Chapitre 18 Effets de la substitution des acides gras saturés par des acides gras polyinsaturés oméga-6 sur le métabolisme des lipoprotéines chez des hommes avec une dyslipoprotéinémie associée à la résistance à l'insuline

Jean-Philippe Drouin-Chartier, André J. Tremblay, Marie-Claude Lépine, Valéry Lemelin, Benoît Lamarche, Patrick Couture

L'article présenté dans ce chapitre s'intitule :

Substitution of dietary omega-6 polyunsaturated fatty acids for saturated fatty acids decreases LDL apoB-100 production rate in men with dyslipidemia associated with insulin resistance: a randomized controlled trial

Cet article est publié dans la revue :

The American Journal of Clinical Nutrition 2018;107(1):26-34.

Résumé

Introduction : La consommation d'AGPI ω-6 en substitution des AGS est recommandée en prévention cardiovasculaire. L'impact de cette substitution sur le métabolisme des lipoprotéines chez des sujets avec une dyslipoprotéinémie associée à la RI n'a jamais été évalué en profondeur.

Objectif : Nous avons évalué l'impact de la substitution des AGS par des AGPI ω-6 sur la cinétique des lipoprotéines avec une apo B et sur l'expression intestinale des gènes clés impliqués dans le métabolisme des lipoprotéines chez des hommes avec une dyslipidémie associée à la RI.

Design : Des hommes dyslipidémiques et RI (n=36) ont été recrutés pour cette étude contrôlée, randomisée, en chassé-croisé et à double-insu. Les sujets ont consommé dans un ordre aléatoire une alimentation contrôlée riche en AGS (AGS : 13,4% de l'énergie ; AGPI ω-6 : 4,0%) et une alimentation contrôlée riche en AGPI ω-6 (AGS : 6,0% ; AGPI ω-6 : 11,3%) pour des périodes de 4 semaines séparées par une période de repos de 4 semaines. À la fin de chacune des périodes expérimentales, la cinétique des lipoprotéines avec une apo B était mesurée en plus de l'expression intestinale des gènes clés impliqués dans le métabolisme des lipoprotéines chez tous les participants.

Résultats : La substitution des AGS pour des AGPI ω-6 n'a pas modifié la clairance (Δ=-3,8%, *P*=0,7) ni le taux de production (Δ=+1,2%, *P*=0,9) des LRT contenant l'apo B-48, bien qu'elle ait diminué l'expression intestinale des gènes *MTP* (Δ=-18,4%, *P*=0,006) et *apo B* (Δ=-16,6%, *P*=0,005). La substitution des AGS par des AGPI ω-6 a diminué la quantité plasmatique de particules LDL (Δ=-7,8%, *P*=0,005). Cette différence était attribuable à une diminution du taux de production de ces lipoprotéines suite à la consommation de la diète AGPI ω-6 (Δ=-10,0%; *P*=0,003).

Conclusions : Cette étude démontre que la substitution des AGS pour des AGPI ω-6 diminue la production et le nombre de particules LDL chez des hommes avec une dyslipidémie associée à la RI.

Title page

Substitution of dietary omega-6 polyunsaturated fatty acids for saturated fatty acids decreases LDL apoB-100 production rate in men with dyslipidemia associated with insulin resistance: a randomized controlled trial

Jean-Philippe Drouin-Chartier[1], André J. Tremblay[1], Marie-Claude Lépine[1], Valéry Lemelin[2], Benoît Lamarche[1], Patrick Couture[1,3]

Affiliations

1- Institute of Nutrition and Functional Foods, Laval University, Quebec City, Quebec, Canada
2- Department of Gastroenterology, CHU de Québec-Université Laval, Quebec City, Quebec, Canada
3- Lipid Research Center, CHU de Québec-Université Laval, Quebec City, Canada

Running head:

Substitution of ω-6 PUFAs for SFAs in insulin resistance

Corresponding author

Patrick Couture, MD, FRCP(C), PhD
Institute of Nutrition and Functional Foods (INAF)
2440, Hochelaga Blvd, Pavillon des Services
Laval University
Quebec City, Canada
G1V 0A6
Phone: 418-654-2106;
E-mail: patrick.couture@crchul.ulaval.ca

Source of support

Supported by the Canadian Institutes of Health Research (CIHR, MOP-106629). The funder had no role to play in the design of the study and in the interpretation of the results.

Trial registration

Clinicaltrials.gov NCT01934543

Abbreviations

ABC: ATP-binding cassette;

ACAC: Acetyl-CoA carboxylase;

ACAT2: Acetyl-CoA acetyltransferase 2;

ANGPTL: Angiopoietin-like;

apo: apolipoprotein;

ApoB48R: apolipoprotein B-48 receptor;

C: cholesterol;

CVD: cardiovascular disease;

DGAT: diglyceride acyltransferase;

FABP2: fatty acid-binding protein 2;

FATP4: fatty acid transport protein 4;

FCR: fractional catabolic rate;

HMR-CoAR: Hydroxymethylglutaryl-CoA reductase;

HNF4α: Hepatocyte nuclear factor 4 alpha;

IR: insulin resistance;

LDLR: LDL receptor;

LRP1: LDL receptor-related protein 1;

MGAT2: Alpha-1,6-mannosyl-glycoprotein 2-beta-N-acetylglucosaminyltransferase;

MTP: Microsomal triglyceride transfer protein;

MUFA: Monounsaturated fatty acid;

NPC1L1: Niemann-Pick C1-like 1;

PCR: Polymerase chain reaction;

PCSK9: Proprotein convertase subtilisin/kexin type 9;

PPAR: Peroxisome proliferator-activated receptor;

PR: production rate;

PS: pool size;

PUFA: Polyunsaturated fatty acid;

SAR1B: SAR1 gene homolog B;

SFA: Saturated fatty acid;

SRB1: Scavenger receptor class B type 1;

SREBP: Sterol regulatory element-binding protein;

TG: triglyceride;

TRL: Triglyceride-rich lipoprotein;

VLDLR: VLDL receptor;

ω: omega.

Abstract

Background: The substitution of omega (*ω*)-6 polyunsaturated fatty acids (PUFAs) for saturated fatty acids (SFAs) is advocated in cardiovascular disease prevention. The impact of this substitution on lipoprotein metabolism in subjects with dyslipidemia associated with insulin resistance (IR) remains unknown.

Objective: In men with dyslipidemia and IR, we evaluated the impact of substituting ω-6 PUFAs for SFAs on the *in vivo* kinetics of apolipoprotein (apo)B-containing lipoproteins and on the intestinal expression of key genes involved in lipoprotein metabolism.

Design: Dyslipidemic and IR men (n=36) were recruited for this double-blind, randomized, crossover, controlled trial. Subjects consumed, in a random order, a fully controlled diet rich in SFAs (SFAs: 13.4% of energy; ω-6 PUFAs: 4.0%) and a fully controlled diet rich in ω-6 PUFAs (SFAs: 6.0%; ω-6 PUFAs: 11.3%) for periods of 4 wk, separated by a 4-wk washout period. At the end of each diet, the *in vivo* kinetics of apoB-containing lipoproteins were measured and the intestinal expression of key genes involved in lipoprotein metabolism was quantified in duodenal biopsies taken from each participant.

Results: The substitution of ω-6 PUFAs for SFAs had no impact on TRL apoB-48 fractional catabolic rate (Δ = -3.8%, *P*=0.7) and production rate (Δ = +1.2%, *P* = 0.9), although it downregulated the intestinal expression of the microsomal triglyceride transfer protein (Δ = -18.4%, *P* = 0.006) and *apoB* (Δ = -16.6%, *P* = 0.005). The substitution of ω-6 PUFAs for SFAs decreased the LDL apoB-100 pool size (Δ = -7.8%; *P* = 0.005). This difference was attributed to a reduction in the LDL apoB-100 production rate after the substitution of ω-6 PUFAs for SFAs (Δ = -10.0%; *P* = 0.003).

Conclusions: This study demonstrates that the substitution of dietary ω-6 PUFAs for SFAs decreases the production and number of LDL particles in men with dyslipidemia and IR. This trial was registered at clinicaltrials.gov as NCT01934543.

Keywords: insulin resistance, lipoprotein metabolism, intestinal mRNA expression, omega-6 polyunsaturated fatty acids, saturated fatty acids

Introduction

A significant proportion of cardiovascular disease (CVD) is attributable to insulin resistance (IR), which affects > 25% of the population in North America.[1, 2] The atherogenic dyslipidemia associated with IR is characterized by both decreased HDL-cholesterol (C) concentrations and an overaccumulation of triglyceride-rich lipoproteins (TRLs).[3] The increased production rate (PR) of both hepatic apolipoprotein (apo) B-100-containing lipoproteins and intestinally derived apoB-48-containing TRLs, as well as the reduced catabolism of these subfractions, cause the overaccumulation of TRLs associated with IR.[4] Recently, our group also showed that subjects with IR present important alterations in the expression of key intestinal genes involved in lipoprotein homeostasis that could play a role in the overall dysregulation of TRL metabolism.[5] This is of significant interest because atherogenic dyslipidemia is undisputedly a major CVD risk factor for populations with IR.[6, 7]

Dietary guidelines advocate the consumption of omega (*ω*)-6 PUFAs instead of SFAs for the prevention of CVD, as they decrease LDL cholesterol concentrations.[8-10] Moreover, acute and chronic dietary studies show that the substitution of *ω*-6 PUFAs for SFAs decreases postprandial lipemia and apoB-48 secretion in insulin-sensitive subjects.[11-14] The cholesterol- and triglyceride-lowering effects of *ω*-6 PUFA consumption in place of SFAs are of particular interest for dyslipidemic and IR subjects. However, data on the impact of this dietary substitution on lipoprotein metabolism in subjects with dyslipidemia associated with IR are limited and mixed.[15-18] Therefore, our ability to define optimal, evidence-based dietary guidelines for this population at high CVD risk remains limited.

The general objective of this study was to characterize the homeostasis of lipoproteins in men with dyslipidemia and IR by examining how the substitution of dietary *ω*-6 PUFAs for SFAs modifies hepatic and intestinal lipoprotein metabolism. The primary objective was to examine the impact of the substitution of dietary *ω*-6 PUFAs for SFAs on the *in vivo* kinetics of apoB-containing lipoproteins. The secondary objective was to examine the impact of the consumption of dietary *ω*-6 PUFAs instead of SFAs on the expression of key intestinal genes that regulate the absorption, synthesis, assembly and secretion of lipids and lipoproteins. We hypothesized that, in dyslipidemic and IR men, the substitution of *ω*-6 PUFAs for SFAs would reduce the PR, stimulate the fractional catabolic rate (FCR) of apoB-containing lipoproteins, and regulate the intestinal expression of key genes involved in lipid and lipoprotein metabolism, as dietary fatty acids are known to affect the transcription of several genes.[19]

Methods

The Laval University Medical Center ethical review committee approved the research protocol. Written consent was obtained from all participants. This trial was registered at clinicaltrials.gov

(NCT01934543). The study was conducted between December 2013 and December 2014 at the Institute of Nutrition and Functional Food (INAF) of Laval University in the Quebec City area (Canada).

Study subjects

Thirty-six, unrelated, dyslipidemic men with IR were recruited. To be enrolled in the study, participants had to be between 18 and 65 y old, had to have fasting plasma TG concentrations ≥1.3 mmol/L (114 mg/dL) and <7.0 mmol/L (619 mg/dL) and fasting insulin concentrations ≥90 pmol/L. Fasting TG and insulin concentrations were measured during two different screening visits. The average concentration of TG and insulin had to meet these criteria. Participants had to have had a stable body weight for at least 3 mo prior to the screening, a BMI ≥25 kg/m^2 and a waist circumference ≥94 cm. Exclusion criteria included smoking (>1 cig/d); alcoholism; illicit drug consumption; extreme dyslipidemias (e.g., familial hypercholesterolemia); type 2 diabetes; a history of CVD or cancer; acute hepatic or renal disease (aspartate aminotransferase/alanine transaminase >1.5 x the upper limit of normal; creatinine levels >176 µmol/L; creatine phosphokinase >2 x the upper limit of normal); HIV; uncontrolled high blood pressure (>160/110 mm Hg); or any other condition that could interfere with participation in the study. Lipid-lowering medication had to be withdrawn 3 wk prior to the first screening visit and abstained from for the duration of the study. The use of antidepressants, antihypertensive drugs or levothyroxine was allowed only if the dose had been stable for at least 3 months prior to the study. Any other drug, dietary supplement or natural health product had to be stopped for the duration of the study. Participants had to notify one of the study coordinators if they had to initiate or modify any medication during the course of the study.

Study design

This study was designed as a double-blind, randomized, controlled, crossover study. Once enrolled, subjects initiated a 2-wk, run-in period to familiarize themselves with the instructions of the study (weeks -2 to 0). At week 0, subjects were randomized to either the SFA-PUFA or PUFA-SFA diet sequence. The randomization was performed with four blocks of 10 subjects with an allocation ratio of 1:1. Subjects with the SFA-PUFA sequence first consumed a fully controlled diet rich in SFAs and low in *ω*-6 PUFAs for 4 wk (weeks 0 to 4). The participants were then subjected to a fully controlled diet rich *ω*-6 PUFAs and low in SFAs diet for 4 wk (weeks 8 to 12) after a 4-wk washout period separating the two experimental diets (weeks 4 to 8). Given the crossover design, participants with the PUFA-SFA sequence consumed the two fully controlled diets in the reverse order. The two experimental diets were consumed under carefully controlled, isocaloric conditions to enable participants to maintain a constant weight during the study. The energy requirements of the study participants were estimated using Harris-Benedict's formula[20] and an assessment of their energy intakes from their completed, validated, web-based, self-administered food frequency questionnaires (FFQs).[21] Body weight was monitored on weekdays before lunch. If a participant's weight changed by ±2 kg from the diet-specific baseline value, the participant's caloric intake was adjusted to stop the

weight variation. Three meals and one snack were provided daily to all participants. A 7-d cyclic menu was used. On weekdays, subjects consumed their lunch meal at the Clinical Investigation Unit at the Institute of Nutrition and Functional Foods under the supervision of a research assistant. Other meals were packed and consumed at home. Participants were instructed to consume all the food, and only the food, that was provided to them. Subjects had free access to caffeine- and calorie-free beverages. Alcohol consumption was forbidden during the experimental diets and limited to ≤2 serving/d and ≤7/week during the run-in and the washout periods. Participants had to complete a daily checklist to identify foods that they had or had not consumed. Dietary compliance was measured using this checklist. Participants had to maintain a constant physical activity level throughout the study. Fasting blood samples were collected at the two screening visits, at the beginning of each diet and on the morning of the kinetic study at the end of each diet (weeks -4, -3, 0, 4, 8, 12). Kinetic studies were conducted on the last day (day 28) of each diet and duodenal biopsies were obtained on the day 26 of each diet. Forty-eight hours prior to the duodenal biopsies and kinetic studies, high-intensity physical activity was forbidden. Participants and coordinators were blinded until the final statistical analyses were conducted.

Composition of the experimental diets

The nutritional composition of the SFA diet was designed to represent the typical US diet while the nutritional composition of the *ω*-6 PUFA diet corresponded to the current US nutritional guidelines.[8] The two experimental diets provided ~35% of energy as fat, ~50% as carbohydrates and ~16% as protein (**Table 1**). The SFA diet contained 13.4% of energy as SFAs, 14.4% as MUFAs and 4.0% as *ω*-6 PUFAs. Dietary fats and SFAs were mainly provided from lard. The *ω*-6 PUFA diet provided 6.0% of energy as SFAs, 14.2% as MUFAs and 11.5% as *ω*-6 PUFAs. Safflower oil instead of lard was used to provide *ω*-6 PUFAs. Other foods were fat free or low in fat in both diets.

In the SFA diet, the use of lard provided an important amount of MUFAs, mainly oleic acid (18:1). To maintain the same MUFA content between the two diets, a small quantity of olive oil was substituted for safflower oil in the *ω*-6 PUFA diet. Thus, the *ω*-6 PUFA content of the *ω*-6 PUFA diet was ~2.0% lower than the SFA content of the SFA diet (11.5% vs. 13.4%). Overall, the main difference between the two experimental diets was the substitution of *ω*-6 PUFAs for SFAs. Other macro- and micronutrients were matched in both diets. The breakfast meal represented ~30% of the daily energy intake and the lunch and dinner meals each provided 35% of the daily energy intake. The composition of the diets was measured using Nutrition Data System for Research software (University of Minnesota). Dietetic technicians prepared the experimental diets throughout the study. Each food or ingredient was weighed with a precision of ±0.1 g.

Fasting plasma lipoprotein, glucose and insulin concentration measurements

Twelve-hour fasting, blood samples were obtained from an antecubital vein. Serum cholesterol and TG concentrations were determined with a Roche/Hitachi MODULAR analyzer (Roche Diagnostics, Indianapolis, IN, USA) using proper reagents. Blood glucose levels were measured by colorimetry and insulin concentrations were measured by electrochemiluminescence (Roche Diagnostics, Indianapolis, IN, USA). Commercial ELISA kits were used to measure levels of apoAV, apoC-II, apoC-III, apoE, angiopoietin-like 3 (ANGPTL3), C-reactive protein (CRP), cholesteryl ester transfer protein (CETP), hepatic lipase (HL), LDL receptor (LDLR), and lipoprotein lipase (LPL). The complete list of manufacturers is presented in the Supplemental Material.

Kinetic studies

To determine the kinetics of apoB-48- and apoB-100-containing lipoproteins, subjects underwent a primed-constant infusion of L-[5,5,5-D_3]leucine while they were maintained in a constant fed state on the last day of each diet. Subjects received 30 small, identical snacks every half hour for 15 h, each containing 1/30th of their estimated daily food intake. The composition of the snacks provided during the kinetic studies was identical to that of the diet. The snacks of the kinetic study performed at the end of the *ω*-6 PUFA diet provided 11.3% of energy as *ω*-6 PUFAs and 6.0% as SFAs. The snacks of the kinetic study performed at the end of the SFA diet provided 4.0% of energy as *ω*-6 PUFAs and 13.4% as SFAs. Blood samples were collected at predetermined times during the test. ApoB-48 and apoB-100 concentrations were measured by commercial ELISA kits (Shibayagi Co. Ltd., Alerchek Inc., respectively). Sample processing, laboratory measurements and lipoprotein kinetic analyses were performed by liquid chromatography-mass spectrometry with multiple reaction monitoring as previously described.[22]

Intestinal biopsies and extraction and quantification of total RNA

Biopsy samples were collected from the second portion of the duodenum during gastroduodenoscopy. Three samples (3 x 3 mm) were collected using single-use biopsy forceps and immediately flash frozen in liquid nitrogen and stored at -80°C before RNA extraction. Intestinal tissue samples were homogenized in 1 mL of QIAzol (Qiagen). RNA was extracted using an RNeasy kit (Qiagen). To eliminate any contaminating DNA, biopsies were treated with an RNase-free DNase set. Total RNA extraction and quantitative real-time polymerase chain reaction (PCR) were performed as previously described.[23] Primer sequences and gene descriptions are available in **Supplemental Table 1**. Quantitative real-time PCR measurements were performed by the CHU de Québec-Université Laval Research Center Gene Expression Platform (Quebec, Canada) and were compliant with MIQE guidelines.

Sample size estimate

A power calculation was conducted using the change in TRL apoB-48 PR as the primary outcome, and this calculation indicated that a sample size of 30 subjects would enable us to detect a clinically significant difference of 37% in TRL apoB-48 PR between the two diets with a power of 80% at a two–sided, 5% significance level. The within-patient SD for TRL apoB-48 PR used for this calculation was 50%, based on previous studies from our group.[24, 25] Studies conducted in healthy subjects (n < 10) reported significant effects on chylomicron metabolism associated with this dietary substitution.[11] A power calculation using the change in LDL apoB-100 FCR as a secondary outcome was also conducted. The within-patient standard deviation was fixed at 35%, based on previous studies from our group.[24, 25] A sample size of 30 subjects would allow us to detect a clinically significant difference of 26% in LDL apoB-100 FCR between the SFA and ω-6 PUFA diets with a power of 80% at a two-sided 5% significance level. This assumption is based on previous studies that reported significant effects on LDL apoB-100 clearance with the substitution of ω-6 PUFAs for SFAs.[12, 26] An estimated attrition rate of 20% was taken into account to determine that 36 subjects had to be recruited.

Statistics

Statistical analyses were performed using mixed models with JMP Pro software version 12.2.0. In the mixed models, treatment was treated as a fixed effect and subjects were treated as a random effect. Treatment sequence and diet-specific baseline values were included in the models as fixed effect covariates. Models for biochemical characteristics, kinetics, and mRNA gene expression were also adjusted for anthropometric characteristics that differed between the two treatments. Only covariates with significant effects were maintained in the models. The covariance structure was adjusted for each dependent variable to increase the fit of the data to the model. The model normality was assessed by the distribution of the scaled residual values. For additional exploratory analyses, a non-parametric Spearman's rank correlation test was performed to evaluate the associations among key intestinal genes involved in cholesterol metabolism with respect to changes in mRNA expression levels following the PUFA diet versus the SFA diet. Differences were considered to be statistically significant at P < 0.05.

Results

A flow chart of the study is presented in **Figure 1**. During the intervention, 5 subjects dropped out of the study for personal reasons (protocol judged too restrictive, n = 2; aversion to foods, n = 3). One subject was randomized although he did not meet the eligibility criteria and was excluded from the study during week 0. Baseline demographic and fasting biochemical characteristics of the 30 subjects who completed the study are presented in **Table 2**. Subjects exhibited features of IR and atherogenic dyslipidemia with elevated HOMA-IR indexes, waist circumferences, and fasting TG concentrations and reduced HDL cholesterol concentrations.

The reported compliance to the experimental diets was very high for both diets (>99%). No difference was observed at the end of the two diets in weight (P = 0.5) and BMI (P = 0.9) of the subjects. Waist circumference was slightly lower at the end of the SFA diet in comparison with the end of the ω-6 PUFA diet (Δ = -0.6 cm, P = 0.02).

The substitution of ω-6 PUFAs for SFAs had no impact on TRL apoB-48 PR, FCR and pool size (PS) (**Table 3**). Kinetic analyses, however, indicated that the substitution of ω-6 PUFAs for SFAs had a significant impact on LDL apoB-100 metabolism. The LDL apoB-100 PS was significantly lower after the ω-6 PUFA diet compared with the SFA diet. This was attributable to the lower LDL apoB-100 PR, as LDL apoB-100 FCR remained unchanged between the two diets. The kinetics of VLDL apoB-100 and of IDL apoB-100 were not modified by the diets. Also, no significant differences between the two diets were observed in the VLDL to LDL and the IDL to LDL conversion rates. Extensive biochemical analyses were conducted to determine if the change in LDL apoB-100 kinetics was associated with a variation in circulating molecules known to impact lipoprotein metabolism (**Table 4**). No changes were observed in the post-diet fasting concentrations of apoA-V, apoE, apoC-II, apoC-III, LPL, HL, ANGPTL3, CETP and LDLR. Finally, the substitution of ω-6 PUFAs for SFAs had no impact on insulin and glucose homeostasis, CRP levels and cholesterol and fasting TG concentrations. Nonetheless, LDL-C concentrations tended to be lower after the ω-6 PUFA diet compared to the SFA diet (P = 0.06).

Figure 2 presents the diet-induced changes in the duodenal mRNA expression levels of key genes involved in lipid metabolism. The substitution of dietary ω-6 PUFAs for SFAs significantly downregulated intestinal expression of hepatic nuclear factor-4α (*HNF4α*: Δ = -8.2 ± 4.6%; P = 0.008) and peroxisome proliferator-activated receptor α (*PPARα*: Δ = -8.6 ± 3.1%, P = 0.009). Among genes involved in lipoprotein assembly and transport, substituting ω-6 PUFAs for SFAs significantly upregulated intestinal expression of the VLDL receptor (*VLDLR*) (Δ = +19.5 ± 9.0%; P = 0.01) and downregulated intestinal expression of microsomal triglyceride transfer protein (*MTP*) (Δ = -18.4 ± 8.3%; P = 0.006), *apoB* (Δ = -16.6 ± 8.4%; P = 0.005), *apoAI* (Δ = -23.1 ± 12.0%; P = 0.002), and SAR1 homolog B (*SAR1B*) (Δ = -9.0 ± 4.7%; P = 0.04). Of the genes involved in cholesterol metabolism and transport, intestinal expression of ATP-binding cassette (*ABC*) *G5* and *ABCG8* were significantly lower after the ω-6 PUFA diet (-20.2 ± 9.9%; P = 0.002 and -17.3 ± 10.0%; P = 0.005, respectively). Similarly, intestinal expression of fatty acid binding protein-2 (*FABP2*) and fatty acid transport protein-4 (*FATP4*) were significantly lower after the ω-6 PUFA diet (Δ = -18.2 ± 8.2%; P = 0.006 and Δ = -12.5 ± 6.1%; P = 0.009, respectively). Finally, intestinal mRNA levels of mannosyl (α-1,6-)-glycoprotein β-1,2-N-acetylglucosaminyltransferase (*MGAT2*) and diacylglycerol O-acyltransferase 1 (*DGAT1*) were significantly lower following the ω-6 PUFA diet compared to the SFA diet (Δ = -6.8 ± 5.3%; P = 0.04 and Δ = -17.1 ± 12.1%; P = 0.01, respectively).

The validity of the duodenal model used was ascertained by correlation between changes in mRNA levels of different genes known to be regulated together. Among others, the diet-induced changes in *HNF4α* mRNA levels were significantly correlated with changes in *FABP2, FATP4, MTP, apoB*, *ABCG5, ABCG8*, *MGAT2* and *DGAT1* (**Supplemental Table 2**).

Discussion

This study evaluated the impact of the substitution of dietary *ω*-6 PUFAs for SFAs on lipoprotein metabolism in men with dyslipidemia associated with IR. Substituting *ω*-6 PUFAs for SFAs had no impact on TRL apoB-48 kinetics, but significantly decreased LDL apoB-100 PR and PS by 10.1% and 7.8%, respectively. The substitution of *ω*-6 PUFAs for SFAs also regulated intestinal expression of several key genes involved in lipoprotein metabolism including *MTP*, *apoB* and *VLDLR*. In summary, this study demonstrated that, in dyslipidemic and IR men, substitution of dietary *ω*-6 PUFAs for SFAs reduces LDL particle number by decreasing production of these lipoproteins.

To our knowledge, this study is the first to evaluate the impact of the substitution of *ω*-6 PUFAs for SFAs on TRL apoB-48 metabolism using tracer analyses in IR subjects. Postprandial overproduction of intestinal TRLs is a major concern for subjects with dyslipidemia and IR because of the atherogenic properties of apoB-48-containing remnant particles.[7, 27] Substitution of *ω*-6 PUFAs for SFAs reduces postprandial lipemia in non-dyslipidemic and insulin sensitive subjects.[11, 12, 14, 28, 29] However, the consumption of various types of dietary fat, including MUFAs, PUFAs, *ω*-3 PUFAs, SFAs, and medium-chain TGs, has also been reported to have a very limited impact on TRL apoB-48 metabolism and postprandial lipemia in subjects with IR.[18, 23, 30, 31] It is well recognized that VLDL apoB-100 PS and insulin sensitivity are the principal determinants of the overproduction of TRL apoB-48 in dyslipidemic and IR subjects.[5, 32] In this context, the absence of impact of *ω*-6 PUFAs on TRL apoB-48 metabolism may result from the neutral effect of *ω*-6 PUFA on VLDL apoB-100 PS and insulin sensitivity. The present study demonstrated that the substitution of *ω*-6 PUFAs for SFAs has no beneficial effects on TRL apoB-48 kinetics and postprandial lipemia in subjects with dyslipidemia associated with IR. Accordingly, accumulating evidence now suggests that the impact of dietary fat on postprandial lipoprotein metabolism and CVD risk factors is attenuated by IR.[33-35]

In comparison with SFAs, PUFAs are known to repress lipogenesis.[19] In this study, the substitution of *ω*-6 PUFAs for SFAs induced a nearly significant downregulation in the intestinal expression of the lipogenic nuclear transcription factor *SREBP1c* (*P*=0.1). Several other genes involved in cholesterol and fatty acid transport (*ABCG5*, *ABCG8*, *FABP2*, *FATP4*), TG synthesis (*MGAT2*, *DGAT1*) and chylomicron assembly and secretion (*apoB*, *MTP* and *SAR1B*) were also downregulated following the *ω*-6 PUFA diet. The concomitant downregulation in the intestinal expression of *PPARs* was unexpected considering that PUFAs are direct agonists of *PPARs*.[19, 36] Considering that IR is associated with a downregulation in *PPAR* expression and with important alterations in fat oxidation

metabolism,[35, 37] it remains likely that the impact of PUFAs on the intestinal expression of *PPARs* was repressed by IR. Overall, changes in the intestinal mRNA levels of key genes involved in intestinal lipid transport, synthesis and oxidation, as well as in chylomicron assembly and secretion following the ω-6 PUFA diet, suggest that the substitution of ω-6 PUFAs for SFAs promoted lipid uptake and retention within enterocytes. However, the absence of change in TRL apoB-48 PR between the two diets contrasts with these numerous changes in gene expression. Considering that plasma free fatty acids have the ability to directly enter enterocytes via the basolateral membrane,[38] exposure to high plasma concentrations of free fatty acids associated with IR probably maintained constant enterocyte lipid content and TRL apoB-48 PR levels between the two diets.[5, 32, 39, 40] Further mechanistic investigations are required to corroborate this hypothesis.

Previous kinetic studies in insulin-sensitive subjects showed that the consumption of ω-6 PUFAs in comparison with SFAs decreased LDL cholesterol concentrations and LDL PS by increasing LDL apoB-100 FCRs.[12, 14, 26, 28, 29, 41] Studies conducted in cell and animal models showed that ω-6 PUFAs increase LPL activity[29] and the expression of hepatic lipoprotein-receptor via PPAR-dependent and independent pathways.[42-44] Conversely, SFAs increase the apoC-III concentration[45] and reduce HL activity[46] and LDLR concentrations,[47] leading to decreased lipoprotein uptake by the liver.[48] However, these mechanisms were all described in insulin sensitive models. In this study, the -7.8% reduction in LDL apoB-100 PS after the ω-6 PUFA diet resulted from the -10.1% decrease in LDL apoB-100 PR. Our results suggest that the reduction in LDL apoB-100 PR could be the outcome of an increased direct clearance of larger TG-rich particles, upstream of LDL production. In that regard, the upregulation of the *VLDLR* gene induced by the substitution of ω-6 PUFAs for SFAs may have increased the uptake of TG-rich apoB-100 containing-particles in the intestine and, potentially, in other peripheral tissues.[49-51] This remains, however, only a hypothesis at this stage. Nonetheless, this study demonstrated that substitution of dietary ω-6 PUFAs for SFAs decreases LDL apoB-100 PS by reducing LDL apoB-100 PR in dyslipidemic and IR men.

The recommendation that patients with dyslipidemia associated with IR or type 2 diabetes should reduce SFA consumption to prevent CVD was recently reaffirmed.[10] However, these guidelines were primarily based on data from studies conducted in an insulin-sensitive population[52-54] and the cardiovascular benefits of a high consumption of ω-6 PUFAs in place of SFAs was challenged by reappraisal of data from the Sydney Diet Heart Study and the Minnesota Coronary Experiment.[55-57] Nonetheless, considering that the LDL apoB-100 particle number is more closely related to coronary risk than the mass of cholesterol within the particles,[58] the reduction in LDL apoB-100 PS measured in our fully controlled, dietary investigation is of particular interest. Our data suggest that the substitution of ω-6 PUFAs for SFAs could provide cardiovascular benefits in the long term for subjects with dyslipidemia associated with IR. Additional investigation in IR subjects on the impact of this

dietary substitution on other cardiometabolic risk factors (e.g. LDL oxidation, inflammation) are required.

The crossover design and fully-controlled diets are major strengths of our dietary intervention and enabled evaluation of the impact of the substitution of *ω*-6 PUFAs for SFAs *per se,* as recommended in dietary guidelines. Additionally, the kinetic analyses and the quantification of intestinal gene expression provided extensive mechanistic explanations of the reported observations. However, the absence of hepatic and peripheral expression of key genes involved in lipoprotein metabolism may limit the interpretation of changes measured at the duodenal level. It cannot be excluded that the limited length of the dietary interventions attenuated the magnitude of some effects. Finally, the generalizability of the results is limited by the fact that only men were included in the present study.

In conclusion, our study showed that the substitution of dietary *ω*-6 PUFAs for SFAs in men with dyslipidemia associated with IR had no impact on TRL apoB-48 kinetics but regulated the intestinal expression of several key genes involved in lipoprotein metabolism. More importantly, this dietary substitution reduced LDL particle numbers by decreasing the production of these particles.

Acknowledgements

The authors are grateful to the study subjects for their excellent collaboration and to the dedicated work of Steeve Larouche, Michèle Valotaire and the staff of the metabolic kitchen.

Conflicts of interest statement

This study was funded by the Canadian Institutes of Health Research (CIHR, MOP-106629). The funder had no role to play in the design of the study and in the interpretation of the results. In the last 5 years, **PC** has received funding from the CIHR, Agriculture and Agri-Food Canada (Growing Forward program supported by the Dairy Farmers of Canada, Canola Council of Canada, Flax Council of Canada, and Dow Agrosciences), Dairy Research Institute, Dairy Australia, Danone Institute, Merck Frosst, Pfizer, Amgen, Sanofi, Kaneka Corporation, and Atrium Innovations. **BL** is Chair of Nutrition at Université Laval, which is supported by private endowments from Pfizer, La Banque Royale du Canada and Provigo-Loblaws. BL has received funding in the last 5 years from the CIHR, the Natural Sciences and Engineering Research Council of Canada, Agriculture and Agri-Food Canada (Growing Forward program supported by the Dairy Farmers of Canada, Canola Council of Canada, Flax Council of Canada, Dow Agrosciences), Dairy Research Institute, Dairy Australia, Danone Institute, Merck Frosst, Pfizer and Atrium Innovations. BL serves as the Chair of the peer-review Expert Scientific Advisory Council of Dairy Farmers of Canada. He is also an advisory board member of the Canadian Nutrition Society and the Conseil pour les initiatives de progrès en alimentation and has served as an advisory expert for the Saturated Fat panel of the Heart and Stroke Foundation of Canada. BL has also received speaker honoraria from the International Chair on

Cardiometabolic Risk, Dairy Farmers of Canada and the World Dairy Platform. **JPDC** is the recipient of doctoral scholarships from the Canadian Institute of Health Research and the Fonds de Recherche du Québec – Santé. JPDC has received speaker honoria from the Dairy Farmers of Canada. AJT, M-CL, VL no conflicts of interest.

Authors' contributions

The author's responsibilities were as follows - PC and BL: designed the study; JPDC, AJT, MCL, VL: conducted the research; JPDC, AJT, BL, and PC: analyzed the data. JPDC, AJT, BL, and PC: wrote the manuscript. PC: had the primary responsibility for the final content of the manuscript. All authors approved the manuscript.

References

1. Ford ES, Giles WH, Dietz WH. Prevalence of the metabolic syndrome among US adults: findings from the third National Health and Nutrition Examination Survey. *JAMA* 2002;287(3):356-9.

2. Malik S, Wong ND, Franklin SS, Kamath TV, L'Italien GJ, Pio JR, Williams GR. Impact of the metabolic syndrome on mortality from coronary heart disease, cardiovascular disease, and all causes in United States adults. *Circulation* 2004;110(10):1245-50.

3. Després JP. Dyslipidaemia and obesity. *Baillieres Clin Endocrinol Metab* 1994;8(3):629-60.

4. Hogue JC, Lamarche B, Tremblay AJ, Bergeron J, Gagne C, Couture P. Evidence of increased secretion of apolipoprotein B-48-containing lipoproteins in subjects with type 2 diabetes. *J Lipid Res* 2007;48(6):1336-42.

5. Couture P, Tremblay AJ, Kelly I, Lemelin V, Droit A, Lamarche B. Key intestinal genes involved in lipoprotein metabolism are downregulated in dyslipidemic men with insulin resistance. *J Lipid Res* 2014;55(1):128-37.

6. Nordestgaard BG, Benn M, Schnohr P, Tybjaerg-Hansen A. Nonfasting triglycerides and risk of myocardial infarction, ischemic heart disease, and death in men and women. *JAMA* 2007;298(3):299-308.

7. McNamara JR, Shah PK, Nakajima K, Cupples LA, Wilson PW, Ordovas JM, Schaefer EJ. Remnant-like particle (RLP) cholesterol is an independent cardiovascular disease risk factor in women: results from the Framingham Heart Study. *Atherosclerosis* 2001;154(1):229-36.

8. Harris WS, Mozaffarian D, Rimm E, Kris-Etherton P, Rudel LL, Appel LJ, Engler MM, Engler MB, Sacks F. Omega-6 fatty acids and risk for cardiovascular disease: a science advisory from the American Heart Association Nutrition Subcommittee of the Council on Nutrition, Physical Activity, and Metabolism; Council on Cardiovascular Nursing; and Council on Epidemiology and Prevention. *Circulation* 2009;119(6):902-7.

9. Mensink RP, Zock PL, Kester ADM, Katan MB. Effects of dietary fatty acids and carbohydrates on the ratio of serum total to HDL cholesterol and on serum lipids and apolipoproteins: a meta-analysis of 60 controlled trials. *Am J Clin Nutr* 2003;77(5):1146-55.

10. 8. Cardiovascular Disease and Risk Management. *Diabetes Care* 2016;39 Suppl 1:S60-71.

11. Weintraub MS, Zechner R, Brown A, Eisenberg S, Breslow JL. Dietary polyunsaturated fats of the W-6 and W-3 series reduce postprandial lipoprotein levels. Chronic and acute effects of fat saturation on postprandial lipoprotein metabolism. *J Clin Invest* 1988;82(6):1884-93.

12. Demacker PN, Reijnen IG, Katan MB, Stuyt PM, Stalenhoef AF. Increased removal of remnants of triglyceride-rich lipoproteins on a diet rich in polyunsaturated fatty acids. *Eur J Clin Invest* 1991;21(2):197-203.

13. Zampelas A, Peel AS, Gould BJ, Wright J, Williams CM. Polyunsaturated fatty acids of the n-6 and n-3 series: effects on postprandial lipid and apolipoprotein levels in healthy men. *Eur J Clin Nutr* 1994;48(12):842-8.

14. Bergeron N, Havel RJ. Influence of diets rich in saturated and omega-6 polyunsaturated fatty acids on the postprandial responses of apolipoproteins B-48, B-100, E, and lipids in triglyceride-rich lipoproteins. *Arterioscler Thromb Vasc Biol* 1995;15(12):2111-21.

15. Summers LK, Fielding BA, Bradshaw HA, Ilic V, Beysen C, Clark ML, Moore NR, Frayn KN. Substituting dietary saturated fat with polyunsaturated fat changes abdominal fat distribution and improves insulin sensitivity. *Diabetologia* 2002;45(3):369-77.

16. Bjermo H, Iggman D, Kullberg J, Dahlman I, Johansson L, Persson L, Berglund J, Pulkki K, Basu S, Uusitupa M, et al. Effects of n-6 PUFAs compared with SFAs on liver fat, lipoproteins, and inflammation in abdominal obesity: a randomized controlled trial. *Am J Clin Nutr* 2012;95(5):1003-12.

17. Heine RJ, Mulder C, Popp-Snijders C, van der Meer J, van der Veen EA. Linoleic-acid-enriched diet: long-term effects on serum lipoprotein and apolipoprotein concentrations and insulin sensitivity in noninsulin-dependent diabetic patients. *Am J Clin Nutr* 1989;49(3):448-56.

18. Masson CJ, Mensink RP. Exchanging saturated fatty acids for (n-6) polyunsaturated fatty acids in a mixed meal may decrease postprandial lipemia and markers of inflammation and endothelial activity in overweight men. *J Nutr* 2011;141(5):816-21.

19. Desvergne B, Michalik L, Wahli W. Transcriptional regulation of metabolism. *Physiol Rev* 2006;86(2):465-514.

20. Harris JA, Benedict FG. A Biometric Study of Human Basal Metabolism. *Proc Natl Acad Sci* 1918;4(12):370-3.

21. Labonte ME, Cyr A, Baril-Gravel L, Royer MM, Lamarche B. Validity and reproducibility of a web-based, self-administered food frequency questionnaire. *Eur J Clin Nutr* 2012;66(2):166-73.

22. Tremblay AJ, Lamarche B, Kelly I, Charest A, Lepine MC, Droit A, Couture P. Effect of sitagliptin therapy on triglyceride-rich lipoprotein kinetics in patients with type 2 diabetes. *Diabetes Obes Metab* 2014;16(12):1223-9.

23. Tremblay AJ, Lamarche B, Labonte ME, Lepine MC, Lemelin V, Couture P. Dietary medium-chain triglyceride supplementation has no effect on apolipoprotein B-48 and apolipoprotein B-100 kinetics in insulin-resistant men. *Am J Clin Nutr* 2014;99(1):54-61.

24. Tremblay AJ, Lamarche B, Cohn JS, Hogue JC, Couture P. Effect of ezetimibe on the in vivo kinetics of apoB-48 and apoB-100 in men with primary hypercholesterolemia. *Arterioscler Thromb Vasc Biol* 2006;26(5):1101-6.

25. 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(7):1463-71.

26. Shepherd J, Packard CJ, Grundy SM, Yeshurun D, Gotto AM, Jr., Taunton OD. Effects of saturated and polyunsaturated fat diets on the chemical composition and metabolism of low density lipoproteins in man. *J Lipid Res* 1980;21(1):91-9.

27. Pal S, Semorine K, Watts GF, Mamo J. Identification of lipoproteins of intestinal origin in human atherosclerotic plaque. *Clin Chem Lab Med* 2003;41(6):792-5.

28. Cortese C, Levy Y, Janus ED, Turner PR, Rao SN, Miller NE, Lewis B. Modes of action of lipid-lowering diets in man: studies of apolipoprotein B kinetics in relation to fat consumption and dietary fatty acid composition. *Eur J Clin Invest* 1983;13(1):79-85.

29. van Schalkwijk DB, Pasman WJ, Hendriks HF, Verheij ER, Rubingh CM, van Bochove K, Vaes WH, Adiels M, Freidig AP, de Graaf AA. Dietary medium chain fatty acid supplementation leads to reduced VLDL lipolysis and uptake rates in comparison to linoleic acid supplementation. *PLoS One* 2014;9(7):e100376.

30. Tremblay AJ, Lamarche B, Hogue JC, Couture P. n-3 Polyunsaturated Fatty Acid Supplementation Has No Effect on Postprandial Triglyceride-Rich Lipoprotein Kinetics in Men with Type 2 Diabetes. *J Diabetes Res* 2016;2016:2909210.

31. Teng KT, Chang CY, Kanthimathi MS, Tan AT, Nesaretnam K. Effects of amount and type of dietary fats on postprandial lipemia and thrombogenic markers in individuals with metabolic syndrome. *Atherosclerosis* 2015;242(1):281-7.

32. Lewis GF, O'Meara NM, Soltys PA, Blackman JD, Iverius PH, Pugh WL, Getz GS, Polonsky KS. Fasting hypertriglyceridemia in noninsulin-dependent diabetes mellitus is an important predictor of postprandial lipid and lipoprotein abnormalities. *J Clin Endocrinol Metab* 1991;72(4):934-44.

33. Lefevre M, Champagne CM, Tulley RT, Rood JC, Most MM. Individual variability in cardiovascular disease risk factor responses to low-fat and low-saturated-fat diets in men: body mass index, adiposity, and insulin resistance predict changes in LDL cholesterol. *Am J Clin Nutr* 2005;82(5):957-63; quiz 1145-6.

34. Chiu S, Williams PT, Dawson T, Bergman RN, Stefanovski D, Watkins SM, Krauss RM. Diets high in protein or saturated fat do not affect insulin sensitivity or plasma concentrations of lipids and lipoproteins in overweight and obese adults. *J Nutr* 2014;144(11):1753-9.

35. Boyle KE, Canham JP, Consitt LA, Zheng D, Koves TR, Gavin TP, Holbert D, Neufer PD, Ilkayeva O, Muoio DM, et al. A high-fat diet elicits differential responses in genes coordinating oxidative metabolism in skeletal muscle of lean and obese individuals. *J Clin Endocrinol Metab* 2011;96(3):775-81.

36. Kliewer SA, Sundseth SS, Jones SA, Brown PJ, Wisely GB, Koble CS, Devchand P, Wahli W, Willson TM, Lenhard JM, et al. Fatty acids and eicosanoids regulate gene expression through direct interactions with peroxisome proliferator-activated receptors alpha and gamma. *Proc Natl Acad Sci* 1997;94(9):4318-23.

37. Li G-S, Liu X-H, Zhu HUA, Huang LAN, Liu Y-L, Ma C-M. Skeletal muscle insulin resistance in hamsters with diabetes developed from obesity is involved in abnormal skeletal muscle LXR, PPAR and SREBP expression. *Exp Ther Med* 2016;11(6):2259-69.

38. Storch J, Zhou YX, Lagakos WS. Metabolism of apical versus basolateral sn-2-monoacylglycerol and fatty acids in rodent small intestine. *J Lipid Res* 2008;49(8):1762-9.

39. Duez H, Lamarche B, Valero R, Pavlic M, Proctor S, Xiao C, Szeto L, Patterson BW, Lewis GF. Both intestinal and hepatic lipoprotein production are stimulated by an acute elevation of plasma free fatty acids in humans. *Circulation* 2008;117(18):2369-76.

40. Fielding BA, Callow J, Owen RM, Samra JS, Matthews DR, Frayn KN. Postprandial lipemia: the origin of an early peak studied by specific dietary fatty acid intake during sequential meals. *Am J Clin Nutr* 1996;63(1):36-41.

41. Turner JD, Le NA, Brown WV. Effect of changing dietary fat saturation on low-density lipoprotein metabolism in man. *Am J Physiol* 1981;241(1):E57-63.

42. Fernandez ML, McNamar DJ. Dietary fat-mediated changes in hepatic apoprotein B/E receptor in the guinea pig: effect of polyunsaturated, monounsaturated, and saturated fat. *Metabolism* 1989;38(11):1094-102.

43. Rumsey SC, Galeano NF, Lipschitz B, Deckelbaum RJ. Oleate and other long chain fatty acids stimulate low density lipoprotein receptor activity by enhancing acyl coenzyme A:cholesterol acyltransferase activity and altering intracellular regulatory cholesterol pools in cultured cells. *J Biol Chem* 1995;270(17):10008-16.

44. Dallongeville J, Bauge E, Tailleux A, Peters JM, Gonzalez FJ, Fruchart JC, Staels B. Peroxisome proliferator-activated receptor alpha is not rate-limiting for the lipoprotein-lowering action of fish oil. *J Biol Chem* 2001;276(7):4634-9.

45. Jackson KG, Wolstencroft EJ, Bateman PA, Yaqoob P, Williams CM. Greater enrichment of triacylglycerol-rich lipoproteins with apolipoproteins E and C-III after meals rich in saturated fatty acids than after meals rich in unsaturated fatty acids. *Am J Clin Nutr* 2005;81(1):25-34.

46. Dreon DM, Fernstrom HA, Campos H, Blanche P, Williams PT, Krauss RM. Change in dietary saturated fat intake is correlated with change in mass of large low-density-lipoprotein particles in men. *Am J Clin Nutr* 1998;67(5):828-36.

47. Mustad VA, Etherton TD, Cooper AD, Mastro AM, Pearson TA, Jonnalagadda SS, Kris-Etherton PM. Reducing saturated fat intake is associated with increased levels of LDL receptors on mononuclear cells in healthy men and women. *J Lipid Res* 1997;38(3):459-68.

48. Jackson KG, Maitin V, Leake DS, Yaqoob P, Williams CM. Saturated fat-induced changes in Sf 60-400 particle composition reduces uptake of LDL by HepG2 cells. *J Lipid Res* 2006;47(2):393-403.

49. Takahashi S, Sakai J, Fujino T, Hattori H, Zenimaru Y, Suzuki J, Miyamori I, Yamamoto TT. The very low-density lipoprotein (VLDL) receptor: characterization and functions as a peripheral lipoprotein receptor. *J Atheroscler Thromb* 2004;11(4):200-8.

50. Tao H, Aakula S, Abumrad NN, Hajri T. Peroxisome proliferator-activated receptor-gamma regulates the expression and function of very-low-density lipoprotein receptor. *Am J Physiol Endocrinol Metab* 2010;298(1):E68-79.

51. Tacken PJ, Hofker MH, Havekes LM, van Dijk KW. Living up to a name: the role of the VLDL receptor in lipid metabolism. *Curr Opin Lipidol* 2001;12(3):275-9.

52. Mensink RP, Katan MB. Effect of dietary fatty acids on serum lipids and lipoproteins. A meta-analysis of 27 trials. *Arterioscler Thromb* 1992;12(8):911-9.

53. Mensink RP, Zock PL, Kester AD, Katan MB. Effects of dietary fatty acids and carbohydrates on the ratio of serum total to HDL cholesterol and on serum lipids and apolipoproteins: a meta-analysis of 60 controlled trials. *Am J Clin Nutr* 2003;77(5):1146-55.

54. Mozaffarian D, Micha R, Wallace S. Effects on coronary heart disease of increasing polyunsaturated fat in place of saturated fat: a systematic review and meta-analysis of randomized controlled trials. *PLoS Med* 2010;7(3):e1000252.

55. Ramsden CE, Zamora D, Leelarthaepin B, Majchrzak-Hong SF, Faurot KR, Suchindran CM, Ringel A, Davis JM, Hibbeln JR. Use of dietary linoleic acid for secondary prevention of coronary heart disease and death: evaluation of recovered data from the Sydney Diet Heart Study and updated meta-analysis. *BMJ* 2013;346:e8707.

56. Ramsden CE, Zamora D, Majchrzak-Hong S, Faurot KR, Broste SK, Frantz RP, Davis JM, Ringel A, Suchindran CM, Hibbeln JR. Re-evaluation of the traditional diet-heart hypothesis: analysis of recovered data from Minnesota Coronary Experiment (1968-73). *BMJ* 2016;353:i1246.

57. Chowdhury R, Warnakula S, Kunutsor S, Crowe F, Ward HA, Johnson L, Franco OH, Butterworth AS, Forouhi NG, Thompson SG, et al. Association of dietary, circulating, and supplement fatty acids with coronary risk: a systematic review and meta-analysis. *Ann Intern Med* 2014;160(6):398-406.

58. Thanassoulis G, Williams K, Ye K, Brook R, Couture P, Lawler PR, de Graaf J, Furberg CD, Sniderman A. Relations of change in plasma levels of LDL-C, non-HDL-C and apoB with risk reduction from statin therapy: a meta-analysis of randomized trials. *J Am Heart Assoc* 2014;3(2):e000759.

Tables

Table 18-1 Composition of the experimental diets

	SFA diet	*ω*-6 PUFA diet
Energy (kcal)	3288 ± 302	3296 ± 299
Alcohol (%)	0.0	0.0
Protein (%)	16.0	16.0
Carbohydrate (%)	49.3	49.7
Lipids (%)	34.7	34.3
Lipids (g)	127.9 ± 11.7	128.2 ± 11.6
SFA (%)	13.4	6.0
SFA (g)	49.4 ± 4.5	22.6 ± 2.1
Butyric acid (4:0) (g)	0.5 ± 0.0	0.3 ± 0.0
Caproic acid (6:0) (g)	0.2 ± 0.0	0.1 ± 0.0
Caprylic acid (8:0) (g)	0.3 ± 0.0	0.2 ± 0.0
Capric acid (10:0) (g)	0.5 ± 0.1	0.3 ± 0.0
Lauric acid (12:0) (g)	1.2 ± 0.1	0.9 ± 0.1
Myristic acid (14:0) (g)	3.0 ± 0.3	1.2 ± 0.1
Palmitic acid (16:0) (g)	28.2 ± 2.6	14.0 ± 1.3
Margaric acid (17:0) (g)	0.0 ± 0.0	0.0 ± 0.0
Stearic acid (18:0) (g)	14.5 ± 1.3	4.8 ± 0.4
Arachidic acid (20:0) (g)	0.1 ± 0.0	0.2 ± 0.0
Behenic acid (22:0) (g)	0.1 ± 0.0	0.1 ± 0.0
MUFA (%)	14.4	14.2
MUFA (g)	52.9 ± 4.9	53.0 ± 4.8
PUFA (%)	4.5	11.8
PUFA (g)	16.6 ± 1.5	44.0 ± 4.0
Linoleic acid (18:2) (g)	14.4 ± 1.3	41.8 ± 3.8
Linolenic acid (18:3) (g)	1.6 ± 0.1	1.6 ± 0.1
Arachidonic acid (20:4) (g)	0.2 ± 0.0	0.2 ± 0.0
Eicosapentanoic acid (20:5) (g)	0.1 ± 0.0	0.1 ± 0.0
Docosapentanoic acid (22:5) (g)	0.1 ± 0.0	0.0 ± 0.0
Docosahexanoic acid (22:6) (g)	0.2 ± 0.0	0.2 ± 0.0
ω-6 PUFA (%)	4.0	11.3
ω-6 PUFA (g)	14.7± 1.4	42.1 ± 3.8
ω-3 PUFA (%)	0.5	0.5
ω-3 PUFA (g)	1.9 ± 0.2	1.9 ± 0.2
PUFA:SFA ratio	0.3	2.0
Trans fatty acid (%)	0.5	0.5
Dietary cholesterol (mg)	328 ± 30	329 ± 30
Fiber (g)	38.2 ± 3.5	38.2 ± 3.5
Sodium (mg)	2934 ± 270	2944 ± 267

[1] Data are presented as the mean ± standard deviation. SFA: saturated fatty acids; ω: omega; PUFA: polyunsaturated fatty acids; MUFA: monounsaturated fatty acids.

Table 18-2 Baseline (week 0) anthropometric and fasting biochemical characteristics of the subjects (n=30)

Characteristics	
Age (year)	39.5 ± 12.2
Weight (kg)	100.4 ± 14.3
BMI (kg/m^2)	32.7 ± 3.6
Waist circumference (cm)	110.8 ± 9.6
Systolic blood pressure (mm Hg)	126 ± 10
Diastolic blood pressure (mm Hg)	75 ± 9
Total-C (mmol/L)	5.39 ± 1.15
TG (mmol/L)	2.44 ± 1.49
HDL-C (mmol/L)	1.09 ± 0.20
LDL-C (mmol/L)	3.12 ± 0.85
Non HDL-C (mmol/L)	4.30 ± 1.10
Total-C/HDL-C	5.06 ± 1.18
Glucose (mmol/L)	5.34 ± 0.56
Insulin (pmol/L)	136 ± 52
HOMA-IR index	4.68 ± 1.91

[1] Data are presented as the mean ± SD. C: cholesterol; TG: triglyceride.

Table 18-3 Kinetic parameters of TRL apoB-48 and VLDL, IDL and LDL apoB-100 of the subjects (n=30) at the end of each diet[1]

	SFA	ω-6 PUFA	%Δ	*P*[2]
TRL apoB-48				
PS (mg)	55.0 ± 32.1	57.4 ± 28.6	+4.2 ± 14.2	0.7
FCR (pools/d)	8.38 ± 4.47	8.07 ± 3.19	-3.8 ± 11.9	0.7
PR (mg/kg/d)	4.05 ± 2.42	4.10 ± 1.44	+1.2 ± 12.6	0.9
VLDL apoB-100				
PS (mg)	596 ± 378	632 ± 423	+6.0 ± 15.3	0.5
FCR (pools/d)	7.79 ± 4.07	7.65 ± 2.62	-1.8 ± 11.3	0.9
PR (mg/kg/d)	39.4 ± 13.5	42.7 ± 13.0	+8.4 ± 8.6	0.2
Direct catabolic rate (%)	18.1 ± 23.4	16.8 ± 20.8	-7.2 ± 31.6	0.7
IDL apoB-100				
PS (mg)	124.9 ± 47.3	118.8 ± 49.1	-4.9 ± 9.9	0.2
FCR (pools/d)	20.5 ± 21.6	19.5 ± 9.4	-4.9 ± 21.0	0.7
PR (mg/kg/d)	23.3 ± 22.2	22.1 ± 9.8	-5.2 ± 18.9	0.7
Direct catabolic rate (%)	73.4 ± 39.9	80.4 ± 35.4	+9.5 ± 13.3	0.3
LDL apoB-100				
PS (mg)	5126 ± 2215	4726 ± 2261	-7.8 ± 11.3	0.005
FCR (pools/d)	0.37 ± 0.12	0.37 ± 0.14	+0.0 ± 8.1	0.9
PR (mg/kg/d)	17.9 ± 7.3	16.1 ± 7.2	-10.1 ± 10.6	0.003
Conversion rate				
VLDL to IDL (%)	48.5 ± 18.9	50.2 ± 18.1	+3.5 ± 9.8	0.6
VLDL to LDL (%)	33.5 ± 16.3	33.0 ± 16.6	-1.5 ± 12.7	0.9
IDL to LDL (%)	26.5 ± 39.9	19.5 ± 35.4	-26.4 ± 36.8	0.3

[1] Diet-specific data are presented as the mean ± SD. Differences between diets are presented as the mean ± SEM. SFA: saturated fatty acids; ω: omega; PUFA: polyunsaturated fatty acids; TRL triglyceride rich-lipoproteins; apo: apolipoprotein; PS: pool size; FCR: fractional catabolic rate; PR: production rate.

[2] *P* values were calculated with mixed models for repeated measures with subjects as a random effect.

Table 18-4 Fasting biochemical characteristics of the subjects (n=30) at the end of each diet[1]

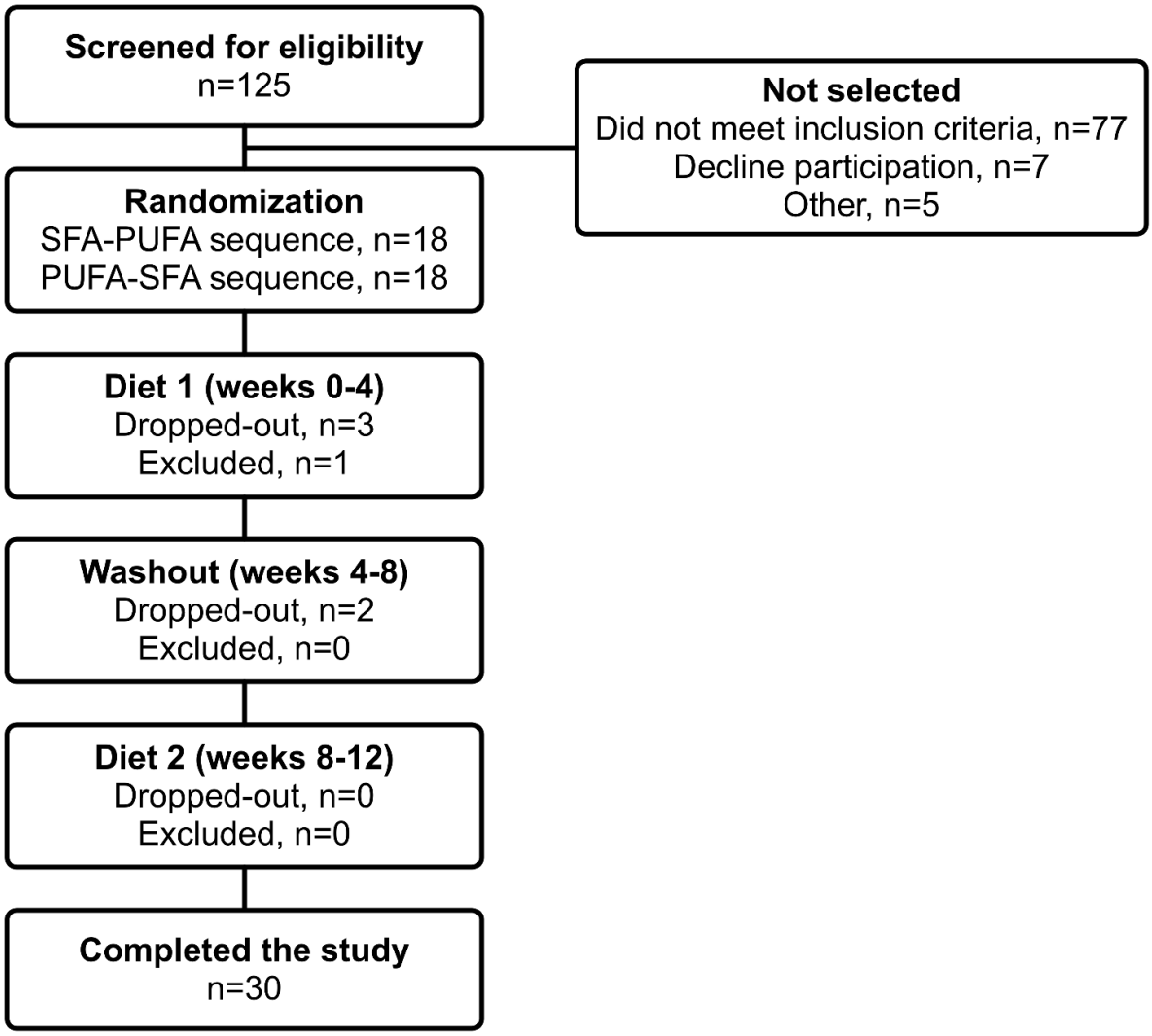
	SFA diet	*ω*-6 PUFA diet	%Δ	*P*[2]
ApoA-V (ng/mL)	58.2 ± 34.9	48.6 ± 21.1	-16.5 ± 12.8	0.1
ApoC-II (µg/mL)	149 ± 48	155 ± 54	+4.0 ± 8.8	0.5
ApoC-III (µg/mL)	144 ± 69	134 ± 54	-6.9 ± 11.1	0.5
ApoE (µg/mL)	37.5 ± 11.5	35.6 ± 8.3	-5.2 ± 6.9	0.2
CETP (ng/mL)	2089 ± 406	1999 ± 379	-4.3 ± 4.8	0.2
HL (ρg/mL)	7907 ± 1312	8178 ± 1354	+3.4 ± 4.4	0.4
LPL (ng/mL)	3899 ± 8943	3963 ± 9100	+1.6 ± 59.7	0.6
ANGPTL3 (ng/mL)	130 ± 31	130 ± 33	0.0 ± 6.37	0.9
LDLR (ng/mL)	61.2 ± 22.9	62.3 ± 17.6	+1.7 ± 8.6	0.3
Glucose (mmol/L)	5.25 ± 0.61	5.21 ± 0.69	-0.8 ± 3.2	0.6
Insulin (ρmol/L)	129 ± 41	133 ± 36	+3.1 ± 7.8	0.3
HOMA-IR index	4.37 ± 1.56	4.43 ± 1.29	+1.4 ± 8.5	0.5
CRP (mg/L)	7.72 ± 15.92	5.87 ± 4.87	-24.0 ± 39.4	0.2
Total-C (mmol/L)	4.91 ± 0.83	4.67 ± 0.86	-4.9 ± 4.5	0.3
TG (mmol/L)	2.02 ± 0.91	1.86 ± 0.77	-7.9 ± 10.9	0.9
HDL-C (mmol/L)	1.02 ± 0.21	1.00 ± 0.17	-2.0 ± 4.9	0.9
LDL-C (mmol/L)	2.96 ± 0.68	2.79 ± 0.73	-5.7 ± 6.4	0.06
Non HDL-C (mmol/L)	3.90 ± 0.82	3.67 ± 0.84	-5.9 ± 5.4	0.4
Total-C/HDL-C	5.01 ± 1.23	4.78 ± 1.15	-4.4 ± 6.2	0.8

[1] Diet-specific data are presented as the mean ± SD. Differences between diets are presented as the mean ± SEM. SFA: saturated fatty acids; ω: omega; PUFA: polyunsaturated fatty acids; Apo: apolipoprotein; ANGPTL3: angiopoietin-like 3; CETP: cholesteryl ester transfer protein; C: cholesterol; CRP: C-reactive protein; HL: hepatic lipase; LDLR: LDL receptor; LPL: lipoprotein lipase; TG: triglyceride.

[2] *P* values were calculated with mixed models for repeated measures with subjects as a random effect.

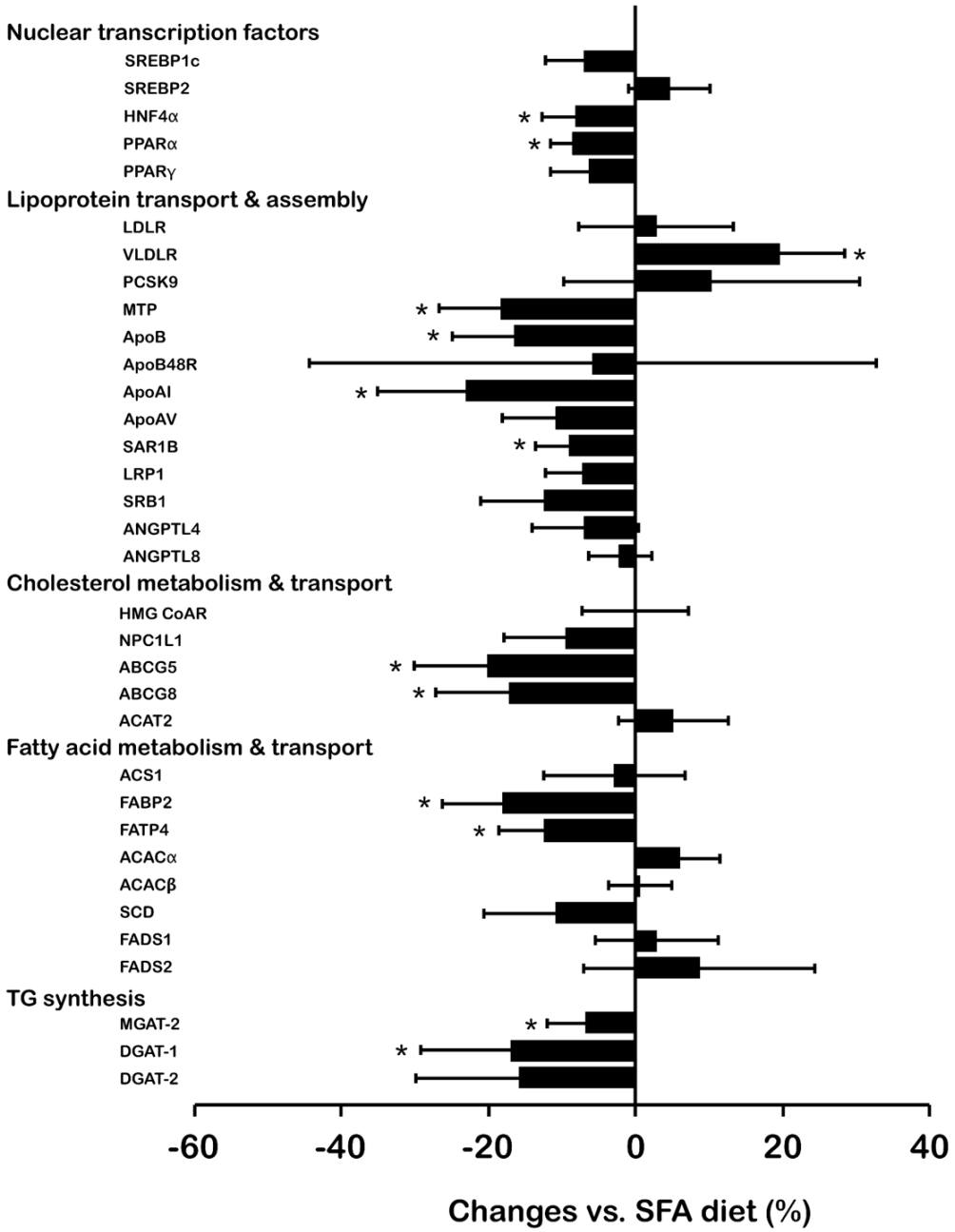
Figures

Figure 18-1 Flow chart of participants



Flow chart of participants. SFA: saturated fatty acids; PUFA: Polyunsaturated fatty acids.

Figure 18-2 Percent changes in intestinal mRNA levels following the ω-6 PUFA diet compared with the SFA diet in men with dyslipidemia and insulin resistance



Percent changes in intestinal mRNA levels following the ω-6 PUFA diet compared with the SFA diet in men with dyslipidemia and insulin resistance (n=30). Intestinal mRNA levels were normalized according to the expression of the house-keeping gene, TATA box binding protein, prior to statistical analyses. Values are presented as the mean change (%) ± SEM. *Significant change vs. SFA diet ($P < 0.05$). *P* values were calculated with mixed models for repeated measures with subjects as a random effect.

Supplemental Material

List of the manufacturers of the ELISA kits:

Assaypro, St. Charles, MO, USA: ApoC-II, apoC-III;

Biocheck Inc., Foster City, CA, USA: CRP;

Cloud-clone Corporation, Houston, TX, USA: CETP, HL;

Cell Biolabs Inc., San Diego, CA, USA: LPL;

R&D systems, Minneapolis, MN, USA: ANGPTL3, LDLR, ApoE;

US Biological, Swampscott, MA, USA: ApoAV.

Supplemental table 18-1 Sequence primers and gene description

Gene Symbol	Description	GenBank	size (pb)	Primer sequence 5'→3' S/AS
DGAT1	Homo sapiens diacylglycerol O-acyltransferase 1 (DGAT1)	NM_012079	135	TGCAGGATTCTTTATTCAGCTCT/CCACCAGGATGCCATACTTGAT
FABP2	Homo sapiens fatty acid binding protein 2, intestinal (FABP2)	NM_000134	137	TCAGGCTGGAATGTAGTGGAGAGA/CAAAACAAAAATTAGCTGGGCACTG
HMGCoAR	Homo sapiens 3-hydroxy-3-methylglutaryl-Coenzyme A reductase (HMGCR), 2 transcripts	NM_000859	195	GGGACCAACCTACTACCTCAG/CGACCTGTTGTGAATCATGTGACTT
ABCG5	Homo sapiens ATP-binding cassette, sub-family G (WHITE), member 5 (ABCG5)	NM_022436	196	AGGCATGCTGAACGCTGTGAATC/TCGGGCAACCTCAGGATGTAA
ABCG8	Homo sapiens ATP-binding cassette, sub-family G (WHITE), member 8 (ABCG8)	NM_022437	269	GGGCAATGCTTTACTATGAACTGGA/ATTGCTGAAGAAGGAGGCCATGT
FATP4	Homo sapiens solute carrier family 27 (fatty acid transporter), member 4 (SLC27A4)	NM_005094	139	TGGCTGCCCTGGTGTACTATG/TTCCGAATCACCACCGTCATG
MGAT2	Homo sapiens mannosyl (alpha-1,6-)-glycoprotein beta-1,2-N-acetylglucosaminyltransferase (MGAT2)	NM_002408	191	TAACCGGCCCGAATACCTCAG/AGGGTACAACTGAATGCTGAAAGGAAA
DGAT2	Homo sapiens diacylglycerol O-acyltransferase 2 (DGAT2), 2 transcripts	NM_032564	215	CCGATGGGTCCAGAAGAAGTT/TCACCAGGGCCTCCATGTACA
MTP	Homo sapiens microsomal triglyceride transfer protein (MTTP), 2 transcripts	NM_000253	210	CAGGGTGGTCTAGCTATTGATATTTC/TGGGTACTGAGAAAACTGCACTGT

APOB	Homo sapiens apolipoprotein B (APOB)	NM_000384	274	CTGCGCAACGAGATCAAGACA/CATGCTGGGAATCGACTTGTGA
LDLR	Homo sapiens low density lipoprotein receptor (LDLR)	NM_000527	193	GCCGTAAGGACACAGCACACAACC/GGAGCACGATGGGGAGGACAAT
PCSK9	Homo sapiens proprotein convertase subtilisin/kexin type 9 (PCSK9), 2 trancripts	NM_174936	172	CAGGGGAGGACATCATTGGTG/TTGGCAGAGAAGTGGATCAGTC
SREBP2	Homo sapiens sterol regulatory element binding transcription factor 2 (SREBF2), 2 transcripts	NM_004599	206	AGGAGAAAGGCGGACAACCCATAATA/CCAGCTTCAGCACCATGTTCTC
HNF4α	Homo sapiens hepatocyte nuclear factor 4, alpha (HNF4A), 10 transcripts	NM_000457	145	GGTGCAGGTGAGCTTGGAGGA/GCCGAAGAGCTTGATGAACTGGAT
ACAT2	Homo sapiens acetyl-Coenzyme An acetyltransferase 2 (ACAT2), 2 transcripts	NM_005891	267	CTGTGGCTCCGGAAGATGTGT/CTCCTGTTCTCAAGTAAGCCAAGTG
NPC1 L1	Homo sapiens NPC1-like 1 (NPC1L1), 2 transcripts	NM_013389	273	GCTGCTGTTTCTCGCCCTGTT/GGGAACTCTGTGGCATACTGGATCT
ACS1	Homo sapiens acyl-CoA synthetase long-chain family member 1 (ACSL1), 5 transcripts	NM_001995	262	GGCAACCCCAAAGGAGCAATG/TTGGAACCACGGGGAAGACAGT
APOA1	Homo sapiens apolipoprotein A-I (APOA1)	NM_000039	213	TGAAGGACCTGGCCACTGTGTA/GGCCCTCTGTCTCCTTTTCCA
SREBP1c	Homo sapiens sterol regulatory element binding transcription factor 1 (SREBF1), 2 transcripts	NM_004176	283	TGCGGAGAAGCTGCCTATCAACC/TTTGTGGACAGCAGTGCGCAGAC
APOB48R	Homo sapiens apolipoprotein B receptor (APOBR)	NM_018690	135	GCCCAGACCCCAACTAAGCAAC/AGGCTTTACAGACCCCGCGTG
ACACA	Homo sapiens acetyl-CoA carboxylase alpha, 5 transcripts	NM_198834	194	CACCGCCAGCTTAAGGACAAC/CGCTGACAAGGTGGAGTGAAT

ACACB	Homo sapiens acetyl-CoA carboxylase beta	NM_001093	138	GCCATGGTAGCCTTCAGGAGAT/CTTGCAGTCATCCTCGGAGTATAG
SCD	Homo sapiens stearoyl-CoA desaturase (delta-9-desaturase)	NM_005063	151	GTCGGGGGAGAGAGTTAGCATGT/ACCCCGAGCCAGGAGAGAAAG
FADS1	Homo sapiens fatty acid desaturase 1	NM_013402	140	CACCCAGCTCCAGGCCACATG/GCACCAGGGGAGCCACTTTGT
FADS2	Homo sapiens fatty acid desaturase 2	NM_004265	234	GCCTTTGTCCTTGCTACCTCTC/GCCCAGAACAAACACGTGCAG
VLDLR	Homo sapiens very low density lipoprotein receptor, 2 transcripts	NM_003383	148	GCAGCAGGCAGTGTAATGGTATC/TGCTCCTGGTTACATACTTTGCTGATA
SAR1B	Homo sapiens SAR1 homolog B (S. cerevisiae)	NM_016103	203	TGGCATTGTATTTCTGGTGGATTGTG/TCCCCTTTCCTGTTGTCTGACCATATA
LRP1	Homo sapiens low density lipoprotein receptor-related protein 1	NM_002332	150	CCGCGAGGACTACATTGAATTTG/TGGGCTCGGTTAATGGACTTTGT
SRB1	Homo sapiens scavenger receptor class B, member 1 (SCARB1), 2 transcripts	NM_005505	129	GTGGGTGAGATCATGTGGGGCTA/GAGCCCAGAGTCGGAGTTGTTGA
G6PD	Homo sapiens glucose-6-phosphate dehydrogenase (G6PD), nuclear gene encoding mitochondrial protein	NM_000402	121	GATGTCCCCTGTCCCACCAACTCTG/GCAGGGCATTGAGGTTGGGAG

Supplemental table 18-2 Correlations between changes in intestinal mRNA levels induced by the substitution of ω-6 PUFAs for SFAs in intestinal genes involved in lipid and lipoprotein metabolism significantly regulated by the experimental diets

	HNF4α	***PPARα***	***VLDLR***	***MTP***	***ApoB***	***ApoA1***	***SAR1B***	***ABCG5***	***ABCG8***	***FABP2***	***FATP4***	***MGAT2***
PPARα	0.47†	---										
VLDLR	-0.32	-0.43*	---									
MTP	0.64‡	0.57†	-0.32	---								
ApoB	0.65‡	0.58‡	-0.47†	0.49†	---							
ApoAI	0.48†	0.65‡	-0.41*	0.76‡	0.58‡	---						
SAR1B	0.74‡	0.50†	-0.34	0.83‡	0.65‡	0.72‡	---					
ABCG5	0.69‡	0.45*	-0.48†	0.59†	0.64‡	0.61‡	0.54†	---				
ABCG8	0.54†	0.46*	-0.43*	0.56†	0.44*	0.65‡	0.53†	0.85‡	---			
FABP2	0.72‡	0.61‡	-0.34	0.85‡	0.57†	0.79‡	0.85‡	0.53†	0.50†	---		
FATP4	0.67‡	0.62‡	-0.53†	0.71‡	0.60‡	0.67‡	0.79‡	0.62‡	0.62‡	0.75‡	---	
MGAT2	0.56†	0.16	-0.07	0.48†	0.38*	0.13	0.58‡	0.41*	0.40*	0.46*	0.55†	---
DGAT1	0.82‡	0.63‡	-0.48†	0.82‡	0.72‡	0.77‡	0.86‡	0.75‡	0.69‡	0.84‡	0.89‡	0.48†

A non-parametric Spearman's rank correlation test was performed to evaluate the associations between key intestinal genes involved in cholesterol metabolism with respect to changes in mRNA expression levels following the PUFA diet vs. the SFA diet. *: $P<0.05$; †: $P<0.01$; ‡: $P<0.001$