Consumption of an Oil Composed of Medium Chain Triacyglycerols, Phytosterols, and N-3 Fatty Acids Improves Cardiovascular Risk Profile in Overweight Women

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Medium chain triacylglycerols (MCT) have been suggested as efficacious in weight management because they possess greater thermogenic qualities relative to long chain triacylglycerols; however, MCT may also increase circulating lipid concentrations, possibly increasing risk of cardiovascular disease (CVD). 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), versus 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 days, with 4 or 8 weeks of washout between phases. Mean plasma total cholesterol concentration was lower ($P < .0001$), by 9.1%, on FctO (4.37 ± 0.20 mmol/L) versus BT (4.80 ± 0.20 mmol/L). Mean plasma low-density lipoprotein (LDL) cholesterol was also lower ($P < .0001$) following FctO (2.39 ± 0.15 mmol/L) versus BT (2.86 ± 0.16 mmol/L), representing a 16.0% difference between diets. High-density lipoprotein (HDL) cholesterol and circulating triacylglycerol concentrations remained unaffected by treatment. Ratios of HDL:LDL and HDL:total cholesterol were higher ($P < .01$) by 22.0% and 11.0%, respectively, on FctO versus BT. Plasma total homocysteine remained unchanged with FctO, but decreased ($P < .05$) with control, hence higher ($P < .05$) end points were observed with FctO (6.95 ± 0.33 μmol/L) versus BT (6.27 ± 0.28 μmol/L). Plasma glutathione increased ($P < .05$) by 0.44 μmol/L with FctO supplementation. In conclusion, despite equivocal effects on homocysteine levels, consumption of a functional oil composed of MCT, phytosterols, and n-3 fatty acids for 27 days improves the overall cardiovascular risk profile of overweight women.

OBESITY IS AN independent risk factor for cardiovascular disease (CVD), the most common cause of mortality and morbidity in North America.[1,2] 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, because MCT increase energy expenditure and fat oxidation relative to long chain triacylglycerols (LCT).[3-6] Medium chain triacylglycerols are rapidly absorbed through the portal circulation[7] and undergo oxidation to carbon dioxide[8,9] or conversion to long chain fatty acids in the liver,[10] thus avoiding deposition in peripheral tissues. Animal studies have indeed shown substantial weight loss following MCT consumption,[11-13] and this has been shown in a supplementation trial,[14] but not in controlled feeding situations in humans.[15]

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),[16-18] lower than those of lauric acid,[17] and intermediate between those of myristic and oleic acids.[19] Conversely, some investigators have reported 8:0 and 10:0 to be similarly cholesterol-raising as palm oil[16,20] and butter.[20] With respect to effects on TAG, short-term MCT supplementation has demonstrated a 3-fold increase following overfeeding[21] and a 42% increase following weight-maintenance feeding[22] in comparison to soybean oil. However, long-term MCT feeding has resulted in unchanged fasting TAG as compared with LCT feeding.[16-20]

Phytosterols have been shown to block the absorption of dietary and endogenously-derived cholesterol from the gut, while being only minimally absorbed themselves.[23] Daily consumption of moderate quantities of phytosterols has been shown to consistently reduce plasma total cholesterol by 5% to 13% and low-density lipoprotein (LDL) cholesterol by 7% to 16%, in both hyper-[24-27] and normocholesterolemic[27-29] individuals, without affecting high-density lipoprotein (HDL) cholesterol or TAG concentrations.

Alpha-linolenic acid (ALA), an n-3 fatty acid found in flaxseed oil, has been shown to undergo conversion[30-32] to the potent hypotriacylglycerolemic[33] 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 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) versus beef tallow as a control fat on circulating lipids, as well as fatty acid metabolism. Because there is some discrepancy in the literature regarding the effects of fish oil on homocysteine (Hcy),[34,35] a secondary objective was to

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measure plasma total Hcy and other aminothiols following dietary fat modification.

SUBJECTS AND METHODS

Subjects

Twenty-two healthy, overweight women were recruited from the surrounding community through newspaper advertising. Enrolled subjects had body mass index (BMI) > 25 kg/m^2, plasma total cholesterol concentration < 7.0 mmol/L, and total circulating TAG concentration ≤ 3.0 mmol/L at screening. Subject body weights were required to have been stable ($\pm 5\%$) for at least 3 months before study entrance. All subjects 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 ≥ 5 times per week or ongoing pregnancy or lactation were excluded. Before study onset, subjects received a complete description of the protocol before signing a consent form in the presence of the study investigators. The experimental protocol was approved by the Human Ethical Review Committee of the Faculty of Agriculture and Environmental Sciences at McGill University.

Study Protocol

The study was a randomized, single-blind, controlled, partially-inpatient clinical trial. A crossover design was used, with two 27-day dietary feeding cycles separated by 8 weeks of washout (4 weeks for 1 subject), during which subjects resumed their habitual diets. Subjects were randomly allocated to receive 1 of 2 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 day 1, 26, and 28 of each dietary phase. Measurements on days 26 and 28 were taken to obtain a better end point estimate of circulating lipid concentrations and to quantify the day-to-day variation in these variables. Because the same menu was assigned to corresponding days across phases, foods consumed preceding day 26 blood draws were identical, except for treatment fat, and similarly for day 28 blood draws. To measure fatty acid excretion, total fecal samples were collected for 3 days 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.

Diets

Experimental diets consisted of prepared North American solid foods, precisely weighed, and based on a 3-day 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 beef tallow (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, Northfield, IL), 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, Vancouver, BC, Canada), were administered at a concentration of 22 mg/kg body weight per day (average daily intake = 1.81 g). Because 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 3 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 1). Average daily cholesterol intakes on FctO and BT diets were 349 mg and 418 mg, respectively. Nonfat and nonsterol constituents were identical across diets.

Table 1. Fatty Acid Composition of Experimental Diets

	Percent of Total Fatty Acids	
	Control Diet	Functional Oil Diet
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
ΣSFA	50.9 ± 0.5	63.8 ± 1.0
ΣMUFA	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

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

Abbreviations: SFA, saturated fatty acids; MUFA, mono-unsaturated fatty acids; P:S, polyunsaturated to saturated ratio.

To provide a targeted energy balance diet, the nutrient intake was adjusted to individual subject energy requirements using the Mifflin equation,[36] to which an activity factor of 1.7 was multiplied to compensate for additional energy needs of active adults.[37] 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 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[38] were met for all vitamins, minerals, fiber, 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), 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.

Analyses

Blood lipid measurement. Blood samples were drawn after a 12-hour overnight fast and at least 24 hours of alcohol abstinence (for day 1) and collected in EDTA-containing Vacutainer (BD, Franklin Lakes, NJ) tubes. Samples were immediately centrifuged at room temperature using a table top centrifuge for 15 minutes at 250 × *g*, and resulting plasma and red blood cell (RBC) subfractions were separated and stored at −80°C until analysis. Plasma total and HDL cholesterol and TAG concentrations were analyzed in quadruplicate with standardized reagents using a VP Autoanalyser (Abbott Laboratories, North Chicago, IL) calibrated as per the standardization protocol of the Canadian Reference Laboratory (1996, Vancouver, BC, Canada). Certification for traceability using this method was maintained through the National Reference System. Measurement of HDL cholesterol in plasma was performed after precipitation of apolipoprotein B with dextran sulphate and magnesium chloride.[39] LDL cholesterol concentrations were calculated using the Friedewald equation.[40] Coefficients of variation (CVs) for replicate analyses of total, HDL cholesterol, and TAG concentrations were 1.4%, 2.3%, and 3.1%, respectively.

Homocysteine and other aminothiols measurements. Total Hcy, cysteine, cysteinylglycine, and glutathione were measured using a modified isocratic high-performance liquid chromatograph (HPLC) with fluorescence detection of derivatized aminothiols as previously described.[41] Aminothiols were reduced and released from proteins by incubation with tri-n-buthylphosphine (10%) in dimethylformamide for 30 minutes at 4°C. Proteins were then precipitated with 0.6 mol/L cold perchloric acid containing EDTA. Derivatization was performed with ammonium 7-fluorobenzo-2-oxa-1,3-diazole-4-sulphonate in sodium hydroxide and potassium borate at 60°C for 60 minutes. After cooling on ice, 10-μL samples were injected into a HPLC (System Gold; Beckman, Fullerton, CA) equipped with an analytical reverse phase column (C18ODS, 250 × 4.6 mm, 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 minutes. Fluorescence detection was performed with a 305-395 nm 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.

Fatty acid composition determination. Individual meals in each 3-day cycle diet, RBC on day 1 and day 28, and 3-day total fecal samples were analyzed in duplicate for fatty acid composition by gas-liquid chromatography after lipid extraction,[42] sodium hydroxide saponification,[43] and boron-trifluoride methylation.[43] Before lipid extraction of RBC samples, 0.1% butylated hydroxytoluene (BHT) was added to minimize peroxidation of long chain PUFA. Total fecal samples collected for 3 days at midpoint of each phase. For fatty acid analyses, freeze dried fecal samples collected within a phase were pooled based on their relative contribution to total fecal mass. One milligram of the internal standard 17:0 was added to samples for quantification of fecal fatty acids.

Derivatized samples were injected via an autoinjector into a gas chromatograph (HP 5890 Series II; Hewlett Packard, Palo Alto, CA) equipped with a flame ionization detector and a 30 m capillary column, with 0.2 mm internal diameter and 0.25 μm film thickness (SP2330; Supelco, Bellefonte, PA). Identification was performed using relative retention times of authentic standards (Supelco). Fatty acid composition of each diet is reported in Table 1. CVs for replicate food total fatty acid recovery, fatty acid content of different meals, and menu cycle days were 12.3%, 6.6%, and 2.6%, respectively.

Statistical Methods

Data are reported as mean ± SEM. Dietary fatty acid composition is presented descriptively, but was not analyzed statistically. Analysis of variance was performed using a mixed model procedure for repeated measures, 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 BT and FctO diets at corresponding times. Separate comparisons between end points (mean of days 26 and 28) were also performed with the mixed model. Endpoint values in tables represent the mean of data obtained on days 26 and 28. Plasma and RBC lipid values for 1 subject were excluded from means on day 1 of the FctO phase, as improper fasting was suggested by TAG concentrations well above the subject's normal range. The level of significance for rejection of the null hypothesis was set at $P < .05$. Version 8.0 of SAS software (SAS Institute, Cary, NC) was used for all statistical analyses.

RESULTS

Twenty-two subjects enrolled and 17 subjects completed both phases of the trial. Women who completed the trial were 44 ± 4 years 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 ± 0.31 MJ/d (2,458 ± 73 kcal/d), and 109.25 ± 3.25 g/d. Four subjects were smokers, and 8 were postmenopausal. For 16 of the subjects who completed the protocol, the washout period was 8 weeks; for logistical reasons, the washout period lasted only 4 weeks for 1 subject.

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 mouthfeel. 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 ($P < .01$) 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 at baseline and endpoint of each feeding period and percent change over time are presented in Table 2. Mean plasma total cholesterol concentration at end point was lower ($P < .0001$) on the FctO versus BT diet (4.37 ± 0.20 mmol/L *v* 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 day 26 of the FctO diet, while there was no change on the BT diet. The mean decrease in total cholesterol after 27 days of FctO consumption was 4.8%.

Mean plasma LDL cholesterol concentration at endpoint was lower ($P < .0001$), by 16.0%, with FctO (2.39 ± 0.15 mmol/L) compared with BT (2.86 ± 0.16 mmol/L) consumption (Fig 1). Relative to baseline, LDL cholesterol concentrations decreased by 0.25 mmol/L on day 26 and 0.28 mmol/L on day 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.

Table 2. Effect of Experimental Diets on Plasma Lipid Concentrations

Plasma Lipid Parameter	Control Diet	Functional Oil Diet
Total cholesterol* (mmol/L)		
Baseline	4.77 ± 0.17	4.58 ± 0.21
Endpoint	4.80 ± 0.20	4.37 ± 0.20†‡
Change (%)	0.6	−4.6§
LDL cholesterol* (mmol/L)		
Baseline	2.76 ± 0.12	2.66 ± 0.15
Endpoint	2.86 ± 0.16	2.39 ± 0.15†‡
Change (%)	3.6	−10.2‡
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‖		
Baseline	0.490 ± 0.029	0.495 ± 0.026
Endpoint	0.481 ± 0.031	0.576 ± 0.036†
Change (%)	−1.8	16.4
HDL:total cholesterol ratio¶		
Baseline	0.279 ± 0.012	0.281 ± 0.010
Endpoint	0.276 ± 0.010	0.304 ± 0.012†
Change (%)	−1.0	8.2
Total triacylglycerols (mmol/L)		
Baseline	1.48 ± 0.12	1.36 ± 0.15
Endpoint	1.37 ± 0.13#	1.42 ± 0.13
Change (%)	−7.4	4.4

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

*Significant main effect of diet, $P < .0001$.

†Significantly different from baseline within dietary phase, $P < .05$.

‡Significantly different from the control diet, $P < .05$.

§Trend toward significant difference from the control diet, $P < .1$.

‖$P < .01$.

¶$P < .05$.

#Trend toward significant difference from baseline within dietary phase, $P < .1$.

HDL cholesterol concentrations remained unchanged during both feeding periods. end point HDL:LDL and HDL:total cholesterol ratios were higher ($P < .01$), by 22.0% and 11.0%, respectively, with FctO versus BT (0.574 ± 0.035 *v* 0.478 ± 0.029 for HDL:LDL cholesterol and 0.304 ± 0.12 *v* 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 day 26 of the BT diet. Percent changes in body weight and BMI were correlated with changes in TAG concentrations ($r = .554$, $P < .001$ for weight, and $r = .544$, $P < 0.01$ for BMI). Separate dietary correlation analysis showed that the association between changes in TAG and body weight was only present in the BT diet ($r = .705$, $P < .01$), but nonexistent in the FctO diet, and likewise for the association between TAG and BMI ($r = .697$, $P < .01$).

Plasma aminothiol concentrations at baseline and endpoint, and percent change over time are presented in Table 3. Mean end point plasma total Hcy concentration was higher ($P < .0001$) on the FctO versus BT diet (6.95 ± 0.33 μmol/L *v* 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 at endpoint of the FctO diet. Cysteine concentrations remained unchanged, although percent variations were marginally different between diets. Cysteinylglycine remained unchanged with BT, but decreased by 1.20 μmol/L at endpoint of FctO consumption. Glutathione increased from baseline at endpoint of the FctO diet by 0.41 μmol/L and did not vary with BT.

Relative fatty acid composition of RBCs on days 1 and 28 of each dietary phase are presented in Table 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 with 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 with 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 days of BT feeding. Functional oil 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

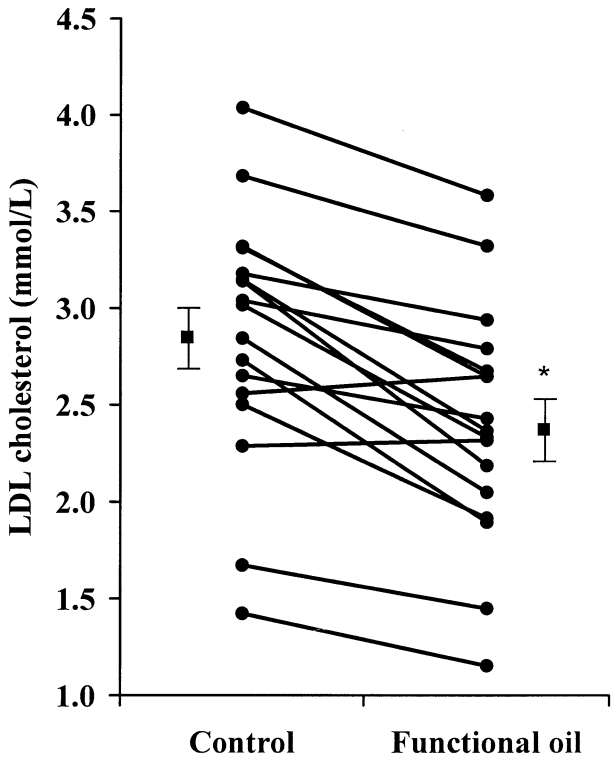

Fig 1. Effect of the control diet and the FctO diet on mean and individual end point (average of days 26 to 28) plasma LDL cholesterol concentrations in overweight women subjects (n = 17); *FctO < control, $P < .0001$).

Table 3. Effect of Experimental Diets on Plasma Aminothiol Concentrations

Plasma Aminothiol and Study Day	Control Diet	Functional Oil Diet
Homocysteine* (μmol/L)		
Baseline	6.68 ± 0.33	6.55 ± 0.33
Endpoint	6.27 ± 0.29†	6.95 ± 0.34‡§
Change (%)	−6.1	6.1‖
Cysteine (μmol/L)		
Baseline	225 ± 7	217 ± 9
Endpoint	221 ± 7	228 ± 9
Change (%)	−1.7 ± 1.8	5.1¶
Cysteinylglycine (μmol/L)		
Baseline	24.9 ± 1.4	24.9 ± 1.3
Endpoint	24.2 ± 1.3	23.5 ± 0.9†
Change (%)	2.8	−5.6
Glutathione (μmol/L)		
Baseline	3.10 ± 0.32	2.93 ± 0.36
Endpoint	3.23 ± 0.27	3.34 ± 0.25
Change (%)	4.2	14

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

*Significant main effect of diet, $P < .0001$.

†Significantly different from baseline within dietary phase: $P < .05$.

‡Significantly different from the control diet, $P < .05$.

§Trend toward significant difference from baseline within dietary phase, $P < .1$.

‖$P < .01$.

¶Trend toward significant difference from the control diet, $P < .1$.

(22:6n-3), 22:5n-3, and the sum of saturated fatty acids remained unchanged on either diet. 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 with BT (0.47 ± 0.09 g/d *v* 0.61 ± 0.08 g/d, respectively, $P = .1$). Changes in plasma cholesterol were not correlated with fecal fatty acid excretion.

DISCUSSION

The present results demonstrate that consumption of a combination of MCT, phytosterols, and n-3 PUFA in a controlled diet for 27 days 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, in the context of weight maintenance, can also be advisable for 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 with the FctO diet. This is unlikely to have confounded plasma cholesterol concentrations because dietary cholesterol is now recognized to have a minor impact on circulating cholesterol values, as opposed to dietary fatty acid type or endogenous cholesterol.[44] Although the BT diet was less palatable for some subjects, this would not have affected the parameters under study, because subjects remained healthy and compliant with food intake. Comparison of the fatty acid profile of the BT diet with results from a recent nutritional survey

Table 4. Fatty Acid Composition of RBCs at Beginning and End of Experimental Diet Supplementations

	Control Diet		Functional Oil Diet	
	Day 1	Day 28	Day 1	Day 28
Percent of total 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
ΣSFA	33.11 ± 0.37	32.60 ± 0.47	32.62 ± 0.55	33.05 ± 0.67
ΣMUFA	22.81 ± 0.41	22.41 ± 0.41	22.94 ± 0.60	22.04 ± 0.40[3]

NOTE. 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, $^2P < .01$, $^7P < .0001$, $^9P < .05$.

[5]Trend toward significant main effect of diet, $P = .0576$.

[3,6,10]Significantly different from day 1 within dietary phase, $^3P < .05$, $^6P < .0001$, $^{10}P < .01$.

[4,8]Significantly different from the control diet at corresponding time points, $^4P < .01$, $^8P < .001$.

show that when compared with recent data on the fat intake of Americans, our BT diet contained more saturated fat (50.9% *v* 33.3% for the typical American diet) and less polyunsaturated fat (7.2% *v* 20.5% for the typical American diet[45]).

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.[24-29] When hypercholesterolemic subjects were fed tall oil phytosterols as part of a controlled diet for 30 days, total and LDL cholesterol were lower by 9.1% and 15.5% compared with placebo.[25] 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% decrease in coronary heart disease risk.[46]

A major concern with MCT feeding in an overweight population is the anticipated increase 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 5.0 g ALA. In contrast to marine sources of n-3 PUFA, flaxseed oil is tasteless, odorless, 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[16-20] and flaxseed oil supplementation.[31,32,47,48] However, TAG were reduced at a high flaxseed oil intake of 60 mL per day for 2 weeks.[49] With background diets low in total and saturated fat, lower ALA intakes were needed to effect TAG lowering.[50] 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,[30,49,48] although small changes in DHA have also been observed.[31] Nevertheless, EPA and DHA should have similar TAG-suppressing abilities.[51]

Reduced dietary fat excretion, perhaps reflecting greater intestinal absorption, was observed on the FctO as compared with the BT diet. The easier and faster digestion and absorption of MCT versus LCT has been documented and is confirmed by MCT use in malabsorption disorders.[50] 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, because the experimental diets were high in folate, vitamin B12, and vitamin B6 (respective mean daily intakes: 409 μg, 4.29 μg, and 2.67 mg). Therefore, the observed higher Hcy end points following FctO versus 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.

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