Clinical Study

n-3 Polyunsaturated Fatty Acid Supplementation Has No Effect on Postprandial Triglyceride-Rich Lipoprotein Kinetics in Men with Type 2 Diabetes

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Dietary n-3 polyunsaturated fatty acids (PUFAs) have been proposed to modulate plasma lipids, lipoprotein metabolism, and inflammatory state and to reduce triglyceride (TG) concentrations. The present double-blind, randomized, placebo-controlled, crossover study investigated the effects of n-3 PUFA supplementation at 3 g/d for 8 weeks on the intravascular kinetics of intestinally derived apolipoprotein (apo) B-48-containing lipoproteins in 10 men with type 2 diabetes. In vivo kinetics of the TG-rich lipoprotein (TRL) apoB-48 and VLDL apoB-100 were assessed using a primed-constant infusion of L-[5,5,5-D3] leucine for 12 hours in a fed state. Compared with the placebo, n-3 PUFA supplementation significantly reduced fasting TG concentrations by $-9.7\%$ ($P=0.05$) but also significantly increased plasma levels of cholesterol (C) (+6.0%, $P=0.05$), LDL-C (+12.2%, $P=0.04$), and HDL-C (+8.4, $P=0.007$). n-3 PUFA supplementation had no significant impact on postprandial TRL apoB-48 and VLDL apoB-100 levels or on the production or catabolic rates of these lipoproteins. These data indicate that 8-week supplementation with n-3 PUFAs in men with type 2 diabetes has no beneficial effect on TRL apoB-48 and VLDL apoB-100 levels or kinetics.

1. Introduction

The characteristic features of dyslipidemia that are associated with insulin-resistant states include elevated plasma triglycerides (TG) due to overaccumulation of TG-rich lipoproteins (TRL) of both intestinal (apoB-48) and hepatic origin (apoB-100), low HDL-cholesterol (C) levels, and the formation of small, dense LDL particles [1]. Several lines of evidence have suggested that apoB-48-containing particles are proatherogenic and are associated with increased cardiovascular disease risk [1]. In this regard, consumption of long-chain n-3 polyunsaturated fatty acids (PUFAs) is an efficacious approach to modify cardiovascular risk by improving dyslipidemia, hypertension, and endothelial function [2]. Supplementation with dietary n-3 PUFA has been consistently shown to reduce TG concentrations [3]. However, the mechanisms responsible for the hypotriglyceridemic effects of n-3 PUFAs have not been fully elucidated. The objective of the current study was to investigate the impact of n-3 PUFAs on the in vivo kinetics of intestinally derived apoB-48-containing lipoproteins and hepatic VLDL apoB-100 in men with type 2 diabetes. We hypothesized that n-3 PUFAs would beneficially affect plasma TRL levels by reducing the production and increasing the catabolism of both intestinal and hepatic lipoproteins.

2. Methods

2.1. Subjects. Ten men with type 2 diabetes as defined by the American Diabetes Association were recruited in Quebec City area to participate in the study. To be included in the study, participants had to have received stable doses of metformin for at least 3 months before randomization. All eligible subjects were required to discontinue their use of lipid-lowering medications for at least 6 weeks before.
Table 1: Characteristics and fasting lipid/lipoprotein profile of the evaluated patients with type 2 diabetes.

| Parameter                   | Placebo (n = 10) | n-3 PUFA (n = 10) | %Δ | P  |
|-----------------------------|------------------|-------------------|----|----|
| Body weight, kg             | 106.1 ± 19.5     | 105.8 ± 21.0      | −0.3| 0.7|
| Body mass index, kg/m²      | 34.1 ± 5.5       | 34.1 ± 6.1        | —  | 0.7|
| Serum                       |                  |                   |    |    |
| Cholesterol, mmol/L         | 4.68 ± 0.80      | 4.96 ± 0.58       | +6.0| 0.05|
| Triglycerides, mmol/L       | 2.58 ± 1.12      | 2.33 ± 0.94       | −9.7| 0.05|
| LDL-cholesterol, mmol/L     | 2.55 ± 0.72      | 2.86 ± 0.47       | +12.2| 0.04|
| HDL-cholesterol, mmol/L     | 0.95 ± 0.17      | 1.03 ± 0.20       | +8.4| 0.007|
| Apolipoprotein B, g/L       | 1.00 ± 0.19      | 1.03 ± 0.11       | +3.0| 0.4 |
| Apolipoprotein AI, g/L      | 1.12 ± 0.18      | 1.15 ± 0.14       | +2.6| 0.4 |
| Glucose homeostasis         |                  |                   |    |    |
| Glucose, mmol/L             | 7.3 ± 1.6        | 8.1 ± 2.2         | +11.0| 0.3|
| Insulin, μmol/L             | 147 ± 86         | 164 ± 109         | +11.9| 0.6|
| HbA1c                       | 0.070 ± 0.010    | 0.072 ± 0.011     | +2.9| 0.1|

PUFA: polyunsaturated fatty acid.
Mean ± SD; % represents the percentage of difference between the two intervention phases.

2. Results

3.1. Demographic Characteristics and Fasting Biochemical Parameters of Subjects. Table 1 shows the demographic characteristics and fasting biochemical parameters of the 10 patients with type 2 diabetes following an 8-week supplementation with either placebo or n-3 PUFA (3 g/d). The mean age of the participants was 54.7 ± 7.6 years. No significant differences were observed in body weight, body mass index, or systolic blood pressure between the two supplementation phases. Supplementation with n-3 PUFA significantly reduced plasma TG concentrations by −9.7% (P = 0.05) but significantly increased levels of plasma cholesterol (+6.0%, P = 0.05), LDL-C (+12.2%, P = 0.04), and HDL-C (+8.4%, P = 0.007). No significant differences in fasting plasma levels of apoB or apoAI were observed between the two treatments. Compared with the placebo, n-3 PUFA supplementation had no significant impact on glucose or
4. Discussion

In the present study, supplementation with n-3 PUFA at a dose of 3 g/d for 8 weeks significantly reduced fasting plasma TG levels but increased plasma cholesterol, LDL-C, and HDL-C concentrations in men with type 2 diabetes compared with a placebo. In addition, n-3 PUFA supplementation had no significant effect on the postprandial secretion and clearance of TRL apoB-48 or VLDL apoB-100 in these subjects.

It is well documented that type 2 diabetes is associated with hypertriglyceridemia and elevated levels of apoB-100 and apoB-48-containing lipoproteins [4]. n-3 PUFA has been used in the treatment of hypertriglyceridemia, as they reduce the hepatic production of VLDL, favor fatty acid oxidation, and enhance VLDL clearance [5]. As expected, n-3 PUFA supplementation for 8 weeks significantly reduced fasting TG levels, which is in agreement with previous observations from other groups [6, 7]. Our results also showed that n-3 PUFA supplementation increased total and LDL-C concentrations in a manner consistent with previous findings [8–10].

Although n-3 PUFA supplementation significantly reduced TG levels in a fasting state, postprandial concentrations of VLDL apoB-100 were not significantly different after the two interventions. Our results showed no significant changes in VLDL apoB-100 fractional catabolic and production rates, a finding that contrasts with previous kinetic studies. Nestel et al. [11] reported that dietary n-3 PUFAs lowered secretion rates of both VLDL apoB and VLDL TG in the absence of changes in the clearance of VLDL. In type 2 diabetic patients, n-3 PUFAs were effective at lowering TG concentrations via the suppression of VLDL production and the stimulation of its conversion into LDL, with no change in the apoB-100-containing lipoprotein clearance rate [12]. Earlier studies on VLDL TG kinetics on subjects who were fed between 10 and 17 g/d of fish oil reported a significant reduction in VLDL TG synthetic rate and an increased clearance rate [13]. In cultured rat hepatocytes, EPA and DHA inhibited VLDL apoB secretion by 31% and 54%, respectively [14]. Moreover, a study in African green monkeys showed that perfused monkey livers had decreased secretion of cholesteryl ester and TG but maintained the same apoB output, suggesting that production of the same number of smaller, TG-depleted apoB particles likely compensated for the decreased availability of TG for VLDL secretion [15].

The impact of n-3 PUFA supplementation on apoB-48 metabolism has been recently investigated. Levy et al. [16] showed that the jejunal secretion of apoB-48 from diabetic and insulin-resistant rats following dietary intake with n-3 PUFAs was significantly reduced, and this was most likely the result of posttranslational degradation. Moreover, apoB mRNA and corresponding apoB secretion were reduced in intestinal/colonic-derived Caco-2 cultured cells when incubated with EPA [16]. In JCR:LA-cp rats, n-3 PUFA treatment resulted in a significant improvement in apoB-48 and TG postprandial response compared with controls [17]. In healthy subjects, n-3 PUFA supplementation (4 g/d) for 4 weeks reduced postprandial TG, apoB-48, and apoB-100 concentrations [5]. However, this is in contrast with our results showing no significant effect of n-3 PUFA supplementation on apoB-48 production and clearance rates.

This study has several strengths. The relatively large number of participants and the robust study design (double-blind, randomized, crossover) increased our statistical power. Our specific inclusion criteria also limit the variability related to the background care provided. The statistical analyses were undertaken in a blinded fashion and according to the a priori defined plan and hypothesis testing. Our study also shows that n-3 PUFA supplementation had no significant impact of postprandial TRL apoB-48 kinetics but significantly modulated both fasting TG and LDL-C concentrations. Several potential mechanisms could explain these observations. (1) The meals provided during the kinetic study did not contain n-3 PUFAs, and this could have influenced postprandial response and attenuated n-3 PUFA effects on VLDL apoB-100 and TRL apoB-48 secretion. Recent studies have suggested that the effect of the meal may in fact overwhelm the
lack of correlation between daily dosages of n-3 PUFA supplementation and increased VLDL apoB-100 and TRL apoB-48 levels or kinetics.

5. Conclusion

n-3 PUFA supplementation for 8 weeks was effective at reducing fasting TG levels in subjects with type 2 diabetes but also increased levels of plasma cholesterol, LDL-C, and HDL-C. However, no significant effect was observed on postprandial VLDL apoB-100 and TRL apoB-48 levels or kinetics.

Abbreviations

Apo: Apolipoprotein  
DHA: Docosahexaenoic acid  
EPA: Eicosapentaenoic acid  
HbA1c: Glycated hemoglobin  
HDL: High-density lipoprotein  
LDL: Low-density lipoprotein  
PUFA: Polyunsaturated fatty acid  
TRL: Triglyceride-rich lipoprotein  
VLDL: Very-low-density lipoprotein.

Disclosure

Benoît Lamarche is the Chair of Nutrition at Laval University.

Conflict of Interests

All authors declare that they have no relevant conflict of interests.

Authors’ Contribution

All of the authors read and approved the final paper. Patrick Couture and Benoît Lamarche designed the research; André J. Tremblay and Jean-Charles Hogue conducted the research; Patrick Couture, Benoît Lamarche, and André J. Tremblay analyzed the data; Patrick Couture, André J. Tremblay, and Benoît Lamarche wrote the paper; and Patrick Couture had primary responsibility for the final content.

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