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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 (PUFAs) on the development of obesity and associated cardiometabolic dysfunction in a murine model. Human APOE3 and APOE4 targeted replacement mice were fed either a control high-fat diet (HFD) or an HFD supplemented with 3% n-3 PUFAs from fish oil (HFD + FO) for 8 wk. We established the impact of intervention on food intake, body weight, 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 PUFAs decreased body-weight 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, 989–997 (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 body-weight 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 (TGs) (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
(10–13). No comprehensive analysis (representing the primary study endpoint) of the impact of APOE genotype on body weight or AT volume or topography is currently available in humans. In APOE targeted replacement (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 PUFAs) 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 refs. 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 an HFD challenge. However, the effects of LC n-3 PUFAs on body weight and insulin sensitivity in humans remain 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 body-weight 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, Hull, United Kingdom).

Animal experimental design and dietary treatments

Forty 13- to 14-wk-old male human APOE3 [B6.129P2-Apoetm2(APOE3)NaeN8] and APOE4 [B6.129P2-Apoetm2(APOE4)NaeN8] TR mice homozygous for the human APOE3 or APOE4 gene (Taconic Farms, Germantown, NY, USA) (22) were randomly allocated to an HFD (45 kcal% from fat, 0.02% (w/w) cholesterol; Research Diets, New Brunswick, NJ, USA) or an HFD with 3% LC n-3 PUFAs (HFD + FO; Research Diets) for 8 wk (n = 10 mice per group) (Supplemental Table S1). An HFD control diet was used to mimic human Western human dietary patterns. The HFD + FO contained a blend of Menhaden FO and docosahexaenoic acid TG (DHAsco oil; DSM Nutritional Products, Columbia, MD, USA), providing 12 g EPA and 18 g DHA per kilogram 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 body weight) (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 Lumexin 200 System (Lumexin, Austin, TX, USA). Plasma levels of the antiflammatory 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 P = 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 was 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 PUFAs (Supplemental Table S3).

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

To investigate whether APOE genotype interacts with dietary LC n-3 PUFAs to determine adiposity and the predisposition to diet-induced obesity, we compared the change in body weight, VAT weight, food intake and efficiency, and fecal lipid content in APOE3 and APOE4 TR mice after an HFD or an HFD + FO for 8 wk. There was a significant effect of diet (P < 0.01) on body-weight gain (Fig. 1A) 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. 1B), 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 PUFAs resulted in an 12% reduction in food intake in APOE3 mice (P < 0.05; Fig. 1C). A lower fecal lipid content (P < 0.05), 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. 1D).

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. 1E).

LC n-3 PUFAs 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 an HFD had approximately 60% higher plasma TG levels. Dietary LC n-3 PUFAs 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 PUFAs decreased plasma TC and HDL-C (P < 0.05).

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

There was no difference in fasting plasma glucose levels (Fig. 2A) between the intervention groups. APOE3 mice fed the HFD had higher fasting plasma insulin levels compared to the mice fed an HFD + FO (P < 0.05; Fig. 2B), which was associated with a higher HOMA-IR (P < 0.05; Fig. 2C). Given the role of the insulin-regulated glucose transporter type 4 [Glut4; solute carrier family 2 (facilitated glucose transporter) member 4 (Slc2a4)] in tissue insulin-stimulated glucose uptake, Glut4 mRNA expression levels in VAT were investigated. APOE3 mice fed an HFD had the lowest Glut4 mRNA levels, but dietary LC n-3 PUFAs resulted in a 5-fold increase in Glut4 mRNA levels (P < 0.05; Fig. 2D). 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. 3A, C). Diet had a significant effect
on plasma insulin levels ($P < 0.001$; Fig. 3B), which was dependent on $APOE$ genotype ($P < 0.05$). $APOE3$ mice fed an HFD had significantly higher plasma insulin levels compared to the mice fed an HFD + FO (Fig. 3B), which was reflected in the plasma insulin AUC ($P = 0.13$; Fig. 3D).

**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 PUFAs 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 an HFD, while the $APOE4$ mice are protected (10, 11). Here, a higher food intake, which implies an increase in appetite

| 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                     | $P$                                  |
| $3.2 \pm 0.1$                       | $3.1 \pm 0.0$                        | $4.0 \pm 0.1$                       | $4.1 \pm 0.3$                        | 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 $G \times D$ interactions using untransformed data and were considered statistically significant when $P < 0.05$. 

Figure 1. Development of diet-induced obesity in $APOE3$ and $APOE4$ TR mice fed HFD or HFD + FO for 8 wk. A) Body weight over 8 wk. Body weight was significantly different between diets but not $APOE$ genotypes ($P < 0.05$, diet; $P = 0.19$, genotype; $P = 0.10$, diet $\times$ genotype; ANOVA). *$P < 0.05$ for $APOE3$-HFD vs. $APOE3$-HFD + FO and $APOE4$-HFD + FO. B) VAT weight at 8 wk. VAT weight was significantly different between diets and $APOE$ genotypes ($P < 0.05$, diet; $P < 0.05$, genotype; $P = 0.94$, diet $\times$ genotype; ANOVA). *$P < 0.05$. C) Average food intake over 8 wk. Average food intake was significantly different between groups ($P < 0.05$, diet $\times$ genotype; Kruskal Wallis). *$P < 0.05$. D) Average food efficiency over 8 wk. E) Relative mRNA expression of $Apoe$ in VAT. Relative mRNA expression of $Apoe$ was significantly different between groups ($P = 0.44$, diet; $P = 0.61$, genotype; $P < 0.001$, diet $\times$ genotype; ANOVA). Data are shown as means ± SEM, $n = 9–10$ mice per group. ***$P < 0.001$. 

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Adipokines/ cytokines
Liver enzymes
Plasma lipid

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

Values are expressed as means ± SEM (\( n = 7–10 \) mice per group). Nonfasting levels of lipids, liver enzymes, and adipokines/cytokines were measured in 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. \( P < 0.05 \).

that has been previously proposed (11), and higher fat absorption, underlie the greater weight gain in APOE3 animals. Dietary LC n-3 PUFAs decreased food intake and body-weight gain especially in APOE3 mice, suggesting that LC n-3 PUFAs may improve satiety and may in part counteract the physiologic effects 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 body-weight 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) Fasting plasma insulin levels (\( P = 0.64 \), diet; \( P = 0.26 \), genotype; \( P < 0.05 \), diet × genotype; ANOVA) were significantly different between groups. C) HOMA-IR index (\( P = 0.58 \), diet; \( P = 0.23 \), genotype; \( P < 0.05 \), diet × genotype; ANOVA) was significantly different between groups. D) Relative mRNA levels of Glut4 (\( P = 0.83 \), diet; \( P = 0.41 \), genotype; \( P < 0.01 \), diet × genotype; ANOVA) were significantly different between groups. *\( P < 0.05 \), **\( P < 0.01 \).](image-url)
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 PUFAs 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 an 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 PUFAs were 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 PUFAs 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

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 were significantly different between diets but not APOE genotypes (P < 0.05, diet; P = 0.90, genotype; P = 0.63, diet × genotype; ANOVA). B) Plasma insulin levels at times 0, 15, and 120 min 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 vs. APOE3-HFD + FO and APOE4-HFD + FO. C) AUC for plasma glucose. D) AUC for insulin. **P < 0.01. Data are shown as means ± SEM, n = 9–10 mice per group.
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 an HFD, which was associated with higher plasma insulin, TG, and NEFA levels. Overall, the increased insulin production to clear plasma glucose in both the briefly starved and non-starved states in the APOE3 mice fed the HFD indicates IGТ 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 findings 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 an 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 PUFAs in APOE3 mice could be the result of the reduction in body weight. 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 PUFAs increased the Glut4 mRNA levels in the VAT of APOE3 mice; although this may reflect increased GLUT4 turnover (45), it likely suggests increased GLUT4 protein levels that could account for the observed beneficial effects on glucose tolerance. Furthermore, LC n-3 PUFAs improve insulin signaling via the insulin receptor by inhibition of the NF-kB 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 PUFAs 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 body weight 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 body weight, 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 PUFAs on body weight 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 body weight, 0.24 kg/m² 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 PUFAs 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 PUFAs in reducing body weight 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.

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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.
