[2, 7, 9] Significant main effect of diet, [2] $p < 0.01$, [7] $p < 0.0001$, [9] $p < 0.05$.

[5] Trend toward significant main effect of diet, $p = 0.0576$.

[3, 6, 10, 12] Significantly different from day 1 within dietary phase, [3] $p < 0.05$, [6] $p < 0.0001$, [10] $p < 0.01$, [12] $p < 0.001$.

[4, 8, 11] Significantly different from the beef tallow diet at corresponding time points, [4] $p < 0.01$, [8] $p < 0.0001$, [11] $p < 0.05$.

Figure 5-1.


4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
LDL cholesterol (mmol/L)
*
Beef Tallow
Functional oil

BRIDGE 5.

The following chapter examines the effects of medium chain triglyceride consumption, in combination with tall oil phytosterols and flaxseed oil, on plasma lipid concentrations. Results from the trial in women show that this combination provides protection against cardiovascular disease compared to a diet rich in beef tallow. However, for the men, the control oil was modified to olive oil. It was thus necessary to establish how the medium chain triglyceride and phytosterol rich functional oil would compare to an oil that is considered to be one of the most heart healthy vegetable oils (Stark and Zecharia, 2002). Also, in addition to the traditional measures of cardiovascular disease risk such as plasma total, low density lipoprotein, and high density lipoprotein cholesterol and triglyceride concentrations, chapter 6 examines the effect of functional oil and olive oil consumption of low density lipoprotein particle size. Characterization of low density lipoprotein particles, in addition to plasma lipid profile, may provide a better indicator of cardiovascular disease risk than plasma lipid profile alone.

Chapter 6. Manuscript 6. ***Submitted to J Nutr.***

CONSUMPTION OF A FUNCTIONAL OIL CONTAINING PHYTOSTEROLS AND MEDIUM CHAIN TRIGLYCERIDE OIL FOR 28 DAYS DECREASES PLASMA LIPID CONCENTRATIONS RELATIVE TO OLIVE OIL AND LEADS TO INCREASED LDL PARTICLE SIZE

Marie-Pierre St-Onge[1], Jean-François Mauger[2], Benoît Lamarche[2], and Peter JH Jones[1]

[1] School of Dietetics and Human Nutrition, McGill University, Ste-Anne-de-Bellevue, Québec, Canada, H9X 3V9

[2] Centre de recherche sur les maladies lipidiques, Centre Hospitalier de l'Université Laval, Université Laval, Ste-Foy, Québec, Canada

Correspondence to: Peter JH Jones

School of Dietetics and Human Nutrition

21 111 Lakeshore Rd.

Ste-Anne-de-Bellevue, Québec

H9X 3V9

Telephone: (514) 398-7547

Fax: (514) 398-7739

e-mail: jonesp@macdonald.mcgill.ca

Supported by Dairy Farmers of Canada and Forbes Medi-Tech Inc.

Short title: Medium chain triglycerides and plasma lipid concentrations

6.1 ABSTRACT

Medium chain triglycerides (MCT) have been proposed as weight lowering agents, however, there is some concern regarding their hyperlipidemic effect. This study evaluated the effects of a combination of MCT oil, phytosterols and flaxseed oil (FctO) on plasma lipid concentrations and low-density lipoprotein (LDL) particle size. Twenty-four healthy overweight men (body mass index 28.2 ± 0.4 kg/m^2), consumed controlled diets designed to maintain weight for two periods of 29 d each. Diets contained 40% of energy as fat, 75% of which was added fat, either FctO or olive oil (OL). Body composition and blood samples were analyzed at baseline and endpoint of each period. Total cholesterol concentrations decreased (-0.68 mmol/L; $p = 0.001$) by 12.5 versus 4.7% with consumption of FctO and OL, respectively ($p = 0.06$ for between diet difference). Similarly, FctO consumption lowered ($p = 0.02$) LDL cholesterol concentrations by 13.9% whereas OL consumption did not. There was no difference in absolute change in LDL cholesterol between FctO and OL consumption. There was a trend ($p = 0.07$) towards increased peak LDL particle size with FctO consumption with no change in proportion of large, medium, or small particles. The change in peak LDL particle size was greater with FctO compared to OL consumption ($p = 0.03$). It can be concluded that a diet containing FctO results in better lipid profile than a control diet rich in OL and leads to larger lipoprotein particle size. Functional oil consumption can therefore help reduce the risk of cardiovascular disease.

Key words: Medium chain triglycerides, phytosterols, cholesterol, low-density lipoprotein cholesterol, lipoproteins

6.2 INTRODUCTION

Medium chain triglycerides (MCT) have been the subject of much research for their purported effects on energy metabolism. In fact, many animal studies (Baba et al., 1982; Geliebter et al., 1983; Lasekan et al., 1992; Rothwell and Stock, 1987) and human trials (Dulloo et al., 1996, Seaton et al., 1986; Scalfi et al., 1991; St-Onge et al., 2003; St-Onge et al., in press; White et al., 1999) have shown that consuming diets rich in MCT increase energy expenditure and fat oxidation. Animal studies have also demonstrated lower body gain and size of fat depots with MCT consumption compared to long chain triglyceride (LCT) consumption. These effects of MCT on energy metabolism have prompted researchers to propose their use in the prevention or treatment of obesity.

Despite potential benefits in EE, triglycerides (TG) containing medium chain fatty acids (MCFA) have been shown to unfavorably alter plasma lipid profiles in humans (Cater et al., 1997; Hill et al., 1989; Swift et al., 1992). Cater et al. (1997) have shown that in men consuming controlled diets rich in either MCFA (octanoate and decanoate), palm oil, or sunflower oil, the palm oil and MCFA containing diets increased total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) concentrations to approximately the same extent. However, the MCFA containing diet also increased TG concentrations to a greater extent than diets containing palm oil and sunflower oil. Swift et al. (1992) have also observed increased TG concentrations with MCT consumption compared to a diet containing mostly LCT. However, these investigators observed no difference in TC and LDL-C concentrations but a decrease in high-density lipoprotein cholesterol (HDL-C) concentrations. After an overfeeding period with a diet containing MCT, Hill et al. (1989)

reported an increase in fasting circulating TG concentrations whereas the same diet containing LCT did not result in any change in TG concentrations. These unfavorable effects of MCT consumption on blood lipid concentrations thus render unhealthy the use of MCT for the prevention of obesity from the cardiovascular disease risk perspective.

In order to make applicable the use of MCT in obesity prevention strategies, a functional oil (FctO) containing a blend of vegetable oils and plant sterols known for their hypocholesterolemic properties was created. This FctO contains mostly MCT oil but also tall oil phytosterols as well as coconut, flaxseed, and olive oil. Flaxseed oil is a rich source of n-3 fatty acids and has been shown in some studies to decrease TC and LDL-C concentrations (Cunnane et al., 1993). Phytosterols have also been shown to reduce TC and LDL-C concentrations by approximately 9 and 13%, respectively (Hendriks et al., 1999; Jones et al., 1999; Jones et al., 2000). We therefore hypothesized that consumption of a diet containing this FctO rich in MCT and phytosterols would result in a favorable lipid profile when compared to a diet containing olive oil (OL) as the main fat source. As a secondary hypothesis, the effects of FctO consumption versus OL on LDL peak particle size and distribution of LDL particle size were evaluated.

5.3 METHODS

5.3.1 Human subjects

Thirty men, aged 26 to 61 y, with TC and TG concentrations below 7.0 mmol/L and 3.0 mmol/L, respectively, who had no history of diabetes, hypothyroidism, hypertension, or other known metabolic disorders, and had a body mass index between 25 and 31 kg/m^2 were

recruited into the study. The experimental protocol was approved by the Ethics Committee of the Faculty of Agriculture and Environmental Sciences at McGill University. All subjects were informed of the protocol and had the opportunity to discuss the procedures with the research coordinator prior to signing a consent form.

5.3.2 Protocol and diet

Subjects were randomly assigned to one of two dietary treatments in a cross-over design. Experimental phases were separated by a 4-wk washout period and differed only in the type of fat incorporated in the diet. Diet composition is shown in **Table 6-1**. Both diets were designed to meet Canadian nutrient recommendations and contained 15, 40, and 45% of protein, fat, and carbohydrates, respectively. Of the total dietary fat, 75% was treatment fat, either FctO or OL; the rest of the fat was provided by foods in the basal diet.

The FctO, was prepared by heating MCT oil and coconut oil and dissolving tall oil phytosterols (64.7% sitosterol, 10.3% sitostanol, 6.7% campesterol, and 1.5% campestanol; Forbes Medi-Tech Inc, Vancouver, Canada) at a concentration of 3.4% of the total weight. The concentration of phytosterols was chosen to provide approximately 22 mg phytosterols/kg body weight daily (Jones et al., 1999) when total FctO consumption was adjusted for each subject's energy intake. Subjects thus consumed an average of 3.38 ± 0.07 g phytosterols/d. Once the phytosterols were dissolved, the oil was left to cool, after which olive oil and flaxseed oil were added. The oil was refrigerated at 4°C until use. **Table 6-2** shows the fatty acid composition of the FctO, as assessed by gas chromatography.

The basal diet consisted of typical North American foods. Diets were designed to maintain weight and energy intake was calculated based on height, weight, and age using the Mifflin equation (Mifflin et al., 1990) and adjusting for activity level by multiplying by a factor of 1.7. Three isoenergetic meals were provided by the staff of the Mary Emily Clinical Nutrition Research Unit (CNRU) of McGill University daily, two of which were consumed under supervision at the CNRU. Subjects consumed the same amount of energy during both treatment phases.

Body composition and energy expenditure were measured at baseline and endpoint of each treatment phase. Whole body magnetic resonance images were acquired at 4 cm intervals using 1 cm slice thickness. The scanner (Siemens, Mississauga, Canada) was land-marked at the L4-L5 vertebrae and at the femoral and humoral heads, allowing for determination of regional body composition. Energy expenditure was assessed using indirect calorimetry. A description of the procedures and data obtained for body composition and energy expenditure measurements is in press (St-Onge et al, in press).

5.3.3 Lipoprotein lipid analyses

Blood samples were collected before breakfast on d 1, 28 and 29 of each experimental phase after a 12 h fast. Samples were immediately centrifuged for 20 min at 1500 rpm and plasma and red blood cells were separated before storing samples at -80°C. Plasma TC, HDL-C and TG concentrations were analyzed using a VP Autoanalyzer and corresponding standards, reagents, and enzymatic kits (Abbott Laboratories, Chicago, USA). Low-density lipoprotein cholesterol concentrations were calculated using formula from Friedewald et al. (1972).

5.3.4 Determination of LDL peak particle density

Nondenaturing 2-16% polyacrylamide gradient gel electrophoresis was performed on whole plasma as described by St-Pierre et al. (2001). Briefly, gels were prepared in batches in our laboratory. Aliquots of 3.5 μL of plasma were mixed with a sampling buffer containing 20% sucrose and 0.25% bromophenol blue in a 1:1 volume ratio. Electrophoresis was performed, after a 15 min pre-run, at 150 V for 3 h. Gels were stained for 1 h with Sudan black (0.07%) and stored in a 0.81% acetic acid 4% methanol solution until analysis. The Imagemaster 1-D Prime computer software (Amersham Pharmacia Biotech, Piscataway, NJ) was used to analyze the gels. A mean LDL particle diameter was also computed using the approach described by Tchernof et al. (1996). Briefly, the mean LDL particle size was calculated by integrating the relative contribution of each LDL particle subclass within a sample and corresponded to the weighted mean of all LDL subclasses. This integrated LDL particle size was calculated as the sum of LDL subspecies' diameter multiplied by its relative proportion. Identification of the major LDL peak shows a coefficient of variation of <2% between assays (St-Pierre et al., 2001). Baseline samples from 2 subjects were lost during processing of LDL particle size determination and therefore, changes in concentrations are reported for 22 subjects whereas endpoint comparisons were done for 24 subjects.

5.3.5 Analysis of data

Baseline values were calculated as the average of screening value and d 1 for each lipid parameter. Endpoint values were calculated as the average concentration of each given parameter on d 28 and 29. Differences between endpoints of each dietary period were tested using paired Student t-test (SAS/STAT version 8, SAS Institute, Cary, USA). Similarly, a

paired Student t-test was used to determine differences between changes in plasma lipid parameters between endpoint and baseline with FctO and OL consumption. Samples from d 1 and d 27 were used to analyze LDL particle size. For lipid concentrations, paired Student's t-tests were used to assess differences in endpoint values as well as differences in change from baseline between FctO and OL dietary periods. Simple correlations were conducted to assess relationships between changes in body composition and changes in blood lipid parameters. Correlations were done on pooled data from the two experimental phases. Upper body adipose tissue (AT) was taken as total AT from images at and above the L4-L5 vertebrae and intramuscular AT (IMAT) consisted of all visible AT around and within muscle fibers but under the muscle fascia. All results are reported as means ± standard error of the mean (SEM). A probability value of 0.05 was used to determine significance of results.

5.4 RESULTS

Twenty-four subjects successfully completed the research trial. Two subjects withdrew from the trial after the first phase for work (n = 1) and health (n = 1) related reasons not pertaining to treatments and three were asked to withdraw from the study for observed non-compliance with the research protocol. Data from one subject were not analyzed due to difficulties with the acquisition of images during the last magnetic resonance imaging scan. All subjects consumed at least two meals per day under supervision at the CNRU. For all meals eaten at home, foods were reported to have been entirely consumed. We thus consider compliance with the study protocol to be acceptable. Body weights decreased by –1.03 ± 0.25 kg with FctO consumption and –0.62 ± 0.29 kg with OL consumption.

Table 6-3 shows plasma lipid values at baseline and endpoint, as well as change from baseline with consumption of FctO and OL. Functional oil consumption resulted in a decrease in TC of 0.68 ± 0.17 mmol/L compared to 0.25 ± 0.17 mmol/L for consumption of the OL diet (**Figure 6-1**). There was a significant change in TC with FctO consumption (-0.85 to -0.50 mmol/L, p = 0.001). Endpoint values for TC were lower (CI 4.30 to 5.11 mmol/L, p = 0.012) after 28 d of consumption of a diet containing FctO compared to one rich in OL (CI 4.58 to 5.46 mmol/L).

Functional oil consumption caused a fall of 0.48 ± 0.19 mmol/L (CI -0.85 to –0.10 mmol/L, p = 0.02) in LDL-C concentrations whereas OL resulted in a drop of 0.15 ± 0.15 mmol/L (CI -0.30 to 0.15 mmol/L, p = 0.16 for between diet difference; Figure 6-1). Low-density lipoprotein cholesterol concentrations were lower (p = 0.03) at the end of the FctO feeding period compared to the OL feeding period (Table 6-3).

Consumption of the FctO did not result in any change in TG concentrations compared to consumption of OL. Endpoint TG values and were not different between FctO and OL consumption periods. Endpoint HDL-C concentrations after each dietary period were similar, however, FctO resulted in a decrease (p = 0.04) in HDL-C from baseline which may be due to slightly higher (p = 0.07) baseline HDL-C values at baseline with the FctO compared to the OL period (Table 6-3).

Table 6-4 shows LDL particle characteristics after consumption of FctO and OL for 28 d. Peak LDL particle size tended to increase (p = 0.07) with FctO consumption from 257.53 ±

0.98 Å to 258.51 ± 0.78 Å. The change in peak LDL particle size with FctO consumption was different from OL (0.90 ± 0.47 Å versus -0.80 ± 0.60 Å, respectively, $p = 0.03$) There was no within diet effect of FctO or OL on LDL integrated size, or proportions of large, medium, or small LDL particles. However, there was a trend ($p = 0.09$) towards greater LDL integrated size at endpoint with FctO compared to OL consumption. Also, the change in proportion of large LDL tended ($p = 0.10$) to be different between FctO and OL consumption with FctO tending to increase the proportion of large LDL whereas OL tended to cause a decrease in large LDL particles.

Correlation analyses showed that change in upper body AT was positively correlated with change in TC ($r = 0.383$, $p < 0.01$) and LDL-C ($r = 0.369$, $p < 0.01$) concentrations, but not with changes in TG and HDL-C concentrations, or LDL particle size. Intramuscular AT was also positively correlated with changes in TC ($r = 0.334$, $p < 0.05$) and LDL-C ($r = 0.306$, $p < 0.05$) concentrations. As was observed with change in upper body AT, there was no correlation between change in IMAT and change in TG and HDL-C concentrations, nor with changes in LDL particle size.

6.5 DISCUSSION

This study shows for the first time that consumption of a combination of MCT oil, phytosterols, and flaxseed oil creates a more beneficial lipid profile and favorably alters LDL particle size compared to OL in mildly hypercholesterolemic men using a randomized crossover design with strictly controlled diets. In addition, only one study to date has examined diets supplemented with phytosterols on LDL particle size (Matvienko et al., 2002)

and only one trial has looked at dietary fatty acid composition in relation to LDL particle size (Kratz et al., 2002). Our results show that, compared to OL, which has been proposed to improve circulatory lipid patterns (Stark and Madar, 2002), FctO caused lower endpoint TC and LDL-C concentrations and led to favorable changes in LDL particle size. Therefore, FctO may be considered a healthy alternative to other dietary fats. Furthermore, this study is the first to establish a link between change in IMAT and change in TC and LDL-C concentrations.

The findings that changes in IMAT are correlated with changes in TC and LDL-C concentrations are highly novel. The few trials that have focused on the metabolic effects of IMAT have examined its relationship with insulin sensitivity (Goodpaster et al., 1997; Goodpaster et al., 2000a; Goodpaster et al., 2000b; Phillips et al., 1996). No previous trial has examined the relationship between IMAT and lipid concentrations. Nevertheless, the trials that have been conducted to date demonstrate that IMAT is a metabolically active depot. Furthermore, IMAT has been shown to be increased in obesity and to respond to weight loss (Goodpaster et al., 2000b). Results obtained in this trial shows that reductions in IMAT are associated with positive effects on plasma TC and LDL-C concentrations.

An important ingredient of the blended FctO was its plant sterol content. Declines in TC and LDL-C concentrations observed in this study are in agreement with data from other studies examining the effects of phytosterols on blood lipid concentrations (Hendriks et al., 1999; Jones et al., 1999; Jones et al., 2000). In a controlled feeding experiment in which subjects consumed 1.8 g tall oil phytosterols/d, it was found that phytosterols caused a decrease in TC

and LDL-C concentrations of 19.5 and 24.4%, respectively, compared to 10.4 and 8.9%, respectively, for the control diet not containing phytosterols (Jones et al., 1999). In other controlled feeding studies, Jones et al. (2000) and Hendriks et al. (1999) found overall decreases in TC of 7.4% and 4.9-6.8%, respectively. These results are similar to those obtained in the present study in which the phytosterol-containing diet (FctO) decreased TC and LDL-C concentrations by 12.6%and 13.9%, respectively. Therefore, it can be assumed that the dose of phytosterols given in this trial, approximately 3.4 g/d, was appropriate and effective in optimizing changes in TC and LDL-C concentrations and was most likely the major factor responsible for the observed changes in TC and LDL-C concentrations.

In this study, FctO did not cause any change in TG concentrations. This is concordant with results observed in previous research examining the effects of plant sterols on plasma lipid concentrations (Hendriks et al., 1999; Jones et al., 1999; Jones et al., 2000). Notwithstanding, the intake of MCT was expected to increase TG concentrations as had been previously observed in humans (Cater et al., 1997; Hill et al., 1989; Swift et al., 1992) although in a hamster model, octanoate had similar effects as cis- and trans-oleate consumption on TG levels (Nicolosi et al., 1998). However, in human work, MCT consumption levels were higher than those in the present study. Effects of MCT consumption on TG may depend on the absolute amount ingested or, as in our study, may have been offset by the presence of other sources of fat. Although flaxseed oil was added to the MCT diet in attempt to diminish TG concentrations, previous studies have also shown the lack of effect of flaxseed n-3 fatty acids in decreasing TG concentrations (Kelley et al., 1993; Layne et al., 1996; Pang et al., 1998). Conversely, others have found that providing large

amounts of flaxseed oil (Singer et al., 1986) or flaxseed oil in combination with a low saturated fat diet (Indu and Ghafoorunissa, 1992), produced a lowering in TG concentrations. When Pedersen et al. (2000) compared diets differing in fat type, it was found that the diet resulting in the most favorable lipid profile was the rapeseed oil diet which contained 6% of total fat as α-linolenic acid. Our FctO diet contained approximately 5% of total fat as flaxseed oil and approximately 3.7% of α-linolenic acid, a level of α-linolenic acid similar to that recommended by de Deckere et al. (1998) to decrease coronary heart disease risk.

Another novel aspect of this study is the analysis of LDL particle characteristics. It has been shown that small, dense LDL are associated with increased risk of coronary heart disease (St-Pierre et al., 2001; Austin et al., 1988; Campos et al., 1992; Lamarche et al., 1997) yet few intervention studies have examined the role of lipid-lowering dietary agents on LDL particle size. The results of this study suggest that consumption of a diet containing MCT, phytosterols, and flaxseed oil may result in a more favorable distribution of LDL subclasses than consumption of a diet rich in OL. A recent trial (Kratz et al., 2002) reported that unsaturated fats, such as those contained in olive, rapeseed and sunflower oils, lower LDL peak particle size relative to saturated fats and do so to a similar extent. Results from the present trial also show that consumption of the OL diet, which is rich in monounsaturated fatty acids, tended to lead to smaller LDL particles than the FctO diet, which contained large amounts of saturated fatty acids. It is therefore possible that the high saturated fatty acid content of the FctO caused the more beneficial change in LDL particle size distribution. Phytosterol consumption has recently been shown not to affect LDL particle size compared to placebo (Matvienko et al., 2002). It is therefore unlikely that phytosterols contained in the

FctO diet had any impact on LDL particle size since phytosterols do not affect TG or HDL-C concentrations (Hendriks et al., 1999; Jones et al., 1999; Jones et al., 2000). Plasma TG concentrations are positively associated with small dense LDL particles while plasma HDL-C concentrations have been shown to be negatively correlated with the small dense LDL phenotype (Tchernof et al., 1996).

Finally, although our study does not allow precise determination of the effects of specific fat-soluble constituents included in the FctO on individual plasma lipid concentrations, it does demonstrate that this combination of oils and plant sterols is healthy from a cardiovascular perspective. Therefore, given the recent interest the in use of MCT as agents which up-regulate energy expenditure and fat oxidation (Dulloo et al., 1996; Seaton et al., 1986; Scalfi et al., 1991; St-Onge et al., 2003; St-Onge and Jones, 2002; White et al., 1999), including the FctO in a typical North American diet in place of oils containing LCT might be expected to produce favorable health effects through actions directed to energy balance and weight control. In conclusion, the present study demonstrates that an MCT-containing oil blend possesses substantial potential to reduce cardiovascular disease risk through its beneficial actions in lipid level modulation and body composition.

6.6 ACKNOWLEDGEMENTS

The help of the staff of the Mary Emily Clinical Nutrition Research Unit is gratefully acknowledged. We would like to thank Isabelle Ruel and Sonia Pomerleau for their help in the determination of LDL-particle size analyses.

6.7 FIGURE LEGEND

Figure 6-1.

Change in plasma total and LDL-cholesterol with FctO (closed squares) and OL (open squares) consumption for 28 d. Values are means ± SEM, n=24. * Significantly different from zero ($p<0.05$) tested using paired Student's t-test.

Table 6-1. Macronutrient composition of the functional oil and olive oil diets.

Nutrient (% of energy)	FctO	OL
Protein	15	15
Carbohydrate	45	45
Fat	40	40
MCT oil	19.5	0
Coconut oil	1.8	0
Canola oil	2.1	0
Olive oil	3.9	30
Flaxseed oil	2.1	0

Table 6-2. Fatty acid composition of the functional oil.

Fatty acid	Functional oil (%)
6:0	0.17
8:0	36.95
10:0	30.35
12:0	3.61
14:0	1.06
16:0	3.52
16:1	0.23
18:0	0.65
18:1	13.81
18:2n-6	4.62
18:3n-3	4.94
20:0	0.05

Table 6-3. Plasma lipid concentrations (mmol/L) with consumption of diets rich in functional oil (FctO) or olive oil (OL) for 28 d.

Diet		TC (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)	TG (mmol/L)
FctO	Baseline	5.38±0.17	1.12±0.06*	3.43±0.18	1.81±0.16
	Endpoint	4.71±0.21*	1.01±0.05	2.96±0.20*	1.61±0.15
	Change	-0.68±0.17**†	-0.11±0.05	-0.48±0.19**	-0.20±0.14
OL	Baseline	5.27±0.23	1.05±0.06	3.41±0.20	1.77±0.15
	Endpoint	5.02±0.23	1.00±0.06	3.26±0.23	1.67±0.20
	Change	-0.25±0.17	-0.05±0.04	-0.15±0.15	-0.10±0.08

* Significant difference between diets, $p < 0.05$.

** Significantly different from baseline.

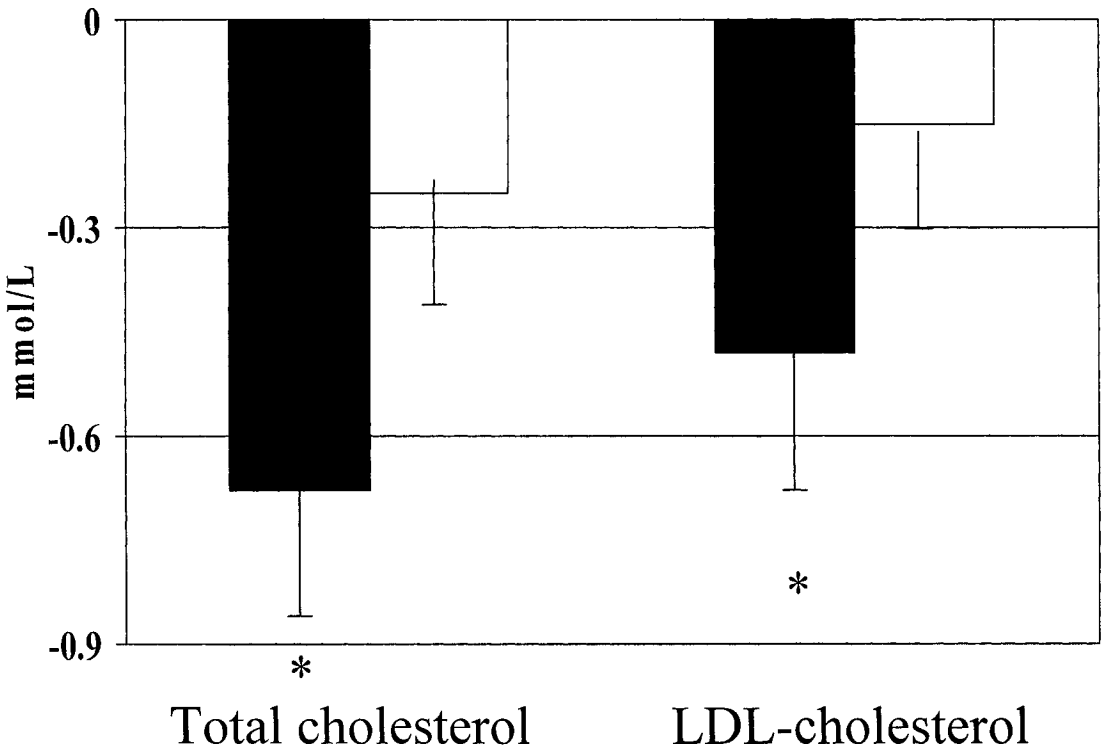
† Trend towards greater change with FctO vs OL, $p = 0.058$

Table 6-4. LDL size and distribution after consumption of functional oil (FctO) and olive oil (OL) for 28 d.

LDL characteristics	FctO	OL	p
LDL peak particle size, Å	258.5 ± 0.8	257.7 ± 0.8	0.15
LDL integrated (mean) size, Å	256.9 ± 0.9	254.5 ± 1.1	0.09
Relative proportions of LDL, %			
<255 Å	42.8 ± 3.1	41.3 ± 3.1	0.45
255-260, Å	20.7 ± 0.9	21.1 ± 1.4	0.77
>260, Å	36.4 ± 3.5	37.5 ± 4.0	0.72
LDL-cholesterol, mmol/L	2.96 ± 0.20	3.26 ± 0.23	0.03
<255 Å	1.22 ± 0.11	1.32 ± 0.12	0.17
255-260, Å	0.61 ± 0.05	0.70 ± 0.07	0.07
>260, Å	1.13 ± 0.14	1.24 ± 0.16	0.37

Values are means ± SEM.

Figure 6-1.



FINAL CONCLUSION AND SUMMARY

Overweight is a growing problem worldwide. In Canada, the prevalence of overweight and obesity was 32.5% and 14.9%, respectively, in the adult population in 2000-2001 (Statistics Canada, 2002). The prevalence of obesity has increased by close to 3 percentage points from the prevalence rate of 1996-1997 (Statistics Canada, 2001). The situation is even more alarming in the United States where overweight status, defined as a body mass index between 25.0 and 29.9 kg/m^2, applies to 33.3% of the adult population. Overweight status increases the risk of developing cardiovascular disease, type II diabetes, hypertension, and other chronic diseases. Therefore, high rates of overweight and obesity result in increased morbidity, which are associated with augmented health care costs. Dietary prevention strategies targeted to promote weight loss are thus necessary. Results from the present research suggest that consumption of our functional oil, rich in medium chain triglycerides could fulfill this role. In fact, over a period of 4 wk, men lost an average of 0.41 kg of body weight more with functional oil compared to olive oil consumption. If the effects of functional oil on energy expenditure are sustainable over long periods, then approximately 5 kg of body weight could be lost over 1 y due to raised diet-induced thermogenesis. If, in addition, satiety is enhanced by functional oil, then greater weight loss can be expected. Prevention of weight gain or enhancement of weight loss would help lower the prevalence of overweight and obesity and would thus have high economic and social impact.

Recent statistics for Canada show that men are more overweight than women (Statistics Canada, 2002). Our results also show that men would benefit from functional oil consumption to a greater extent than women. Functional oil consumption resulted in

enhanced energy expenditure which resulted in a significant lowering of adiposity, preferentially upper body adiposity. Upper body fatness is considered to be linked to greater risks of developing type II diabetes as well as cardiovascular disease and other chronic illnesses. Since men tend to deposit fat centrally, this aspect of weight loss with functional oil consumption is of utmost importance. The rise in energy expenditure observed with functional oil consumption in women was not sufficient to induce greater weight loss when compared to beef tallow consumption. When our data was compared to previous trials in women and men, we noticed that our results were similar to those obtained in earlier trials in women and that these were lower than what had been observed in trials conducted in men. This may be due to the smaller quantity of medium chain triglyceride consumed by women, who require less energy, or possibly due to a gender difference in the way medium chain triglycerides are metabolized.

Our research has demonstrated that a functional oil rich in medium chain triglycerides, flaxseed oil and phytosterols not only increases energy expenditure, leads to beneficial body composition changes but also improves plasma lipid profile. These effects of functional oil make it a suitable candidate for characterization as a functional food. A functional food is a food that is similar in appearance to a conventional food but that provides demonstrated health benefits or reduces the risk of chronic disease above and beyond their basic nutritional functions. Our functional oil meets this definition since it increases thermic effect of food and post-prandial fat oxidation to a greater extent than a conventional long chain triglyceride-containing oil, helps to reduce body fatness, and reduces plasma lipid concentrations. These

effects can lead to lower risk of developing obesity and cardiovascular disease, 2 prevalent debilitating conditions in Western societies.

Health claims relating a functional food to disease prevention are permitted in the US and several other countries globally, but are not yet allowed in Canada. Health Canada does not allow any manufacturer to allude that a food product can heal, treat, or prevent disease or any undesirable health status. However, although the Canadian legislation does not permit health claims, manufacturers must be ready for future changes to take effect since Health Canada is currently studying the possibility of permitting certain health claims on foods. Several types of foods could incorporate our functional oil to effect functionality and hence be eligible for health claims. Creamy salad dressings, soft margarines, baked goods and crackers would be excellent media for functional oil. The oil is without taste or odour and therefore would not impart any flavour to foods in which it is added. It would, however, be highly unlikely that this oil could be sold as cooking oil since phytosterols tend to precipitate out of solution in cold storage and lead to a depot at the bottom of the bottle. This results in an oil of clouded appearance, which may not be acceptable to the general population, accustomed to clear, liquid oils.

Since this oil is not yet available, other sources of medium chain triglycerides and phytosterols may be seeked. Conventional foods exist that naturally contain medium chain triglycerides and phytosterols. Medium chain triglycerides can be found in small quantities in coconut oil and dairy fats. Phytosterols are incorporated into functional foods in several countries including the United States and Australia. However, since these functional foods

are not permitted in Canada, it is not possible to find foods containing added amounts of phytosterols in Canada. Nevertheless, several plant oils, such as corn oil and soybean oil, are rich sources of phytosterols. Also, diets rich in plant foods can provide as much as 1 g of phytosterols daily. Flaxseed oil can be easily found in grocery and health food stores.

In conclusion, data presented in this thesis demonstrate that:

1. medium chain triglyceride consumption, as part of a precisely controlled diet, results in increased energy expenditure and fat oxidation relative to long chain triglyceride consumption in both women and men;
2. the effects of medium chain triglyceride consumption on energy expenditure and fat oxidation are not transient and last over a 4 wk period;
3. medium chain triglyceride consumption results in greater loss of adiposity in men but not women. This effect of medium chain triglycerides can be attributed to the effects observed on energy expenditure;
4. medium chain triglyceride consumption tends to result in lower food intake compared to long chain triglycerides;
5. combining phytosterols and flaxseed oil to medium chain triglycerides leads to diminished concentrations of plasma total and low density lipoprotein cholesterol;
6. combining phytosterols and flaxseed oil to medium chain triglycerides does not affect plasma high density lipoprotein cholesterol and triglyceride concentrations;
7. combining phytosterols and flaxseed oil to medium chain triglycerides does not affect low density lipoprotein particle size.

LIMITATIONS OF THE THESIS

Both trials conducted for this thesis were strictly controlled. Food items were precisely weighed, all meals were isoenergetic and energy intake was constant over the feeding periods. This allowed us to control energy intake and quantities of fat consumed. As a result, the only difference between the two diets was the quality of the oil included in the diet and any result obtained could easily be ascribed to the oil tested. However, the study design employed to test our objectives does not represent a free living situation in which energy and macronutrient intakes vary from meal-to-meal and day-to-day. Therefore, it is unknown whether these results would be obtained in a free-living population. It can be hypothesized that subjects would compensate for the energy imbalance by consuming more calories and thus no weight loss would be achieve. On the other hand, previous studies and our subgroup analysis with men showed that medium chain triglyceride consumption leads to greater satiety and lower food intake than long chain triglyceride consumption. If this is true, then it can be hypothesized that a greater amount of weight loss would be observed with free-living medium chain triglyceride consumption since energy intake would be decreased and energy expenditure increased. In chapter 1, we have deduced, from existing literature, that the amount of weight lost could range from 0.45 to 1.35 kg/mo, when examining the least and most optimistic scenarios, respectively.

Furthermore, the controlled nature of the trials prevented us from studying the effects of medium chain triglyceride consumption over an extended period of time. Longer periods of controlled feeding can lead to non-compliance due to the monotony and repetitiveness of the diet. Four weeks was considered long enough to detect the small difference in body

composition with magnetic resonance imaging in men, but was not sufficiently long to allow detection of these slight differences in women.

With respect to the results of our analyses of plasma lipid concentrations, due to the multifaceted nature of the medium chain triglyceride-containing diet, we cannot ascribe the observed effects on plasma lipids to one particular component of the diet. Therefore, it is not known whether feeding medium chain triglyceride oil would have altered plasma lipids and whether the effects observed on total and low density lipoprotein cholesterol concentrations were entirely due to the phytosterols that were added to the oil. Likewise for triglyceride concentrations; it cannot be ascertained that the lack of change in triglyceride concentrations was prevented by the addition of flaxseed oil to the medium chain triglyceride-containing diet. The lack of change in triglyceride concentrations may also have been due to a lack of effect of medium chain triglycerides in increasing triglyceride concentrations.

Despite these limitations, this thesis demonstrated that an oil combining medium chain triglycerides, phytosterols, and flaxseed oil leads to greater energy expenditure and fat oxidation in overweight women and men and results in decreased levels of adiposity in men compared to consumption of an oil rich in long chain triglycerides. Furthermore, the blend of medium chain triglycerides, phytosterols, and flaxseed oil results in a more favourable lipid profile compared to baseline. Therefore, consumption of the functional oil may fulfill a dual role in the prevention of obesity and cardiovascular disease. It can thus be proposed that medium chain triglycerides be used in weight management as part of dietary prevention strategy against obesity.

REFERENCES

1. Al Makdessi S, Andrieu JL, Bacconin A, Fugier JC, Herilier H, Faucon G. Assay of lipids in dog myocardium using capillary gas chromatography and derivatization with boron trifluoride and methanol. J Chromatogr 1985;339:25-34.
2. Anderson JW, Konz EC, Frederich RC, Wood CL. Long-term weight-loss maintenance: a meta-analysis of US studies. Am J Clin Nutr 2001;74:579-84.
3. Appendix A-11-e: Average daily energy requirement of adults whose occupational work is classified as light, moderate, or heavy, expressed as a multiple of basal metabolic rate, in Shils ME, Olson JA, Shike M, Ross AC (ed): Modern nutrition in health and disease, 9th ed. Baltimore, MD, Williams & Wilkins, 1999.
4. Asakura L, Lottenberg AMP, Neves MQTS, et al. Dietary medium-chain triacylglycerol prevents the postprandial rise of plasma triacylglycerols but induces hypercholesterolemia in primary hypertriglyceridemic subjects. Am J Clin Nutr 2000;71:701-5.
5. Austin MA, Breslow JL, Hennekens CH, Buring JE, Willet WS, Krauss RM. Low-density lipoprotein subclass patterns and risk of myocardial infarction. JAMA 1988;260:1917-21.
6. Baba N, Bracco EF, Hashim SA. Enhanced thermogenesis and diminished deposition of fat in response to overfeeding with diet containing medium chain triglyceride. Am J Clin Nutr 1982;35:678-82.
7. Babayan, VK. Medium chain triglycerides and structured lipids. Lipids 1987;22:417-20.
8. Bach AC, Babayan V K. Medium-chain triglycerides: an update. Am J Clin Nutr

1982;36:950-62.

9. Bach AC, Ingenbleek Y, Frey A. The usefulness of dietary medium-chain triglycerides in body weight control: Fact or fancy? J Lipid Res 1996;37:708-26.

10. Barbera R, Peracchi M, Cesana B, Bianchi PA, Basilisco G. Sensations induce by medium and long chain triglycerides: role of gastric tone and hormones. Gut 2000;46:32-6.

11. Bell L, Jones PJH, Telch J, Clandinin MT, Pencharz PB. Prediction of energy needs for clinical studies. Nutr Res 1985;5:123-9.

12. Bendixen H, Flint A, Raben A, et al. Effect of 3 modified fats and a conventional fat on appetite, energy intake, energy expenditure, and substrate oxidation in healthy men. Am J Clin Nutr 2002;75:47-56.

13. Binnert C, Pachiaudi C, Beylot M, Hans D, et al. Influence of human obesity on the metabolic fate of dietary long- and medium-chain triacylglycerols. Am J Clin Nutr 1998;68:595-601.

14. Bourque C, St-Onge M-P, Papamandjaris AA, Cohn JS, Jones PJH. Consumption of a functional oil composed of medium chain triacylglycerols, phytosterols and n-3 fatty acids improves the overall cardiovascular risk profile of overweight women. Metabolism 2003 (in press).

15. Bray GA, Lee M, Bray TL. Weight gain of rats fed medium-chain triglycerides is less than rats fed long-chain triglycerides. Int J Obes 1980;4:27-32.

16. Brownell KD, Jeffrey RW. Improving long-term weight loss: pushing the limits of treatment. Behav Ther 1987;18:353-74.

17. Calloway DH, Kurtz GW, McCullen JJ, Thomas LV. The absorbability of natural and modified fats. Food Res 1956;21:621-9.

18. Campos H, Genest JJ, Blijlevens E, et al. Low density lipoprotein particle size and coronary artery disease. Arterioscler Thromb 1992;12:187-95.

19. Carnielli VP, Sulkers EJ, Moretti C, et al. Conversion of octanoic acid into long-chain saturated fatty acids in premature infants fed a formula containing medium-chain triglycerides. Metabolism Clin Exper 1994;43:1287-92.

20. Cater NB, Heller HJ, Denke MA. Comparison of the effects of medium-chain triacylglycerols, palm oil, and high oleic acid sunflower oil on plasma triacylglycerol fatty acids and lipid and lipoprotein concentrations in humans. Am J Clin Nutr 1997;65:41-5.

21. Crozier G, Bois-Joyeux B, Chanez M, Girard J, Peret J. Metabolic effects induced by long-term feeding of medium-chain triglycerides in the rat. Metabolism 1987;36:807-14.

22. Cunnane SC, Ganguli S, Menard C, Liede AC, et al. High alpha-linolenic acid flaxseed (Linum usitatissimum): Some nutritional properties in humans. Br J Nutr 1993;69:443-53.

23. de Deckere EAM, Korver O, Verschren PM and Katan MB. Health aspects of fish and n-3 polyunsaturated fatty acids from plant and marine origin. Eur J Clin Nutr 1998;59:749-53.

24. DeLany JP, Windhauser MM, Champagne CM, Bray GA. Differential oxidation of individual dietary fatty acids in humans. Am J Clin Nutr 2000;72:905-11.

25. Denbow DM, Van Krey HP, Lacy MP, Watkins BA The effect of triacylglycerol

chain length on food intake in domestic fowl. Physiol Behav 1992;51:1147-50.

26. Dulloo AG, Fathi M, Mensi N, Girardier L. Twenty-four-hour energy expenditure and urinary catecholamines of humans consuming low-to-moderate amounts of medium-chain triglycerides: a dose-response study in human respiratory chamber. Eur J Clin Nutr 1996;50:152-8.

27. Durand P, Fortin LJ, Lussier-Cacan S, Davignon J, Blache D. Hyperhomocysteinemia induced by folic acid deficiency and methionine load – applications of a modified HPLC method. Clin Chim Acta 1996;252:83-93.

28. Feinle C, Rades T, Otto B, Fried M. Fat digestion modulated gastrointestinal sensations induced by gastric distension and duodenal lipid in humans. Gastroenterol 2001;120:1100-7.

29. Ferraro RT, Eckel RH, Larson DE, Fontvieille AM, Rising R, Jensen DR, Ravussin E. Relationship between skeletal muscle lipoprotein lipase activity and 24-hour macronutrient oxidation. J Clin Invest 1993;92:441-5.

30. Flatt JP, Ravussin E, Acheson KJ, Jequier E. Effects of dietary fat on postprandial substrate oxidation and on carbohydrate and fat balances. J Clin Invest 1985;76:1019-24.

31. Flegal KM, Carroll MD, Ogden CL, Johnson CL. Prevalence and trends in obesity among US adults, 1999-2000. JAMA 2002;288:1723-7.

32. Folch J, Lees M, Stanley GHS. A simple method for isolation and purification of total lipids from animal tissues. J Biol Chem 1957;226:497-509.

33. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972;18:499-502.

34. Furuse M, Choi YH, Mabayo RT, Okumura JI. Feeding behavior in rats fed diets containing medium chain triglyceride. Physiol Behav 1992;52:815-7.

35. Galuska D, Serdula M, Parnuk E, Siegel P, Byers T. Trends in overweight among US adults from 1987-1993: a multistate telephone survey. Am J Pub Health 1996;86:1729-35.

36. Garrow JS. Energy balance and obesity in man. Elsevier Biomedical: Oxford, UK, 1978.

37. Gasic S, Schneider B, Waldhausl W. Indirect calorimetry: variability of consecutive baseline determinations of carbohydrate and fat utilization from gas exchange measurements. Horm Metab Res 1997;29:12-5.

38. Geliebter A, Torbay N, Bracco E, Hashim SA, Van Itallie TB. Overfeeding with medium-chain triglyceride diet results in diminished deposition of fat. Am J Clin Nutr 1983;37:1-4.

39. Goh YK, Jumpsen JA, Clandinin MT. Effect of omega 3 fatty acid on plasma lipids, cholesterol and lipoprotein fatty acid content in NIDDM patients. Diabetologia 1997;40:45-52.

40. Goodpaster BH, Thaete FL, Kelley DE. Thigh adipose tissue distribution is associated with insulin resistance in obesity and in type 2 diabetes mellitus. Am J Clin Nutr 2000a;71:885-92.

41. Goodpaster BH, Theriault R, Watkins SC, Kelley DE. Intramuscular lipid content is increased in obesity and decreased by weight loss. Metabolism 2000b;49:467-72.

42. Goodpaster BH, Thaete FL, Simoneau J-A, Kelley DE. Subcutaneous abdominal fat and thigh muscle composition predict insulin sensitivity independently of visceral fat. Diabetes 1997;46:1579-85.

43. Grimsgaard S, Bonaa KH, Hansen JB, Nordoy A. Highly purified eicosapentaenoic acid and docosahexeanoic acid in human have similar triacylglycerol-lowering effects but divergent effects on serum fatty acids. Am J Clin Nutr 1997;66:649-59.

44. Groff JL, Gropper SS. Advanced Nutrition and Human Metabolism. Wadsworth Thomson Learning: Belmont, 2000.

45. Grundt H, Nilsen DW, Hetland O, Mansoor MA, Aarsland T, Woie L. Atherothrombogenic risk modulation by n-3 fatty acids was not associated with changes in homocysteine in subjects with combined hyperlipidaemia. Thromb Haemost 81:561-565, 1999.

46. Harris WS. n-3 Fatty acids and serum lipoproteins: Human studies. Am J Clin Nutr 1997;65(5 Suppl):1645S-54S.

47. Hashim SA, Tantibhedyangkul P. Medium chain triglyceride in early life: effects on growth of adipose tissue. Lipids 1987;22:429-34.

48. Health Canada, Department of National Health and Welfare. Recommended nutrient intakes for Canadians. Ottawa, Canada, 1990.

49. Heart and Stroke Foundation, Health Statistics, Division of Statistics Canada and Health Canada. Heart disease and stroke in Canada. Ottawa, Canada, 1997.

50. Hendriks HFJ, Weststrate JA, van Vliet T, Meijer GW. Spreads enriched with three different levels of vegetable oil sterols and the degree of cholesterol lowering in normocholesterolaemic and mildly hypercholesterolaemic subjects. Eur J Clin Nutr 1999;53:319-27.

51. Hensrud DD. Dietary treatment and long-term weight loss and maintenance in type 2 diabetes. Obes Res 2001;9(suppl 4):349S-53S.

52. Hill AJ, Blundell JE. Nutrients and behaviour: research strategies for the investigation of taste characteristics, food preferences, hunger sensations and eating patterns in man. J Psych Res 1982;17:203-12.

53. Hill JO, Peters JC, Lin D, Yakubu F, Greene H, Swift L. Lipid accumulation and body fat distribution is influenced by type of dietary fat fed to rats. Int J Obes 1993;17:223-36.

54. Hill JO, Peters JC, Swift LL, et al.: Changes in blood lipids during six days of overfeeding with medium or long chain triglycerides. J Lipid Res 1990;31:407-16.

55. Hill JO, Peters JC, Yang D, et al. Thermogenesis in humans during overfeeding with medium-chain triglycerides. Metabolism 1989;38:641-8.

56. Hyvonen L, Lampi AM, Varo P, Kavistoinen O. Fatty acid analysis, TAG equivalents as net fat value, and nutritional attributes of commercial fats and oils. J Food Compos Anal 1993;6:24-40.

57. Indu M, Ghafoorunissa. n-3 Fatty acids in Indian diets-comparison of the effects of precursor (alpha-linolenic acid) vs product (long chain n-3 polyunsaturated fatty acids). Nutr Res 1992;12:569-82.

58. Johnson RC, Young SK, Cotter R, Lin L, Rowe WB. Medium-chain-triglyceride lipid emulsion: Metabolism and tissue distribution. Am J Clin Nutr 1990;52:502-8.

59. Jones PJ, MacDougall DE, Ntanios F, Vanstone CA. Dietary phytosterols as cholesterol-lowering agents in humans. Can J Physiol Pharmacol 1997;75:217-27.

60. Jones PJH, Ntanios FY, Raeini-Sarjaz M, Vanstone CA. Cholesterol-lowering efficacy of a sitostanol-containing phytosterol mixture with a prudent diet in hyperlipidemic men. Am J Clin Nutr 1999;69:1144-50.

61. Jones PJH, Papamandjaris AA. Lipids: Cellular metabolism: p. 104-114 Bowman BA, Russell RM, ed. Present knowledge in nutrition, 8th ed. ILSI Press, Washington, DC, 2001.

62. Jones PJH, Pencharz PB, Clandinin MT. Whole body oxidation of dietary fatty acids; implications for energy utilization. Am J Clin Nutr 1985;42:769-77.

63. Jones PJ, Raeini-Sarjaz M, Ntanios FY, Vanstone CA, Feng JY, Parsons WE. Modulation of plasma lipid levels and cholesterol kinetics by phytosterol versus phytostanol esters. J Lipid Res 2000;41:697-705.

64. Kaplan RJ, Greenwood CE. Poor digestibility of fully hydrogenated soybean oil in rats: a potential benefit of hydrogenated fats and oils. J Nutr 1998;128:75-80.

65. Katch F, McArdle W (eds). Nutrition, weight control, and exercise. Lea and Febiger: Philadelphia, 1993.

66. Kelley DS, Nelson GJ, Love JE, et al. Dietary α-linolenic acid alters tissue fatty acid composition but not blood lipids, lipoprotein, or coagulation status in humans. Lipids 1993;28:533-7.

67. Kratz M, Gülbahçe E, von Eckardstein A, et al. Dietary mono- and polyunsaturated fatty acids similarly affect LDL size in healthy men and women. J Nutr 2002;132:715-8.

68. Kuczmarski R, Flegal K, Campbell S, Johnson C. Increasing prevalence of overweight among US adults. JAMA 1994;272:205-11.

69. Lamarche B, Tchernof A, Moorjani S, et al. Small, dense low-density lipoprotein particles as a predictor of the risk of ischemic heart disease in men. Circulation 1997;95:69-75.

70. Lasekan JB, Rivera J, Hirvonen MD, Keesey RE, Ney DM. Energy expenditure in rats maintained with intravenous or intragastric infusion of total parenteral nutrition solutions containing medium- or long-chain triglyceride emulsions. J Nutr 1992;122:1483-92.

71. Lavau MM, Hashim SA. Effect of medium chain triglyceride on lipogenesis and body fat in the rat. J Nutr 1978;108:613-20.

72. Layne KS, Goh YK, Jumpsen JA, Ryan EA, Chow P, Clandinin MT. Normal subjects consuming physiological levels of 18:3(n-3) and 20:5(n-3) from flaxseed or fish oils have characteristic differences in plasma lipid and lipoprotein fatty acid levels. J Nutr 1996;126:2130-40.

73. Lia A, Andersson H, Mekki N, Juhel C, Senft M, Lairon D. Postprandial lipemia in relation to sterol and fat excretion in ileostomy subjects given oat bran and wheat test meals. Am J Clin Nutr 1997;66:357-65.

74. Littell RC, Henry PR, Ammerman CB. Statistical analysis of repeated measures data using SAS procedures. J Anim Sci 1998;76:1216-31.

75. Lusk G. The elements of the science of nutrition. WB Saunders Co: Philadelphia, 1928.

76. Maas MIM, Hopman WPM, Katan MB, Jansen JBMJ. Release of peptide YY and inhibition of gastric acid secretion by long-chain and medium-chain triglycerides but not by sucrose polyester in men. Eur J Clin Invest 1998;28:123-30.

77. Maas MIM, Hopman WPM, Katan MB, Jansen JBMJ. Inhibition of gastrin-stimulated gastric acid secretion by medium-chain triglycerides and long-chain triglycerides in healthy young men. Regulatory Peptides 1996;66:203-10.

78. Mabayo RT, Furuse M, Murai A, Okumura JI. Interactions between medium-chain and long-chain triacylglycerols in lipid and energy metabolism in growing chicks. Lipids 1994;29:139-44.

79. Maggio CA, Koopmans HS. Food intake after intragastric meals of short-, medium-, or long-chain triglyceride. Physiol Behav 1982;28:921-6.

80. Mantzioris E, James MJ, Gibson RA, Cleland LG. Dietary substitution with an alpha-linolenic acid-rich vegetable oil increases eicosapentanoic acid concentrations in tissues. Am J Clin Nutr 1994;59:1304-9.

81. Matsuo T, Matsuo M, Kasai M, Takeuchi H. Effects of a liquid diet supplement containing structured medium- and long-chain triacylglycerols on bodyfat accumulation in healthy young subjects. Asia Pacific J Clin Nutr 2001;10:46-50.

82. McLaughlin J, Luca MG, Jones MN, D'Amato M, Dockray GJ, Thompson DG. Fatty acid chain length determines cholecystokinin secretion and effect on human gastric motility. Gastroenterol 1999;116:46-53.

83. Mifflin MD, St-Jeor ST, Hill LA, Scott BJ, Daugherty SA, Koh YO. A new predictive equation for resting energy expenditure in healthy individuals. Am J Clin Nutr 1990;51:241-7.

84. Miettinen TA, Puska P, Gylling H, Vanhanen H, Vartiainen E. Reduction of serum cholesterol with sitostanol-ester margarine in a mildly hypercholesterolemic population. N Engl J Med 1995;333:308-12.

85. Mitsiopoulos N, Baumgartner RN, Lyons W, Heymsfield SB, Gallagher D, Ross R. Cadaver validation of magnetic resonance imaging and computerized tomography measurement of human skeletal muscle. J Appl Physiol 1998;85:115-22.

86. Moghadasian MH, Frohlich JJ. Effects of dietary phytosterols on cholesterol metabolism and atherosclerosis: clinical and experimental evidence. Am J Med 1999;107:588-94.

87. Nicolosi RJ, Wilson TA, Rogers EJ and Kritchevsky D. Effects of specific fatty acids (8:0, 14:0, cis-18:1, trans-18:1) on plasma lipoproteins, early atherogenic potential, and LDL oxidative properties in the hamster. J Lipid Res 1998;39:1972-80.

88. Olszewski AJ, McCully KS. Fish oil decreases serum homocysteine in hyperlipemic men. Coron Artery Dis 1993;4:53-60.

89. Pang D, Allman-Farinelli MA, Wong T, Barnes R, Kingham KM. Replacement of linoleic acid with alpha-linolenic acid does not alter blood lipids in normolipidaemic men. Br J Nutr 1998;80:163-7.

90. Papamandjaris AA, White MD, Jones PJH. Components of total energy expenditure in healthy young women are not affected after 14 days of feeding with medium- versus long-chain triglycerides. Obes Res 1999;7:273-80.

91. Pedersen A, Baumstark MW, Marckmann P, Gylling H and Sanström B. An olive oil-rich diet results in higher concentrations of LDL cholesterol and a higher number of LDL subfraction particles than rapeseed oil and sunflower oil diets. J Lipid Res 2000;41:1901-11.

92. Pelletier X, Belbraouet S, Mirabel D, Mordret F, Perrin JL, Pages X, Debry G. A diet moderately enriched in phytosterols lowers plasma cholesterol concentrations in normocholesterolemic humans. Ann Nutr Metab 1995;39:291-5.

93. Phillips DIW, Caddy S, Illic V, et al. Intramuscular triglyceride and muscle insulin sensitivity: evidence for a relationship in nondiabetic subjects. Metabolism 1996;45:947-50.

94. Popkin BM, Siega-Riz AM, Haines PS, Jahns L: Where's the fat? Trends in U.S. diets 1965-1996. Prev Med 32:245-254, 2001.

95. Ratkowsky DA, Evans MA, Alldredge JR. Chapter 3: The 2-treatment, 2-period, 2-sequence design, Cross-over experiments: Design, analysis, and application, Statistics: Textbooks and monographs: vol 135, p. 77-120. Marcel Dekker, New York, NJ, 1993.

96. Reed GW, Hill JO. Measuring the thermic effect of food. Am J Clin Nutr 1996;63:164-9.

97. Ross R. Magnetic resonance imaging provides new insights into the characterization of adipose and lean tissue distribution. Can J Physiol Pharmacol 1996;74:778-85.

98. Ross R, Leger L, Morris D, de Guise J, Guardo R. Quantification of adipose tissue by MRI: relationship with anthropometric variables. J Appl Physiol 1992;72:787-95.

99. Ross R, Rissanen J, Pedwell H, Clifford J, Shragge P. Influence of diet and exercise on skeletal muscle and visceral adipose tissue in men. J Appl Physiol 1996;81:2445-55.

100. Ross R, Shaw KD, Martel Y, de Guise M, Avruch L. Adipose tissue distribution measured by magnetic resonance imaging in obese women. Am J Clin Nutr 1993;57:470-5.

101. Rothwell NJ, Stock MJ. Stimulation of thermogenesis and brown fat activity in rats fed medium chain triglyceride. Metabolism 1987;36:128-30.

102. Scalfi L, Coltorti A, Contaldo F. Postprandial thermogenesis in lean and obese subjects after meals supplemented with medium-chain and long-chain triglycerides. Am J Clin Nutr 1991;53:1130-3.

103. Seaton TB, Welle SL, Warenko MK, Campbell RG. Thermic effect of medium-chain and long-chain triglycerides in man. Am J Clin Nutr 1986;44:630-4.

104. Sevenhuysen GP, Fieldhouse P, Young TX, McNeil C, Gelskey DE, McDonald SM. The Manitoba Nutrition Survey: fat consumption data. Can J Pub Health 1993;84:389-93.

105. Shils ME, Olson JA, Shike M, Ross AC (eds). Appendix A-11-e: Average daily energy requirement of adults whose occupational work is classified as light, moderate, or heavy, expressed as a multiple of basal metabolic rate, Modern nutrition in health and disease, 9th ed. Williams & Wilkins, Baltimore, MD, 1999.

106. Simon E, Fernandez-Quintela A, Del Puy Portillo M, Del Barrio AS. Effects of medium-chain fatty acids on body composition and protein metabolism in overweight rats. J Physiol Biochem 2000;56:337-46.

107. Singer P, Berger I, Wirth M, Godicke W, Jaeger W, Voigt S. Slow desaturation and elongation of linoleic and alpha-linolenic acids as a rationale of eicosapentanoic acid-rich diet to lower blood pressure and serum lipids in normal, hypertensive and hyperlipemic subjects. Prostaglandins Leukot Med 1986;24:173-93.

108. Snyder WS, Cook MJ, Nasset ES, Karhausen LR, Howells GP, Tipton IH. Report of the task group on reference man. Pergamon: Oxford, UK, 1975.

109. Stark AH and Zecharia M. Olive oil as a functional food: Epidemiology and nutritional approaches. Nut Rev 2002;60:170-6.

110. Statistics Canada. Health Indicators. 2001;3.

111. Statistics Canada. Health Indicators. 2002;1.

112. St-Onge M-P, Bourque C, Jones PJH, Ross R, Parsons WE. Medium- versus long-chain triacylglycerides for 27 days increases fat oxidation and energy expenditure without resulting in changes in body composition in overweight women. Int J Obes 2003;27:95-102.

113. St-Onge M-P, Bourque C, Papamandjaris AA, Jones PJH. Consumption of medium chain triglycerides versus long chain triglycerides over 4 weeks increases energy expenditure and fat oxidation in obese women. Ann Nutr Metab 2001;45 (suppl 1):89 (abstract).

114. St-Onge M-P, Jones PJH. Medium chain triglyceride consumption increases energy expenditure relative to long chain triglyceride in overweight men. Am J Clin Nutr 2002a;75 (suppl 2):340S (abstract).

115. St-Onge M-P, Ross R, Jones PJH. Medium chain triglyceride consumption for 28 days improves blood lipid profile, increases energy expenditure, and leads to smaller

body fat compartments compared to olive oil consumption in overweight men. Circulation 2002b;106(suppl II):II-742.

116. St-Onge M-P, Ross R, Parsons WD, Jones PJH. Consumption of a functional oil containing medium chain triglycerides by overweight men increases energy expenditure and decreases body adiposity compared to a diet rich in olive oil. Obes Res (in press).

117. St-Pierre AC, Ruel IL, Cantin B, et al. Comparison of various electrophoretic characteristics of LDL particles and their relationship to the risk of ischemic heart disease. Circulation 2001;104:2295-9.

118. Stubbs RJ. The use of visual analog scales to assess motivation to eat in human subjects: a review of their reliability and validity with evaluation of new hand-held computerized systems for temporal tracking of appetite ratings. Brit J Nutr 2000;84:405-15.

119. Stubbs RJ, Harbron CG. Covert manipulation of the ration of medium- to long-chain triglycerides in isoenergetically dense diets: effect on food intake in ad libitum feeding men. Int J Obes 1996;20:435-44.

120. Swift LL, Hill JO, Peters JC, Greene HL. Medium-chain fatty acids: Evidence for incorporation into chylomicron triglycerides in humans. Am J Clin Nutr 1990;52:834-6.

121. Swift LL, Hill JO, Peters JC, Green HL. Plasma lipids and lipoproteins during 6 d of maintenance feeding with long-chain, medium-chain, and mixed-chain triglycerides. Am J Clin Nutr 1992;56:881-6.

122. Tammi A, Ronnemaa T, Gylling H, et al. Plant stanol ester margarine lowers serum total and low-density lipoprotein cholesterol concentrations of healthy children: The STRIP project. J Pediatr 2000;136:503-10.

123. Tchernof A, Lamarche B, Prud'homme D, et al. The dense LDL phenotype: Association with plasma lipoprotein levels, visceral obesity, and hyperinsulinemia in men. Diabetes Care 1996;19:629-37.

124. Temme EHM, Mensink RP, Hornstra G. Effects of medium chain fatty acids (MCFA), myristic acid, and oleic acid on serum lipoproteins in healthy subjects. J Lipid Res 1997;38:1746-54.

125. The Lipid Research Clinics Coronary Primary Prevention Trial results: II. The relationship of reduction inincidence of coronary heart disease to cholesterol lowering. JAMA 251:365-374, 1984.

126.

127. Thomas LE, Saeed N, Hajnal JV, Brynes A, Goldstone AP, Frost G, Bell JD. Magnetic resonance imaging of total body fat. J Appl Physiol 1998;85:1778-85.

128. Tsai YH, Park S, Kovacic J, Snook JT. Mechanisms mediating lipoprotein responses to diets with medium-chain triglyceride and lauric acid. Lipids 1999;34:895-905.

129. Tsuji H, Kasai M, Takeucchi H, Nakamura M, Okazaki M, Kondo K. Dietary medium-chain triacylglycerols suppress accumulation of boyd fat in a double-blind, controlled trial in healthy men and women. J Nutr 2001;131:2853-9.

130. US Department of Health and Human Services, National Institutes of Health, National Heart, Lung, and Blood Institute: Clinical guidelines on the identification,

evaluation, and the treatment of overweight and obesity in adults: The evidence report. Bethesda, MD:1998. [NIH Publication No. 98-4083.]

131. Van Wymelbeke V, Himaya A, Louis-Sylvestre J, Fantino M. Influence of medium-chain and long-chain triacylglycerols on the control of food intake in men. Am J Clin Nutr 1998;68:226-34.

132. Van Wymelbeke V, Louis-Sylvestre J, Fantino M. Substrate oxidation and control of food intake in men after a fat-substitute meal compared with meals supplemented with an isoenergetic load of carbohydrate, long-chain triacylglycerols, or medium-chain triacylglycerols. Am J Clin Nutr 2001; 74:620-30.

133. Wardlaw GM, Snook JT, Park S, et al. Relative effects on serum lipids and apolipoproteins of a caprenin-rich diet compared with diets rich in palm oil/palm-kernel or butter. Am J Clin Nutr 1995;61:535-42.

134. Warnick GR, Benderson J, Albers JJ. Dextran sulfate-Mg^{2+} precipitation procedure for quantitation of high-density-lipoprotein cholesterol. Clin Chem 1982;28:1379-88.

135. White MD, Papamandjaris AA, Jones PJH. Enhanced postprandial energy expenditure with medium-chain fatty acid feeding is attenuated after 14 d in premenopausal women. Am J Clin Nutr 1999;69:883-9.

136. Yost TJ, Eckel RH. Hypocaloric feeding in obese women: metabolic effects of medium-chain triglyceride substitution. Am J Clin Nutr 1989;49:326-30.

APPENDIX

Ethics Review Form (Trial 1 women)

Comparison of a short and medium chain fat/plant sterol containing oil versus a typical oil on weight control and circulating lipid levels in obese and non-obese women

CONSENT BY SUBJECT OF RESEARCH PROTOCOL

Protocol #:_____ Patient Name:

Comparison of a short and medium chain fat/plant sterol containing oil versus a typical oil on weight control and circulating lipid levels in obese and non-obese women.
School of Dietetics and Human Nutrition, Macdonald Campus, McGill University.
Researchers: Dr. Peter Jones, Marie-Pierre St. Onge, Christine Bourque.
Phone:
Contact Physician: Dr. William Parsons, Phone:

I, _____, the undersigned, hereby consent to participate as a subject in the above-named research project conducted by McGill University. The nature of the procedure or treatment, its risks and/or benefits, and possible alternatives, follow:

I. NATURE AND DURATION OF PROCEDURE:

The aim of the study is to examine how different types of fat in the diet, specifically medium chain triglycerides enriched with plant sterols or long chain triglycerides, influence body composition, energy expenditure, and cholesterol levels in the body. A blood sample (10 ml or two teaspoons) will be taken for the laboratory to confirm the absence of health abnormalities and to measure your blood lipid levels. Your body composition will be assessed using bioelectric impedance analysis (BIA). Electrodes will be attached to one hand and foot, and an undetectable impulse through your body will measure your body composition. You will also need to ensure that you refrain from consuming any lipid lowering drugs for at least 8 weeks prior to initiation of the study. Your weight must be stable prior to entrance into the study.

When you start the clinical trial, you will consume test diets provided by the Metabolic Kitchen at the Mary Emily Clinical Nutrition Research Unit for 2 periods of 28 days. A minimum four week break will separate each diet, where you will resume consumption of your typical intake. Each test diet will contain normal foods and be fed to you as three meals per day. One diet will contain beef tallow as the main fat source. The other diet will contain a combination of butter, medium chain triglycerides, and coconut oil as the main fat source. This diet will also contain 1.6g/day of phytosterols, a material resembling cholesterol obtained from plant sources. These plant sterols are tasteless and odourless.

CONSENT BY SUBJECT OF RESEARCH PROTOCOL

During each 28 day dietary treatment phase, you will be required to live at the Research Unit. You will be provided with a bed and personal area. The Research Unit will be staffed 24h per day to ensure your safety and security. You will be required to remain at the Research Unit for the duration of each 28 day phase. All meals will be prepared and served to you at the Research Unit.

At the beginning (day 1) and end (day 28) of each of the 28 dietary treatment periods, you will be taken to the Montreal Neurological Institute to have your body composition assessed for fat and lean tissue using an imaging technique. This procedure requires you to lie quietly for approximately 30 minutes while a magnetic field is passed over your body to assess your body composition. Also at the beginning and end of each trial you will be asked to provide 30 ml of blood for cholesterol level and blood analysis. In addition on day 1 and day 28, the amount of energy your body requires to function and digest food will be measured. For 30 minutes before breakfast and for 6 hours after breakfast on those days, you will be required to lie quietly under a ventilated plastic hood while your energy consumption is measured. In the middle of each dietary treatment phase, day 13-15 inclusive, you will be asked to deliver fecal samples to assess nutrient absorption and energy balance.

The total blood volume required for the entire study will be 130 ml.

II POTENTIAL RISKS AND/OR BENEFITS:

The dietary fats used in the preparation of the diets have all been approved for human consumption. The plant sterol mixture added to the diet at the proposed level has been shown to have no negative side effects on health in previous animal and human experiments. A slight chance exists that you may experience mild stomach upset at the beginning of each dietary phase as your body adjusts to the new fat source. In case you feel any discomfort during the experimental trial a physician, Dr. Parsons, will be available to contact at any time. Dr. Parsons can be reached at

The substance of the project and procedures associated with it have been fully explained to me, and all experimental procedures have been identified. I have had the opportunity to ask questions concerning any and all aspects of the project and any procedures involved. I am aware that I may refuse to participate as well as withdraw my consent at any time. I acknowledge that no guarantee or assurance has been given by anyone as to results to be obtained. Confidentiality of records concerning my involvement in this project will be maintained in an appropriate manner.

CONSENT BY SUBJECT OF RESEARCH PROTOCOL

I understand that, in compensation for the inconvenience of the study schedule, I will receive $1,400 at completion of the trial. I will also be given access to my results concerning the blood lipid level assessment and body composition determination when they become available. If I decide to withdraw before completion, I will receive an appropriate pro-rated fraction of this amount.

I acknowledge receiving a copy of this consent form and all appropriate attachments.

Doctor Signature of Subject

Witness

Date Time

Ethics Review Form (Trial 2, men)

Comparison of a short and medium chain fat/plant sterol containing oil versus a typical oil on weight control and circulating lipid levels in overweight men

CONSENT BY SUBJECT OF RESEARCH PROTOCOL

Protocol #:_____ Patient Name:

Comparison of a short and medium chain fat/plant sterol containing oil versus a typical oil on weight control and circulating lipid levels in overweight men.
School of Dietetics and Human Nutrition, Macdonald Campus, McGill University.
Researchers: Dr. Peter Jones, Marie-Pierre St-Onge.
Phone:
Contact Physician: Dr. William Parsons, Phone:

I, _____, the undersigned, hereby consent to participate as a subject in the above-named research project conducted by McGill University. The nature of the procedure or treatment, its risks and/or benefits, and possible alternatives, follow:

I. NATURE AND DURATION OF PROCEDURE:

The aim of the study is to examine how different types of fat in the diet, specifically medium chain triglycerides enriched with plant sterols or long chain triglycerides, influence body composition, energy expenditure, and cholesterol levels in the body. A blood sample (10 ml or two teaspoons) will be taken for the laboratory to confirm the absence of health abnormalities and to measure your blood lipid levels. You will also need to ensure that you refrain from consuming any lipid lowering drugs for at least 8 weeks prior to initiation of the study. Your weight must be stable prior to entrance into the study.

When you start the clinical trial, you will consume test diets provided by the Metabolic Kitchen at the Mary Emily Clinical Nutrition Research Unit for 2 periods of 28 days. A minimum four week break will separate each diet, where you will resume consumption of your typical intake. Each test diet will contain normal foods and be fed to you as three meals per day. One diet will contain olive oil as the main fat source. The other diet will contain a combination of olive oil, medium chain triglycerides, coconut oil, canola oil and flaxseed oil as the main fat sources. This diet will also contain 1.6 g/day of phytosterols, a material resembling cholesterol obtained from plant sources. These plant sterols are tasteless and odourless.

CONSENT BY SUBJECT OF RESEARCH PROTOCOL

During each 28 day dietary treatment phase, you will be required to consume all meals prepared and served to you at the Research Unit.

At the beginning (day 1) and end (day 29) of each of the 28 dietary treatment periods, you will be taken to the Montreal Neurological Institute to have your body composition assessed for fat and lean tissue using an imaging technique. This procedure requires you to lie quietly for approximately 40 minutes while a magnetic field is passed over your body to assess your body composition. Also on days 1, 27 and 29, you will be asked to provide 20 ml of blood for cholesterol level and blood analysis. In addition on days 2 and 28, the amount of energy your body requires to function and digest food will be measured. For 30 minutes before breakfast and for 6 hours after breakfast on those days, you will be required to lie quietly under a ventilated plastic hood while your energy consumption is measured. In the middle of each dietary treatment phase, days 15-18 inclusive, you will be asked to deliver fecal samples to assess nutrient absorption and energy balance.

The total blood volume required for the entire study will be 130 ml.

II POTENTIAL RISKS AND/OR BENEFITS:

The dietary fats used in the preparation of the diets have all been approved for human consumption. The plant sterol mixture added to the diet at the proposed level has been shown to have no negative side effects on health in previous animal and human experiments. A slight chance exists that you may experience mild stomach upset at the beginning of each dietary phase as your body adjusts to the new fat source. In case you feel any discomfort during the experimental trial a physician, Dr. Parsons, will be available to contact at any time. Dr. Parsons can be reached at

The substance of the project and procedures associated with it have been fully explained to me, and all experimental procedures have been identified. I have had the opportunity to ask questions concerning any and all aspects of the project and any procedures involved. I am aware that I may refuse to participate as well as withdraw my consent at any time. I acknowledge that no guarantee or assurance has been given by anyone as to results to be obtained. Confidentiality of records concerning my involvement in this project will be maintained in an appropriate manner.

CONSENT BY SUBJECT OF RESEARCH PROTOCOL

I, _____, have read the above description with one of the investigators, _____. I fully understand the procedures, advantages and disadvantages of the study, which have been explained to me. I understand that, in compensation for the inconvenience of the study schedule, I will receive $1,000 in two installments of $500. I will also be given access to my results concerning the blood lipid level assessment and body composition determination when they become available. If I decide to withdraw before completion or should the study be terminated early, I will receive an appropriate pro-rated fraction of this amount.

I acknowledge receiving a copy of this consent form and all appropriate attachments and agree to be contacted be a member of the Research Ethics Committee.

Investigator Signature of Subject

Witness

Date Time

Montreal Neurological Hospital

MAGNETIC RESONANCE

Patient Name: _____
Medicare #: _____
Physician Name: _____
Date: _____

QUESTIONNAIRE AND CONSENT FORM

It is of the ultimate importance for the patient safety that this questionnaire be filled out by the physician and the patient, and attached to the request for services.

1. Previous surgery (type and date) _____

_____

2. Does the patient have any of the following?

	YES	NO
Cardiac pacemaker	____	____
Surgical clip on an aneurysm or othe vessel	____	____
Surgical clip or valve on the heart	____	____
Prostheses (please specify type and location)	____	____
_____		
Implants (please specify type and location)	____	____
_____		
Metal or metallic fragments in any part of the body	____	____
(please specify)_____		

3. Is the patient pregnant? ____ ____

All of my questions regarding this exam have been satisfactorily answered.

I hereby give consent to Magnetic Resonance examination.

_____ _____ _____

Patient signature Physician signature Date

SATIETY QUESTIONNAIRE

IMMEDIATELY AFTER BREAKFAST

1. How hungry do you feel?

Not at all hungry — As hungry as I have ever been

2. How full do you feel?

Not at all full — As full as I have ever been

3. How strong is your desire to eat

Very weak — Very strong

4. How much do you think you could eat now?

Nothing at all — A large amount

5. Urge to eat

No urge to eat — Strong, want to eat now, waiting is very uncomfortable)

6. Preocupation with thoughts on food

No thoughts on food — Very preoccupied, difficult to concentrate on other things

SATIETY QUESTIONNAIRE

2 HOURS AFTER BREAKFAST

7. How hungry do you feel?

Not at all hungry — As hungry as I have ever been

8. How full do you feel?

Not at all full — As full as I have ever been

9. How strong is your desire to eat

Very weak — Very strong

10. How much do you think you could eat now?

Nothing at all — A large amount

11. Urge to eat

No urge to eat — Strong, want to eat now, waiting is very uncomfortable)

12. Preocupation with thoughts on food

No thoughts on food — Very preoccupied, difficult to concentrate on other things

SATIETY QUESTIONNAIRE

4 HOURS AFTER BREAKFAST

13. How hungry do you feel?

Not at all hungry — As hungry as I have ever been

14. How full do you feel?

Not at all full — As full as I have ever been

15. How strong is your desire to eat

Very weak — Very strong

16. How much do you think you could eat now?

Nothing at all — A large amount

17. Urge to eat

No urge to eat — Strong, want to eat now, waiting is very uncomfortable)

18. Preocupation with thoughts on food

No thoughts on food — Very preoccupied, difficult to concentrate on other things

School of Dietetics and Human Nu
McGill University, Macdonald Campus

facsimile transmittal

To: KAREN KING Date: JULY 3rd, 2002

Fax:

From: HADIE-RICKE ST-ONGE Pages (including this one): 1

Tel:

School of Dietetics and Human Nutrition

Fax:

E-mail:

Message:

DEAR MS. KING:

I WOULD LIKE TO REPUBLISH ALL OF THIS ARTICLE:

ST-ONGE M-P AND JONES PJH. PHYSIOLOGICAL EFFECTS OF MEDIUM CHAIN TRIGLYCERIDES: POTENTIAL AGENTS IN THE PREVENTION OF OBESITY. J NUTR 2002; 132: 329-332.

THIS ARTICLE WILL BE F
PH.D THESIS.

THANK YOU,

MONTREAL NEUROLOGICAL INSTITUTE AND HOSPITAL

INSTITUT ET HÔPITAL NEUROLOGIQUES DE MONTRÉAL

A Teaching and Research Institute of McGill University

Institut d'enseignement et de recherche de l'Université McGill

October 14, 1999

Dr. Peter J. Jones
Dietetics and Human Nutrition
McGill University
Macdonald-Stewart Building

Bruce Pike, Ph.D.
Coordinator - McConnell Brain Imaging Centre
Tel:
Fax:

Douglas C. Arnold, MD

D. Louis Collins, Ph.D.

Alain Dagher, MD

Mirko Diksic, Ph.D.

Alan C. Evans, Ph.D.

Robert Gjedde, MD

Ernst Meyer, Ph.D.

C.J. Thompson, Ph.D.

Dear Dr. Jones:

RE: Comparison of a short and medium chain fat/plant sterol <<designer>> oil versus a typical oil on weight control and circulating lipid levels in obese and non-obese women.

At its meeting on October 5, 1999, the MR Research Committee reviewed the protocol you submitted to perform fMRI studies on 36 subjects over a two-year period. During the first year you will study 18 of these subjects. Approval has been granted by the committee.

As you have already received approval from the MacDonald College Ethics Committee for this study, please contact Dr. Pokrupa, Chair of the MNI Ethics Committee to verify whether it is necessary that the study be reviewed by this committee. In addition, as the MR consent forms were added to the protocol after the MacDonald College Ethics Committee had reviewed the protocol, we recommend that these forms be brought to the committee's attention.

All scanning for this project will be performed on the Siemens 1.5 T scanner and scheduling of scans should be done via the MRI receptionist (X 8510). Any questions concerning billing should be directed to Elizabeth Kofron of NSI (X 1903).

Sincerely,

Bruce Pike. Ph.D.
Chair, MR Research Committee

cc: Mr. Bill Brodie
Mrs. Elizabeth Kofron
Mr. Gilles Leroux

CENTRE D'IMAGERIE CEREBRALE McCONNELL BRAIN IMAGING CENTRE

McGill

School of Dietetics and Human Nutrition
Faculty of Agricultural and Environmental Sciences
McGill University
Macdonald Campus

École de diététique et nutrition humaine
Faculté des sciences de l'agriculture et de l'environnement
Université McGill
Campus Macdonald

/dietetic

CERTIFICATION OF ETHICAL ACCEPTABILITY FOR RESEARCH INVOLVING HUMAN SUBJECTS

A review committee consisting of:

Position	Field of Research
L. Prichard	Lay Member
E. Idziak, Associate Professor	Natural Resource Sciences
L.E. Phillip, Associate Professor	Animal Science
P. Ribeiro, Assistant Professor	Parasitology
S. Kubow, Associate Professor	Human Nutrition
W. Parsons, M.D.	Medical Advisor

has examined the application for funds in support of a project entitled:

Comparison of a Short and Medium Chain Fat/Plant Sterol "Designer" Oil Versus a Typical Oil on Weight Control and Circulating Lipid Levels in Obese and Non-obese Women.

As proposed by Peter J. Jones, McGill University. Robert R. Ross, Queen's University. (Applicant) to Dairy Farmers of Canada & Forbes Medi-tech Inc. (Granting agency, if any)

and consider the experimental procedures, as outlined by the applicant, to be acceptable on ethical grounds for research involving human subjects.

Comments:

99/07/19
Date

Chairperson of Ethics Committee

Dean's Representative

MCGILL UNIVERSITY
FACULTY OF AGRICULTURAL AND ENVIRONMENTAL SCIENCES

CERTIFICATE OF ETHICAL ACCEPTABILITY FOR RESEARCH INVOLVING HUMANS

The Faculty of Agricultural and Environmental Sciences Ethics Review Committee consists of 4 members nominated by the Faculty of Agricultural and Environmental Sciences Nominating Committee and elected by Faculty, an appointed member from the community and an individual versed in ethical issues.

The undersigned considered the application for certification of the ethical acceptability of the project entitled:

COMPARISON OF MEDIUM CHAIN FAT / PLANT STEROL OIL VERSUS A TYPICAL OIL ON WEIGHT CONTROL AND CIRCULATING LIPID LEVELS

as proposed by:

Applicant's Name P JONES

Supervisor's Name _____

Applicant's Signature _____

Supervisor's Signature _____

Degree / Program / Course _____

Granting Agency _____

The application is considered to be:

A Full Review _____

An Expedited Review _____

A Renewal for an Approved Project X

A Departmental Level Review _____
Signature of Chair / Designate

The review committee considers the research procedures and practices as explained by the applicant in this application, to be acceptable on ethical grounds.

1. Prof. Robin Beech
Department of Parasitology
Psychology

_____
Signature / date

2. Prof. Peter Jones
School of Dietetics and Human Nutrition

_____
Signature / date

3. Prof. Paula Ribeiro
Department of Parasitology
Studies

_____
Signature / date

4. Dr. W. Parsons
Ste. Anne's Hospital

_____
Signature / date

5. Member of the Community – L. Prichard

_____
Signature / date

Revised May, 1999

Centre universitaire de santé McGill
McGill University Health Centre

19 December 2000

Peter J Jones, PhD
School of Dietetics and Human Nutrition
Macdonald Campus
McGill

Meeting of 2000.12.18

5.c. JONP 1999/1 **Comparison of a Short and Medium Chain Fat/Plant Sterol Oil versus a Typical Oil on Weight Control and Circulating Lipid Levels in Obese and Non-Obese Women**

The board approves renewal of the above protocol and consent forms for a period of one year.

Attached please find an appendix outlining routine conditions of the approval.

Yours very truly,

Ronald Pokrupa, MD, Chair
Research Ethics Board
/ve

Wildash, Trace

From:
Sent: 28 January 2003 16:26
To:
Subject: copyright

To whom it may concern:

I would like to obtain permission to publish the following article:

Medium- versus long-chain triglycerides for 27 days increases fat oxidation
and energy expenditure without resulting in changes in body composition in
overweight women. Int J Obes 2003;27:95-102.

in my Ph.D thesis. I am the first author of this article. I have the approval of my co-authors for this request.

Sincerely yours,

TO:
Marie-Pierre St-Onge, Ph.D

NATURE PUBLISHING GROUP granted permission for reproduction of the attached material with the following conditions:
a) the source of the materials be acknowledged by a full reference to the original article
b) permission to reproduce the material is obtained (as far as possible) from the author(s) of the original article(s)

Date 28/1/03

TRACE WILDASH, Rights and Reprints Manager
NATURE PUBLISHING GROUP

Memorandum

Ethics certificates for the studies conducted for the thesis entitled: "Effect of medium versus long chain triglyceride consumption on energy expenditure, substrate oxidation and body composition in overweight men and women" will be sent to the thesis in the following weeks due to extenuating circumstances. In addition, due to these same circumstances, a signed waiver will be sent concurrently by Dr. Peter Jones, co-author of a published manuscript included in the above-mentioned thesis.

Marie-Pierre St-Onge
Ph.D Candidate