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

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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.


## 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 [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

*Abbreviations:* 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.

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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 $\beta$-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 levels 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 homozygous mice 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.

**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 |
| 6Ratio *n*-6/*n*-3 PUFA | 13 | 100 |

[a] Tecklad Diet 2018 (Harlan Laboratory, Indianapolis, IN).
[b] Diet D04042202 (Research Diets, Inc., New-Brunswick, NJ).

### 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 was 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 $\beta$-actin (1:10,000, Cell Signaling Technology, Danvers, MA). Membranes were washed with TBST, immunoblotted with a horseradish peroxidase linked secondary anti-rabbit Ab (1:10,000, 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 $\beta$-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 s cycles to break cell membranes and then centrifuged at 5000*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 analyze 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).