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Site-specific effects of apolipoprotein E expression on diet-induced obesity and white adipose tissue metabolic activation

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ABSTRACT

Apolipoprotein E (APOE) has been strongly implicated in the development of diet induced obesity. In the present study, we investigated the contribution of brain and peripherally expressed human apolipoprotein E3 (APOE3), the most common human isoform, to diet induced obesity.

In our studies APOE3 knock-in (*Apoe3*$^{knock\text{-}in}$), Apoe-deficient (*apoe*$^{-/-}$) and brain-specific expressing APOE3 (*Apoe3*^{brain}) mice were fed western-type diet for 12 week and biochemical analyses were performed. Moreover, AAV-mediated gene transfer of APOE3 to *apoe*$^{-/-}$ mice was employed, as a means to achieve APOE3 expression selectively in periphery, since peripherally expressed APOE does not cross blood brain barrier (BBB) or blood-cerebrospinal fluid barrier (BCSFB).

Our data suggest a bimodal role of APOE3 in visceral white adipose tissue (WAT) mitochondrial metabolic activation that is highly dependent on its site of expression and independent of postprandial dietary lipid deposition.

Our findings indicate that brain APOE3 expression is associated with a potent inhibition of visceral WAT mitochondrial oxidative phosphorylation, leading to significantly reduced substrate oxidation, increased fat accumulation and obesity. In contrast, peripherally expressed APOE3 is associated with a notable shift of substrate oxidation towards non-shivering thermogenesis in visceral WAT mitochondria, leading to resistance to obesity.

1. Introduction

Apolipoprotein e (Apoe) is a major protein of the lipoprotein transport system involved in plasma metabolism and clearance of triglyceride-rich lipoproteins from circulation [1,2]. In human it is found in three natural isoforms, APOE2, APOE3 and APOE4 [2,3] with APOE3 being the most frequent allele [2–8]. In mouse, lipoprotein-bound Apoe is a natural ligand for the low density lipoprotein receptor (Ldlr) and Ldlr-related protein 1 (Lrp1) [9–11]. APOE3 and APOE4 bind LDLR with a much higher affinity than APOE2 [12], while all three isoforms have similar binding affinities for LRP1 [11]. Numerous studies showed that APOE is associated with the development and progression of coronary heart disease in humans [4,13–17]. The important role of Apoe in the development of dyslipidemia and atherosclerosis was confirmed in mice with the creation of the Apoe-deficient (apoe$^{-/-}$) mouse [18].

Relatively recently, work in experimental mice also provided a link between Apoe and obesity. Work by Chiba et al. [19] showed that Leptin and Apoe double deficient mice (*ob/ob x apoe*$^{-/-}$) are resistant to body weight and adipose tissue gain when fed a high-fat/high-cholesterol diet, despite an increase in their plasma VLDL levels. In another

Abbreviations: ALT, Alanine transaminase; Apoa1, Mouse apolipoprotein A-I; Apoa2, Mouse apolipoprotein A-II; Apoc1, Mouse apolipoprotein C-I; Apoc3, Mouse apolipoprotein C-III; Apoe, Mouse apolipoprotein E; APOE2, Human apolipoprotein E2; APOE3, Human apolipoprotein E3; APOE4, Human apolipoprotein E4; *apoe*$^{-/-}$, Apoe deficient mouse; *Apoe3*$^{knock\text{-}in}$, APOE3 knock-in mouse; *Apoe3*^{brain}, Brain-specific expressing APOE3 mouse; AAV-E3, APOE3-expressing adenoassociated virus; AST, aspartate transaminase; BAT, brown adipose tissue; BBB, blood-brain barrier; BCSFB, blood-cerebrospinal fluid barrier; BRITE, BRown Into whiTE; CNS, central nervous system; Cox4, Mouse cytochrome *c* oxidase subunit 4; CytC, Mouse cytochrome *c*; *dgat-1*, Diacylglycerol acyltransferase gene 1; *fasn*, Fatty acid synthase gene; GFAP, astrocyte-specific glial fibrillary acidic protein promoter; HDL, High density lipoprotein; LDLR, Human low density lipoprotein receptor; Ldlr, Mouse low density lipoprotein receptor; LRP1, Human low density lipoprotein receptor related protein 1; Lrp1, Mouse low density lipoprotein receptor related protein 1; pfu, Plaque forming units; PNS, peripheral nervous system; *ppar-gamma*, Peroxisome proliferator-activated receptor gamma gene; *rps18*, Ribosomal RNA 18 gene; RT-PCR, Real-Time polymerase chain reaction; S.E.M., standard error of the mean; UCF, ultracentrifugation; Ucp1, Mouse uncoupling protein 1; VLDL, very low density lipoprotein; WAT, white adipose tissue

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study, Huang et al. [20] reported that Apoe-deficient (*apoe*$^{-/-}$) mice accumulate less body fat content and possess smaller adipocytes compared to wild type C57BL/6 controls. Hofmann and coworkers [21] extended further this observation by showing that *apoe*$^{-/-}$ mice fed high-fat-high-sucrose diabetogenic diet for 24 weeks were resistant to diet-induced obesity and exhibited improved glucose uptake by muscle and brown adipose tissue. The role of Apoe in obesity was confirmed in the *Ay/*$^{+}$ mice [22] where deficiency in Apoe prevented the development of obesity, with decreased fat accumulation in the liver and adipose tissues [22]. Along the same line, data from our laboratory [23] showed that mice expressing human APOE3 in place of mouse Apoe (*Apoe3*$^{knock\text{-}in}$) fed the standard western-type diet for 24 weeks were more sensitive to diet-induced obesity and related metabolic dysfunctions than C57BL/6 mice, while *apoe*$^{-/-}$ mice were resistant to the development of these conditions.

Adipose organ is made of white adipose tissue (WAT) that is mainly responsible for lipid storage, and brown adipose tissue (BAT) that is mainly responsible for non-shivering thermogenesis. It has been proposed that under certain circumstances WAT may be metabolically activated into BRITE (BRown Into whiTE) adipose tissue to produce heat via thermogenesis [24–26]. Increased thermogenesis is a result of increased mitochondrial metabolism and in particular elevated uncoupling protein 1 (Ucp1) activity that mediates the metabolic conversion of free fatty acids to heat [24,26,27]. Metabolic activation of WAT into BRITE is considered as the ultimate goal for treating morbid obesity and numerous experimental approaches are under development towards this goal, though to this date the molecular targets for such intervention remain ill-defined.

In the present study we sought to investigate the effects of brain and peripheral expression of Apoe on WAT and BAT mitochondrial metabolic function and obesity. We focused our work on APOE3, the most common human isoform [2–8], and studied *apoe*$^{-/-}$, *Apoe3*$^{knock\text{-}in}$, and brain-specific expressing APOE3 (*Apoe3*^{brain}) mice along with *apoe*$^{-/-}$ mice infected with an APOE3-expressing adenoassociated virus (AAV-E3). Since Apoe cannot cross blood-brain barrier (BBB) or blood-cerebrospinal fluid barrier (BCSFB) [28], these models allowed to evaluate the role of peripheral versus brain-specific effects of APOE3 in diet-induced obesity.

2. Material and methods

2.1. Animals

The *apoe*$^{-/-}$ mice [29] and the mice expressing APOE3 specifically in astrocytes under the control of the astrocyte-specific glial fibrillary acidic protein (GFAP) promoter (*Apoe3*^{brain}) [30] were purchased from Jackson Labs (Bar Harbor, Maine, USA). In the *Apoe3*^{brain} mice, APOE3 expression is contained in astrocytes and the neuropil throughout development and into the adult period, as assessed by immunocytochemistry and immunoblot analysis in several GFAP-APOE3 [30]. Due to a minor promoter leakage, trace amounts of APOE3 may also be found in periphery, though they are not sufficient to lower peripheral cholesterol levels [30]. The *Apoe3*$^{knock\text{-}in}$ mice [31] were purchased from Taconic Farms. All mice were back-crossed on the C57BL/6 genetic background for at least 9 generations. Male mice 10–12 weeks old were caged individually and allowed unrestricted access to food and water under a 12 h light/dark cycle. To ensure similar average starting body weights, cholesterol and triglyceride levels, groups of mice were formed after determining the fasting body weights, cholesterol and triglyceride levels of the individual mice. All the groups were fed standard western-type diet (Mucedola SRL, Milano, Italy) that contains 4.5 kcal/g [17.3% protein, 48.5% carbohydrate, 21.2% fat, 0.2% cholesterol (0.15% added, 0.05% from fat source)] for the indicated times. Administration of 1×10^8 pfu of the recombinant APOE3 expressing adenoassociated virus (AAV-E3) to *apoe*$^{-/-}$ mice was performed by intravenous injection in the tail vain, as described previously [32–34]. AAV-E3 was a gift of Prof. Vassilis I. Zannis. All animal studies were contacted according to the European Union guidelines of the *Protocol for the Protection and Welfare of Animals*. For an initial rough estimation of sample size regarding body-weight gain, we used an online statistical tool (http://www.stat.ubc.ca/~rollin/stats/ssize/n2.html) where mu1 was set at 28, mu2 was set at 40, σ value was set at 10, a was set at 0.05 and desired power was set at 0.75. This gave us a sample estimation of 10 mice per group. As the study progressed it became apparent that we could obtain statistically significant results with fewer animals and sample size was adjusted appropriately in order to abide by the 3Rs rule of the *Protocol for the Protection and Welfare of Animals*. The work was authorized by the appropriate committee of the Laboratory Animal Center of The University of Patras Medical School and the Veterinary Authority of the Prefecture of Western Greece.

2.2. Determination of body weight and daily food consumption

Every four weeks, food intake was assessed during a 7-day period, as described previously [23,35]. Food intake for each group was then reported as the mean of all measurements obtained during the course of the experiment ± standard error of the mean. At the beginning (week 0) and at the end of the study (week 12) mice were fasted overnight (~16 h), then briefly anesthetized using isofluorane and weighted on a Mettler® precision microscale.

2.3. Measurement of plasma total cholesterol, triglyceride, alkaline transaminase and aspartate transaminase levels

Following a 16-hour fasting period plasma samples were isolated from the experimental mice and plasma cholesterol and triglyceride levels were measured as described previously [36]. Total cholesterol levels were assessed spectrophotometrically in plasma samples, using the DiaSys Cholesterol FS kit (ref# 11300, Diagnostic Systems, GmbH, Holzheim, Germany) according to manufacturers' instructions and as described previously [36]. Triglyceride levels were also assessed spectrophotometrically in plasma samples using the DiaSys Triglycerides FS kit (ref# 15710, Diagnostic Systems, GmbH, Holzheim, Germany) according to manufacturers' instructions [37,38]. The measurement of plasma alkaline transaminases (ALT) and aspartate transaminases (AST) were performed in a Reflotron system (Roche, Switzerland) according to the manufacturer's instructions.

2.4. Isolation of mitochondria

Mitochondria from BAT and visceral WAT were isolated by a modification of the method of Commins et al. [39], as described previously [40]. Briefly, BAT and the contralateral fat pad from each depot site were dissected and weighted immediately after animals were euthanized. Tissues were minced in ice-cold sucrose buffer [0.25 M sucrose and 5.0 mM N-Tris, i.e., (hydroxymethyl)methyl-2-aminoethanesulfonic acid buffer, pH 7.2; Sigma-Aldrich]; diluted to 10% and 5% (w/v), respectively, in sucrose buffer; and homogenized with a glass-Teflon homogenizer. Homogenates were then centrifuged at 22,500*g* for 20 min, and the pellet was resuspended in ice-cold sucrose buffer. After a low-speed spin at 850 *g* for 10 min, the supernatant, containing crude mitochondria, was decanted to a fresh tube and spun for 20 min at 48,000 *g*. The pelleted mitochondria were then resuspended in 2 ml solubilization buffer containing 20 mM Tris (pH 8), 1 mM EDTA, 100 mM NaCl, and 0.9% sodium cholate (Sigma-Aldrich) and incubated on ice for 30 min. Mitochondria samples were then recentrifuged at 48,000 *g* for 30 min. The pellet was resuspended in solubilization buffer containing 1% Triton X-100 and incubated on ice for 30 min. The suspension was recentrifuged at 48,000 *g*, and the supernatant was retained for protein assay and Western blotting. The protein concentration of each mitochondrial sample was determined using the DC™