The FASEB Journal article fj.201600921RR. Published online December 2, 2016.

The effect of dietary fish oil on weight gain and insulin sensitivity is dependent on *APOE* genotype in humanized targeted replacement mice

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ABSTRACT: We investigated the independent and interactive impact of the common *APOE* genotype and marine n-*3* polyunsaturated fatty acids (PUFA) on the development of obesity and associated cardiometabolic dysfunction in a murine model. Human *APOE3* and *APOE4* targeted replacement mice were fed either a high-fat control diet (HFD) or a HFD supplemented with 3% n-*3* PUFA from fish oil (HFD + FO) for 8 wk. We established the impact of intervention on food intake, bodyweight, and visceral adipose tissue (VAT) mass; plasma, lipids (cholesterol and triglycerides), liver enzymes, and adipokines; glucose and insulin during an intraperitoneal glucose tolerance test; and *Glut4* and *ApoE* expression in VAT. HFD feeding induced more weight gain and higher plasma lipids in *APOE3* compared to *APOE4* mice ($P < 0.05$), along with a 2-fold higher insulin and impaired glucose tolerance. Supplementing *APOE3*, but not *APOE4*, animals with dietary n-*3* PUFA decreased bodyweight gain, plasma lipids, and insulin ($P < 0.05$) and improved glucose tolerance, which was associated with increased VAT *Glut4* mRNA levels ($P < 0.05$). Our findings demonstrate that an *APOE3* genotype predisposes mice to develop obesity and its metabolic complications, which was attenuated by n-*3* PUFA supplementation.—Slim, K. E., Vauzour, D., Tejera, N., Voshol, P. J., Cassidy, A., Minihane, A. M. The effect of dietary fish oil on weight gain and insulin sensitivity is dependent on *APOE* genotype in humanized targeted replacement mice. FASEB J. 31, 000–000 (2017). www.fasebj.org

KEY WORDS: diabetes · glucose · *Glut4* · IL-10 · IPGTT

Apolipoprotein E (APOE) is a multifunctional protein expressed in many organs and cell types, in particular hepatocytes, astrocytes, and macrophages (1). In humans, 2 nonsynonymous single nucleotide polymorphisms in the *APOE* gene, 388T/C (rs429358) and 526C/T (rs7412), give rise to 3 common allelic variants, *APOE2, APOE3,* and *APOE4.* Although the etiology is only partially understood, an *APOE4* genotype (~25–30% of white populations) is traditionally considered detrimental; it is associated with reduced longevity (2), increased prevalence of Alzheimer disease (3), and a modestly increased risk of cardiovascular diseases attributed to higher plasma lipid levels (4) relative to the common *APOE3/E3* genotype. Emerging evidence, mainly from rodent studies, indicates that the *APOE* genotype may influence bodyweight gain and obesity risk.

Adipocytes have the capacity to synthesize significant amounts of APOE, which is involved in the expansion of adipose tissue (AT) (5, 6). The expression of APOE increases linearly upon differentiation of adipocytes (7), where it is essential for the accumulation of triglycerides (TG) (8). $APOE^{-/-}$ mice have a lower fat mass and impaired plasma TG clearance (9). Information on the impact of *APOE* genotype; on AT accumulation, morphology, and function; and how it interacts with adiposity to modulate the associated metabolic profile (altered insulin and glucose metabolism, and adipokine and inflammatory status) is currently limited and inconsistent

ABBREVIATIONS: ALP, alkaline phosphatase; ALT, alanine aminotransferase; APOE, apolipoprotein E; AST, aspartate aminotransferase; AT, adipose tissue; AUC, area under the curve; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; ER, endoplasmic reticulum; FO, fish oil; HDL-C, HDL-cholesterol; HFD, high-fat diet; HOMA, homeostatic model assessment; IGT, impaired glucose tolerance; IPGTT, intraperitoneal glucose tolerance test; IR, insulin resistance; LC, long chain; NEFA, nonesterified fatty acid; PPAR-γ, peroxisome proliferator-activated receptor γ; PUFA, polyunsaturated fatty acid; qRT-PCR, quantitative RT-PCR; TC, total cholesterol; TG, triglycerides; TR, targeted replacement; VAT, visceral adipose tissue

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doi: 10.1096/fj.201600921RR
This article includes supplemental data. Please visit *http://www.fasebj.org* to obtain this information.

(10–13). No comprehensive analysis (representing the primary study endpoint) of the impact of *APOE* genotype on bodyweight or AT volume or topography is currently available in humans. In targeted replacement (*APOE* TR) mice, which retain the murine regulatory sequences but solely express the human *APOE3* or *APOE4* genes, Huebbe *et al.* (11) reported *APOE3* as the thrifty genotype associated with greater weight gain and abdominal AT mass after a high-fat diet (HFD). The lower weight gain in *APOE4* animals was coupled with higher AT fatty acid mobilization and up-regulated fatty acid β-oxidation genes in skeletal muscle (11).

The long-chain n-3 polyunsaturated fatty acids (LC n-3 PUFA) eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are found in oily fish and fish oil (FO) supplements, have been shown to exert potent anti-obesogenic and insulin-sensitizing effects in murine models [reviewed in (14, 15)]. In various studies, LC n-3 PUFA supplementation has been shown to reduce the accumulation of body fat (16) and to prevent or reverse insulin resistance (IR) (17) after a HFD challenge. However, the effects of LC n-3 PUFA on bodyweight and insulin sensitivity in humans remains controversial (reviewed in refs. 18, 19). The considerable intra- and interstudy variability and lack of consistency in the response is likely to be in part due to the genetic variability both within and between populations. Although the role of APOE in adipocyte metabolism is well defined and the *APOE* genotype has been shown to determine LC n-3 PUFA status (20) and the response of plasma lipids to LC n-3 PUFA intervention (21), the impact of genotype on the responsiveness of adiposity and its associated phenotype to altered LC n-3 PUFA intake are unknown.

Using the HFD-fed *APOE3* and *APOE4* TR mouse models, in addition to characterizing *APOE* genotype–adiposity associations, we investigated the impact of LC n-3 PUFA supplementation on bodyweight gain, visceral adipose tissue (VAT) mass, glucose tolerance, and biomarkers of insulin action according to *APOE* genotype status.

MATERIALS AND METHODS

Study approval

All experimental procedures and protocols used in this study were reviewed and approved by the Animal Welfare and Ethical Review Body and were conducted within the provisions of the Animals (Scientific Procedures) Act of 1986 and the *Guiding Principles for Preparing for and Undertaking Aseptic Surgery* (Laboratory Animal Science Association).

Animal experimental design and dietary treatments

Forty 13- to14-wk-old male human *APOE3* [B6.129P2-Apoe$^{tm2(APOE*3)Mae}$ N8] and *APOE4* [B6.129P2-Apoe$^{tm2(APOE4)Mae}$ N8] TR mice homozygous for the human *APOE3* or *APOE4* gene (Taconic Farms, Germantown, NY, USA) (22) were randomly allocated to a HFD (45 kcal% from fat, 0.02% [w/w] cholesterol; Research Diets, New Brunswick, NJ, USA) or a HFD with 3% LC n-3 PUFA (HFD + FO; Research Diets) for 8 wk (n = 10 mice per group) (Supplemental Table S1). A HFD control diet was used to mimic human Western human dietary patterns. The HFD + FO contained a blend of Menhaden FO and docosahexaenoic acid triglyceride (DHAsco oil; DSM Nutritional Products, Columbia, MD, USA), providing 12 g EPA and 18 g DHA per kg of diet (Supplemental Table S1). Mice were maintained in a controlled environment (21°C; 12 h light–dark cycle; light from 7:00 AM). Food pellets were replaced every other day to avoid oxidation of the bioactive components.

At the end of wk 8, animals were sedated with a mixture of isoflurane (1.5%), nitrous oxide (70%), and oxygen (30%), and blood samples were collected by cardiac puncture into lithium–heparin–coated microtubes (Sarstedt, Leicester, United Kingdom), followed by transcardiac perfusion of ice-cold saline containing heparin (10 U/ml; Sigma-Aldrich, St. Louis, MO, USA). Plasma samples were isolated by centrifugation at 2000 *g* for 10 min, and the VAT was snap frozen and stored at −80°C.

Intraperitoneal glucose tolerance test

Forty-eight hours before the animals were humanely killed, an intraperitoneal glucose tolerance test (IPGTT) was performed. After a 16-h overnight brief starvation period, which was used to deplete liver glycogen stores and minimize variability in blood glucose, a baseline tail blood sample was collected before mice received an intraperitoneal injection of D-glucose (2 g/kg bodyweight) (23). After injection, blood samples (2 μl) were collected at 10, 20, 30, 60, and 120 min, and glucose concentrations were determined using an AlphaTrak 2 glucometer (Abbott Laboratories, Lake Bluff, IL, USA). Additional blood samples (20 μl) for insulin determination were collected at baseline and at 15 and 120 min into EDTA-coated Microvette tubes (Sarstedt). Tubes were centrifuged at 2000 *g* for 15 min at room temperature, and resulting plasma samples were snap frozen and stored at −80°C. Insulin concentrations were determined using a commercial ELISA kit following the manufacturer's instructions (Crystal Chem, Downers Grove, IL, USA). Whole-body IR was estimated using the homeostatic model assessment (HOMA)-IR index. Area under the curve (AUC) was calculated by the trapezoidal method (24).

Biochemical analysis

Plasma total cholesterol (TC), HDL-cholesterol (HDL-C), TG, alanine aminotransferase (ALT), alkaline phosphatase (ALP), and aspartate aminotransferase (AST) concentrations were measured using commercial IL Test assays (Instrumentation Laboratory UK, Warrington, United Kingdom), and non-esterified fatty acid (NEFA) levels were measured using the commercial colorimetric assay (Randox Laboratories, Crumlin, United Kingdom) on the ILab-650 analyzer (Instrumentation Laboratory UK). Plasma levels of the liver enzymes ALT, ALP, and AST were assessed to evaluate liver function. Plasma non-HDL-C levels, consisting of LDL-cholesterol plus very low density lipoprotein-cholesterol, were calculated by subtracting HDL-C from TC. The plasma levels of the 2 major adipokines involved in appetite and metabolic control, leptin and adiponectin, were analyzed with commercial ProcartaPlex Simplex Immunoassays (eBiosciences, Hatfield, United Kingdom) using the Luminex 200 System (Luminex, Austin, TX, USA). Plasma levels of the antiinflammatory and insulin sensitizing cytokine IL-10 were analyzed using a commercial Mouse IL-10 ELISA Ready–Set–Go kit (eBiosciences). The moisture content of 50 mg of total feces was determined, with total lipid content expressed on a dry weight basis (25). Total lipids were extracted from 50 to 200 mg of epididymal AT with chloroform/methanol (2:1 v/v) containing 0.01% butylated hydroxytoluene (Sigma-Aldrich) as an antioxidant (26). Fatty acid analysis of total lipids was conducted as previously described (27).

RNA isolation and real-time PCR

Total RNA was isolated from VAT using Ambion Trizol reagents (Thermo Fisher Scientific, Loughborough, United Kingdom). The primer sequences are given in Supplemental Table S2. One microgram of total RNA was treated with DNase I (Thermo Fisher Scientific) and used for cDNA synthesis using Oligo (dT) primers and SuperScript II Reverse Transcriptase (Thermo Fisher Scientific). Real-time quantitative RT-PCR (qRT-PCR) reactions were performed with Precision qPCR MasterMix (PrimerDesign, Southampton, United Kingdom) using SYBR green detection technology on the Applied Biosystems 7500 Real-Time PCR system (Thermo Fisher Scientific). Results are expressed as relative quantity scaled to the average across all samples per target gene and normalized to the reference genes actin, β (*Actb*), ATP synthase, H + transporting mitochondrial F1 complex, β subunit (*Atp5b*), and glyceraldehyde-3-phosphate dehydrogenase (*Gapdh*).

Statistical analysis

All values are presented as means ± SEM. A power calculation was conducted to calculate the number of animals needed to detect a standardized mean difference of 1.45 for a 2-sample comparison of means with power of 90% at significance level of $\alpha = 0.05$, which yielded 10 mice per *APOE* genotype × diet group. Data were analyzed by 2-way ANOVA followed by comparisons of *APOE3*-HFD, *APOE3*-HFD + FO, *APOE4*-HFD, and *APOE4*-HFD + FO groups with Bonferroni correction when a significant overall genotype × diet interaction were detected. Data with multiple timepoints were analyzed by repeated-measures ANOVA. Data not following a normal distribution (food intake and ALT) detected by the Kolmogorov-Smirnov test, or having unequal variances detected by Levene's test, were assessed by nonparametric tests. Statistical analysis of the mRNA expression levels was done with log-transformed values. All statistical analyses were performed by SPSS 18.0 (IBM SPSS, Chicago, IL, USA), with the threshold of significance of $P < 0.05$.

RESULTS

The expected FO-induced enrichment of AT EPA and DHA was observed, with a genotype × diet interaction evident for EPA ($P < 0.05$) but not for DHA or total LC n-3 PUFA (Supplemental Table S3).

LC n-*3* PUFA modulate the *APOE3* genotype predisposition to higher weight gain and increased VAT expansion

To investigate whether *APOE* genotype interacts with dietary LC n-3 PUFA to determine adiposity and the predisposition to diet-induced obesity, we compared the change in bodyweight, VAT weight, food intake and efficiency, and fecal lipid content in *APOE3* and *APOE4* TR mice after a HFD or a HFD + FO for 8 wk. There was a significant effect of diet ($P < 0.01$) on bodyweight gain (**Fig. 1*A***) and a trend toward a different effect according to *APOE* genotype ($P = 0.10$), with more weight gain in *APOE3*-HFD relative to *APOE3*-HFD + FO and *APOE4*-HFD + FO from 3 wk onward.

There was a significant effect of diet ($P < 0.05$) and *APOE* genotype ($P < 0.05$) on VAT weight (Fig. 1*B*), which was 17% higher in *APOE3* mice compared to *APOE4* mice, and 9% higher in mice fed the HFD + FO compared to the HFD.

APOE3 mice fed the HFD had 20% higher food intake compared to the other 3 groups, and dietary LC n-3 PUFA resulted in an 12% reduction in food intake in *APOE3* mice ($P < 0.05$; Fig. 1*C*). A lower fecal lipid content ($^{+/-}$ FO), indicative of higher lipid absorption, was evident in *APOE3* animals (**Table 1**).

Food efficiencies were not significantly different between groups, although there was a trend toward lower values after HFD + FO feeding (Fig. 1*D*).

APOE3 mice fed the HFD had 25-fold lower *Apoe* mRNA levels in VAT compared to the other 3 groups, with a significant increase after FO intervention (Fig. 1*E*).

LC n-*3* PUFA attenuation of blood lipids is dependent on *APOE* genotype

Diet interacted with *APOE* genotype to determine plasma TG ($P < 0.001$), TC ($P < 0.001$), non-HDL-C ($P < 0.001$), and NEFA ($P < 0.05$) (**Table 2**). Compared to the other groups, *APOE3* mice fed a HFD had approximately 60% higher plasma TG levels. Dietary LC n-3 PUFA decreased plasma TG, TC, HDL-C, non-HDL-C, and NEFA levels in *APOE3* mice ($P < 0.05$). In *APOE4* mice, dietary LC n-3 PUFA decreased plasma TC and HDL-C ($P < 0.05$).

LC n-*3* PUFA decrease ALT and increase IL-10 in mice

Higher plasma ALT levels after the HFD were evident in *APOE3* mice, with FO feeding reducing the concentration by 2.5-fold in *APOE3* animals only ($P < 0.05$). There was no effect of genotype or diet on plasma ALP and AST levels (Table 2). A significant overall FO induced increase in IL-10 was evident, with increases significant in *APOE4* mice only ($P = 0.027$, Table 2). There were no significant genotype- or diet-mediated differences in the plasma adiponectin or leptin levels.

LC n-*3* PUFA mitigate IR through increased VAT expression of *Glut4* in *APOE3* mice

There was no difference in fasting plasma glucose levels (**Fig. 2*A***) between the intervention groups. *APOE3* mice fed the HFD had higher fasting plasma insulin levels compared to the mice fed a HFD + FO ($P < 0.05$; Fig. 2*B*), which was associated with a higher HOMA-IR ($P < 0.05$; Fig. 2*C*). Given the role of the insulin-regulated glucose transporter Glut4 in tissue insulin-stimulated glucose uptake, *Glut4* mRNA expression levels in VAT were investigated. *APOE3* mice fed a HFD had the lowest *Glut4* mRNA levels, but dietary LC n-3 PUFA resulted in a 5-fold increase in *Glut4* mRNA levels ($P < 0.05$; Fig. 2*D*). The beneficial effect of FO on *Glut4* expression was not observed in *APOE4* mice.

There was no difference in the plasma glucose levels during the IPGTT (**Fig. 3*A*, *C***). Diet had a significant effect

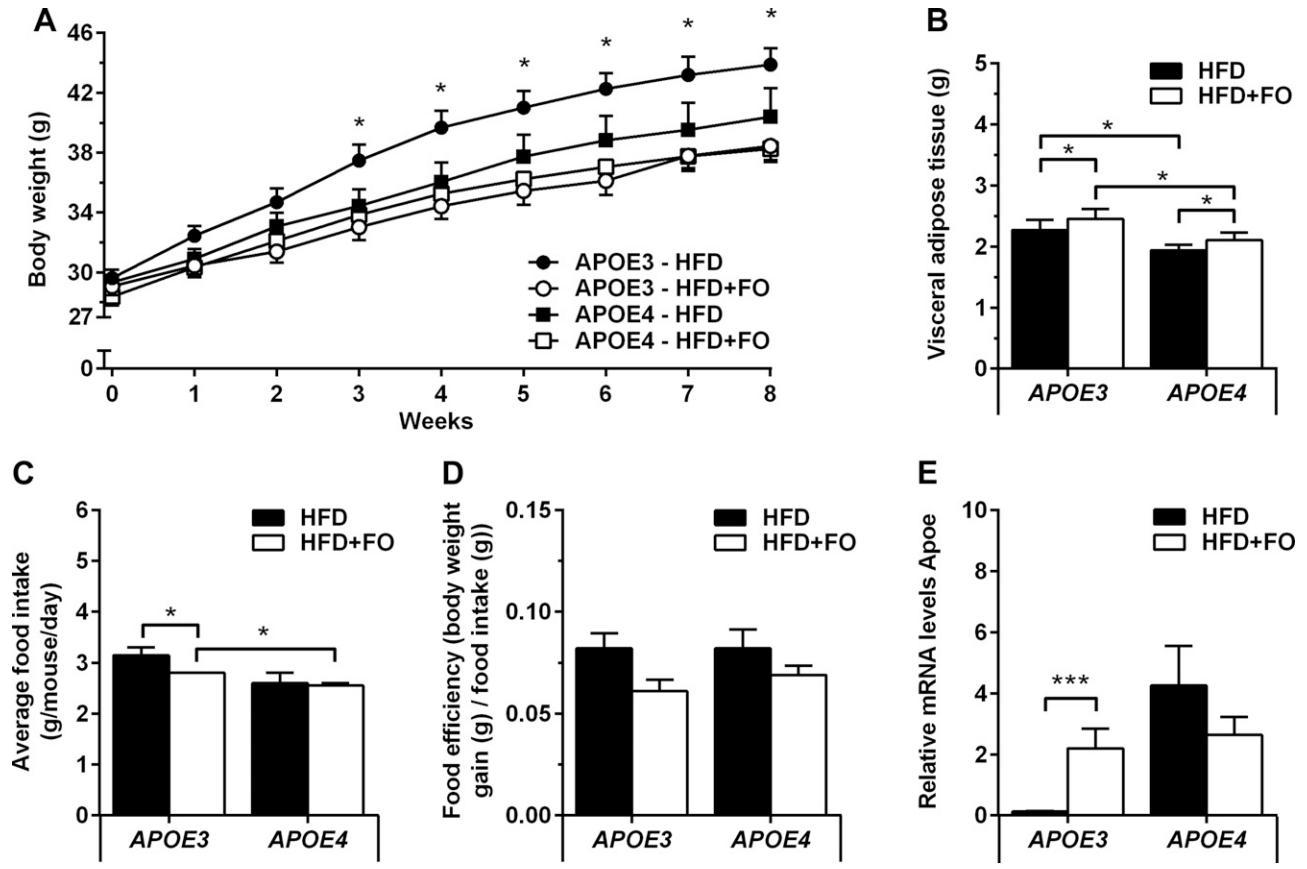

Figure 1. Development of diet-induced obesity in *APOE3* and *APOE4* TR mice fed HFD or HFD + FO for 8 wk. *A*) Bodyweight over 8 wk. Bodyweights were significantly different between diets but not *APOE* genotypes ($P < 0.05$, diet; $P = 0.19$, genotype; $P = 0.10$, diet × genotype; ANOVA). *$P < 0.05$ *APOE3*-HFD is different from *APOE3*-HFD + FO and *APOE4*-HFD + FO. *B*) VAT weight at 8 wk. VAT weights were significantly different between diets and *APOE* genotypes ($P < 0.05$, diet; $P < 0.05$, genotype; $P = 0.94$, diet × genotype; ANOVA). *$P < 0.05$. *C*) Average food intake over 8 wk. Average food intake was significantly different between groups ($P < 0.05$, diet × genotype; Kruskal Wallis). *$P < 0.05$. *D*) Average food efficiency over 8 wk. *E*) Relative mRNA expression of apolipoprotein E (*Apoe*) in VAT. Relative mRNA expression of *Apoe* was significantly different between groups ($P = 0.44$, diet; $P = 0.61$, genotype; $P < 0.001$, diet × genotype; ANOVA). Data are shown as means ± SEM, $n = 9$–10 mice per group. ***$P < 0.001$. APOE3-HFD is different to other groups. APOE, apolipoprotein E; HFD, high-fat diet; HFD + FO, HFD containing 30 g EPA + DHA/kg of diet.

on plasma insulin levels ($P < 0.001$; Fig. 3*B*), which was dependent on *APOE* genotype ($P < 0.05$). *APOE3* mice fed a HFD had significantly higher plasma insulin levels compared to the mice fed a HFD + FO (Fig. 3*B*), which was reflected in the plasma insulin AUC ($P = 0.13$; Fig. 3*D*).

DISCUSSION

With overweight and obesity affecting almost 2 billion people worldwide, there is widespread interest in the identification of dietary strategies for weight management and the mitigation of its metabolic complications (28). The cardiovascular benefits of LC n-3 PUFA are well described (29), but their effects on adiposity and insulin sensitivity remain controversial. Our findings demonstrate that an *APOE3* genotype predisposes mice to develop obesity and its metabolic complications, which was attenuated by n-3 PUFA supplementation.

Consistent with 2 previous studies using this mouse model, we showed that *APOE3* animals develop diet-induced obesity, dyslipidemia, and IR while eating a HFD, while the *APOE4* mice are protected (10, 11). Here, a higher food intake, which implies an increase in appetite that has

TABLE 1. *Total lipid content (% dry weight basis) of fecal samples after 8 wk of feeding*

APOE3-HFD	*APOE3*-HFD + FO	*APOE4*-HFD	*APOE4*-HFD + FO	*P*
3.2 ± 0.1	3.1 ± 0.0	4.0 ± 0.1	4.1 ± 0.3	0.001, G

Values are expressed as means ± SEM for 3 extractions from pooled feces samples of 10 animals per group. ANOVA was conducted to establish significance of impact of *APOE* genotype (G), diet (D), and G × D interactions using untransformed data and were considered statistically significant when $P < 0.05$.

TABLE 2. *Plasma biochemistry in APOE3 and APOE4 mice after 8 wk of feeding*

Biochemistry	APOE3-HFD	APOE3-HFD + FO	APOE4-HFD	APOE4-HFD + FO	*P*		
					G	D	G × D
Plasma lipid							
TG (mg/dl)	128 ± 10^{a}	71 ± 6^{b,c}	81 ± 9^{b}	84 ± 5^{b,c}	<0.05	<0.01	<0.001
TC (mg/dl)	137 ± 3^{a}	91 ± 4^{b,d}	119 ± 5^{c}	100 ± 5^{b,d}	<0.05	<0.01	<0.001
HDL-C (mg/dl)	116 ± 3^{a}	99 ± 7^{b}	107 ± 6^{a}	82 ± 4^{c}	<0.05	<0.001	NS
Non-HDL-C (mg/dl)	20 ± 2^{a}	10 ± 1^{b}	12 ± 1^{b,c}	17 ± 2^{a,c}	NS	NS	<0.001
NEFA (mM)	1.7 ± 0.1^{a,c}	0.7 ± 0.1^{b}	1.3 ± 0.2^{a,c}	0.9 ± 0.1^{b,c}	NS	<0.001	<0.05
Liver enzymes							
ALT (IU/L)	49 ± 8^{a}	21 ± 1^{b}	31 ± 8^{a,b}	17 ± 1^{b}	<0.05	<0.01	<0.001
AST (IU/L)	106 ± 12	123 ± 21	105 ± 14	79 ± 7	NS	NS	NS
ALP (IU/L)	63 ± 4	49 ± 3	62 ± 5	62 ± 5	NS	NS	NS
Adipokines/ cytokines							
IL-10 (pg/ml)	301 ± 55^{a}	378 ± 43 ^{a,b}	278 ± 35^{a}	536 ± 138^{b}	NS	<0.05	NS
Adiponectin (μg/ml)	194 ± 22	183 ± 29	207 ± 22	214 ± 18	NS	NS	NS
Leptin (ng/ml)	6.7 ± 0.5	5.1 ± 0.5	5.5 ± 0.8	4.4 ± 0.4	NS	NS	NS

Values are expressed as means ± SEM (n = 7–10 mice per group). Lipids, liver enzymes, and adipokines/cytokines were measured in nonfasted plasma samples. Two-way ANOVA followed by Bonferroni correction was conducted to establish the significance of the impact of *APOE* genotype (G), diet (D), and G × D interactions on plasma lipids, liver enzymes, and adipokines/cytokines. Letters (*a–c*) indicate differences between groups. $P < 0.05$.

been previously proposed (11), and higher fat absorption, underlie the greater weight gain in *APOE3* animals. Dietary LC n-3 PUFA decreased food intake and bodyweight gain especially in *APOE3* mice, suggesting that LC n-3 PUFA may improve satiety and may in part counteract the physiologic effect of the *APOE3* genotype. Leptin is an important AT-derived regulator of food intake, which acts on receptors in the hypothalamus to decrease hunger and stimulate satiety. Higher circulating levels of leptin have been associated with an increase in body mass index and body fat in humans (30) and have been shown to increase during prolonged hyperinsulinemia (31). Although we observed a trend toward reduced leptin after FO feeding, it did not reach statistical significance.

APOE3 mice fed the HFD had a higher average food intake and lipid absorption but a similar overall food efficiency compared to the other 3 groups, which is in accordance with their greater bodyweight gain. However, on the HFD + FO diet, a lack of difference in weight gain in *APOE3* relative to *APOE4* animals despite higher food

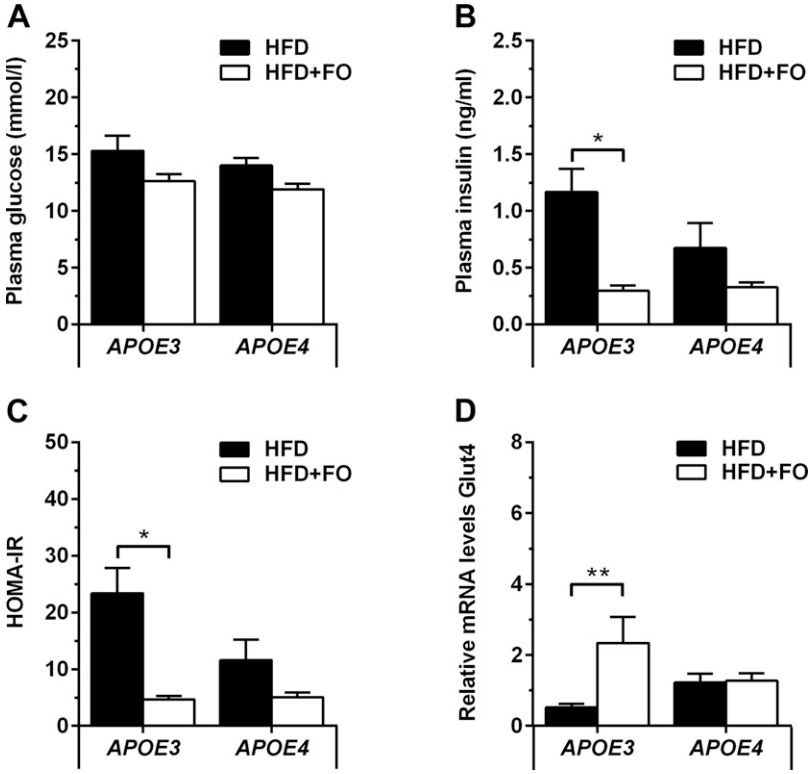

Figure 2. Markers of insulin sensitivity in the unfed state of *APOE3* and *APOE4* TR mice after 8 wk on HFD or HFD + FO. *A*) Plasma glucose. *B*) Plasma insulin. *C*) HOMA-IR index. *D*) Relative mRNA levels of insulin-regulated glucose transporter *Glut4* in VAT. Data are shown as means ± SEM, n = 9–10 mice per group. Fasting plasma insulin (P = 0.64, diet; P = 0.26, glucose; $P < 0.05$, diet × genotype; ANOVA), HOMA-IR (P = 0.58; P = 0.23; $P < 0.05$, diet × genotype; ANOVA), and relative mRNA levels of *Glut4* (P = 0.83; P = 0.41; $P < 0.01$, diet × genotype; ANOVA) were significantly different between groups. *$P < 0.05$, **$P < 0.01$. *Glut4*, solute carrier family 2 (facilitated glucose transporter) member 4 (*Slc2a4*).

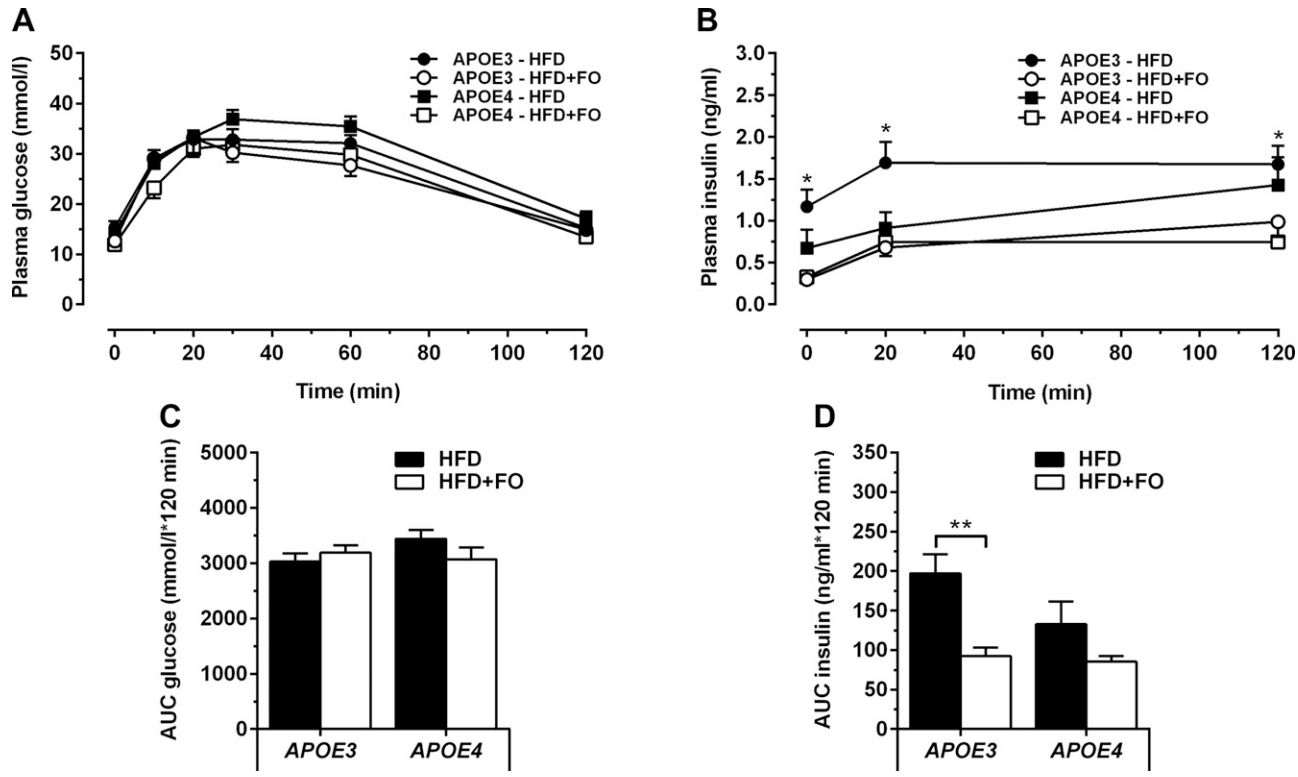

Figure 3. Glucose and insulin levels during an IPGTT performed at 8 wk in *APOE3* and *APOE4* TR mice fed HFD or HFD + FO. *A*) Plasma glucose levels at times 0, 10, 20, 30, 60, and 120 min. *B*) Plasma insulin levels at times 0, 15, and 120 min. *C*) AUC for plasma glucose. *D*) AUC for insulin. Data are shown as means ± SEM, n = 9–10 mice per group. Plasma glucose levels were significantly different between diets but not *APOE* genotypes ($P < 0.05$, diet; $P = 0.90$, genotype; $P = 0.63$, diet × genotype; ANOVA). Plasma insulin levels were significantly different between diets, which was dependent on *APOE* genotype ($P < 0.001$, diet; $P = 0.08$, genotype; $P < 0.05$, diet × genotype; ANOVA). *$P < 0.05$ *APOE3*-HFD is different from *APOE3*-HFD + FO and *APOE4*-HFD + FO.

intake, higher lipid absorption, and similar food efficiency is indicative of a higher energy expenditure. This is unlikely to be attributed to greater activity in these mice, as physical activity—although contributing to daily energy expenditure—has no effect on total energy expenditure in mice housed below their thermoneutrality (32). Two recent studies indicated that in mice, the *APOE4* genotype is associated with an increased basal mitochondrial uncoupling and fatty acid oxidation compared to the *APOE3* genotype (11, 33). LC n-3 PUFA increase mitochondrial biosynthesis and oxidative capacity, as well as fatty acid oxidation (14). Thus, we speculate that in *APOE3* mice, although having a lower basal rate, an LC n-3 PUFA induction of mitochondrial activity may explain the lower weight gain in the group fed a HFD + FO relative to the HFD group, and the apparent higher energy expenditure compared to *APOE4* mice fed the HFD + FO. Further studies are needed to fully elucidate differences in mitochondrial function between *APOE3* and *APOE4* mice and their response to dietary lipids.

The *APOE4* genotype was associated with a lower VAT mass compared to the *APOE3* genotype in the current study. APOE is involved in AT expansion, with its expression levels increasing on differentiation and correlating with the lipid content in adipocytes (7, 34). A potential mechanism linking *APOE* genotype to adipogenesis signaling pathways is endoplasmic reticulum (ER) stress, which is increasingly recognized as one of the underlying causes of metabolic dysfunction in obesity (35). Nutritional excess and an accumulation of unfolded and misfolded proteins within the ER are 2 known causes of the ER stress triggering unfolded protein response, which activates metabolic and inflammatory signaling pathways (35). The APOE4 protein has a lower protein stability and is increasingly recognized as an abnormally folded protein in the ER compared to the APOE3 protein. Although Dose *et al.* (36) recently reported little influence of *APOE* genotype on hepatic stress response pathways, an *APOE4* genotype resulted in an increased ER stress response in both macrophages and astrocytes (37). The increased ER stress would have a negative effect on the formation of new adipocytes (adipogenesis). This theory is supported by findings from Arbones-Mainar *et al.* (10), who showed that *APOE4* mice had fewer but larger adipocytes after 8 wk of HFD feeding compared to *APOE3* mice, suggesting that formation of new adipocytes rather than adipocyte TG accumulation is contributing to the reduced AT expansion in *APOE4* mice. Dietary LC n-3 PUFA was associated with a higher VAT mass in both *APOE3* and *APOE4* mice in the current study, which could be mediated by peroxisome proliferator-activated receptor γ (PPAR-γ) signaling. LC n-3 PUFA function as ligands for PPAR-γ, with PPAR-γ being both sufficient and essential for adipogenesis, as well as required for the initiation and maintenance of the

differentiated state in adipocytes (38) and associated with increased APOE (39).

In this study, *APOE3* but not *APOE4* mice developed IR and impaired glucose tolerance (IGT) when fed a HFD, which was associated with higher plasma insulin, TG, and NEFA levels. Overall, the increased insulin production to clear plasma glucose in both the fasting and the nonfasting state in the *APOE3* mice fed the HFD indicates IGT and reduced insulin sensitivity. Consistent with a greater human blood mononuclear cell inflammatory cytokine response to FO according in human *APOE4* carriers (40), here higher FO-induced IL-10 was evident in *APOE4* animals, which may have contributed to the insulin-sensitizing effects. Congruent with this idea, it has been shown that mice treated with IL-10 did not become insulin resistant when exposed to either IL-6 or lipid infusions (41). Furthermore, Lumeng *et al.* (42) have reported that IL-10-treated 3T3L1 adipocytes are protected from TNF-induced cellular IR.

Our finding are not consistent with the only previous study reporting that *APOE4* and not *APOE3* mice develop IGT after 8 wk of HFD feeding (10). The reasons for these apparently contradictory findings are unknown, but they may relate to differences in the composition of the diets. Our diet contained no sucrose, whereas Arbones-Mainar *et al.* (10) used a HFD containing a high (34.1%) sucrose content, with sucrose known to promote the development of IR and diabetes.

Although metabolic interdependency cannot be established, the observed prevention of IR and IGT by dietary LC n-3 PUFA in *APOE3* mice could be the result of the reduction in bodyweight. The insulin-regulated GLUT4 plays an important role in the insulin-stimulated glucose uptake in muscle and AT (43), with its protein levels being regulated at the transcriptional level (44). We demonstrated that LC n-3 PUFA increased the *Glut4* mRNA levels in the VAT of *APOE3* mice, which, although may reflect increased GLUT4 turnover (45), likely suggests increased GLUT4 protein levels that could account for the observed beneficial effects on glucose tolerance. Furthermore, LC n-3 PUFA improve insulin signaling *via* the insulin receptor by inhibition of the NF-κB and JNK pathways, which results in more effective insulin-stimulated GLUT4 translocation from intracellular vesicles to the cell membrane and increased glucose uptake (46). Surprisingly, the beneficial effects of LC n-3 PUFA on *Glut4* mRNA levels were not observed in *APOE4* mice.

The strengths of the current study were the use of a diet-induced obesity approach relevant to human obesity and dietary patterns, and the use of IPGTT to assess insulin sensitivity.

Human data on the association between *APOE* genotype and bodyweight are currently inconsistent (47–52). Limited studies suggest that *APOE* genotype interacts with body mass index to determine metabolic parameters such as plasma cholesterol, glucose, and insulin (13, 48). Although studies in *APOE* TR mice increasingly show an effect of *APOE* genotype on bodyweight, the effect in humans is likely to be more modest. Furthermore, human studies investigating energy metabolism according to *APOE* genotype are currently lacking; more research is needed in this area. The beneficial effects of LC n-3 PUFA on bodyweight and composition in humans remain controversial, although a recent meta-analysis indicated that LC n-3 PUFA supplementation results in a modest 590 g reduction in bodyweight, 0.24 kg/m^2 reduction in body mass index, 0.8 cm decrease in waist circumference, and 0.5% decrease in body fat in adults over 3 wk to 3 yr intervention periods (53). Although subtle, such effects on weight maintenance and adiposity could contribute to amelioration of the typical gain of 2 kg per decade evident in adult populations (54). The effect of LC n-3 PUFA on glucose homeostasis and insulin sensitivity in humans is also inconsistent (18, 19), which is likely to be in large part attributable to genetic variability of participants, with the current study indicating that *APOE* genotype may be important. Although *APOE* genotype has been shown to affect LC n-3 PUFA metabolism and bioavailability (20, 33, 55) and to determine the plasma lipid response to LC n-3 PUFA supplementation (21, 56), its impact on the response of glucose and insulin is currently unknown. Future prospective cohort and intervention studies in humans (or retrospective analyses of existing cohorts) that capture *APOE* genotype status are therefore warranted to determine whether the efficacy of LC n-3 PUFA in reducing bodyweight and improving insulin sensitivity varies according to this common genotype.

In summary, our results indicate that although the *APOE4* allele has been associated with risk of cardiovascular disease, in an obesogenic environment, it may be protective against weight gain and its associated metabolic complications. Furthermore, we propose that in those with the common *APOE3/E3* genotype, LC n-3 PUFA intervention may mitigate the adiposity and loss of insulin action associated with a high fat intake. These findings are of wide public health relevance, given the prevalence of the *APOE3* and *APOE4* alleles. An important direction for future research is to reproduce these findings in humans. FJ

ACKNOWLEDGMENTS

This project was funded by a University of East Anglia Faculty of Medicine and Health Sciences Graduate School studentship and a Biotechnology and Biological Sciences Research Council Institute Strategic Program grant (BB/J004545/1).

AUTHOR CONTRIBUTIONS

K. E. Slim, D. Vauzour, P. J. Voshol, A. Cassidy, and A. M. Minihane conceived and designed the study; K. E. Slim, D. Vauzour, and N. Tejera performed the animal intervention and sample analyses. K. E. Slim, D. Vauzour, P. J. Voshol, N. Tejera, and A. M. Minihane interpreted the data. K. E. Slim, D. Vauzour, and A. M. Minihane drafted the article. All authors revised the article, critically reviewed it for intellectual content, and approved the final version.

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Received for publication August 10, 2016.
Accepted for publication November 14, 2016.

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FASEB J published online November 28, 2016
Access the most recent version at doi:10.1096/fj.201600921RR