Apolipoprotein E isoforms disrupt long-chain fatty acid distribution in the plasma, the liver and the adipose tissue of mice.

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Running title: *APOE* genotype on tissue lipid profiles of transgenic mice

Abstract

Evidences suggest that omega-3 fatty acid (*n*−3 PUFA) metabolism is imbalanced in apolipoprotein E epsilon 4 isoform carriers (*APOE4*). This study aimed to investigate *APOE* genotype-dependant modulation of FA profiles, protein and enzyme important to fatty acid (FA) metabolism in the adipose tissue, the liver and the plasma using human *APOE*-targeted replacement mouse-model (N = 37). FA transport (FATP) and binding (FABP) protein levels in tissues and concentrations of liver carnitine palmitoyltransferase 1 (CPT1) were performed. *N-3* PUFA concentration was >45% lower in the adipose tissue and liver of *APOE4* mice compared to *APOE3* mice. In *APOE4* mice, there were higher levels of FATP and FABP in the liver and higher FATP in the adipose tissue compared to *APOE2* mice. There was a trend towards higher CPT1 concentrations in *APOE4* mice compared to *APOE3* mice. Therefore, since *APOE*-isoform differences were not always in line with the unbalanced n–3 PUFA profiles in organs, other proteins may be involved in maintaining n–3 PUFA homeostasis in mice with different *APOE*-isoforms.

Keywords: Transport and binding proteins, *n*–3 PUFA metabolism, cellular FA uptake and degradation, *APOE4* carriers.

Abbreviations used: AA, arachidonic acid; Ab, antibody; AD, Alzheimer's disease; ALA, alpha-linolenic acid; ApoE, apolipoprotein E; CPT1, Carnitine palmitoyltransferase 1; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FA, fatty acid; FABP, fatty acid binding protein; FATP, fatty acid transport protein; LCFA, long-chain fatty acids; *n*–3 PUFA, omega-3 fatty acid; *n*–6 PUFA, omega-6 fatty acid.

1. INTRODUCTION

Fatty acids (FA), notably long-chain fatty acids (LCFA) such as docosahexaenoic acid (DHA, 22:6), are essentials for brain growth as well as for brain health maintenance [1]. Imbalance in FA metabolism has been associated with neurological diseases such as depression and Alzheimer's disease (AD) [2]. AD is the most common type of dementia (around 50–60% of all cases) and ranked fifth as leading cause of death in American population aged 65 years and over [3]. This disease results from a combination of non-modifiable factors (i.e. genetic factors) and reversible factors such as diet [4]. Most important genetic risk factor of AD is carrying one or two allele(s) of the apolipoprotein E ε4 (*APOE4*) [5-7]. Fatty fish intake containing omega-3 FA (*n*–3 PUFA), such as DHA is suggested to reduce the risk of developing AD but this link seems to hold only in the non-carriers of *APOE4* [5-7]. Moreover, compared to non-carriers of *APOE4* (i.e. *APOE2* and *APOE3*), consumption of *n*–3 PUFA, such as DHA, fails to reduce the risk of cognitive decline among *APOE4* carriers [5, 8]. This could potentially be explained by a disturbed DHA metabolism in *APOE4* carriers, supported by lower DHA content in the brain of *APOE4* animals and humans [9, 10].

Higher levels of DHA have also been reported in the plasma of human carrying the *APOE4* allele [11, 12]. However, the mechanisms explaining why DHA homeostasis could be imbalanced in *APOE4* carriers are still unknown. One hypothesis is that *APOE* genotype modulates expression of key fatty acid handling proteins thereby impairing transport and uptake of FA by peripheral organs such as adipose tissue and liver. These two tissues are important players in lipid metabolism because they constantly exchange FA with blood. Therefore, plasma FA profile pictures the balance of uptake and release

of FA from hepatic and adipose cells [13]. Key proteins are involved in the transport, release and uptake of plasma FA towards peripheral organs: fatty acid transport proteins (FATP) and fatty acid binding proteins (FABP). FATPs are transmembrane transport proteins necessary for efficient uptake of FA by cells [14]. Once bound to FATPs, FABPs act as chaperon proteins to reduce the hydrophobic nature of LCFAs and ease their transport within the cells towards specific metabolic routes [15, 16]. For example, upon activation by hepatic acyl-CoA synthase, newly formed LCFA-CoAs are trapped inside hepatocytes and may be directed by FABP toward the mitochondria for β-oxidation to produce energy [17]. Carnitine palmitoyltransferase 1 (CPT1) is currently recognized as the key limiting enzyme initiating FA oxidation [18]. Overall, FATPs and FABPs regulate LCFA transport, uptake and release by tissues, as CPT1 regulates their catabolism [2, 14, 15, 18]. FATP1 and FABP4 are mainly found in adipose tissue whereas FATP5 and FABP1 are highly expressed in the liver [14, 15]. FATPs and FABPs partner together for efficient LCFA uptake by cells [19].

The aim of the present study was to investigate whether *APOE* genotypes disrupt FA profile in the adipose tissue and the liver and whether this is explained by different level of FABPs, FATPs and CTP1 in these tissues.

2. METHODS

2.1 Animals

Male and female APOE-targeted replacement mice expressing human *APOE* genotypes were purchase at Taconic (Hudson, NY). Animals were breed in order to obtain colonies of mice homozygous for human *APOE2*, *APOE3*, or *APOE4* on a C57/BL6 background (N=10–14/genotype group). This mouse model was first created by Sullivan et al [20] to study human *APOE3* phenotype *in vivo* and is currently recognized as a useful *in vivo* model to study the role of human apoE on lipid metabolism. *APOE*-targeted replacement mice have phenotypes similar to those found in humans [21], such as high blood cholesterol and LDL-cholesterol levels in *APOE4* mice and high levels of plasma triglycerides and cholesterol in *APOE2* mice [22].

From weaning to 4-month of age, mice were fed a commercial chow diet to prevent any neurodevelopmental problems coming from dietary deficiency. At 4-month of age, mice were switched to a low-fat diet (low *n*–3 PUFA/*n*–6 PUFA) until sacrifice. The low-fat diet had the following composition: 66.0% (w/w) of proteins, 20.3% (w/w) of carbohydrates and 5.0% (w/w) of lipids (Table 1). In order to investigate the influence of age on *n*–3 PUFA metabolism according to *APOE* genotype, necropsies was performed on mice of either 8.5 or 12-months of age. At sacrifice, mice were perfused in the heart with 50 ml of ice-cold 0.1 M PBS buffer after deep anesthesia with ketamine/xylazine. The adipose tissue, the liver and the plasma were collected within minutes and rapidly frozen on dry ice. Organs and plasma were stored at –80°C until further analysis. The animal protocol was performed in accordance with the Canadian

Council on Animal Care and was approved by the Comité d'éthique de la recherche du CHUQ-Centre hospitalier de l'Université Laval.

2.2 Fatty acid analysis

Total lipids were extracted from adipose tissue (10 mg), liver (100 mg) and plasma (100 µL), using 2:1 chloroform–methanol as described by Folch *et al.* [23]. Lipids were extracted from organs in a glass potter and from plasma in a glass tube. After collecting the organic phase, total lipids were saponified for releasing the FA from cholesteryl esters and glycerolipids [10]. Non-esterified FAs were thereafter transmethylated using 14% boron trifluoride–methanol (Sigma, St. Louis, MO). FA profiles were determined by gas chromatography as previously described [24].

2.3 Proteins and Western blot analysis

Total proteins were extracted from adipose tissue and liver and homogenized using glass potters in a solution containing 50 mM Tris–HCL (pH = 7.4), 2.5 mM EDTA, 150 mM NaCl, 1% Triton and a freshly added protease inhibitor cocktail (Roche Diagnostics, Indianapolis, IN). After 15 min on ice, samples were centrifuged at 13,000 rpm for 15 min at 4°C. Protein concentrations were assessed using bicinchoninic acid (BCA) Protein Assay kit (Thermo Fisher Scientific Inc., Waltham, MA). 15 µg of proteins were denatured with SDS blue buffer (New England Biolabs, Ipswich, MA), loaded on a 10% sodium dodecyl sulfate-polyacrylamide electrophoresis gel and transferred onto a 0.2 µM polyvinylidene difluoride (PVDF) membrane (Bio–Rad, ON, Canada). Membranes were blocked with 5% milk–0.01% TBS-Tween (TBST) for 30 min at room temperature, then incubated overnight at 4°C with primary antibodies (Ab)

against FATP1 (1:1000, Abcam Inc., Cambridge, MA), FABP4 (1:1000, Cayman Chemical, Ann Arbor, MI), FATP5 (1:500, Santa Cruz Biotechnology Inc., Dallas, TX), FABP1 (1:1000, Cell Signaling Technology, Danvers, MA) and β-actin (1:10000, Cell Signaling Technology, Danvers, MA). Membranes were washed with TBST, immunoblotted with a horseradish peroxidase linked secondary anti-rabbit Ab (1:10000, Cell Signaling Technology, Danvers, MA) followed by chemiluminescence reagents (chemiluminescence ECL kits, Perkin Elmer, Waltham, MA). Densitometry was analyzed using ImageJ software (U.S. National Institutes of Health) and results were expressed in ratio to β-actin.

2.4 Liver CPT1 quantification

Liver samples were weighed before homogenization. Livers (100 mg) were homogenized in 1 mL of 1X PBS buffer (pH = 7.4) using an eppendorf micropestle. The resulting suspension was sonicated for a total of 3 × 5 sec cycles to break cell membranes and then centrifuged at 5,000 × g for 5 min at 4°C. Supernates were removed, diluted (1:500 and 1:800) and assay immediately. Liver CPT1 isoform A levels were analyse using a highly sensitive (1.56 pg/mL) and quantitative sandwich enzyme-linked immunosorbant assay (ELISA) test kit for mouse CPT1 liver isoform enzyme (CPT1a; Cusabio, Wuhan, China; [CV%] < 10 %). All standards and samples were assayed in duplicate. Average of duplicate readings was used for calculating concentrations using a four parameter logistic (4-PL) curve-fit model (MasterPlex ® EX expression analysis module, Hitachi Software, San-Francisco, CA).

2.5 Statistical analysis

Normal distribution and homogeneity of variance were evaluated by performing Levene's test (parametric or non-parametric) before further statistical analysis. All data were analysed for statistical differences using ANOVA or Kruskal-Wallis non-parametric analysis of variance in SPSS version 22.0 (IBM Corp., Armonk, NY). When significant differences were found, pairwise comparisons (Fisher's LSD or Mann-Whitney U tests) were performed in order to assess statistical differences between genotype groups. Pearson correlations and multiple regression models were used to investigate associations between tissue-specific levels of FA handling proteins (FABPs and FATPs) and concentration in LCFAs. The average of duplicate readings for each liver homogenate samples was used to asses CTP1 concentrations according to *APOE* genotype. *P* values < 0.05 were considered statistically significant, and *P* values for trends were set as < 0.08. Data are presented as means ± SEMs or as % compared to control mice, namely *APOE*3 (Equation 1).

Equation 1:

$$\text{Compared to control (\%)} = (\text{APOE4} \div \text{APOE3}) \times 100$$

3. RESULTS

3.1 Mice groups

The study groups had N=10 *APOE2* mice, N=13 *APOE3* mice, N=14 *APOE4* mice and of N=8 Wild-type C57BL/6 mice. At sacrifice, the mean weight for *APOE2* mice was 43.2 g ± 7.6 g, 37.2 g ± 7.0 g for *APOE3*, 34.5 g ± 6.7 g for *APOE4* and 44.8 g ± 6.0 g for Wild-Type mice. There was no age-difference in any of the studied outcomes, thus mice of 8.5 or 12-months of age were pooled by *APOE* genotype in further analysis.

3.2 Fatty acid profiles

FA profile of the adipose tissue (Table 2), the liver (Table 3) and the plasma (Table 4) of transgenic mice was modified by *APOE* genotype. There were limited differences in the FA profile of adipose tissue, liver and plasma of *APOE3* mice, compared to Wild-type C57BL/6 mice (WT). *APOE3* mice were henceforth designated as the control group since they express the most common *APOE* isoform and this allele is not associated to any human disease [21].

In the adipose tissue of *APOE4* mice, alpha-linolenic (ALA; 18:3 *n*–3) and DHA were both significantly lower than in *APOE3* mice, whereas 16:1 *n*–7 was significantly higher (Table 2). In the adipose tissue and the liver of *APOE4* mice, ALA levels were respectively 56% ($P = 0.036$) and 14% ($P < 0.001$) the levels of *APOE3* mice. In the liver of *APOE4* mice, DHA was 66% lower than the level of *APOE3* mice. All other fatty acids measured in the liver did not differ according to *APOE* genotype. In the adipose tissue and the liver of *APOE4* mice, *n*–3 PUFA concentrations were 53% lower compared to *APOE3* mice (P values ≤ 0.003). Total FA concentration in the plasma of

135 *APOE2* mice was approximately 5 fold higher than *APOE3* and *APOE4* mice supporting
136 hyperlipidemia in *APOE2* mice as previously described [22]. In this study, plasma total
137 FA was calculated by the addition of the measured FA expressed in concentrations.
138 Plasma samples from *APOE2* mice were cloudy and milky compared to the plasma of
139 WT, *APOE3* and *APOE4* mice.

140

3.3 FATP1 and FABP4 regulation in adipose tissues

141 There was a weak, but significant *APOE* genotype effect on FATP1/*β*-actin ratio
142 (Fig. 1A, left panel; $P = 0.047$). In *APOE4* mice, FATP1 level was at least 30% higher
143 compared to the other genotypes. There was no significant *APOE* genotype effect for the
144 ratio of FABP4/*β*-actin in the adipose tissue (Fig. 1A, right panel). There were significant
145 negative correlations between adipose tissue concentrations (μg/mg) of total *n*–3 PUFA
146 and FATP1 levels ($r = -0.431$, $P = 0.003$). There were no correlation between FABP4
147 and any of the FA analysed in the adipose tissue neither was there between the levels of
148 FATP1 or FABP4 and plasma FA profile. Using multiple regression analysis, LCFA
149 concentrations (μg/mg) in adipose tissue were the only significant predictor of variation
150 in FATP1 levels ($R^2 = 19.9\%$, $P = 0.006$). There was no relationship between FABP4 and
151 the FA profile of adipose tissue. Plasma FA concentration was unable to predict any
152 change in FATP1 or FABP4 protein levels (data not shown).

3.4 FATP5 and FABP1 regulation in the liver

There was a significant *APOE* genotype effect on FATP5/β-actin ratio and FABP1/β-actin ratio in the liver (*P* values of 0.037 and 0.031 respectively). FATP5 and FABP1 levels were more than 2 fold higher in *APOE4* mice compared to *APOE2* mice (Fig. 1B). Levels of FATP5 and FABP1 were also significantly higher in *APOE3* mice compared to *APOE2*. There was no correlation between FA concentrations in the liver and FATP5 or FABP1 levels. However, the plasma concentration of total *n*–3 PUFA was negatively correlated with FATP5 ($r = -0.455$, $P = 0.044$). Similarly, the plasma concentrations of AA was negatively correlated to FABP1 ($r = -0.618$, $P = 0.004$). Using multiple regression analysis, FATP5 was able to predict variation in the plasma concentrations of DHA ($R^2 = 25.4\%$, $P = 0.024$). Similarly, FABP1 levels predicted variation in the plasma concentration of AA ($R^2 = 38.2\%$, $P = 0.004$) (data not shown).

3.5 Liver-type CTP1 levels according to *APOE* genotype

There was a trend towards a genotype effect for the CPT1 in the liver ($P = 0.073$). Although, there was no significant genotype effect on CPT1, its mean concentration seemed to be higher in *APOE4* mice (1.92 ± 0.10) compared to *APOE2* (1.59 ± 0.14) and *APOE3* mice (1.45 ± 0.22) (Fig. 2). Using Mann-Whitney U test to assess difference between two samples, concentration of CPT1 in the liver of *APOE4* mice was 21% higher than *APOE3* mice ($P = 0.038$).

4. DISCUSSION

This study reports lower concentrations of *n*–3 PUFA in the adipose and the liver of *APOE4* mice compared to *APOE3* mice. There was an *APOE* isoform-dependant effect for the levels of FATP in the liver and the adipose tissue and for FABP in the liver and a trend towards an *APOE* isoform-dependant effect on CPT1 concentration in the liver.

The exact mechanism by which LCFAs are up-taken by cells is not clearly established and is still source of debates. Mitchell and Hatch [2] suggested a model involving the collaboration of four families of FA-handling proteins, among which FATPs and FABPs are major contributors. Briefly, LCFAs interact with membrane FATPs to be transported from the exoplasmic side to the cytoplasmic side of the cell membrane. The crucial mechanistic importance of FATPs for LCFAs internalization, form blood into organs and tissues, was confirmed through the use of FATP5 [25] and FATP1 [26] knockout (KO) mice models. Since FATP transport is bidirectional, FABP must then interact with internalized LCFAs in order to prevent their efflux back into circulation. Cytosolic tracking FABPs thereafter direct LCFAs towards metabolic routes such as mitochondrial *β*-oxidation [2, 14, 15, 27]. Thus, it was anticipated that the higher levels of FATPs and FABPs would result in higher LCFA uptake by tissues. In this study, there was a negative correlation between FATP5 levels and plasma LCFAs concentrations, independently of *APOE* genotypes. However, there was lower ALA, DHA and *n*–3 PUFA in the liver and the adipose tissue of *APOE4* mice compared to *APOE3* mice. Therefore, we sought to determine whether this could be linked to higher *β*-oxidation of *n*–3 PUFA based on our previous results in humans which suggested

192 higher β-oxidation of DHA in *APOE4* carriers [11]. Therefore, we quantified CPT1
193 concentration in the liver according to *APOE* genotype. There was a trend towards an
194 *APOE* isoform-dependant effect on CPT1 level where *APOE4* mice tended to have 21%
195 higher CPT1 levels than *APOE3* mice. Considering that ALA is a preferred substrate for
196 β-oxidation in humans and animals, [28-30], our results support the hypothesis of higher
197 FA β-oxidation since *APOE4* mice had significantly lower levels of ALA in the liver
198 compared to *APOE3* mice. However, DHA is usually highly conserved in animals and in
199 humans based on our previous studies, but the lower level of DHA in the liver of *APOE4*
200 mice support that there may be a shift in FA preference towards β-oxidation pathway,
201 explaining why DHA seems to be more catabolized in *APOE4* carriers [11].

202 The importance of the association between LCFAs degradation and *APOE*
203 genotype resides in the recent association between low hepatic DHA and AD. Astarita *et*
204 *al* [31, 32] reported lower DHA levels in the liver of AD patients compared to control
205 subjects, which suggested an association between hepatic DHA homeostasis and
206 cognition. Since carrying an allele of *APOE4* is currently recognized as the most
207 important risk factor of AD [5-7] higher β-oxidation of *n*–3 PUFAs may compromise the
208 availability of DHA to support normal brain functions. It may also explain the lower
209 brain level of DHA (%) in 13-months *APOE4* mice compared to *APOE2* mice reported
210 by Vandal *et al* [9]. However, it seems possible to rebalance DHA homeostasis by
211 providing a diet rich in DHA to *APOE4* carriers [33]. This needs to be further
212 investigated since β-oxidation of DHA in *APOE4* carriers fed 3 g/d of *n*–3 PUFAs was
213 lower compared to pre-supplementation [11], conversely to non-carriers for which
214 feeding the supplement increases β-oxidation of DHA compared to baseline [34].

This study reported that the uptake of circulating LCFAs by *APOE2* mice was apparently inefficient and that CPT1 concentrations in the liver tended to be lower in *APOE2*-expressing animals compared to *APOE4* (Fig. 2). These results are in line with the hypertriglyceridemia generally associated with *APOE2* homozygous human carriers [22] and humanized *APOE2* mice [35], as well as with the phenotype reported for CPT1 KO mouse model [36]. Indeed, in this study, *APOE2* mice add almost 5-fold higher total plasma lipids and plasma was white rather than light transparent yellow like the plasma of the other genotypes. As previously reported, lower hepatic and intramuscular β-oxidation have been report in the brain CPT1c isoform KO mice as well as elevated triacylglycerol content in liver and muscle in these mice [36]. Not much information is available in literature concerning the liver CPT1a isoform KO mice model for two major reasons: 1) complete inhibition of CPT1a enzyme is lethal to mice [37]; 2) CPT1a isoform bears a highly homologous primary sequence with its other known isoforms (CPT1b, muscle type isoform; CPT1c, brain type isoform) which gene-inactivation are non-lethal [38].

Using the same mouse model and the same diet, Vandal et al [9] recently reported 40% higher relative % of n-3 PUFA and 34% higher DHA in plasma total lipids of *APOE3* and *APOE4* mice compared to *APOE2* of 13-months of age. Similarly, in this study, the relative % of n-3 PUFA was nearly 40% higher in 8.5 to 12-month *APOE3* mice than *APOE2* mice, but there was no difference between *APOE4* and *APOE2* mice (data not shown). The same pattern was observed for the relative % of DHA in plasma, with more than 30% higher DHA in *APOE3* mice compared to *APOE4* and *APOE2* (data not shown). *APOE2* mice have hyperlipidemia [35], supporting why in this study *APOE2*

mice had 5 times higher plasma total lipids than *APOE3* mice. Therefore, it seems more appropriate to express results in concentration rather than relative % of total FA when using the *APOE*-targeted replacement mouse model since relative % may mask potential higher lipid content in organs and plasma of these mice.

There is now a need for defining whether FA preference for β-oxidation is *APOE* isoform-dependent and whether it is possible to rebalance FA kinetics and metabolism in *APOE4* carriers through high dose supplementation with DHA and/or EPA. This pathway could contribute in explaining why *APOE4* carriers are at higher risk of developing AD. Unfortunately, we did not evaluate mice cognition in the present study and therefore, we cannot confirm this speculation − i.e. cognitive impairments in *APOE4* mice [39, 40]. Also, the activity of FATPs, FABPs and CPT1 remains to be further investigated since only the levels, and not activities, were measured in the present study. Moreover, results obtained in humanized mice may not be translatable to humans and thus, caution is recommended with regard to interpretation of data obtained in humans. However, in support to the humanized mouse model used in our study, Raffaï *et al* [41] reported that introduction of apoE4 domain interaction into endogenous mouse *APOE* gene (i.e. through substitution of Thr-61 by Arg-61 resulted in a phenotype similar to that found in humans homozygous for *APOE4* allele.

In conclusion, this study showed an *APOE* isoform-dependant effect on adipose tissue, liver and plasma *n*–3 PUFAs concentrations. Similarly, *APOE*-isoforms significantly modulated FATP levels in the adipose tissue and the liver and FABP levels in the liver. However, these *APOE* isoform-dependencies were not always in line with the unbalanced *n*–3 PUFA profiles in organs. Therefore, besides *APOE*-isoform differences

261 in FATP and FABP, other proteins are involved in maintaining *n*–3 PUFA homeostasis in

262 mice with different *APOE*-isoforms.

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TABLE 1

Macronutrients composition of the diets fed from weaning to 4-month and from 4 to 13-months of age.

	Diets at months (all mice, N= 45)	
	0-4 [a]	4-13 [b]
Energy (kcal/g)	3.4	3.9
Proteins (%, w/w)	18.9	20.3
Carbohydrates (%, w/w)	57.3	66.0
Fat (%, w/w)	6	5
ALA (g/kg)	4.1	0.4
EPA (g/kg)	0	0
DHA (g/kg)	0	0
LA (g/kg)	52.3	36.0
AA (g/kg)	0	0
Ratio *n*−6/*n*−3 PUFA	13	100

[a] Tecklad Diet 2018 (Harlan Laboratory, Indianapolis, IN)

[b] Diet D04042202 (Research Diets, Inc., New-Brunswick, NJ)

TABLE 2

Fatty acid concentrations in the adipose tissue of *APOE*-targeted replacement mice carrying human *APOE* isoforms or of Wild-type C57/BL6 mice [a]

	ADIPOSE TISSUE (µg/mg of tissue)				*P* values
	APOE2	*APOE3*	*APOE4*	WT	(for genotype)
16:0	162.9 ± 8.8	147.4 ± 5.7	170.6 ± 9.7	145.4 ± 6.6	0.092
18:0	11.1 ± 0.8 ^{A}	14.8 ± 1.4 ^{AB}	16.7 ± 1.9 ^{B}	11.9 ± 0.7 ^{A}	0.049
16:1 *n*−7	58.8 ± 6.2 ^{A}	43.3 ± 5.1 ^{A}	64.0 ± 5.5 ^{B}	47.6 ± 2. 7 ^{A}	0.023
18:1 *n*−9	299.8 ± 14.1	282.2 ± 9.6	307.7 ± 8.8	274.6 ± 8.9	0.118
18:2 *n*−6	340.3 ± 25.2	355.8 ± 14.5	307.8 ± 24.3	317.9 ± 14.5	0.323
20:4 *n*−6	3.8 ± 0.3	3.7 ± 0.1	3.9 ± 0.2	3.3 ± 0.3	0.378
Total *n*−6 PUFA	344.5 ± 25.3	360.9 ± 14.6	312.8 ± 24.5	321.3 ± 14.7	0.322
18:3 *n*−3	11.7 ± 2.2 ^{AB}	16.0 ± 1.4 ^{A}	8.9 ± 2.1 ^{B}	14.7 ± 1.6 ^{A}	0.031
22:6 *n*−3	0.4 ± 0.3 ^{B}	1.2 ± 0.4 ^{A}	0.3 ± 0.1 ^{B}	ND	0.014
Total *n*−3 PUFA	12.0 ± 2.3 ^{A}	17.3 ± 1.6 ^{A}	9.2 ± 2.2 ^{B}	14.7 ± 1.6 ^{AB}	0.025
Ratio *n*−6/*n*−3 PUFA	24.7 ± 1.3	27.3 ± 1.5	37.4 ± 8.0	22.6 ± 1.3	0.325

[a] Mice were homozygous for human apolipoprotein E epsilon 2 (*APOE2*), or apolipoprotein E epsilon 3 (*APOE3*), or apolipoprotein E epsilon 4 (*APOE4*) and mice carrying endogenous murin-*APOE* gene (Wild-type; WT).

ND: Not detected, Statistical differences were performed by a One-Way ANOVA or non-parametric analysis of variance (SPSS Statistics software, NY, USA). Significance was set as $P < 0.05$ and the trend at $P < 0.08$. Fisher's LSD or Mann-Whitney U post hoc analyses were performed to assess differences between genotype-groups. Different superscript letters within the same line indicate significant difference by *APOE* genotype.

TABLE 3

Fatty acid concentrations in the liver of *APOE*-targeted replacement mice carrying human *APOE* isoforms or of Wild-type C57/BL6 mice [a]

	LIVER (µg/mg of tissue)				*P* values
	APOE2	*APOE3*	*APOE4*	WT	(for genotype)
16:0	25.4 ± 3.5	26.5 ± 6.9	19.6 ± 2.4	28.2 ± 4.0	0.638
18:0	3.0 ± 0.2	3.2 ± 0.2	3.1 ± 0.2	2.8 ± 0.2	0.378
16:1 *n*−7	6.7 ± 1.3	8.7 ± 3.4	5.2 ± 0.9	7.5 ± 1.3	0.701
18:1 *n*−9	33.7 ± 5.0	36.7 ± 12.2	33.9 ± 5.0	42.3 ± 5.9	0.917
18:2 *n*−6	21.5 ± 1.8	21.3 ± 4.5	16.7 ± 2.0	18.1 ± 2.2	0.637
20:4 *n*−6	5.3 ± 0.4	5.3 ± 0.5	4.9 ± 0.4	4.6 ± 0.5	0.718
Total *n*−6 PUFA	27.9 ± 2.2	27.6 ± 5.1	22.5 ± 2.5	23.8 ± 2.7	0.654
18:3 *n*−3	0.4 ± 0.1 ^{B}	0.7 ± 0.1 ^{A}	0.1 ± 0.1 ^{B}	0.3 ± 0.1 ^{B}	0.001
22:6 *n*−3	2.4 ± 0.3 ^{A}	2.7 ± 0.3 ^{A}	1.8 ± 0.1 ^{B}	2.1 ± 0.2 ^{AB}	0.030
Total *n*−3 PUFA	2.8 ± 0.4 ^{AB}	3.7 ± 0.4 ^{A}	2.0 ± 0.2 ^{B}	2.4 ± 0.3 ^{B}	0.004
Ratio *n*−6/*n*−3 PUFA	13.5 ± 3.2	7.3 ± 0.7	12.5 ± 1.5	10.0 ± 0.4	0.051

[a] Mice were homozygous for human apolipoprotein E epsilon 2 (*APOE2*), or apolipoprotein E epsilon 3 (*APOE3*), or apolipoprotein E epsilon 4 (APOE4) and mice carrying endogenous murin-*APOE* gene (Wild-type; WT).

Statistical differences were performed by a One-Way ANOVA or non-parametric analysis of variance (SPSS Statistics software, NY, USA). Significance was set as $P < 0.05$ and the trend at $P < 0.08$. Fisher's LSD or Mann-Whitney U post hoc analyses were performed to assess differences between genotype-groups. Different uppercase superscript letters within the same line indicate significant difference by *APOE* genotype.

TABLE 4

Fatty acid concentrations in the plasma of *APOE*-targeted replacement mice carrying human *APOE* isoforms or of Wild-type C57/BL6 mice [a]

	PLASMA (µg/mL of tissue)				*P* values
	APOE2	*APOE3*	*APOE4*	WT	(for genotype)
16:0	1862.5 ± 299.4 [B]	377.4 ± 40.9 [A]	545.9 ± 105.7 [A]	499.6 ± 36.2 [A]	<0.001
18:0	839.3 ± 107.9 [B]	273.1 ± 18.3 [A]	391.6 ± 67.3 [A]	337.8 ± 30.3 [A]	<0.001
16:1 *n*−7	334.5 ± 85.7 [B]	40.1 ± 8.7 [A]	64.6 ± 19.4 [A]	77.5 ± 12.7 [A]	<0.001
18:1 *n*−9	2826.1 ± 642.4 [B]	308.3 ± 45.7 [A]	473.1 ± 86.4 [A]	456.6 ± 69.7 [A]	<0.001
18:2 *n*−6	3326.7 ± 625.1 [B]	642.6 ± 86.1 [A]	715.0 ± 140.3 [A]	663.1 ± 38.5 [A]	<0.001
20:4 *n*−6	1085.9 ± 168.4 [B]	379.8 ± 52.3 [A]	396.6 ± 44.6 [A]	487.6 ± 128.1 [A]	<0.001
Total *n*−6 PUFA	4557.1 ± 797.9 [B]	1066.5 ± 124.8 [A]	1170.6 ± 132.0 [A]	1237.0 ± 144.4 [A]	<0.001
18:3 *n*−3	78.5 ± 31.5 [B]	6.6 ± 1.6 [A]	6.8 ± 2.7 [A]	ND	0.002
20:5 *n*−3	29.3 ± 14.5 [B]	10.2 ± 2.72 [A]	4.9 ± 1.9 [A]	9.3 ± 2.2 [A]	0.090
22:6 *n*−3	23.7 ± 11.6 [B]	7.4 ± 2.0 [A]	6.3 ± 2.3 [A]	5.9 ± 2.2 [A]	<0.001
Total *n*−3 PUFA	420.9 ± 107.3 [B]	128.0 ± 12.0 [A]	133.5 ± 26.6 [A]	163.1 ± 23.1 [A]	0.001
Ratio *n*−6/*n*−3 PUFA	15.7 ± 4.2	9.2 ± 1.4	10.7 ± 1.1	8.0 ± 0.8	0.134

[a] Mice were homozygous for human apolipoprotein E epsilon 2 (*APOE2*), or apolipoprotein E epsilon 3 (*APOE3*), or apolipoprotein E epsilon 4 (*APOE4*) and mice carrying endogenous murin-*APOE* gene (Wild-type; WT).

ND: Not detected, Statistical differences were performed by a One-Way ANOVA or non-parametric analysis of variance (SPSS Statistics software, NY, USA). Significance was set as $P < 0.05$ and the trend at $P < 0.08$. Fisher's LSD or Mann-Whitney U post hoc analyses were performed to assess differences between genotype-groups. Different uppercase superscript letters within the same line indicate significant difference by *APOE* genotype.

LEGEND OF FIGURES

FIG 1. (**A.**) Levels of fatty acid transport protein and binding protein of the adipose tissue (i.e. FATP1 and FABP4), over β-actin. (**B.**) Levels of fatty acid transport protein and binding protein of the liver (i.e. FATP5 and FABP1), over β-actin. FATP and FABP levels were obtained by Western blot and results are means ± SEM. For each proteins measured (i.e. FATP or FABP), three representative bands (N = 3) per genotype are presented in order to show the intragroup variations. Levels of tissue specific FATP and FABP in the adipose tissue and in the liver were analysed for statistical differences using non-parametric analysis of variance in SPSS (IBM Corp., Armonk, NY). Significant genotype effect was found for FATP1 (P = 0.047) in the adipose tissue and FATP5 (P = 0.037) and FABP1 (P = 0.031) in the liver. Pairwise comparisons were performed using Mann-Whitney U tests. P values < 0.05 were considered statistically significant.

FIG 2. Liver carnitine palmitoyltransferase 1 (CPT1) concentrations (ng/mg) measured by enzyme-linked immunosorbent assay (ELISA). Results are presented in means ± SEM. Liver concentrations of CPT1 were analysed for statistical differences using ANOVA analysis of variance in SPSS (IBM Corp., Armonk, NY). There was a trend towards a genotype effect (P value = 0.073). *Pairwise comparison (Fisher's LSD test) reported significant differences between *APOE4* and *APOE3* mice (P = 0.032). Dotted line is used to indicate the genotype trend (P value < 0.08).