APOLIPOPROTEIN E (epsilon) genotype has a greater impact on apoB-48 than apoB-100 responses to dietary fat manipulation– insights from the SATgenε study

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Abbreviations: %E, percentage of energy; ANOVA, analysis of variance; apo, apolipoprotein; AUC, area under the curve; CHO, carbohydrate; CVD, cardiovascular disease; DHA, docosahexaenoic acid; LF, low fat; HDL-C, HDL cholesterol; HSF, high saturated fat; HSF-DHA, HSF diet with DHA; IAUC, incremental AUC; LDL-C, LDL

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cholesterol; maxC, maximum concentration; RP, retinyl palmitate; S_f, Svedberg flotation rate; SFA, saturated fatty acid; TAG, triacylglycerol; TRL, TAG-rich lipoprotein.

Keywords: *APOE* genotype; dietary fat quantity, docosahexaenoic acid; saturated fat, triacylglycerol

Abstract

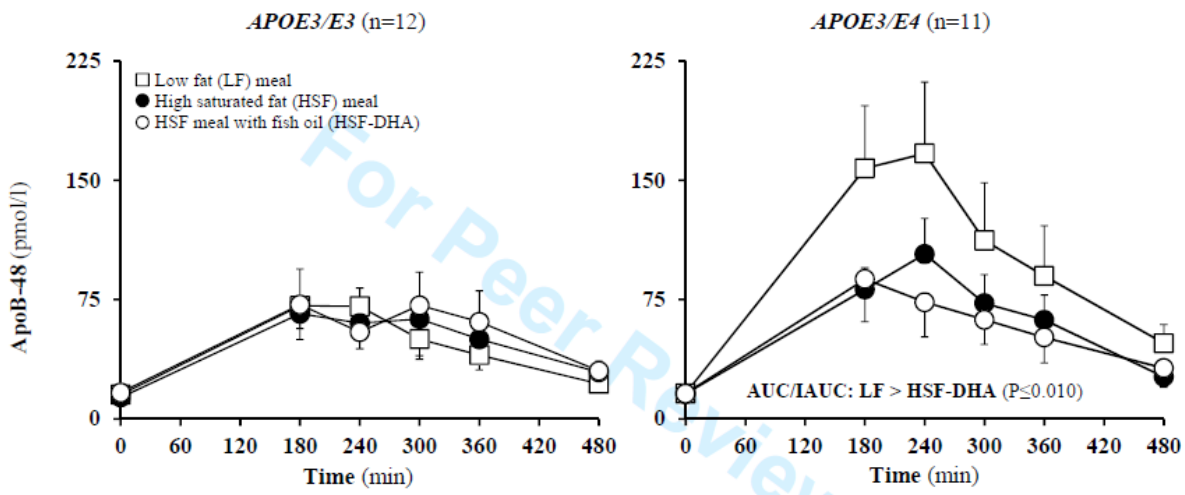
Scope: To determine the contribution of intestinally and liver-derived lipoproteins to the postprandial plasma triacylglycerol (TAG) response in *APOE3/E3* and *E3/E4* individuals following chronic dietary fat manipulation.

Methods and Results: In sequential order, participants (n=12 *E3/E3*, n=11 *E3/E4*) followed low fat (LF); high-fat, high-saturated fat (HSF); and HSF with 3.45 g/day docosahexaenoic acid (HSF-DHA) diets, each for 8 weeks. After each dietary period, an acute test meal with a macronutrient profile representative of the dietary intervention was consumed. Apolipoprotein (apo)B isoforms were determined in isolated TAG-rich lipoprotein fractions (S_f>400, S_f 60-400 and S_f 20-60) by specific ELISA. A genotype*meal/diet interaction for the S_f>400 fraction apoB-48 response (P<0.05) was observed, with higher concentrations reached after the LF than HSF-DHA meal in *E4* carriers. This finding was associated with a lower TAG content of the S_f>400 particles. Fasting S_f 60-400 and 20-60 apoB-48 concentrations were also significantly higher in *E4* carriers. No impact of genotype on the apoB-100 responses was evident.

Conclusion: Our study revealed marked effects of dietary fat composition on the S_f>400 apoB-48 response and particle TAG content in *E4* carriers relative to the 'wild-type' *E3/E3* genotype, which suggest *APOE* genotype is a potential modulator of chylomicron particle synthesis.

Molecular Nutrition and Food Research graphical abstract text

We determined the effects of chronic dietary fat manipulation on postprandial triacylglycerol-rich lipoprotein metabolism according to apolipoprotein (*APO)E* genotype. *APOE4* carriers showed greater changes in the number (apoB-48) and particle composition of intestinally derived lipoproteins circulating following diets/meals of varying fat content and composition than the *E3/E3* group. Our findings suggest a potential role of *APOE* genotype in chylomicron synthesis.



1. Introduction

The concentration of triacylglycerol (TAG) in the fasting and postprandial (fed) state has been consistently associated with cardiovascular disease (CVD) risk, although causality has not been fully established [1]. The contribution of the liver-derived TAG-rich lipoproteins (TRL), VLDL and their remnants, to the pathogenesis of atherosclerosis has been well described [2-4]. Elevated levels of chylomicrons and chylomicron remnants which circulate

following a meal have also been directly and indirectly related to CVD risk. However, different mechanisms are thought to operate for the liver and intestinally-derived TRL [1]. Yet, insight into the atherogenicity of these intestinally-derived TRL have been slower to emerge due to methodological difficulties associated with the determination of their inherent apolipoprotein, apolipoprotein (apo)B-48, in plasma and TRL fractions [5].

The *APOE* (epsilon) genotype is considered to be an important genetic determinant of the inter-individual variation in postprandial lipid metabolism [6-8]. with higher TAG concentrations reported in *E4* carriers relative to the 'wild-type' *E3/E3* group [9]. However, little is known about the contribution of intestinally (apoB-48) and liver (apoB-100) derived TRL to the total TAG response according to *APOE* genotype. Of the studies conducted [10-13], *APOE* genotyping has been performed retrospectively resulting in differences in age, male/female ratio and subject numbers between the *E3/E3* and *E3/E4* study groups, which makes the independent impact of genotype difficult to establish with certainty. Using a prospective genotyping approach in the SATgenε study, we aimed to determine for the first time, both the postprandial TRL apoB-48 and B-100 responses to meals of varying fat composition in *E3/E3* and *E3/E4* men, matched for age and BMI.

2. Methods

2.1 Subjects and postprandial protocol

Details of the SATgenε dietary intervention [8] and postprandial study [9] have been published elsewhere. The study was given a favourable ethical opinion for conduct by the University of Reading Research Ethics committee (project 08/62) and is registered at Clinicaltrials.gov I.D NCT0138. All subjects gave informed consent prior to participation in

the study. Briefly 23 men prospectively recruited according to *APOE* genotype (n=12 *E3/E3* and n=11 *E3/E4*) underwent a postprandial investigation at the end of the 8 week prescribed iso-energetic low fat (LF) diet, high fat, high saturated fat (HSF) diet and HSF diet with 3.45 g/day of docosahexaenoic acid (HSF-DHA). The macronutrient composition of the diets and test meals are shown in Table 1. After an overnight fast, the subjects attended the clinical investigation unit where an indwelling cannula was inserted into a vein in the forearm, and a fasting blood sample (0 min) was taken. The test meal was given in the form of a warm chocolate drink containing the test oils with toast and jam, and consumed within 20 min. For isolation of the TRL fractions, Svedberg flotation rate (S_f)>400 (predominately chylomicrons), S_f 60-400 (large TAG-rich VLDL1) and S_f 20-60 (smaller TAG-poor VLDL2), blood was collected at 180, 240, 300, 360 and 480 min post meal.

2.2 TRL isolation and apoB analysis

Blood samples and TRL fractions were isolated as previously described [9]. ApoB-48 and B-100 were measured in the TRL fractions using specific in-house ELISAs [14]. For the apoB-100 ELISA, some modifications to the original method were made. Briefly, the ELISA plate was coated with 1 mg/ml of a 1:1 mixture of apoB-100 monoclonal antibodies 4G3 and 5E11 (Ottawa Heart Institute Research Corporation, University of Ottawa) in 0.1 M bicarbonate-carbonate buffer (pH 9.6) for 16 h at 4°C. After washing with 0.02 M PBS containing 0.05% (by volume) Tween 20 and 0.1% BSA (PBSBT), the plate was blocked with 150 µl of 0.02M PBS, 0.025% (by volume) Tween 20 and 3% BSA at 37°C for 1 h. A 9 point standard curve was prepared by serial dilution of LDL (density=1.019-1.063 g/ml, Source Bioscience, Nottinghamshire, UK) in PBSBT to produce a concentration range of apoB-100 from 1.25 µg/ml to 5 ng/ml. The S_f>400 fraction was diluted 1:6, the S_f 60-400 fraction 1:1000 and S_f 20-60 fraction 1:2000 in PBSBT prior to the addition to the plate. Standards, samples and

quality controls (100 µl/well) were added in duplicate to the plate and incubated for 2 h at 37°C. The plate was washed, 100 µl of goat anti-apoB antibody conjugated to horse radish peroxidase (Source Bioscience) was added to the plate at a final dilution of 1:20,000, and incubated for a further 2 h at 37°C. After washing the plate, 100 µl of 3, 3', 5, 5'-tetramethylbenzamidine substrate (Sigma, Dorset, UK) was added for 30 min and reaction stopped by the addition of 1M HCL. The absorbance was read at 450 nm. The inter-assay CVs for the apoB-48 and apoB-100 assays were less than 10%.

2.3 Statistical analysis

Details of the sample size calculation for the postprandial study have been previously described [9]. Data were analysed using PASW statistics 18 (SPSS Inc, Chicago, IL). All data were checked for normality and log transformed where necessary before statistical analysis. Repeated measures analysis of variance (ANOVA) determined the effects of the meals on the postprandial time course responses, with 'meal' and 'time' as the within-subject factors and 'genotype' as the between-subjects factor. A one-within, one-between repeated measures ANOVA analysed differences in the fasting concentrations and postprandial summary measures between the genotype groups. For a significant main effect of 'diet/meal', Student's paired t-tests were performed, with a Holm's sequential Bonferroni correction applied to correct for significant pairwise differences. Data are presented as mean ± SEM. Values of $P \leq 0.05$ were taken as significant.

3. Results

3.1 ApoB-48 responses

The impact of the test meals on the postprandial apoB-48 response in the S_f>400 fraction (predominately chylomicrons) was influenced by *APOE* genotype (meal*genotype

interaction, *P*=0.030), with higher apoB-48 concentrations following the LF compared with the HSF-DHA meal in the *E3/E4* group only (Fig.1). This was reflected in the greater AUC (44%), IAUC (53%) and maxC (53%) after the LF than HSF-DHA meal ($P \leq 0.010$; Table 2). ApoB-48 responses were not different between meals in the *E3/E3* group (Fig. 1 and Table 2).

Since meal*genotype interactions were not evident for the apoB-48 responses in the S_f 60-400 and S_f 20-60 TRL fractions, data for the two genotype groups within each TRL fraction were combined to determine the overall effects of the diet/meals on the fasting and postprandial responses. In the S_f 60-400 fraction, fasting apoB-48 concentrations were higher after the LF than HSF-DHA diet (*P*=0.006; Supplementary Table 1). A higher postprandial time course response, AUC, IAUC and maxC was also observed after the LF than HSF and HSF-DHA meals ($P \leq 0.017$; Supplementary Fig.1 and Supplementary Table 1). Only the apoB-48 IAUC (found to be higher after the HSF compared with the LF meal) was influenced by meal composition in S_f 20-60 TRL fraction (*P*=0.005; Supplementary Table 1).

Independent of the diet/meals, *APOE* genotype was shown to influence fasting apoB-48 concentrations. Compared with the wild-type *E3/E3* group, *E4* carriers had higher concentrations in both the S_f 60-400 (46.5 vs. 63.5 pmol/l; *P*=0.048) and S_f 20-60 (47.8 vs 78.1 pmol/l; *P*=0.003) fractions. A genotype effect was also observed for maxC reached after the meals in the S_f 20-60 fraction, with on average higher apoB-48 concentrations in the *E3/E4* (160 pmol/l) than *E3/E3* (122 pmol/l) group (*P*=0.035).

3.2 ApoB-100 responses

Independent of genotype, diet/test meal composition only influenced fasting and postprandial apoB-100 concentrations in the S_f 60-400 TRL fraction ($P<0.001$; Supplementary Table 1). Fasting concentrations were 52% and 42% lower after the HSF-DHA than LF and HSF diets,

respectively. The postprandial time response profile, AUC and maxC were also lower after HSF-DHA compared with the other test meals (P≤0.005; Supplementary Fig. 1 and Supplementary Table 1). There was no impact of diet/meal composition or genotype on fasting apoB-100 concentration or postprandial responses determined in the S_f>400 and S_f 20-60 fractions.

3.3 S_f>400 fraction TRL composition

As each chylomicron particle has only one apoB-48 molecule, the TAG, cholesterol and apoE concentrations [9] were expressed as a ratio of the apoB-48 concentration in order to establish particle composition and gain insight into possible mechanisms underlying the differences in the S_f>400 fraction apoB-48 responses between the meals in the *E4* carriers. There was a significant meal*genotype interaction for the TAG:apoB-48 ratio (an indicator of particle TAG content) of the S_f>400 particles after the meal (*P*=0.015), with a higher ratio observed after the HSF than LF meal in the *E4* carriers only (*P*=0.009; Fig.2). The IAUC for the postprandial TAG:apoB-48 ratio was also lower after the LF meal than following the HSF and HSF-DHA meals (*P*≤0.023). Differences were not evident in the postprandial TAG:apoB-48 ratio between meals in the *E3/E3* group. Meal composition and genotype did not influence the cholesterol:apoB-48 or apoE:apoB-48 ratios (data not shown).

4. Discussion

An *APOE4* genotype (20-25% Caucasians) has been associated with reduced longevity [15] and increased CVD risk [16], with an impact on fasting and postprandial lipid metabolism likely to significantly contribute to the higher CVD incidence. Here, in the SATgenε study, we observed a greater responsiveness of the S_f>400 fraction apoB-48 response to diet/meals of varying fat content and composition in *E4* carriers, with little impact of *APOE* genotype on TRL apoB-100 responses.

Only three studies have determined postprandial TRL apoB-48 and apoB-100 responses according to *APOE* genotype [10, 11, 13], but findings are inconsistent. In two studies [10, 11], similar fasting and postprandial TRL apoB-48:B-100 ratios and retinyl palmitate (RP, an indirect marker of chylomicrons and their remnants) concentrations were observed in response to high fat meals (100-105 g) in *E3/E3* and *E4* carriers. Using stable isotopes (a primed constant infusion of deuterated leucine) in the fed state, Welty et al. [13] reported *E3/E4* individuals to have a significantly lower postprandial TRL (d<1.006 g/ml) apoB-100 production rate after small meals (each equivalent to 1/20th of their daily food intake) representative of an American diet (36% fat) whereas the TRL apoB-48 pool size and clearance of chylomicron remnants were not different between the genotype groups. In contrast to previous studies which genotyped retrospectively, we prospectively recruited according to *APOE* genotype and matched the groups for age and BMI, two important determinants of the postprandial lipaemic response [17, 18]. *E4* carriers had higher fasting S_f 60-400 and S_f 20-60 apoB-48 concentrations, and reached higher postprandial (maxC) concentrations in the S_f 20-60 fraction than *E3/E3* individuals, with apoB-100 responses similar in the genotype groups. The technique used to measure intestinally-derived lipoproteins may provide an explanation for the inconsistent findings on the effects of *APOE* genotype on TRL apoB responses. Early studies [10, 11] included RP in the test meal to label the lipid moiety of chylomicrons and determined RP responses in plasma and TRL fractions. However, discordance in the data from studies comparing apoB-48 (the integral protein component of chylomicrons) with RP responses, has highlighted limitations of the latter as a specific marker of chylomicron remnants [19]. Furthermore, the isolation of only TAG-rich and TAG-poor TRL fractions in the earlier studies compared with the three distinct TRL fractions of progressively increasing density and decreasing particle size [20] in the current

study, may have masked the specific effects of *APOE* genotype on TRL apoB-48 metabolism.

A novel aspect of our study design is the use of test meals which were representative of the fat content and composition of the intervention diet, providing a more physiological assessment of the effects of diets with varying fat composition on postprandial lipaemia. As far as the authors are aware, only one other study has determined the effects of habitual dietary fat manipulation on TRL apoB responses according to *APOE* genotype [12]. Following a high-fat PUFA-rich diet for 29 days, fasting concentrations of TRL apoB-48 and B-100 were similar in the *E3/E3* and *E3/E4* groups, with *E4* carriers showing a delayed clearance of both apoB-48 and B-100 TRL remnants after the test meals, irrespective of whether they were enriched in SFA (PUFA/SFA ratio = 0.2) or PUFA (PUFA/SFA ratio = 1.3). In the present study, greater postprandial changes in apoB-48, but not B-100 in response to the test meal fat content and composition were evident in *E4* carriers. In particular, higher S_f>400 apoB-48 responses and IAUC after the LF than HSF-DHA meal are suggestive of either a greater production and/or impaired clearance of chylomicrons after the low fat, high carbohydrate test meal.

Insulin plays a pivotal role in chylomicron metabolism by regulating apoB-48 secretion, the activity of the hydrolytic enzyme lipoprotein lipase and the expression of hepatic remnant receptors [21, 22]. Acutely, insulin secretion has been shown to be influenced by test meal fat composition [23]. In the current study, as we have previously reported, the insulin and glucose responses to the meals were similar within each genotype group [9], indicating that differences in insulin action do not contribute to the marked effects of meal composition on apoB-48 responses in *APOE4* carriers. Animal studies have reported gut hormones, in particular glucagon-like peptide 2 to play an important role in stimulating intestinal lipid absorption and chylomicron production [24-26] but findings in humans are

limited [27]. The impact of *APOE* genotype on gut hormone production is currently unknown.

Previous reports by ourselves [14] and others [28, 29] have suggested that the meal fat composition could influence the lipid and apolipoprotein content of TRL particles which has been proposed to affect their rate of metabolism in the circulation. A notable difference in the TAG content (TAG:apoB-48 ratio) of the postprandial S_f>400 TRLs after the meals was evident, with a lower TAG content and smaller incremental change in the TAG enrichment of the TRL particles after the LF relative to the high-fat meals. Since TRL particle size is determined primarily by the TAG content, the smaller chylomicrons circulating after the LF than HSF-DHA meal may have been more resistant to hydrolysis by lipoprotein lipase which could result in delayed clearance and provide an explanation for the greater S_f>400 fraction apoB-48 response relative to the HSF-DHA meal. Although the apoE content of the S_f>400 particles was not different, the apoE isoform (E3 versus E4) present on the surface of the TRLs may have also influenced their metabolism in the circulation. In particular, apoE3 shows a greater preference for smaller TAG-poor TRL whereas apoE4 binds specifically to larger TAG-rich particles [30, 31] and is associated with a more atherogenic lipoprotein profile [30]. We speculate that the larger TAG-rich chylomicrons circulating after the HSF-DHA meal (than LF meal) were more enriched with apoE4, facilitating a more rapid conversion to smaller denser remnant particles than after the LF meal. However, similar S_f 20-60 fraction apoB-48 responses (representing small chylomicrons and chylomicron remnants) were observed after the HSF-DHA and LF meals, which were lower relative to the HSF meal. It is unclear from our data whether differences in production rate and clearance of these particles from the circulation or rates of conversion between S_f>400 to S_f 60-400 and S_f 20-60 fractions were responsible for these findings.

With the increasing recognition of the contribution of raised levels of intestinally-derived lipoproteins after a fat containing meal to CVD risk, there is considerable interest in the effects of diet on postprandial fat handling and TRL composition. Our study has revealed marked effects of the LF diet/meal on S_f>400 apoB-48 responses in *E4* carriers relative to the HSF-DHA diet/meal, which was associated with a notable difference in the estimated TAG content of the chylomicron particles. Although the postprandial S_f>400 TAG time response profiles were comparable after the LF, HSF and HSF-DHA diet/meals, our findings indicate that consumption of the lower fat and higher carbohydrate diet may have impacted on pathways regulating chylomicron synthesis and particle lipid composition, particularly in *APOE3/E4* individuals. Our novel findings are of interest and potentially lend support to a recent study in men classified with the metabolic syndrome [32] which proposed that the enterocyte does not just process dietary TAG but may also upregulate pathways for the synthesis of fatty acids (de novo lipogenesis) during higher intakes of carbohydrate. The *APOE* genotype now represents one of the few common genotypes consistently associated with longevity, chronic disease risk and responsiveness to dietary manipulation. Therefore, further studies, in particular kinetic studies using stable isotopes, are warranted to confirm our findings and provide insights into mechanisms underlying the effects of *APOE* genotype on intestinal fat absorption, chylomicron synthesis and metabolism.

Author Contributions

AMM, KGJ, JAL and CMW designed the study; SL and ACW were responsible for running the study and managing the clinical visits, and ACW performed the TRL isolation; KGJ performed the apoB-48 and apoB-100 analysis, conducted the statistical analysis and wrote the manuscript with JAL and AMM. All authors approved the final version of the manuscript.

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Conflict of interest statement

The authors had no conflicts of interest.

5. References

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FIGURE LEGENDS

Figure 1: Mean ± SEM for the apoB-48 responses in the Svedberg flotation rate (S_f)>400 fraction after consumption of test meals representative of the low fat diet (LF, open squares), high saturated fat diet (HSF, closed circles) and HSF diet with fish oil (HSF-DHA, open circles) in the *APOE3/E3* (*n* = 12) and *APOE3/E4* (*n* = 11) groups. For the *E3/E4* group, there was a significant test meal (*P*=0.032) and time (*P*<0.001) effect, with a trend for a meal*time interaction (*P*=0.077).

FIGURE 1

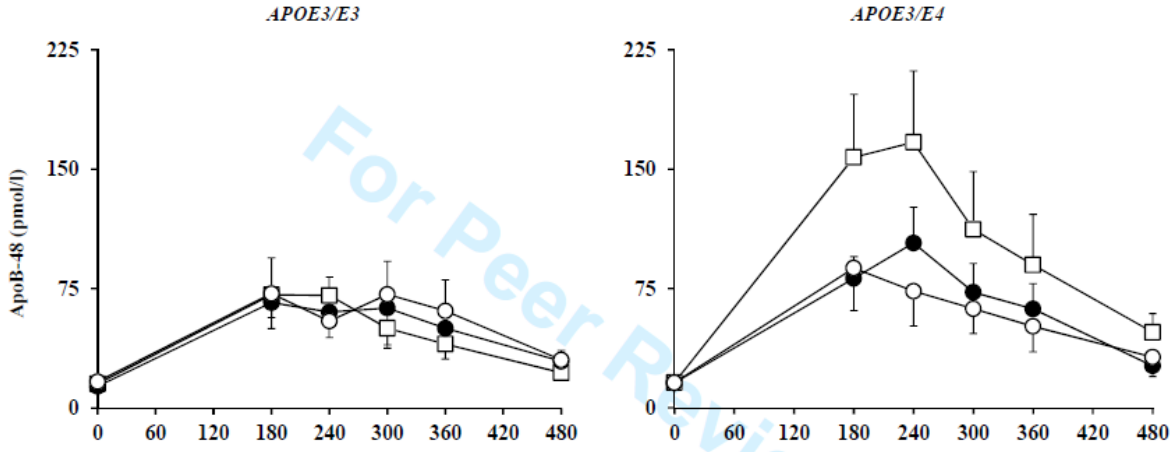

Figure 2: Mean ± SEM for the change in triacylglycerol (TAG) content of the Svedberg flotation rate (S_f)>400 fraction lipoproteins (presented as number of TAG molecules per apoB-48 particle) after consumption of test meals representative of the low fat diet (LF, open squares), high fat, high saturated fat diet (HSF, closed circles) and HSF diet with fish oil (HSF-DHA, open circles) in the *APOE3/E3* (*n* = 12) and *APOE3/E4* (*n* = 11) groups. For the *E3/E4* group, there was a significant test meal (*P*=0.018) and time (*P*<0.001) effect.

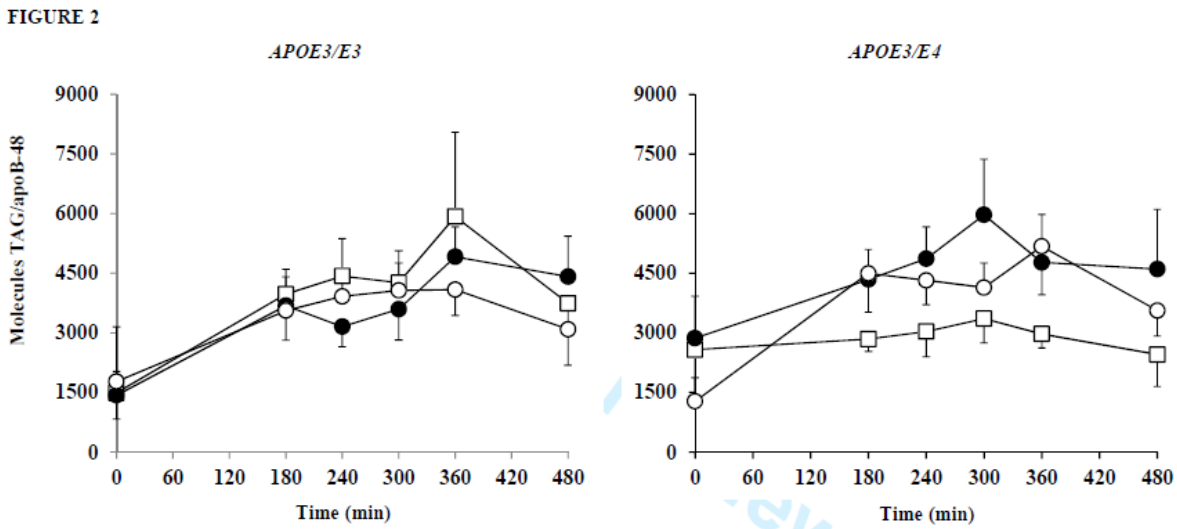

Table 1: Macronutrient composition of the diets and test meals

Composition	Diets[†]			Test meals		
	LF	HSF	HSF-DHA	LF	HSF	HSF-DHA
%Energy from fat	24.0	38.0	38.0	27.5	37.8	37.8
SFA	8.0	18.0	18.0	8.3	18.0	18.3
MUFA	8.0	12.0	12.0	12.2	12.2	12.2
PUFA	6.0	6.0	6.0	6.4	6.4	6.6
%Energy from CHO	55.0	45.0	45.0	55.0	45.0	45.1
%Energy from protein	17.0	17.0	17.0	17.0	17.0	17.0

[†]Target daily intake. Abbreviations: CHO, carbohydrate; HSF, high fat, high saturated fat diet; HSF-DHA, HSF diet with 3.45 g/day docosahexaenoic acid; LF, low fat; SFA, saturated fatty acid.

Table 2: Postprandial summary measures for the S_f>400 fraction apoB-48 responses in the *APOE3/E3* and *APOE3/E4* groups

	Test meals			ANOVA
	LF	**HSF**	**HSF-DHA**	meal (P=)
APOE3/E3 (n=12)				
Fasting (pmol/l)	15.2 ± 2.3	13.5 ± 2.3	16.5 ± 2.6	0.815
AUC	22.1 ± 3.2	22.8 ± 4.5	25.0 ± 5.6	0.585
IAUC	14.8 ± 3.1	16.3 ± 4.2	17.0 ± 5.3	0.857
maxC (pmol/l)	95 ± 13	104 ± 25	107 ± 28.0	0.831
APOE3/E4 (n=11)				
Fasting (pmol/l)	15.9 ± 2.3	15.7 ± 1.8	15.8 ± 2.3	0.954
AUC	48.0 ± 10.6*	28.9 ± 5.1	26.6 ± 7.5	0.007
IAUC	40.4 ± 10.7*	21.4 ± 5.0	19.0 ± 7.2	0.034
maxC (pmol/l)	201 ± 50†	117 ± 21	95 ± 26	0.003

Values represent mean ± SEM. Abbreviations: AUC, area under the curve; HSF, high fat, high saturated fat diet; HSF-DHA, HSF diet with 3.45 g/day docosahexaenoic acid; IAUC, incremental AUC; LF, low fat ; maxC, maximum concentration. AUC and IAUC are expressed as pmol/l x 480 min.

*$P \leq 0.01$, compared with HSF-DHA meal, †$P \leq 0.013$, compared with HSF and HSF-DHA meals.