Dietary medium-chain triglyceride supplementation has no effect on apolipoprotein B-48 and apolipoprotein B-100 kinetics in insulin-resistant men[1–3]

André J Tremblay, Benoît Lamarche, Marie-Ève Labonté, Marie-Claude Lépine, Valéry Lemelin, and Patrick Couture

ABSTRACT

Background: Medium-chain triglyceride (MCT) supplements are used by clinicians to treat patients with severe hypertriglyceridemia who are at risk of pancreatitis. However, the potential mechanisms underlying the effects of MCT on triglyceride-rich lipoprotein (TRL) metabolism have not yet been thoroughly examined in humans.

Objective: This double-blind randomized crossover study compared the impact of 4 wk of supplementation with 20 g MCT oil/d or 20 g corn oil/d on the kinetics of apolipoprotein (apo) B-48–containing TRLs and apo B-100–containing very-low-density lipoprotein (VLDL), as well as on the expression of key intestinal genes involved in lipid metabolism in 28 obese, insulin-resistant men.

Design: The in vivo kinetics of TRL apo B-48 and VLDL apo B-100 were assessed by using a primed-constant infusion of L-[5,5,5-D_3]leucine for 12 h in the fed state. Real-time polymerase chain reaction quantification was performed on duodenal biopsy samples taken at the end of each phase of supplementation.

Results: Compared with corn oil, MCT supplements had no significant effect on plasma lipoprotein profile or TRL apo B-48 and VLDL apo B-100 kinetics. Positive correlations were observed between the intestinal expression of several key genes involved in lipoprotein metabolism in a subgroup of participants (n = 16) after MCT supplementation. However, there was no difference between MCT and the corn oil control supplement in the intestinal messenger RNA expression levels of these key genes.

Conclusion: These data indicate that short-term supplementation with MCT has a neutral effect on TRL apo B-48 and VLDL apo B-100 kinetics and on the intestinal expression of genes involved in lipid and fatty acid metabolism in men with insulin resistance. This trial was registered at www.clinicaltrials.gov as NCT01806142. *Am J Clin Nutr* 2014;99:54–61.

INTRODUCTION

Several lines of evidence have indicated that men and women with insulin resistance (IR)[4] have a 2- to 5-fold higher risk of developing cardiovascular disease than do people without IR (1–4). Hypertriglyceridemia associated with IR has been attributed to a combination of increased hepatic production and reduced clearance of VLDL particles (5, 6). Previous studies have also suggested that IR is associated with an elevated production rate of intestinally derived lipoproteins and contributes to the overall hypertriglyceridemic state in IR individuals (7, 8). This relation is of significant interest because there is now convincing evidence indicating that elevated concentrations of intestine-derived lipoproteins are associated with an increased risk of cardiovascular disease (9). Chylomicrons are too large to enter the subendothelial space, but once hydrolyzed by the lipoprotein lipase, chylomicron remnants <700 angstroms are small enough to migrate into the intima and to participate in the development of atherosclerotic lesions (10). Chylomicron remnants are chemically modified and have been shown to impair normal endothelial function (11) and to accumulate in the subendothelial space in the same way that apolipoprotein (apo) B-100–containing lipoproteins do (12, 13).

Medium-chain triglycerides (MCTs) are composed of fatty acids with 6–12 carbons and are found primarily in coconut and palm kernel oils (14). MCTs do not enter the lymphatic and peripheral circulation as chylomicrons but are transported to the liver directly via the portal circulation, where they may affect fuel metabolism (15). It is well recognized that MCTs have been clinically effective in treating patients with severe hypertriglyceridemia associated with familial hyperchylomicronemia (16, 17). However, the clinical efficacy of MCTs in patients with other forms of lipoprotein disorders remains controversial. Several studies have shown a significant increase in fasting plasma triglyceride concentrations in subjects consuming ≥40% of their energy in the form of MCTs (18–20), whereas other studies using lower doses of MCTs (20–60 g/d or 12–20% of energy intake) have shown no adverse changes in plasma triglycerides (21–24). Asakura et al (23) showed that a test meal containing 25% MCT oil (40 g fat/m^2)

[1] From the Institute of Nutrition and Functional Foods (AJT, BL, M-ÈL, M-CL, and PC), the Lipid Research Centre, Centre hospitalier de l'Université Laval (CHUL) Research Centre (AJT and PC), and the Department of Gastroenterology, CHUL (VL), Laval University, Quebec City, Canada.

[2] Supported by the Canadian Institutes of Health Research (MOP-219879). PC is the recipient of a Research Award from the Fonds de Recherche du Quebec-Santé (FRQS). BL is the Chair Professor in Nutrition, Functional Food and Cardiovascular Health for the Canada Research Chair Program. M-ÈL is the recipient of doctoral scholarships from the Canadian Institutes of Health Research and the FRQS.

[3] Address correspondence and requests for reprints to P Couture, Institute of Nutrition and Functional Foods (INAF), 2440 Hochelaga Boulevard, Pavillon des Services, Laval University, Quebec City, PQ, Canada, G1V 0A6. E-mail: patrick.couture@crchul.ulaval.ca.

[4] Abbreviations used: apo, apolipoprotein; IR, insulin resistance; MCT, medium-chain triglyceride; PR, production rate; TRL, triglyceride-rich lipoprotein.

Received June 18, 2013. Accepted for publication July 23, 2013.

First published online October 30, 2013; doi: 10.3945/ajcn.113.068767.

significantly reduced postprandial triglyceride concentrations by 36% in patients with primary hypertriglyceridemia. In the present study, we investigated the impact of MCT supplementation on the relations between intestinally derived apo B-48–containing lipoprotein kinetics and the expression of key intestinal genes involved in lipid and lipoprotein metabolism in nondiabetic, IR men. Gene expression studies were conducted by using a human duodenal biopsy model (25). We hypothesized that MCT supplementation would lead to beneficial changes in the postprandial metabolism of triglyceride-rich lipoproteins (TRLs).

SUBJECTS AND METHODS

Subjects

Twenty-eight nondiabetic, IR men from the Quebec City area were recruited to participate in the study. The IR subjects had to have plasma triglyceride concentrations >1.7 mmol/L, HDL-cholesterol concentrations <1.1 mmol/L, plasma insulin concentrations >90 ρmol/L, and a waist circumference >94 cm. Subjects were excluded if they had elevated blood pressure (>140/90 mm Hg), monogenic hyperlipidemia such as familial hypercholesterolemia, plasma triglyceride concentrations >4.5 mmol/L, a recent history of alcohol or drug abuse, diabetes mellitus, or a history of cancer. Furthermore, none of the participants were first- or second-degree relatives. The study consisted of a 1-wk screening period followed by two 4-wk double-blind, crossover intervention periods with corn oil (20 g/d) or MCT oil (20 g/d) supplementation. The supplementation periods occurred in random order, and there was a 4-wk washout period between the 2 phases. The participants were instructed to consume 2 snacks per day, each containing either 10 g of corn oil or 10 g of MCT oil, while maintaining their normal diet and physical activities. The snacks provided to the participants included banana muffins, chocolate cookies, and granola bars. All of these snacks had similar nutritional composition and contained 10 g of MCT oil or corn oil, 250 kcal, 14.5 g of total fat, 27.9 g of carbohydrates, 4.3 g of protein, and 51 mg of cholesterol. Fasting blood samples were obtained at the end of each treatment. Kinetic studies using primed-constant infusion of deuterated leucine were performed in all of the participants, and duodenal biopsies were performed in a subgroup of 16 participants after each phase. The MCT oil was purchased commercially (Nestlé). The research protocol was approved by the Laval University Medical Center Ethical Review Committee, and written informed consent was obtained from each subject. This trial was registered at clinicaltrials.gov as NCT01806142.

Experimental protocol for in vivo stable isotope kinetics

To determine the kinetics of TRL apo B-48 and VLDL apo B-100, the subjects underwent a primed-constant infusion of L-[5,5,5-D_3]leucine while they were in a constant fed state. Starting at 0700, the subjects received 30 small, identical cookies every half hour for 15 h, each containing up to 1/30th of their estimated daily food intake based on the Harris-Benedict equation (26), with 15% of calories from protein, 45% from carbohydrates, and 40% from fat (containing 20 g of corn oil or 20 g of MCT oil depending on the supplementation period), as well as 85 mg of cholesterol/1000 kcal. At 1000, with 2 intravenous lines in place, one for the infusate and one for blood sampling, L-[5,5,5-D_3] leucine (10 μmol/kg body weight) was injected as a bolus intravenously and then by continuous infusion (10 μmol · kg body weight^{-1} · h^{-1}) over a 12-h period. Blood samples (20 mL) were collected at hours 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 11, and 12.

Characterization of plasma lipids and lipoproteins

Twelve-hour fasting venous blood samples were obtained from an antecubital vein before the beginning of the kinetic study. Serum was separated from blood cells by centrifugation at 3000 rpm for 10 min at 4°C. Serum cholesterol and triglyceride concentrations were determined with a Roche/Hitachi Modular analyzer (Roche Diagnostics) using Roche Diagnostics reagents. The VLDL (TRL) ($d < 1.006$ g/mL) fraction was isolated from fresh plasma obtained with Vacutainer tubes containing EDTA (0.1% final concentration) by ultracentrifugation (27), and HDL cholesterol was measured as previously described (28). Serum apo B and HDL apo A-I concentrations were measured by using a Behring Nephelometer BN-100 (Behring Diagnostic) with reagents and calibrators (Dade Behring) provided by the manufacturer.

Quantification and isolation of apo B-48 and apo B-100

The apo B concentration in VLDL (TRL) was determined by performing a noncompetitive ELISA using immunopurified polyclonal antibodies (Alerchek Inc) to calculate the respective pool size. The CV for the apo B assay ranged between 6% and 10% depending on the region of the standard curve. Apo B-100 and apo B-48 were then separated by SDS-PAGE according to standardized procedures (29). Briefly, 100 μL of the TRL fractions was mixed with 50 μL of 3% SDS sample buffer and subjected to electrophoresis in 3–10% linear gradient polyacrylamide slab mini-gels. The gels were stained for 2–3 h in 2.5% Coomassie blue R-250 and destained overnight. On the basis of the assumption that both apo B-100 and apo B-48 have the same chromogenicity, the relative proportions of apo B-100 and apo B-48 were assessed by scanning each gel with laser densitometry (30). We scanned lipoprotein fractions at 3 different time points to calculate ratios and to estimate the average concentrations of apo B-100 and apo B-48 by using the total apo B concentration.

Isotopic enrichment determinations

The apo B-48 and apo B-100 bands were excised from polyacrylamide gels, and the bands were hydrolyzed in 6 N HCl at 110°C for 24 h (31). Trifluoroacetic acid and trifluoroacetic anhydride (1:1) were used as derivatization reagents for the amino acids before the analyses were performed by using a Hewlett-Packard 6890/5973 gas chromatograph/mass spectrometer (32). The isotopic enrichment (%) and tracer:tracee ratios (%) were calculated from the observed ion current ratios (33). The isotopic enrichment of leucine in the apolipoproteins was expressed as the tracer:tracee ratio (%) by using standardized formulas (33).

Kinetic analysis

The kinetics of TRL apo B-48 and VLDL apo B-100 were derived by using a multicompartmental model that has been previously described (34). We assumed that the enrichment of the precursor pool was stable and used the TRL apo B-48 and VLDL apo B-100 plateau

of the isotopic enrichment data as the forcing function to drive the appearance of the tracer into apo B-48 and apo B-100 (31). Under steady state conditions, the fractional catabolic rate is equivalent to the fractional synthetic rate. The apo B production rates (PRs) were determined by using the formula PR ($mg \cdot kg^{-1} \cdot d^{-1}$) = [fractional catabolic rate (pools/d) × apo B concentration (mg/dL) × plasma volume (L)]/body weight (kg) (35). The plasma volume was estimated at 4.5% of body weight. The SAAM II program (SAAM Institute) was used to fit the model to the observed tracer data.

Intestinal biopsies

Biopsies were obtained from the second portion of the duodenum during gastroduodenoscopy. Six biopsy samples were collected by using multiple-sample, single-use biopsy forceps, immediately flash-frozen in liquid nitrogen, and stored at −80°C before RNA extraction.

Total RNA extraction, RNA quantification, and quantitative real-time polymerase chain reaction

The intestinal biopsy tissue samples were homogenized in 1 mL of Qiazol (Qiagen) and were extracted by using an RNeasy kit (Qiagen). The tissue samples were also treated with an RNase-free DNase set to eliminate any contaminating DNA. Total RNA was then eluted into 100 μL RNase-free H_2O and stored at −80°C. RNA quantification and quantitative real-time polymerase chain reaction were performed as described (36).

Power calculations and statistical analysis

This study was designed to provide adequate statistical power to investigate the MCT-induced changes in the kinetics of TRL apo B-48 as the primary outcome. There are currently no data on the effect of MCTs on TRL apo B-48 kinetics in humans. However, previous data showed that hypertriglyceridemic subjects consuming an MCT-supplemented diet ($40\ g \cdot m^{-2} \cdot d^{-1}$) for 2 wk had a 36% reduction in postprandial triglyceride concentrations compared with subjects consuming corn oil (23). Our power analyses suggest that with a final sample size of 28 subjects in this 2-treatment crossover study, the probability is 81% that the study will detect a treatment difference at a one-sided 5% significance level, if the true difference in the TRL apo B-48 PR between placebo and MCT supplementation is 35%. This is based on the assumption that the within-patient SD (50%) is greater in magnitude than the main effect per se (35%).

Student's paired *t* tests were used to compare the effects of MCTs on the fasting lipid-lipoprotein profile, kinetic parameters, and mRNA expression. Mixed models with proper interaction terms were used to assess potential interactions between variables.

Spearman's correlation coefficients were determined to assess the significance of the associations. Differences were considered significant at $P \leq 0.05$. All analyses were performed by using JMP statistical software (version 10.0; SAS Institute).

RESULTS

Demographic characteristics and fasting biochemical variables of subjects

Demographic characteristics and fasting biochemical variables of the 28 subjects after a 4-wk intervention with either corn oil or MCT supplementation are shown in **Table 1**. The mean (±SD) age of the participants was 37.8 ± 10.3 y. No significant differences were observed between the 2 intervention conditions in body weight, waist circumference, or systolic and diastolic blood pressure. MCT supplementation had no significant impact on the fasting lipid/lipoprotein profile compared with corn oil supplementation.

TABLE 1
Characteristics and fasting lipid/lipoprotein profiles of the 28 participants at the end of each intervention phase[1]

	Corn oil (n = 28)	MCT oil (n = 28)	%Δ	P
Body weight (kg)	102.9 ± 15.2[2]	102.8 ± 15.4	−0.1	0.7
Waist circumference (cm)	112.0 ± 11.6	111.8 ± 11.4	−0.2	0.4
Systolic blood pressure (mm Hg)	126.6 ± 10.9	129.4 ± 12.8	+2.2	0.2
Diastolic blood pressure (mm Hg)	76.1 ± 9.8	76.6 ± 10.8	+0.7	0.8
Serum				
Cholesterol (mmol/L)	5.07 ± 0.92	5.25 ± 0.84	+3.6	0.2
Triglycerides (mmol/L)	2.22 ± 0.91	2.28 ± 1.13	+2.7	0.7
apo B (g/L)	1.03 ± 0.19	1.06 ± 0.20	+2.9	0.3
VLDL				
Cholesterol (mmol/L)	0.86 ± 0.46	0.90 ± 0.56	+4.7	0.7
Triglycerides (mmol/L)	1.53 ± 0.80	1.59 ± 0.98	+3.9	0.7
apo B (g/L)	0.12 ± 0.05	0.13 ± 0.06	+8.3	0.4
LDL				
Cholesterol (mmol/L)	3.25 ± 0.80	3.40 ± 0.89	+4.6	0.1
Triglycerides (mmol/L)	0.33 ± 0.11	0.35 ± 0.15	+6.1	0.5
apo B (g/L)	0.91 ± 0.18	0.93 ± 0.20	+2.2	0.4
HDL				
Cholesterol (mmol/L)	0.96 ± 0.23	0.95 ± 0.20	−1.0	0.7
Triglycerides (mmol/L)	0.35 ± 0.10	0.34 ± 0.11	−2.9	0.6
apo A-I (g/L)	1.25 ± 0.16	1.22 ± 0.14	−2.4	0.2

[1] Student's paired *t* test for continuous measures was used to assess the differences between the 2 dietary interventions. apo, apolipoprotein; MCT, medium-chain triglyceride; %Δ, the percentage difference between the 2 groups.
[2] Mean ± SD (all such values).

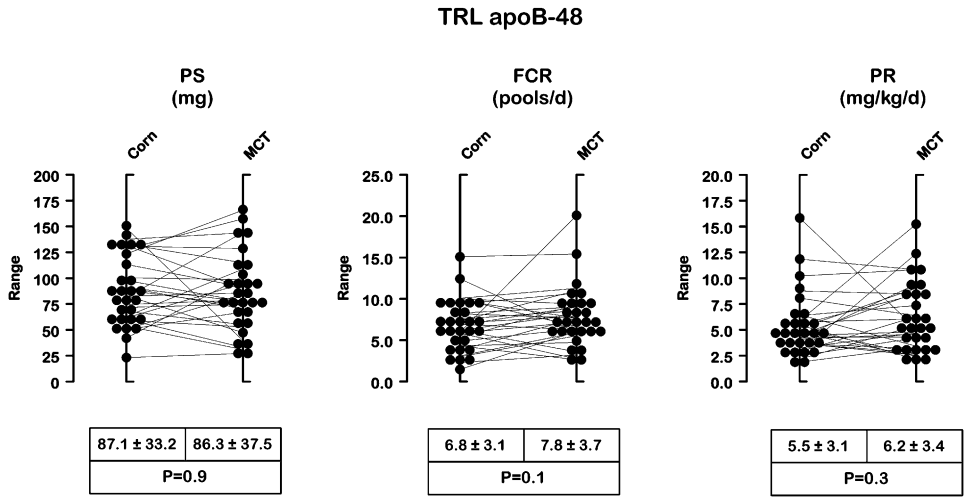

FIGURE 1. Individual responses (corn oil compared with MCT supplementation) of the 28 participants. Values for triglyceride-rich lipoprotein apo B-48 PS, FCR, and PR are shown along with their means ± SDs. Student's paired *t* test for continuous measures was used to assess the differences between the 2 dietary interventions. apo, apolipoprotein; FCR, fractional catabolic rate; MCT, medium-chain triglyceride; PR, production rate; PS, pool size.

Kinetics of TRL apo B-48 and VLDL apo B-100

Analyses of the deuterated plasma amino acids and the lipid/lipoprotein measurements indicated that plasma leucine enrichments, as well as plasma triglyceride and TRL apo B-48 concentrations, remained constant over the course of the infusion (data not shown). Detailed kinetic information obtained by conducting a multicompartmental model analysis is summarized in **Figures 1** and **2**. Compared with corn oil supplementation, MCT supplementation had no significant effect on the pool size and kinetics of TRL apo B-48 (Figure 1) or VLDL apo B-100 (Figure 2), suggesting that 20 g MCTs/d for 4 wk did not improve postprandial lipemia or apo B-100 metabolism in IR men.

Intestinal mRNA levels

As shown in **Table 2**, intestinal *SREBP2* gene expression after MCT supplementation was strongly correlated with the mRNA levels of several genes known to be regulated by this nuclear transcription factor: *HNF4α* ($r = 0.51$, $P < 0.05$), *HMGCoAR* ($r = 0.75$, $P < 0.001$), *ACAT2* ($r = 0.51$, $P < 0.05$), *NPC1L1* ($r = 0.66$, $P < 0.05$), *ACS1* ($r = 0.74$, $P < 0.001$), *LDLR* ($r = 0.67$, $P < 0.05$), and *PCSK9* ($r = 0.60$, $P < 0.05$). *HNF4α* was also positively associated with *HMGCoAR* ($r = 0.57$, $P < 0.05$), *NPC1L1* ($r = 0.86$, $P < 0.0001$), and *ACS1* ($r = 0.66$, $P < 0.05$). The intestinal mRNA expression of *FATP4* and *FABP2*, 2 genes involved in fatty acid metabolism, was also positively correlated ($r = 0.71$, $P < 0.05$). However, no significant association was observed between *MTP* and apo B intestinal mRNA expression. Finally, the PR of TRL apo B-48 was positively correlated with the intestinal mRNA levels of *SREBP2* ($r = 0.55$, $P < 0.05$), *PCSK9* ($r = 0.61$, $P < 0.05$), *HMGCoAR* ($r = 0.61$, $P < 0.05$), *ACS1* ($r = 0.60$, $P < 0.05$), and *ACAT2* ($r = 0.57$, $P < 0.05$). Similar correlations were observed using the data collected after corn oil supplementation (ie, the control condition). Mixed models with proper interaction terms have shown no evidence of a carryover effect of the MCT oil supplementation (data not shown).

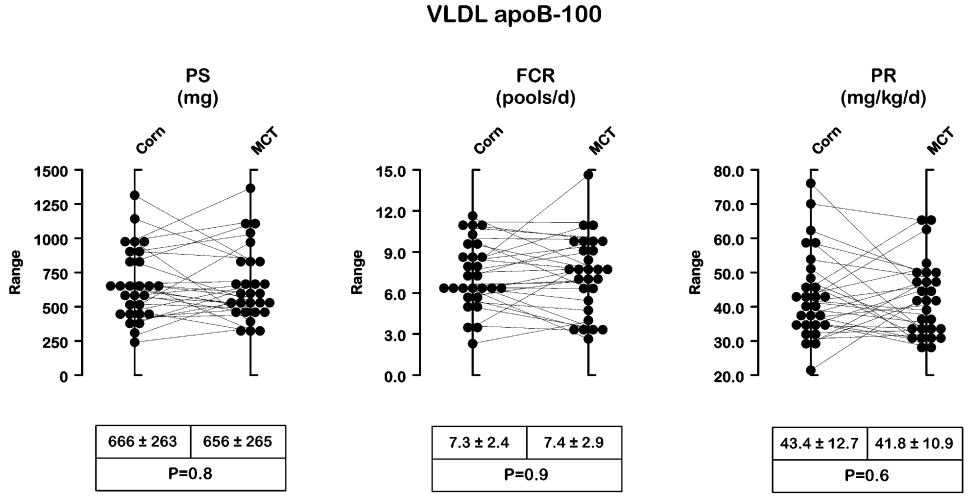

FIGURE 2. Individual responses (corn oil compared with MCT supplementation) of the 28 participants. Values for VLDL apo B-100 PS, FCR, and PR are shown along with their means ± SDs. Student's paired *t* test for continuous measures was used to assess the differences between the 2 dietary interventions. apo, apolipoprotein; FCR, fractional catabolic rate; MCT, medium-chain triglyceride; PR, production rate; PS, pool size.

TABLE 2
Summary of the correlations between the mRNA levels of key intestinal genes after MCT supplementation[1]

	SREBP1c	*SREBP2*	*HNF4α*	*HMGCoAR*	*ACAT2*	*NPC1L1*	*ACS1*	*FABP2*	*FATP4*	*MTP*	*APOB*	*LDLR*
SREBP2	0.44											
HNF4α	0.80†	0.51*										
HMGCoAR	0.51*	0.75†	0.57*									
ACAT2	−0.14	0.51*	−0.05	0.47								
NPC1L1	0.55*	0.66*	0.86‡	0.62*	0.15							
ACS1	0.59*	0.74†	0.66*	0.77†	0.31	0.70*						
FABP2	0.45	0.12	0.62*	0.33	0.06	0.61*	0.19					
FATP4	0.60*	0.47	0.90‡	0.58*	−0.06	0.91‡	0.59*	0.71*				
MTP	0.26	−0.25	0.55*	−0.14	−0.52*	0.35	−0.11	0.59*	0.59*			
APOB	0.74*	0.49	0.91‡	0.52*	−0.05	0.89‡	0.69*	0.53*	0.83‡	0.37		
LDLR	0.65*	0.67*	0.41	0.55*	0.26	0.39	0.43	0.25	0.32	−0.17	0.35	
PCSK9	0.12	0.60*	0.11	0.62*	0.69*	0.10	0.35	−0.05	0.06	−0.35	0.01	0.41

[1] $n = 16$. The correlation analyses were performed by using Spearman's rank order test. *$P < 0.05$, †$P < 0.001$, ‡$P < 0.0001$. MCT, medium-chain triglyceride.

Finally, we investigated whether MCT supplementation was associated with variations in the expression of the major genes involved in intestinal lipid and lipoprotein metabolism in individuals with IR. As shown in **Table 3**, MCT supplementation had no significant impact on gene expression compared with corn oil. These results suggest that MCT supplementation, at a dose of 20 g/d for 4 wk, does not alter the intestinal expression of key nuclear transcription factors and target genes involved in the metabolism of cholesterol, fatty acids, triglycerides, and lipoproteins.

DISCUSSION

In the present study, MCT supplementation at a dose of 20 g/d for 4 wk had no effect on fasting and postprandial lipemia compared with the control condition (corn oil supplementation). Moreover, MCT supplementation had no significant impact on the mRNA expression of several key intestinal genes involved in lipid and lipoprotein metabolism. These results suggest that short-term consumption of a moderate amount of MCT has no impact on the oversecretion of TRL apo B-48 generally observed in subjects with IR and does not affect the mRNA gene expression at the enterocyte level.

Previous studies have produced conflicting results regarding the effects of MCTs on plasma lipids. In the present study, MCT supplementation had no significant impact on plasma cholesterol and triglyceride concentrations. These results align with previous findings in overweight subjects that showed that MCTs do not increase triglyceride or total and LDL-cholesterol concentrations when they account for 12–20% of the total energy intake (21, 22, 24). Asakura et al (23) showed that MCT consumption up to a maximum of 24 g/d for 2 wk (compared with corn oil) was not associated with any change in fasting triglyceride concentrations, but that plasma cholesterol concentrations increased. Furthermore, 2 different studies by Nosaka et al (37, 38) showed that there was no significant change in plasma cholesterol and triglyceride concentrations after an MCT intervention for 12 wk and 4 wk, respectively. Woollett et al (39) detected no change in serum concentrations of total, LDL, or HDL cholesterol after 30 d of MCT supplementation in hamsters. These findings contrast with the findings of other studies that found a detrimental effect of MCT on plasma cholesterol and triglyceride concentrations. It is possible that the MCT dose used in the various studies may have confounded the results of earlier studies in which MCTs increased triglyceride or cholesterol concentrations. Indeed, 2 studies in which the subjects consumed 40% of their energy in the form of MCTs at either 100% or 150% of weight-maintenance energy requirements reported significant increases in triglyceride concentrations (42% and 200%, respectively) (19, 20). Cater et al (18) reported a significant increase in triglyceride concentrations after 3 wk of MCT oil consumption at 43% of energy intake compared with equivalent amounts of palm oil and high-oleic sunflower oil. In 17 healthy young men who replaced part of their habitual dietary fat intake with 70 g MCTs, Tholstrup et al (40) showed that plasma triglycerides increased by 22% and that plasma cholesterol concentratons increased by 11%. In a rat model, Geelen et al (41) showed that MCT oil consumption significantly increased plasma triglyceride concentrations compared with corn oil. The dose-dependent impact of MCTs on plasma lipid profile has been emphasized by Swift et al (20), who found that subjects who consumed a lower dose of MCTs had no significant increase in fasting triglycerides compared with subjects consuming higher doses. Thus, it may be possible that a high dietary intake of MCTs (>60 g/d or 20% of energy intake) is required to elicit adverse changes in plasma triglyceride and cholesterol concentrations.

MCTs are fairly hydrophilic and thus are rapidly absorbed across the intestinal brush border without the intervention of micellar solubilization (42, 43). Once absorbed, these compounds are not reesterified and incorporated into the nascent chylomicron; rather, they pass directly into portal circulation, from which they are extracted by the liver. A study by Swift et al (44) in which 93% of dietary fat calories were MCTs showed that the mass of chylomicron triglycerides produced was approximately one-fifth the mass present in subjects who consumed a diet with long-chain triglycerides. The reduced chylomicron formation is the basis for the use of MCTs in the treatment of both type I and type V hyperlipidemia (45). Only one report has evaluated the effects of MCTs on postprandial lipid concentrations in patients with primary hypertriglyceridemia (23). In that study, consumption of 24 g MCT oil/d for 4 wk did not change fasting plasma triglyceride concentrations. However, when a test meal containing 25% MCT oil (40 g fat/m^2 body surface area) was administered to the participants, postprandial triglyceride concentrations were

TABLE 3
Intestinal mRNA expression of key genes involved in lipid/lipoprotein metabolism in men with IR[1]

Gene	Corn oil (n = 16)	MCT oil (n = 16)	%Δ	P
	No. of copies/100,000 copies HPRT1	*No. of copies/100,000 copies HPRT1*		
Nuclear transcription factors				
SREBP1c	36,406 ± 16,574[2]	30,858 ± 11,034	−15.2	0.3
SREBP2	86,578 ± 41,432	74,275 ± 31,241	−14.2	0.3
HNF4α	138,327 ± 44,761	12,4825 ± 53,720	−9.8	0.5
PPARα	96,316 ± 29,274	90,571 ± 33,893	−6.0	0.6
PPARγ	92,284 ± 21,120	99,875 ± 26,721	+8.2	0.4
Cholesterol metabolism and transport				
HMGCoAR	151,690 ± 41,472	146,018 ± 41,042	−3.7	0.6
ACAT2	161,167 ± 38,089	156,927 ± 30,035	−2.6	0.5
NPC1L1	33,554 ± 13,302	32,923 ± 18,273	−1.9	0.9
ABCG5	125,405 ± 48,846	132,313 ± 56,096	+5.5	0.6
ABCG8	33,978 ± 15,080	32,939 ± 13,108	−3.1	0.8
ABCG1	29,555 ± 14,766	30,277 ± 16,342	+2.4	0.9
ABCA1	3371 ± 1528	3011 ± 1277	−10.7	0.4
Fatty acid metabolism and transport				
ACACα	3275 ± 1417	2808 ± 1191	−14.3	0.3
ACACβ	11,362 ± 3691	10,599 ± 4150	−6.7	0.6
SCD1	220,742 ± 114,265	211,079 ± 96,842	−4.4	0.8
FADS1	40,575 ± 17,200	37,258 ± 13,714	−8.2	0.4
FADS2	36,731 ± 31,667	29,570 ± 17,581	−19.5	0.3
ACS1	42,939 ± 15,585	37,136 ± 16,870	−13.5	0.4
FABP2	658,503 ± 258,407	639,153 ± 239,487	−2.9	0.8
FATP4	92,067 ± 33,838	86,557 ± 40,911	−6.0	0.7
Triglyceride synthesis				
MGAT2	62,724 ± 13,910	59,342 ± 16,830	−5.4	0.5
DGAT1	197,815 ± 60,914	200,748 ± 78,117	+1.5	0.9
DGAT2	153,861 ± 87,525	148,487 ± 86,225	−3.5	0.8
Lipoprotein assembly and transport				
MTP	1,797,150 ± 1,013,857	1,720,401 ± 811,451	−4.3	0.6
APOB	877,287 ± 291,529	828,459 ± 364,197	−5.6	0.6
APOAI	15,638,749 ± 7,967,734	14,776,895 ± 7,207,641	−5.5	0.6
LDLR	59,865 ± 30,709	59,804 ± 32,992	−0.1	0.9
VLDLR	5523 ± 4094	4776 ± 3422	−13.5	0.2
PCSK9	11,996 ± 10,131	9220 ± 7823	−23.1	0.3
SAR1β	241,063 ± 44,114	242,726 ± 40,891	+0.7	0.9
LRP1	127,631 ± 41,881	125,186 ± 54,954	−1.9	0.9
APOB48R	403 ± 152	351 ± 183	−12.9	0.3
SRB1	117,785 ± 53,372	102,565 ± 46,278	−12.9	0.3

[1] Student's paired t test for continuous measures was used to assess the differences between the 2 dietary interventions. IR, insulin resistance; %Δ, the percentage difference between the 2 groups.
[2] Mean ± SD (all such values).

significantly reduced over an 8-h period compared with the participants who consumed corn oil. The authors suggested that this reduction in postprandial triglyceride concentrations might be attributable to a reduction in chylomicrons and VLDL-cholesterol formation or to the enhancement of the TRL clearance rate. However, these results (from participants in a fed state) contrast with those found in the current study, most likely because of the amount of MCT oil consumed in the test meal. In fact, the same amount of MCT oil (20 g) over the 15-h period of the kinetic study was consumed, compared with >40 g for the test meal used in the other study in which 24 g MCT/d was used for the 4-wk intervention. Moreover, the fasting plasma triglyceride concentrations in the participants in the Asakura et al study (23) were 3 times those of the participants in the present study, which may partially explain the beneficial effect of MCT oil on postprandial triglycerides that they observed. In contrast, a study that compared serum lipid concentrations in 25 men with a BMI (in kg/m^2) ≥23 who received a single dose of either 10 g of MCTs or long-chain triglycerides showed a significant reduction in postprandial serum and chylomicron triglyceride concentrations (46). Swift et al (20) also reported that MCT oil significantly decreased postprandial triglycerides after healthy men consumed a test meal containing 40% fat (~44 g). Finally, a previous study showed that the intestinal apo B-48 mass in newborn swine was significantly reduced after they were fed a diet containing 48% MCTs (47). In the present study, there was no significant change in the TRL apo B-48 pool size in the fed state after MCT supplementation. The production and clearance rates of TRL apo B-48 were not affected by MCT supplementation compared with corn oil supplementation. In addition, the kinetic VLDL apo B-100 variables did not change after MCT supplementation. Therefore, it seems that the dose of MCTs plays an important role on the postprandial response to MCT intake.

We also investigated the effect of MCT supplementation on the expression of several key genes involved in lipid and lipoprotein metabolism at the enterocyte level by using intestinal biopsy samples collected at the end of each intervention phase. Consistent with the lack of a change in plasma lipids, MCT supplementation did not produce any significant changes in the intestinal mRNA expression of genes involved in the transport and metabolism of fatty acids and lipoproteins. No previous studies have investigated gene expression at the enterocyte level after MCT supplementation. However, Geelen et al (41) showed that MCT consumption increased the hepatic activities of acetyl-CoA carboxylase, fatty acid synthase, and diacylglycerol acyltransferase in rats. These changes would be expected to increase hepatic triglyceride production and VLDL secretion. It has been suggested that MCT-rich diets, especially in amounts that exceed caloric needs, might increase de novo fatty acid synthesis and enhance fatty acid elongation activity in the liver (48, 49), with the consequence of increasing hepatic triglyceride production and VLDL secretion, thus elevating plasma triglyceride concentrations. It has been reported that the activity of 3-hydroxy-3-methylglutaryl-CoA reductase is reduced in rats treated with MCTs (50).

In conclusion, the results of the present study show that 20 g supplemental MCTs/d for 4 wk had no effect on plasma lipids, TRL apo B-48– and apo B-100–containing lipoprotein kinetics, or the intestinal expression of genes involved in lipoprotein and fatty acid metabolism in IR subjects.

The authors are grateful to the subjects for their excellent collaboration and for the dedicated work of Danielle Aubin, Steve Larouche, Michèle Valotaire, Johanne Marin, and Pascal Dubé.

The authors' responsibilities were as follows—PC and BL: designed the research study; AJT, M-CL, and VL: conducted the research; PC, BL, M-ÈL, and AJT: analyzed the data; PC, BL, and AJT: wrote the manuscript; and PC: had primary responsibility for the final content. All of the authors read and approved the final manuscript. None of the authors reported a conflict of interest.

REFERENCES

1. Isomaa B, Almgren P, Tuomi T, Forsen B, Lahti K, Nissen M, Taskinen MR, Groop L. Cardiovascular morbidity and mortality associated with the metabolic syndrome. Diabetes Care 2001;24:683–9.
2. Howard BV, Criqui MH, Curb JD, Rodabough R, Safford MM, Santoro N, Wilson AC, Wylie-Rosett J. Risk factor clustering in the insulin resistance syndrome and its relationship to cardiovascular disease in postmenopausal white, black, Hispanic, and Asian/Pacific Islander women. Metabolism 2003;52:362–71.
3. Resnick HE, Jones K, Ruotolo G, Jain AK, Henderson J, Lu W, Howard BV. Insulin resistance, the metabolic syndrome, and risk of incident cardiovascular disease in nondiabetic American Indians: the Strong Heart Study. Diabetes Care 2003;26:861–7.
4. Lakka HM, Laaksonen DE, Lakka TA, Niskanen LK, Kumpusalo E, Tuomilehto J, Salonen JT. The metabolic syndrome and total and cardiovascular disease mortality in middle-aged men. JAMA 2002;288:2709–16.
5. Lewis GF, Carpentier A, Adeli K, Giacca A. Disordered fat storage and mobilization in the pathogenesis of insulin resistance and type 2 diabetes. Endocr Rev 2002;23:201–29.
6. Chan DC, Barrett PH, Watts GF. Recent studies of lipoprotein kinetics in the metabolic syndrome and related disorders. Curr Opin Lipidol 2006;17:28–36.
7. Hogue JC, Lamarche B, Tremblay AJ, Bergeron J, Gagné C, Couture P. Evidence of increased secretion of apolipoprotein B-48-containing lipoproteins in subjects with type 2 diabetes. J Lipid Res 2007;48:1336–42.
8. Duez H, Lamarche B, Uffelman KD, Valero R, Cohn JS, Lewis GF. Hyperinsulinemia is associated with increased production rate of intestinal apolipoprotein B-48-containing lipoproteins in humans. Arterioscler Thromb Vasc Biol 2006;26:1357–63.
9. Kolovou GD, Anagnostopoulou KK, Daskalopoulou SS, Mikhailidis DP, Cokkinos DV. Clinical relevance of postprandial lipaemia. Curr Med Chem 2005;12:1931–45.
10. Nordestgaard BG, Tybjaerg-Hansen A. IDL, VLDL, chylomicrons and atherosclerosis. Eur J Epidemiol 1992;8(suppl 1):92–8.
11. Doi H, Kugiyama K, Ohgushi M, Sugiyama S, Matsumura T, Ohta Y, Nakano T, Nakajima K, Yasue H. Remnants of chylomicron and very low density lipoprotein impair endothelium-dependent vasorelaxation. Atherosclerosis 1998;137:341–9.
12. Twickler TB, Dallinga-Thie GM, Cohn JS, Chapman MJ. Elevated remnant-like particle cholesterol concentration: a characteristic feature of the atherogenic lipoprotein phenotype. Circulation 2004;109:1918–25.
13. Proctor SD, Mamo JC. Intimal retention of cholesterol derived from apolipoprotein B100- and apolipoprotein B48-containing lipoproteins in carotid arteries of Watanabe heritable hyperlipidemic rabbits. Arterioscler Thromb Vasc Biol 2003;23:1595–600.
14. Wanten GJ, Naber AH. Cellular and physiological effects of medium-chain triglycerides. Mini Rev Med Chem 2004;4:847–57.
15. Babayan VK. Medium chain triglycerides and structured lipids. Lipids 1987;22:417–20.
16. Rouis M, Dugi KA, Previato L, Patterson AP, Brunzell JD, Brewer HB, Santamarina-Fojo S. Therapeutic response to medium-chain triglycerides and omega-3 fatty acids in a patient with the familial chylomicronemia syndrome. Arterioscler Thromb Vasc Biol 1997;17:1400–6.
17. Shirai K, Kobayashi J, Inadera H, Ohkubo Y, Mori S, Saito Y, Yoshida S. Type I hyperlipoproteinemia caused by lipoprotein lipase defect in lipid-interface recognition was relieved by administration of medium-chain triglyceride. Metabolism 1992;41:1161–4.
18. 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.
19. Hill JO, Peters JC, Swift LL, Yang D, Sharp T, Abumrad N, Greene HL. Changes in blood lipids during six days of overfeeding with medium or long chain triglycerides. J Lipid Res 1990;31:407–16.
20. Swift LL, Hill JO, Peters JC, Greene 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.
21. St-Onge MP, Lamarche B, Mauger JF, Jones PJ. Consumption of a functional oil rich in phytosterols and medium-chain triglyceride oil improves plasma lipid profiles in men. J Nutr 2003;133:1815–20.
22. Bourque C, St-Onge MP, Papamandjaris AA, Cohn JS, Jones PJ. Consumption of an oil composed of medium chain triacyglycerols, phytosterols, and N-3 fatty acids improves cardiovascular risk profile in overweight women. Metabolism 2003;52:771–7.
23. Asakura L, Lottenberg AM, Neves MQ, Nunes VS, Rocha JC, Passarelli M, Nakandakare ER, Quintao EC. 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.
24. St-Onge MP, Bosarge A, Goree LL, Darnell B. Medium chain triglyceride oil consumption as part of a weight loss diet does not lead to an adverse metabolic profile when compared to olive oil. J Am Coll Nutr 2008;27:547–52.
25. Tremblay AJ, Lamarche B, Lemelin V, Hoos L, Benjannet S, Seidah NG, Davis HR Jr, Couture P. Atorvastatin increases intestinal expression of NPC1L1 in hyperlipidemic men. J Lipid Res 2011;52:558–65.
26. Harris J, Benedict F. A biometric study of basal metabolism in man. Washington, DC: Carnegie Institution, 1919.
27. Havel RJ, Eder H, Bragdon J. The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. J Clin Invest 1955;34:1345–53.
28. Albers JJ, Warnick GR, Wiebe D, King P, Steiner P, Smith L, Breckenridge C, Chow A, Kuba K, Weidman S, et al. Multi-laboratory comparison of three heparin-Mn2+ precipitation procedures for estimating cholesterol in high-density lipoprotein. Clin Chem 1978;24:853–6.
29. Kotite L, Bergeron N, Havel RJ. Quantification of apolipoproteins B-100, B-48, and E in human triglyceride-rich lipoproteins. J Lipid Res 1995;36:890–900.

30. Zilversmit DB, Shea TM. Quantitation of apoB-48 and apoB-100 by gel scanning or radio-iodination. J Lipid Res 1989;30:1639–46.
31. Welty FK, Lichtenstein AH, Barrett PH, Dolnikowski GG, Schaefer EJ. Human apolipoprotein (apo) B-48 and apoB-100 kinetics with stable isotopes. Arterioscler Thromb Vasc Biol 1999;19:2966–74.
32. Dwyer KP, Barrett PH, Chan D, Foo JI, Watts GF, Croft KD. Oxazolinone derivative of leucine for GC-MS: a sensitive and robust method for stable isotope kinetic studies of lipoproteins. J Lipid Res 2002;43:344–9.
33. Cobelli C, Toffolo G, Bier DM, Nosadini R. Models to interpret kinetic data in stable isotope tracer studies. Am J Physiol 1987;253:E551–64.
34. Tremblay AJ, Lamarche B, Hogue JC, Couture P. Effects of ezetimibe and simvastatin on apolipoprotein B metabolism in males with mixed hyperlipidemia. J Lipid Res 2009;50:1463–71.
35. Cohn JS, Wagner D, Cohn S, Millar J, Schaefer E. Measurement of very low density and low density lipoprotein apolipoprotein (apo) B-100 and high density lipoprotein apo A-I production in human subjects using deuterated leucine: effect of fasting and feeding. J Clin Invest 1990;85:804–11.
36. Tremblay AJ, Lamarche B, Guay V, Charest A, Lemelin V, Couture P. Short-term, high-fat diet increases the expression of key intestinal genes involved in lipoprotein metabolism in healthy men. Am J Clin Nutr 2013;98:32–41.
37. Nosaka N, Maki H, Suzuki Y, Haruna H, Ohara A, Kasai M, Tsuji H, Aoyama T, Okazaki M, Igarashi O, et al. Effects of margarine containing medium-chain triacylglycerols on body fat reduction in humans. J Atheroscler Thromb 2003;10:290–8.
38. Nosaka N, Kasai M, Nakamura M, Takahashi I, Itakura M, Takeuchi H, Aoyama T, Tsuji H, Okazaki M, Kondo K. Effects of dietary medium-chain triacylglycerols on serum lipoproteins and biochemical parameters in healthy men. Biosci Biotechnol Biochem 2002;66:1713–8.
39. Woollett LA, Spady DK, Dietschy JM. Mechanisms by which saturated triacylglycerols elevate the plasma low density lipoprotein-cholesterol concentration in hamsters: differential effects of fatty acid chain length. J Clin Invest 1989;84:119–28.
40. Tholstrup T, Ehnholm C, Jauhiainen M, Petersen M, Hoy CE, Lund P, Sandstrom B. Effects of medium-chain fatty acids and oleic acid on blood lipids, lipoproteins, glucose, insulin, and lipid transfer protein activities. Am J Clin Nutr 2004;79:564–9.
41. Geelen MJ, Schoots WJ, Bijleveld C, Beynen AC. Dietary medium-chain fatty acids raise and (n-3) polyunsaturated fatty acids lower hepatic triacylglycerol synthesis in rats. J Nutr 1995;125:2449–56.
42. Sallee VL, Dietschy JM. Determinants of intestinal mucosal uptake of short- and medium-chain fatty acids and alcohols. J Lipid Res 1973;14:475–84.
43. Westergaard H, Dietschy JM. The mechanism whereby bile acid micelles increase the rate of fatty acid and cholesterol uptake into the intestinal mucosal cell. J Clin Invest 1976;58:97–108.
44. 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.
45. Furman RH, Howard RP, Brusco OJ, Alaupovic P. Effects of medium chain length triglyceride (MCT) on serum lipids and lipoproteins in familial hyperchylomicronemia (dietary fat-induced lipemia) and dietary carbohydrate-accentuated lipemia. J Lab Clin Med 1965;66:912–26.
46. Kasai M, Maki H, Nosaka N, Aoyama T, Ooyama K, Uto H, Okazaki M, Igarashi O, Kondo K. Effect of medium-chain triglycerides on the postprandial triglyceride concentration in healthy men. Biosci Biotechnol Biochem 2003;67:46–53.
47. Wang H, Zhan R, Hunter F, Du J, Black D. Effect of acute feeding of diets of varying fatty acid composition on intestinal apolipoprotein expression in the newborn swine. Pediatr Res 1996;39:1078–84.
48. Kritchevsky D, Tepper SA. Influence of medium-chain triglyceride (MCT) on cholesterol metabolism in rats. J Nutr 1965;86:67–72.
49. Leveille GA, Pardini RS, Tillotson JA. Influence of medium-chain triglycerides on lipid metabolism in the rat. Lipids 1967;2:287–94.
50. Takase S, Morimoto A, Nakanishi M, Muto Y. Long-term effect of medium-chain triglyceride on hepatic enzymes catalyzing lipogenesis and cholesterogenesis in rats. J Nutr Sci Vitaminol (Tokyo) 1977;23:43–51.