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Effect of Omega-3 Fatty Acid Supplementation on the Postprandial Metabolism of Apolipoprotein(a) in Familial Hypercholesterolemia

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Aim: Lipoprotein(a) (Lp(a)) is a low-density lipoprotein-like particle containing apolipoprotein(a) (apo(a)) that increases the risk of atherosclerotic cardiovascular disease (ASCVD) in familial hypercholesterolemia (FH). Postprandial redistribution of apo(a) protein from Lp(a) to triglyceride-rich lipoproteins (TRLs) may also increase the atherogenicity of TRL particles. Omega-3 fatty acid (ω3FA) supplementation improves postprandial TRL metabolism in FH subjects. However, its effect on postprandial apo(a) metabolism has yet to be investigated.

Methods: We carried out an 8-week open-label, randomized, crossover trial to test the effect of ω3FA supplementation (4 g/day) on postprandial apo(a) responses in FH patients following ingestion of an oral fat load. Postprandial plasma total and TRL-apo(a) concentrations were measured by liquid chromatography with tandem mass spectrometry, and the corresponding areas under the curve (AUCs) (0–10h) were determined using the trapezium rule.

Results: Compared with no ω3FA treatment, ω3FA supplementation significantly lowered the concentrations of postprandial TRL-apo(a) at 0.5 (–17.9%), 1 (–18.7%), 2 (–32.6%), and 3 h (–19.2%) ($P<0.05$ for all). Postprandial TRL-apo(a) AUC was significantly reduced with ω3FA by 14.8% ($P<0.05$). By contrast, ω3FA had no significant effect on the total AUCs of apo(a), apoC-III, and apoE ($P>0.05$ for all). The decrease in postprandial TRL-apo(a) AUC was significantly associated with changes in the AUC of triglycerides (r=0.600; $P<0.01$) and apoB-48 (r=0.616; $P<0.01$).

Conclusions: Supplementation with ω3FA reduces postprandial TRL-apo(a) response to a fat meal in FH patients; this novel metabolic effect of ω3FA may have implications on decreasing the risk of ASCVD in patients with FH, especially in those with elevated plasma triglyceride and Lp(a) concentrations. However, the clinical implications of these metabolic findings require further evaluation in outcome or surrogate endpoint trials.

Key words: Atherosclerotic cardiovascular disease, Familial hypercholesterolemia, Fish oil, Lipoprotein(a), Postprandial dyslipidemia

Introduction

Familial hypercholesterolemia (FH) is a dominantly inherited disorder commonly due to mutations in the low-density lipoprotein (LDL) receptor that cause markedly elevated plasma LDL-

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cholesterol concentrations and increased risk of atherosclerotic cardiovascular disease (ASCVD)[1-4]. Despite treated with high-intensity statins and/or ezetimibe, a significant proportion of FH patients remains at increased lipoprotein-mediated residual risk of ASCVD[3-6]. This relates to not achieving LDL-cholesterol targets as well as to abnormalities in the metabolism of triglyceride-rich lipoproteins (TRLs) and lipoprotein(a) (Lp(a))[7, 8].

Abnormal metabolism of TRL may lead to the development of ASCVD in FH owing to prolonged accumulation of TRLs in the circulation, including very-low-density lipoprotein (VLDL)-apolipoprotein (apo) B-100 and apoB-48-containing chylomicrons and their remnants in the circulation[7, 8]. TRL remnants are atherogenic, contributing to inflammation, oxidative stress, endothelial dysfunction, and foam cell formation[9]. Postprandial redistribution of apo(a) protein from Lp(a) to TRLs particles may also increase the atherogenicity of TRL particles[10, 11].

Elevated Lp(a) enhances ASCVD and calcific aortic valve disease (CAVD) in FH owing to its atherogenic, thrombogenic, and proinflammatory properties[12-16]. Recent studies have also reported that Lp(a) is associated with lipid and apolipoprotein profiles and metabolic factors, such as triglycerides, apoB, apoE, and proprotein convertase subtilisin/kexin type 9 [17]. Lp(a) is a highly polymorphic particle consisting of an LDL-like particle and a protein moiety consisting of apoB-100 covalently bound to apo(a)[18]. The metabolism of Lp(a) in humans is poorly understood. Experimental evidence suggests that the apoB-100-apo(a) complex within Lp(a) particles has high affinity for TRL particles[10, 11]. A significant proportion of Lp(a) particles can bind non-covalently to TRLs in the hypertriglyceridemic state[19]. The Lp(a)-TRL complex may aggravate the pathogenesis of ASCVD in FH. Understanding the metabolism of apo(a) within the TRL fraction in the postprandial state is important to further elucidate the role of Lp(a) in atherosclerosis, as this remains unexplained.

Fish oil is a rich source of long-chain omega-3 fatty acids (ω3FAs), primarily eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)[20]. Compelling evidence suggests that ω3FA supplementation protects against ASCVD through multiple mechanisms, including improvements in hypertriglyceridemia, blood pressure, and inflammation[20-22]. We have previously reported that ω3FA supplementation lowered fasting and postprandial concentrations of triglycerides, VLDL-apoB-100, and apoB-48 in FH patients[23]. Whether ω3FA supplementation also improves the postprandial response of Lp(a) particle concentration in FH patients remains to be investigated.

In the present study, we investigate the responses of total apo(a) and TRL-apo(a) concentrations to an oral fat load. We hypothesized that ω3FA supplementation lowers the postprandial response of plasma TRL-apo(a) in FH patients on statin and/or ezetimibe treatment.

Methods

Subjects and Clinical Protocol

Full details of the study design and protocol have been previously published[23]. Briefly, a total of 20 patients (10 men and 10 women) with FH aged 18–70 years (body mass index [BMI] <40 kg/m^2) completed the study. FH was defined by the presence of a pathogenic mutation in the *LDLR* gene (definite FH) and/or a Dutch Lipid Clinic Network criteria score of more than 8 (phenotypic definite FH)[5, 6]. Eligible patients were on statins (or statin plus ezetimibe) at recruitment and throughout the duration of the study. In this 8-week open-label, randomized, crossover trial, all FH patients were randomized into one of the two following groups: no ω3FA treatment (i.e. standard care only) or an 8-week treatment period of 4 g/day ω3FA supplementation (Omacor® 46% EPA and 38% DHA in ethyl ester form; Abbott Products Pty Ltd.) with an 8-week washout period between each intervention. The study was approved by the Human Research Ethics Committee of the Royal Perth Hospital and informed written consent was obtained from all subjects.

Comparison with Non-FH Controls

For comparison, 10 healthy non-FH subjects (5 men and 5 women, age 60±7 years, BMI 29.8±4.9 kg/m^2, systolic blood pressure 124±13 mmHg, diastolic blood pressure 76±7 mmHg, plasma triglycerides 1.6±0.8 mmol/L, and total cholesterol 5.3±0.7 mmol/L on no drug treatment) were also recruited from the community via newspaper advertisement. in which the fat load test was performed in one occasion only.

Postprandial Oral Fat Load Test

Subjects were admitted to the metabolic ward in the morning after a 14-h fast. They were studied in a semi-recumbent position and allowed to drink only water after the test meal. After a baseline fasting blood sample was collected, a liquid-formulated high fat test meal was consumed within 2 min (a total of 4800 kJ, 130 g of fat, 17 g of protein, and 21 g of carbohydrates), following which blood samples were

obtained at 0.5, 1, 2, 3, 5, 6, 8, and 10 h. The subjects were then given a snack and allowed to go home. Postprandial oral fat loading test were repeated after the 8-week intervention period.

Biochemical Measurements

Fasting blood samples were collected at the end of each treatment period. Briefly, fasting whole venous blood samples collected in EDTA were immediately centrifuged at 1500×g for 15 min at 4℃. Plasma was collected and stored at −80℃. Plasma lipid and glucose concentrations were measured using enzymatic methods (Roche Diagnostics Australia, Castle Hill, NSW, Australia). LDL-cholesterol was estimated using the Friedewald calculation. Fasting insulin was measured using a chemiluminescent immunometric assay (Abbott Diagnostics, North Ryde, NSW, Australia), and insulin resistance was estimated using the homeostasis model assessment (HOMA). Plasma hsCRP was measured using a high-sensitivity polyclonal antibody assay (Abbott Laboratories, Abbott Park, IL, USA). Plasma apoB-48 levels were measured using an enzyme immunoassay kit (Fujirebio, Tokyo, Japan). The TRL fraction was isolated from 3.5 mL of plasma by ultracentrifugation (Optima XL-100K; Beckman Coulter, Australia) at a density of less than 1.006 g/mL (40,000 rpm, 16 h, 4℃). Plasma VLDL-apoB-100 was measured in the TRL fraction using an ELISA kit (Mabtech, Nacka, Sweden). The kit is specific for apoB-100 and does not recognize apoB-48. Total Lp(a) mass concentration was measured using an automated latex enhanced immunoassay (Quantia Lp(a) assay and standards; Abbott Laboratories). The fasting and postprandial apo(a) in the plasma and TRL fraction (density <1.006 g/mL) were determined as particle number in nmol/L using trypsin proteolysis and subsequent analysis of proteotypic peptides by liquid chromatography with tandem mass spectrometry (LC-MS/MS), as described previously[24, 25]. Non–TRL-apo(a) was calculated as the difference between plasma total apo(a) and TRL-apo(a) concentrations. Fasting and postprandial apoC-III and apoE concentrations (total and apoB-containing lipoprotein [LpB] fractions), as well as apoE phenotyping, were also determined by LC-MS/MS, as previously described[24, 25]. Postprandial metabolism was quantified by calculating the area under the curve (AUC) for plasma total apo(a), TRL-apo(a), and non-TRL-apo(a) (0–10 h) using the trapezium rule.

Statistical Analyses

All data were analyzed using the SPSS 26 (SPSS, Chicago, IL, USA) software. Data are reported as mean±SEM, unless specified otherwise. Skewed variables were log-transformed. The groups were compared using independent *t*-tests or Fisher's test.. Carryover effects of the crossover design were estimated using general linear modeling (PROC GLM SAS 9.2; SAS Institute). If the carryover effect was not statistically significant, then the data from the two periods (i.e no ω3FA treatment period vs ω3FA treatment period) was compared using a paired t test to estimate the treatment effect of ω3FA supplementation in our FH patients. Associations were examined using a simple linear regression method. Significance was defined at the 5% level using a two-tailed test.

Results

Baseline Characteristics

On average, the FH patients were middle aged (53.3±3.0 years), non-obese (BMI 27.0±1.4 kg/m^2), and normotensive (systolic blood pressure 124±2.9 mmHg and diastolic blood pressure 69.3±1.9 mmHg). None of the FH patients were current smokers. Seventeen patients were genetically diagnosed with FH (pathogenic mutations in the *LDLR* gene) and the other 3 had a DLCN score of more than 8 (phenotypic definite FH). Thirteen of the 20 subjects carried the apoE3/E3 phenotype, 4 the apoE3/E4 phenotype, 2 the apoE2/E3 phenotype, and 1 the apoE2/E4 phenotype. Nine patients were on rosuvastatin (12.5–40 mg/day), 8 on atorvastatin (40–80 mg/day), and 3 on simvastatin (80 mg/day). Thirteen patients were also on ezetimibe (10 mg/day), 9 on aspirin, 3 on anti-hypertension medication, and 4 reported a history of coronary artery disease (CAD).

Body Weight, Blood Pressure, and Biochemical Characteristics

The effect of ω3FA supplementation on body weight, blood pressure, plasma lipid and lipoprotein concentrations, and postprandial triglycerides, VLDL-apoB-100 and apoB-48 total AUCs in 20 FH patients has been previously reported[23]. Briefly, body weight, waist circumference, and BMI did not change significantly during the intervention ($P>0.05$ for all). ω3FA supplementation significantly lowered the systolic and diastolic blood pressures, fasting plasma triglycerides, and plasma apoB-100, VLDL-apoB-100, and apoB-48 concentrations ($P<0.05$ for all). ω3FA supplementation also lowered plasma apoE concentration, albeit not significantly ($P=0.053$). Total cholesterol, LDL-cholesterol, hsCRP, apoC-III, and Lp(a) concentrations were not significantly altered with ω3FA supplementation, nor were the glucose

Table 1. Effects of ω-3FA supplementation on clinical and biochemical characteristics in 20 FH patients

	No ω-3FA Treatment	ω-3FA	*P* value
Weight (kg)	79.1 ± 3.6	79.0 ± 3.5	0.800
Waist circumference (cm)	90.5 ± 2.9	90.4 ± 3.1	0.884
Body mass index (kg/m^2)	27.0 ± 1.4	27.0 ± 1.3	0.702
Systolic blood pressure (mmHg)	124 ± 2.9	117 ± 3.4	0.009
Diastolic blood pressure (mmHg)	69.3 ± 1.9	65.1 ± 1.9	0.006
Total cholesterol (mmol/L)	4.58 ± 0.27	4.20 ± 0.16	0.069
LDL-cholesterol (mmol/L)	2.81 ± 0.29	2.54 ± 0.16	0.204
Triglycerides (mmol/L)	1.30 ± 0.14	1.05 ± 0.09	0.011
ApoB (g/L)	0.83 ± 0.06	0.76 ± 0.03	0.038
VLDL-apoB-100 (mg/L)	77.2 ± 6.2	56.5 ± 4.9	0.001
ApoB-48 (mg/L)	8.77 ± 1.72	5.64 ± 0.81	0.026
ApoC-III (mg/L)	85.5 ± 7.5	81.7 ± 7.6	0.627
ApoE (mg/L)	66.3 ± 5.1	59.4 ± 4.3	0.052
Hs-CRP (mg/L)	1.90 ± 0.54	1.79 ± 0.54	0.602
Lipoprotein(a) (g/L)	0.44 ± 0.11	0.42 ± 0.10	0.134
Glucose (mmol/L)	5.19 ± 0.10	5.32 ± 0.11	0.122
Insulin (mU/L)	7.79 ± 1.00	8.81 ± 0.94	0.249
HOMA score	1.81 ± 0.24	2.09 ± 0.22	0.174

Data presented as Mean ± SEM; HOMA, homeostatic model assessment; the values of clinical and biochemical characteristics were determined at the end of each 8 week treatment period.

and insulin concentrations and HOMA score **(Table 1)**. ω3FA supplementation was also significantly associated with a decrease in postprandial triglyceride and VLDL-apoB-100 total AUCs (–19% and –26%, respectively, $P<0.01$). The postprandial apoB-48 total AUC (0–10 h) was significantly reduced with ω3FA supplementation (–30%, $P<0.05$). The AUCs of apoC-III and apoE (total and LpB fraction) did not change significantly during the intervention ($P>0.05$ for all) **(Supplementary Table 1)**.

Postprandial Responses of Total and TRL-Apo(a)

Fig. 1 shows the postprandial responses of plasma total apo(a) and TRL-apo(a) to the fat load (0–10 h) before and after 8 weeks of ω3FA supplementation. In the no ω3FA treatment period, there was a small but significant increase in the plasma total apo(a) concentration following the first hour of the fat load ($P<0.05$), which then progressively decreased, returning to the baseline levels at 10 h **(Fig. 1A)**. Following the oral fat load, there was a significant increase in the plasma TRL-apo(a) concentration, which reached a maximum at 2 h postprandially ($P<0.05$) followed by a return approximately to baseline levels at 10 h **(Fig. 1B)**. When expressed as the percentage of the total apo(a) protein, the proportion of apo(a) in TRL at 0.5 (23.7% ± 11.2%), 1 (23.9% ± 12.6%), 2 (25.8% ± 15.0%), and 3 h (24.5% ± 11.0%) was significantly higher than that at baseline (19.9% ± 6.7%; $P<0.05$ in all). Compared with baseline levels, there was no significant difference in the plasma concentration of apo(a) in the non-TRL fraction following the oral fat load ($P>0.05$, data not shown), except for a significant increase in apo(a) concentration at 1 h ($P<0.05$) **(Supplementary Table 2)**

The fasting total apo(a) concentration was significantly associated with the AUC of TRL-apo(a) (r=0.809; $P<0.001$). The fasting TRL-apo(a) concentration was also significantly associated with the AUC of TRL-apo(a) (r=0.949; $P<0.001$). The AUCs of total apo(a) or TRL-apo(a) were not associated with the AUCs for plasma triglycerides, VLDL-apoB-100, apoB-48, apoC-III, and apoE (data not shown).

Comparison of Postprandial Responses of Total and TRL-Apo(a) between FH and Non-FH Subjects

Age, body weight, BMI, and systolic and diastolic blood pressures were not significantly different between the FH patients and non-FH controls ($P>0.05$ for all). Eight of the control subjects carried the apoE3/E3 genotype and two the apoE3/E4 phenotype. The frequency of the apoE phenotype was not significantly different between the groups ($P>0.05$). While there was also no significant difference in the fasting plasma total apo(a) concentrations between the FH and control groups ($P>0.05$), FH patients

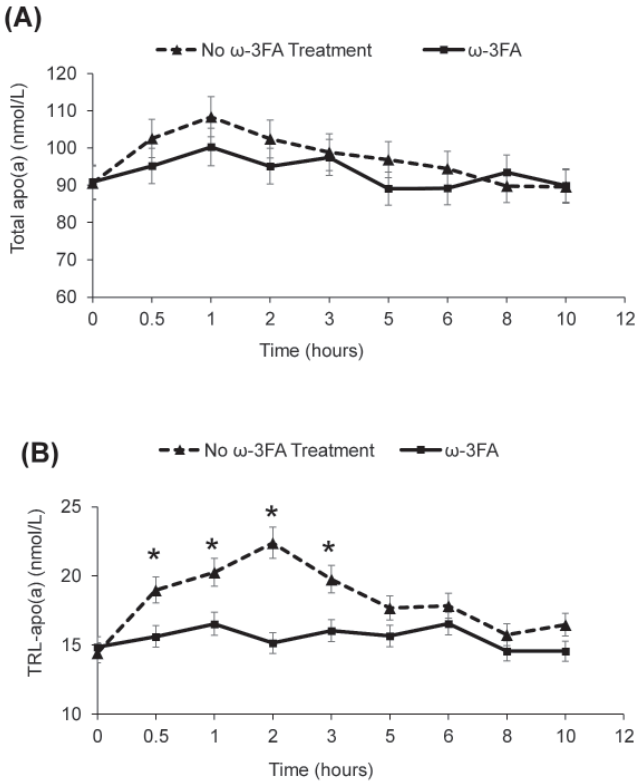

Fig. 1. Plasma total apolipoprotein(a) (A) and triglyceride-rich lipoprotein-apo(a) (B) responses (mean ± SEM) to the fat load before and after W-3 FA supplementation

Apo(a): apolipoprotein(a); TRL: triglyceride-rich lipoprotein
*$P<0.05$ compared with no treatment group at the corresponding time points

Table 2. Plasma apolipoprotein(a) concentration and area under curves in the two groups

		Control ($n=10$)	FH ($n=20$)	P value
Apolipoprotein(a)*				
Total	Concentration	92.7 ± 112	86.4 ± 97.7	0.875
	AUC	973 ± 1152	917 ± 1009	0.893
TRL	Concentration	5.7 ± 8.1	14.4 ± 9.7	0.022
	AUC	60.1 ± 94.1	182 ± 125	0.012

Mean ± SD; AUC: area under curve; TRL: triglyceride-rich lipoprotein; FH: familial hypercholesterolemia
*Concentration expressed as nmol/L and AUC as nmol/L •10 h

had a higher fasting plasma TRL-apo(a) concentration ($P<0.05$) **(Table 2)**. FH patients also had a higher percentage of apo(a) in the TRL fraction (19.9% ± 6.7% vs. 5.9% ± 2.1%; $P<0.001$) and showed a greater postprandial TRL-apo(a) AUC response to the fat load than the non-FH controls ($P<0.05$). However, there was no significant difference in the postprandial total apo(a) AUC response between the groups ($P>0.05$). The concentration and total AUCs of apoE, but not apoC-III, were significantly higher in FH patients than in the non-FH controls **(Supplementary Table 3)**.

*ω*3FA Intervention

The postprandial responses in the plasma total apo(a) and TRL-apo(a) to the fat load are shown in **Table 3**. Compared with the no *ω*3FA treatment period, *ω*3FA supplementation reduced the rise in total and TRL-apo(a) concentrations in response to the fat load at 0.5 (–17.9%), 1 (–18.7%), 2 (–32.6%),

Table 3. Effects of ω-3FA supplementation on postprandial total apo(a) (A) and TRL-apo(a) (B) concentrations in the FH patients

(A)	Total apo(a) (nmol/L)			*P* value*
	No ω-3FA Treatment	ω-3FA	%	
0h	86.4 ± 21.8	86.6 ± 21.6	0.2	0.884
0.5h	97.8 ± 25.4†	90.7 ± 22.2	-7.3	0.229
1h	103 ± 27.3†	95.6 ± 23.8	-7.2	0.422
2h	97.6 ± 21.5	90.7 ± 20.3	-7.1	0.255
3h	94.2 ± 23.4	92.9 ± 23.9	-1.4	0.705
5h	92.4 ± 23.1	85.0 ± 20.2	-8.0	0.146
6h	90.0 ± 21.9	85.0 ± 21.9	-5.6	0.266
8h	85.6 ± 22.1	89.1 ± 24.9	4.1	0.537
10h	85.5 ± 21.1	85.7 ± 21.0	0.2	0.938
AUC	917 ± 226	888 ± 222	-3.2	0.191
(B)	TRL-apo(a) (nmol/L)			*P* value*
	No ω-3FA Treatment	ω-3FA	%	
0h	14.4 ± 2.2	14.8 ± 2.4	2.8	0.566
0.5h	19.0 ± 3.1†	15.6 ± 2.3	-17.9	0.027
1h	20.3 ± 3.6†	16.5 ± 2.5	-18.7	0.013
2h	22.4 ± 4.5†	15.1 ± 2.3	-32.6	0.029
3h	19.8 ± 3.4†	16.0 ± 2.5	-19.2	0.047
5h	17.7 ± 3.1	15.7 ± 2.5	-11.3	0.200
6h	17.8 ± 2.9	16.5 ± 2.7	-7.3	0.265
8h	15.7 ± 2.3	14.6 ± 2.0	-7.0	0.419
10h	16.5 ± 2.5	14.5 ± 2.2	-12.1	0.156
AUC	182 ± 29	155 ± 22	-14.8	0.024

Data presented as mean ± SEM;
apo(a): apolipoprotien(a); AUC: area-under curve; TRL: triglyceride-rich lipoprotein
*P values compared with no treatment group using t-test.
Bold values denote statistically significance at the $P<0.05$
†$P<0.05$ compared with fasting levels at 0hr

and 3 h (–19.2%) ($P<0.05$ in all). There was no significant effect on the fasting total apo(a) and TRL-apo(a) with ω3FA supplementation ($P>0.05$ for both). ω3FA supplementation lowered postprandial plasma total apo(a) AUC (–3.2%), although not significantly ($P=0.191$). Postprandial TRL-apo(a) AUC (0–10 h) was significantly reduced by 15% with ω3FA supplementation ($P<0.05$). There was no significant effect on fasting concentration and postprandial AUC for non-TRL-apo(a) with ω3FA supplementation (data not shown; $P>0.05$ for both). ω3FA supplementation had no significant effect on fasting and postprandial apo(a) in the non-TRL fraction ($P>0.05$, **Supplementary Table 2**).

The percentage change in TRL-apo(a) AUC with ω3FA supplementation was significantly associated with the corresponding percentage changes in the AUCs of triglycerides (**Fig. 2A**; r=0.600; $P=0.007$) and apoB-48 (**Fig. 2B**; r=0.616; $P=0.005$), but not with the AUCs of VLDL-apoB-100, apoC-III, and apoE with ω3FA supplementation ($P>0.05$ for all, data not shown).

Discussion

We demonstrated that FH patients had impaired postprandial TRL-apo(a) response to a fat load and that these abnormalities were partially corrected by ω 3FA supplementation. This was reflected in the decrease in the total AUC of TRL-apo(a). We also found that the percentage change in TRL-apo(a) AUC with ω3FA supplementation was significantly associated with the corresponding changes in the AUCs of triglycerides and apoB-48, but not with those of VLDL-apoB-100, apoC-III, and apoE.

Previous Studies

Several studies have examined the metabolism of

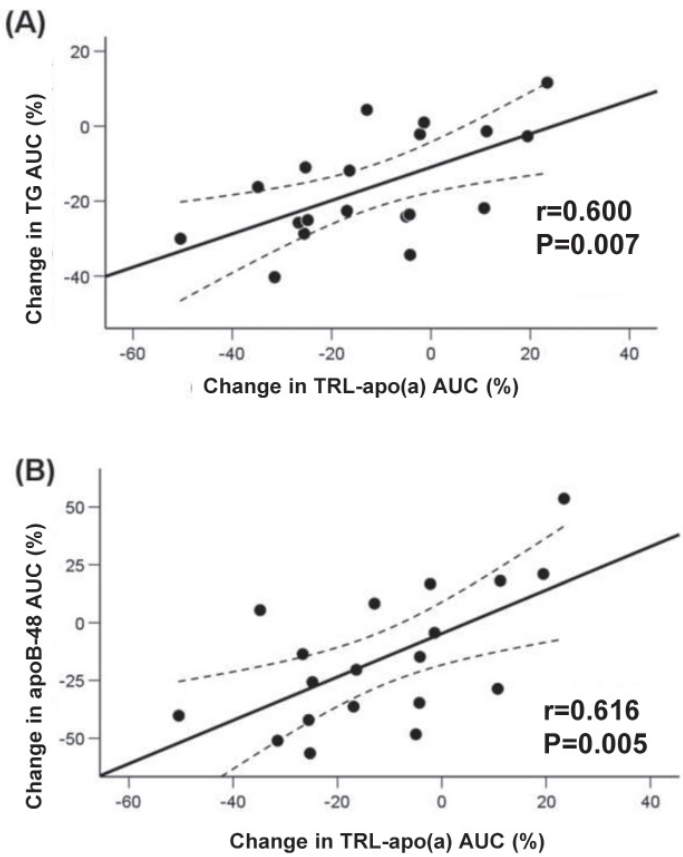

Fig. 2. Association between percentage change in triglyceride-rich lipoprotein-apo(a) area-under curve (AUC) and (A) triglyceride AUC and (B) apoB-48 AUC with ω-3 FA supplementation

Plots displayed as mean ± 95%CI.
Apo: apolipoprotein; AUC: area-under curve; TG: triglyceride; TRL: triglyceride-rich lipoprotein.

apo(a) in non-FH subjects, with conflicting results showing increased, decreased, or no change in Lp(a) concentration following a fat load[26-31]. We have previously reported that plasma Lp(a) concentrations did not significantly change after oral fat load in statin-treated patients with type 2 diabetes[32]. However, none of these studies specifically examined the metabolism of TRL-apo(a) in the postprandial state. In a study of 20 normolipemic men with a wide range of apo(a), Cohn *et al.* found that TRL-apo(a), but not total apo(a), increased in the postprandial state following a fat-rich meal[10]. In another study of four healthy individuals with an elevated level of plasma Lp(a), Marcoux *et al.* also found that there was a rise in TRL-apo(a) in the postprandial state[11], consistent with another report in patients with CAD[29]. However, owing to the small sample size, the increase did not reach statistical significance. The aforementioned discrepant findings might be explained by the small sample sizes and differences in subject characteristics, types of fat load, and study protocols (time points for blood sampling). Our present data add to previous studies by investigating the postprandial responses for total apo(a) and TRL-apo(a) in subjects with FH. We also examine the effect of high-dose ω3FA supplementation (4 g/day) on postprandial total and TRL-apo(a) in response to a fat load in FH patients against a background of cholesterol-lowering treatment (i.e., statin with or without ezetimibe).

TRL-apo(a) metabolism in FH

Fasting State

Experimental evidence suggests that the TRL-apo(a) complex is particularly atherogenic. This relates to an accumulation of lipids in macrophages[33], delayed chylomicron remnant clearance[34], and increased arterial uptake of apo(a) via VLDL receptors[19]. Several mechanisms have been proposed for the presence of apo(a) in TRL. It is possible that apo(a) is secreted by the liver as a component of VLDL[35]. Alternatively, the apoB-100-apo(a) complex

within the Lp(a) particle may non-covalently bind to TRLs in the circulation[11]. It is noteworthy that the existence of non-covalent apo(a)-apoB-100 complexes within the secretory pathway in HepG2 cells has recently been reported[36]. The distribution of plasma apo(a) in the TRL fraction is affected by different physiological conditions. Approximately 2%–5% of plasma apo(a) is found in the TRL fraction in healthy normolipidemic individuals[19]. By contrast, up to 40%–70% of plasma apo(a) can be bound to TRL in patients with hypertriglyceridemia[19]. In the present study, we found that FH patients had a higher percentage of apo(a) detected in the TRL fraction (~20%) than non-FH controls. This level is comparable to that observed in an earlier study showing that 17% of plasma apo(a) was associated with TRLs in patients with coronary heart disease[29]. However, the precise mechanism for the association of apo(a) to TRLs in the fasted state is poorly understood; whether decreased LDLR activity and/or statin use in FH has a role in it remains to be elucidated.

Postprandial State

Consistent with previous studies[10, 11], we found that the plasma concentration of TRL-apo(a) increased significantly following fat load. The observation that fasting total and TRL-apo(a) levels were significantly associated with the AUC of TRL-apo(a) suggests that the rise in TRL-apo(a) in response to a fat load depends on the fasting apo(a) concentration. It is possible that TRL-apo(a) are secreted directly by the liver in response to the fat load[37]. The fed state could also preferentially cause increased secretion of larger TRLs, particularly chylomicron particles (density < 1.006), thereby allowing more apo(a) to bind newly synthesized or secreted TRL particles. These potential mechanisms are supported by our results showing a small but significant increase in the plasma total apo(a) concentration following the first hour of fat load. An alternative explanation for the increase in apo(a) in postprandial TRL involves a simple transfer mechanism of apo(a) from non-TRL to TRL particles[10]. However, given that there was no significant decrease in the plasma concentration of apo(a) in the non-TRL fraction following fat load, this second explanation appears less likely. Nonetheless, this needs to be confirmed in a study with a larger sample size. Accordingly, we also found that our FH patients had impaired postprandial response for TRL-apo(a) (total AUC) compared with non-FH controls. Whether the higher total AUC of TRL-apo(a) was attributable to increased production and/or impaired catabolism of TRL-apo(a) in the fasting and postprandial conditions requires investigation.

Both apoC-III and apoE play a critical role in TRL metabolism[38, 39]. We previously showed that the metabolism of Lp(a) is modulated by apoE in the post-absorptive state[25]. In the present study, we found no association between the AUCs of total apo(a) or TRL-apo(a) and the corresponding AUC for apoC-III or apoE in FH patients. This observation suggests that apoC-III and apoE do not play a significant role in the regulation of Lp(a) metabolism in the postprandial state. Statins can regulate the metabolism of apoC-III and apoE [40, 41]; however, whether this accounts for the negative postprandial findings in our study remains to be confirmed. The effect of the apoE phenotype, particularly the presence of apoE2, may also influence the metabolism of TRL-apo(a)[42]. Since the number of FH patients with the E2 phenotype was small in our study (n=3), statistical comparison of the concentration and AUC of TRL-apo(a) between FH patients with and without the apoE2 phenotype was not possible. The impact of APOE genotypes on the metabolism of TRL-apo(a) merits further investigation.

Mode of Action of ω3FA Supplementation

The precise mechanism(s) of action of ω3FA supplementation lowering postprandial AUC of TRL-apo(a) is not clear. There is no evidence demonstrating the direct effect of ω3FA on the hepatic secretion and clearance of apo(a). We have previously demonstrated that ω-3FA supplementation can improve TRL metabolism by decreasing the secretion of apoB-48 in obese subjects[43]. As discussed earlier, the mechanisms by which TRL-apo(a) concentrations increased following a fat load may involve increase in the secretion of larger TRLs. Consistently, the observed improvement in postprandial TRL-apo(a) metabolism following the first 2 h of fat load is likely to be a consequence of a decrease in the hepatic and/or intestinal secretion of postprandial TRLs with ω3FA supplementation. This is also supported by the observed significant association between the percentage change in TRL-apo(a) AUC with those in the AUCs of triglycerides and apoB-48 following treatment with ω3FAs. However, we did not find a correlation between the percentage change in TRL-apo(a) AUC and the corresponding percentage change in the AUCs of VLDL-apoB-100 with ω3FA supplementation. Hence, it is possible that the apo(a) (free or with apoB-100 complex) in TRLs may preferentially bind to chylomicron remnants, follow their fate, and thus be removed from the circulation by the remnant receptor pathway. Consistent with

this, experimental data show that chylomicrons can bind more Lp(a) per particle than VLDL[19]. This speculation merits further investigation. The lack of treatment effect of ω3FA supplementation on the postprandial AUCs of apoC-III or apoE also did not support any potential mechanistic effect of ω3FA supplementation on postprandial TRL-apo(a) catabolism.

Study Limitations

Our study has several limitations. The sample size was relatively small; hence, we cannot not exclude the possibility that our results showing a significant difference in fasting plasma TRL-apo(a), but not in total apo(a) concentrations, between the FH and control groups might be caused by a type II statistical error. Nevertheless, our findings were consistent with another report in patients with CAD[29]. In addition, we might have been unable to detect a significant change in the postprandial total and non-TRL-apo(a) AUCs. In the present study, we found that the fasting total apo(a) concentration was significantly associated with the AUC of TRL-apo(a). Given that low apo(a) isoform size is associated with high plasma Lp(a) level and high risk for ASCVD[44], variations in apo(a) isoform size may affect the non-covalent binding of apo(a) to TRLs. Thus, determination of apo(a) isoform size may help elucidate its impact on the postprandial metabolism of TRL-apo(a). The composition of the fat meal in the current study may confound the effect of ω3FA supplementation on postprandial TRL-apo(a)[45]. Hence, our results might have been different if we employed a test meal with a different composition (e.g., low-fat or mixed meal). We did not specifically study FH patients with elevated plasma concentrations of Lp(a). In a subgroup analysis of six FH patients with elevated Lp(a) (>0.5 g/L), we found that ω3FA supplementation reduced the AUC of TRL-apo(a) (337±41 vs. 271±31 nmol/L; $P<0.05$). Hence, we consider that our findings could also generally be applied to subjects ith elevated Lp(a) concentrations, but this needs to be confirmed in a larger population. We did not measure apo(a) concentration specifically in the VLDL and chylomicron fractions, which might have helped clarify the mechanism of action of ω3FAs on apo(a) metabolism in the postprandial state. Further investigations should examine the relationship between apo(a) isoform and the postprandial metabolism of TRL-apo(a) and the corresponding effects of ω3FA supplementation [46].

Conclusions

FH is associated with an extremely high risk of ASCVD, not all of which is explained by elevation in LDL-cholesterol[6, 8, 12, 47]. Lp(a) can aggravate the development of ASCVD and CAVD in FH. Our data suggest that a significant portion of apo(a) can be distributed in the TRL fraction both in the fasting and postprandial states, which may increase the atherogenicity of TRL particles; whether this is magnified in patients with elevated Lp(a) remains to be confirmed. Our study provides a novel mechanistic insight into the favorable effect of high-dose ω3FA supplementation on TRL-apo(a) metabolism in FH patients on maximally tolerated statin therapy and ezetimibe. Whether the effect of ω3FA supplementation on TRL-apo(a) metabolism is seen in other high-risk conditions other than FH, such as in chylomicronemia syndrome and diabetes mellitus, needs confirmation. Moreover, the cadiovascular benefit of TRL-apo(a) reduction in the Reduction of Cardiovascular Events with EPA-Intervention Trial also merits further investigation[48]. In view of the different effects of DHA and EPA on both TRL and LDL metabolism[49], it may be of interest to examine the role of pure DHA and EPA on postprandial apo(a) metabolism. Future studies should examine the effects of new agents, such as inhibitors of angiopoietin-like protein 3 and antisense oligonucleotides against apo(a), in addition to statin therapy on postprandial TRL-apo(a) metabolism[50].

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Contributors

DCC, JP and GFW designed the study. DC, JP and GFW conducted the study. QY, MC, DCC, VB, JP and MK analysed the data. QY, DCC and GFW drafted the manuscript. All authors reviewed and approved the manuscript.

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Competing Interests

GFW has received honoraria for advisory boards and research grants from Amgen, Arrowhead, Esperion, AstraZenca, Kowa, Novartis, Pfizer, Sanofi and Regeneron.

Data Availability

The protocol/data that support the findings of this study are available from the corresponding author on reasonable request.

Clinical Trial Registration

https://www.clinicaltrials.com/ NCT01577056

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Supplementary Table 1. Effects of ω-3FA supplementation on postprandial area under curves (AUCs) for postprandial triglycerides, very-low-density lipoprotein (VLDL), apolipoproteinB-48 (apoB-48), apolipoprotein C-III (apoC-III) and apolipoprotein E (apoE) responses to the fat load

	No ω-3FA Treatment	ω-3FA
Triglycerides (mmol/L•10 h)	27.8 ± 4.7	22.3 ± 3.3**
VLDL-apoB-100 (mg/L•10 h)	964 ± 79	702 ± 53***
ApoB-48 (mg/L•10 h)	190 ± 38	137 ± 21*
ApoC-III (mg/L•10 h)		
Total	800 ± 82.8	730 ± 79.3
LpB fraction	472 ± 41	452 ± 53
ApoE (mg/L•10 h)		
Total	704 ± 78	611 ± 45
LpB fraction	380 ± 44	345 ± 28

Data presented as mean ± SEM; LpB: apoB-containing lipoprotein
*: $P<0.05$, **: $P<0.01$, ***: $P<0.001$ compared with no ω-3FA treatment group using *t*-test.

Supplementary Table 2. Effects of ω-3FA supplementation on postprandial apo(a) concentration in non-TRL fraction in the FH patients

	Non-TRL apo(a) (nmol/L)			*P* value*
	No ω-3FA Treatment	ω-3FA	%	
0h	76.1 ± 21.0	76.0 ± 20.6	-0.1	0.958
0.5h	83.6 ± 24.0	79.6 ± 21.2	-4.8	0.505
1h	88.2 ± 26.0†	83.7 ± 23.0	-5.1	0.648
2h	80.0 ± 18.8	80.0 ± 18.9	-0.0	0.991
3h	79.1 ± 21.5	81.4 ± 22.7	+2.9	0.582
5h	79.2 ± 21.2	73.4 ± 19.0	-7.3	0.238
6h	76.6 ± 20.1	72.6 ± 20.9	-5.2	0.415
8h	74.0 ± 21.5	78.9 ± 24.9	+6.6	0.450
10h	73.1 ± 20.0	75.4 ± 20.5	+3.1	0.591
AUC	781 ± 210	776 ± 213	-0.6	0.842

Data presented as mean ± SEM;
apo(a): apolipoprotien(a); AUC: area-under curve; TRL: triglyceride-rich lipoprotein
Non-TRL apo(a) was calculated by substracting TRL-apo(a) from total apo(a) concentration
*P values compared with no treatment group using *t*-test.
Bold values denote statistically significance at the $P<0.05$
†$P<0.05$ compared with fasting levels at 0hr

Supplementary Table 3. Plasma apolipoprotein C-III and apolipoprotein E concentration and area under curves in the two groups

		Control ($n=10$)	FH ($n=20$)	*P* value
Apolipoprotein C-III*				
Total	Concentration	74.1 ± 33.5	85.5 ± 33.7	0.387
	AUC	673 ± 300	800 ± 370	0.356
Apolipoprotein E*				
Total	Concentration	35.1 ± 16.1	66.3 ± 22.6	0.001
	AUC	322 ± 140	704 ± 350	0.003

Mean ± SD; AUC: area under curve; FH: familial hypercholesterolemia
*Concentration expressed as mg/L and AUC as mg/L •10 h