Apolipoprotein E2 Accentuates Postprandial Inflammation and Diet-Induced Obesity to Promote Hyperinsulinemia in Mice

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Genetic studies have revealed the association between the ε2 allele of the apolipoprotein E (apoE) gene and greater risk of metabolic diseases. This study compared C57BL/6 mice in which the endogenous mouse gene has been replaced by the human APOE2 or APOE3 gene (APOE2 and APOE3 mice) to identify the mechanism underlying the relationship between ε2 and obesity and diabetes. In comparison with APOE3 mice, the APOE2 mice had elevated fasting plasma lipids and insulin levels and displayed prolonged postprandial hyperlipidemia accompanied by increased granulocyte number and inflammation 2 h after being fed a lipidd-rich meal. In comparison with APOE3 mice, the APOE2 mice also showed increased adiposity when maintained on a Western-type, high-fat, high-cholesterol diet. Adipose tissue dysfunction with increased macrophage infiltration, abundant crown-like structures, and inflammation were also observed in adipose tissues of APOE2 mice. The severe adipocyte dysfunction and tissue inflammation corresponded with the robust hyperinsulinemia observed in APOE2 mice after being fed the Western-type diet. Taken together, these data showed that impaired plasma clearance of apoE2-containing, triglyceride-rich lipoproteins promotes lipid redistribution to neutrophils and adipocytes to accentuate inflammation and adiposity, thereby accelerating the development of hyperinsulinemia that will ultimately lead to advanced metabolic diseases.

Apolipoprotein E (apoE) is a 34-kDa protein found in plasma associated with several classes of lipoproteins with a primary function in cholesterol and lipid transport (1). It is expressed in most cell types, including hepatocytes, smooth muscle, macrophages, adipocytes, and the central nervous system (1). In addition to facilitating lipid transport between various tissues and organs, apoE also has lipid transport–independent functions, such as the modulation of cell signaling, oxidation, and enzyme activation (2). Both lipid transport–dependent and –independent functions of apoE can modulate the progression and severity of a wide spectrum of metabolic diseases. The human APOE gene exists with three major polymorphic alleles. The most common allele, ε3, which imparts metabolic benefits due to the anti-inflammatory and antioxidative properties of apoE3. The ε4 allele, with a frequency of ~15%, encodes apoE4, which is proinflammatory and thus increases the risk for both cardiovascular disease and neurodegenerative disorders such as Alzheimer disease (1,2).

The relationship between the ε2 allele with metabolic disease risk is less clear. Typically, ε2 carriers have lower plasma cholesterol levels (3) but tend to have higher plasma triglyceride levels and are prone to developing type III dyslipoproteinemia (3,4), putting them at greater risk for metabolic-associated diseases. Although several independent studies have failed to identify an association between ε2 mutation and risk of type 2 diabetes (5–8), genetic studies in two other populations have revealed the association of the ε2 allele with higher BMI (odds ratio 3.55) and waist circumference (odds ratio 3.3) (4,9). A large-scale meta-analysis combining data from 30 independent studies showed that ε2 carriers have a moderately increased risk of developing type 2 diabetes (10). Diabetic ε2 carriers also have a twofold increased risk and severity of coronary artery disease compared with ε3 patients with diabetes (11,12). In nondiabetic patients, the ε2 allele is an independent risk factor for end-stage renal disease, peripheral vascular diseases such as cerebrovascular disease and ischemia of lower extremity arteries, and cardiovascular atherosclerosis (13–16). The mechanism(s) underlying the contribution of apoE2 toward obesity, diabetes, and peripheral vascular disease has not been elucidated.

A well-established role of apoE is its ability to bind LDL receptor family proteins to mediate clearance of triglyceride-rich lipoproteins from circulation (1). ApoE2 is defective in binding to the LDL receptor (1). Although the majority of ε2 homozygotes have normal or even lower plasma cholesterol levels (1), almost all heterozygous and homozygous ε2 carriers have elevated triglyceride levels due to impaired hepatic clearance of triglyceride-rich lipoproteins, including the chylomicron remnants derived postprandially after a fatty meal (1,17). With increasing evidence suggesting that the postprandial increase of plasma triglyceride levels promotes systemic inflammation (18–23), which increases the risk of metabolic diseases (22,23), it is reasonable to postulate that the elevated metabolic disease risk associated with the ε2 allele may be due to sustained postprandial inflammation as a consequence of delayed postprandial triglyceride-rich lipoprotein clearance. To test this hypothesis, this study compared fatty meal–induced postprandial inflammation between mice in which the endogenous mouse apoE gene has been replaced by either the human APOE2 or APOE3 gene. The consequence of chronic high-fat feeding on tissue inflammation and obesity/diabetes development between APOE2 and APOE3 gene replacement mice was also explored.
RESEARCH DESIGN AND METHODS

Animals and diets. Gene replacement mice, in which the endogenous mouse apoE gene has been replaced at the same locus with the human APOE2 or APOE3 gene (24,25), hereafter designated as APOE2 and APOE3 mice, were purchased from Taconic (Hudson, NY), where they were back-crossed to C57BL6 background through nine and eight generations, respectively. The human APOE genotypes in these animals were confirmed by restriction iso- typing after gene amplification as previously described (26). The mice were fed either chow (Teklad, Madison, WI) or a Western-type, high-fat, high-cholesterol diet containing 21.2% fat and 0.2% cholesterol (TD8137; Teklad). Animals were maintained under controlled environmental conditions with free access to food and water. All animal protocols were approved by the University of Cincinnati Institutional Animal Use and Care Committee.

Weight and adiposity measurements. Age-matched APOE2 and APOE3 mice were housed according to their genotype with one to three mice per cage. Food consumption was monitored daily over a 1-week period. No obvious difference in the average amounts of food consumed per animal was observed regardless of housing conditions. Body weight and adiposity measurements were performed every 2 weeks. Weights were obtained using a Denver 300 K scale. Adiposity measurements were obtained using an EchoMRI Whole-Body Composition Analyzer (Echo Medical Systems, Houston, TX), as described previously (27).

Blood chemistry. Blood was collected from mice after an overnight fast. Blood glucose was determined with an Accu-Chek Active Glucometer (Roche Applied Science, Indianapolis, IN). Plasma insulin levels were measured with the Ultra Sensitive Rat Insulin ELISA kit (Crystal Chem, Chicago, IL). Plasma adiponectin levels were measured by mouse Adiponectin/Acrp30 DuoSet ELISA (R&D Systems, Minneapolis, MN). Plasma levels of leptin and other cytokines were determined using MILLIPLEX MAP Mouse Adipokine Panel (Millipore, St. Charles, MO). Plasma cholesterol and triglycerides were determined using Infinity cholesterol and triglyceride kits (Thermo Fisher Scientific, Middle- town, NJ). Samples from each mouse were analyzed individually except for lipoprotein separation, in which two separately pooled samples, each with 0.25 mL plasma from three animals in each group, were subjected to fast-performance liquid chromatography (FPLC) gel filtration on two Superose 6 columns connected in series. Individual fractions in the FPLC were analyzed based on cholesterol content and by Western blot analysis with goat anti-human apoB (Millipore) and rabbit anti-human apoE (Daklo) antibodies at 1:5,000 dilutions, as described previously (28).

Postprandial lipid clearance. Mice were fasted overnight and then fed a bolus lipid-rich meal (15 mL olive oil and 0.2 μCi [14C]triolein per gram body weight) by stomach gavage. Blood (50 μL) was obtained from the tail vein before and 15, 30, 60, 120, and 180 min afterward. Plasma was obtained after centrifugation and used to measure triglyceride levels and determine radioactivity by liquid scintillation counting.

Glucose tolerance and insulin sensitivity tests. A glucose solution was administered orally by stomach gavage to chow-fed APOE2 and APOE3 mice (2 g/kg body weight) after an overnight fast. Insulin sensitivity was monitored by intraperitoneal injection of 0.75 units pig insulin per gram body weight after a 10-h fast. Blood was obtained from the tail vein before and every 15 min after glucose or insulin administration to measure glucose levels.

Adipose tissue histology. Subcutaneous (inguinal) and visceral (gonadal) adipose tissue were fixed in isoionic neutral 4% paraformaldehyde solutions prior to embedding in paraffin. Three 5-μm sections from different levels of each depot obtained from each mouse (four APOE2 and six APOE3 mice) were analyzed for adipocyte size and crown-like structures after staining with hemotoxylin and eosin. Adipocyte sizes were determined as adipocyte area of aggregates of nucleated cells surrounding individual adipocytes. Crown-like structure density was obtained by counting the total number in each section compared with the total number of adipocytes.

RNA quantification. Total RNA was isolated with TRIzol reagent (Invitrogen) and treated with Turbo DNase (Applied Biosystems/Ambion, Austin, TX). cDNA was generated using the iScript cDNA Synthesis Kit (Bio-Rad Laboratories, Hercules, CA). Quantitative real-time PCR was performed on a StepOnePlus Fast Thermocycler using Fast SYBR Green Master Mix (Applied Biosystems, Carlsbad, CA) with primer sequences as shown in Table 1.

Flow cytometry. Flow cytometry analysis was performed on a Guava EasyCyte MHT System and analyzed using Guava InCyte (Millipore, Hayward, CA). In brief, mice fasted overnight were given an oral gavage of 200 μL olive oil. Blood was collected from the submandibular vein in heparinized tubes before and 2 h after the gavage. Erythrocytes were lysed with RBC lysis buffer (eBio- science, San Diego, CA) for 10 min at room temperature twice and then washed once in flow cytometry staining buffer (FCSB; Ca2+-/Mg2+-free Hanks’ balanced salt solution with 0.3% NaCl, and 1% BSA [fraction V; Sigma]). FCSB was used for all washes and antibody incubations unless otherwise indicated. All remaining incubations were carried out at 4°C. Non-specific binding was blocked using CD16/32 antibody (eBioScience, San Diego, CA). Cells were washed and incubated for 45 min with various extracellular-labeling antibodies at 1:25 dilution and then fixed in 2% paraformaldehyde solution overnight. Cells were permeabilized with BD Cytofix/Cytoperm Plus Kit (BD Biosciences, Franklin Lakes, NJ), incubated with 1:25 dilutions of antibodies specific for intracellular markers, and subjected to flow cytometry analysis. Conjugated antibodies to nitric oxide synthase (NOS2), Lys6G, and CD11b were purchased from BD Biosciences. For neutral lipid staining, leukocytes were incubated with HCS RapidTOX (Invitrogen) at 1:100 for 1 h prior to flow cytometry analysis. The magnitude of lipid accumulation was determined based on green fluorescent intensity measured at 485-nm excitation and 518- nm emission. Adipose tissue macrophage content was assessed based on positive staining of stromal-vascular cells with F4/80 antibodies (eBioScience) after enzymatic tissue disaggregation with type I collagenase (Worthington, Lakewood, NJ).

Statistics. Statistical analyses were performed using Microsoft Excel spreadsheet or SigmaPlot version 11.0 software. Data are expressed as means ± SDs, except where noted in the figure legend. Differences were considered significant at P < 0.05 based on ANOVA analyses with Bonferroni post hoc test or Student t test.

RESULTS

Prolonged postprandial hypertriglyceridemia in APOE2 mice. Male APOE2 and APOE3 mice were placed on either a basal rodent chow and/or fed a Western-type, high-fat, high-cholesterol diet beginning at 6 weeks of age. Fasting plasma lipid levels were determined after 4 weeks. Consistent with results reported previously (27,28), APOE2 mice have significantly higher plasma triglyceride and cholesterol levels compared with the APOE3 mice under both dietary conditions (Fig. 1, A and B). Interestingly, plasma triglyceride levels in APOE2 mice fed the basal diet were higher and their plasma cholesterol levels were similar to APOE3 mice fed the Western-type diet. Hyper- lipidemia was further exaggerated in APOE2 mice after feeding the Western-type diet (Fig. 1A and B). When the mice maintained on basal diet were challenged with

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<tr>
<th>Gene</th>
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<th>Antisense primer</th>
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<td>CTCATGCTTGGCAGTTC</td>
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<td>F4/80</td>
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<tr>
<td>NOS2</td>
<td>TTCCACCATGTTC</td>
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TABLE 1
Primer sequences used for RT-PCR amplification of RNA
a bolus lipid-rich meal of olive oil containing $[^{14}C]$triolein, delayed plasma clearance of the radiolabeled lipids, indicative of defective triglyceride-rich lipoprotein clearance, was observed in APOE2 mice (Fig. 1C). The impaired clearance of postprandial, triglyceride-rich lipoproteins resulted in robust postprandial hyperlipidemia in the APOE2 mice (Fig. 1D). Analysis of lipoproteins in chow- and Western diet–fed mice under both fasting and postprandial conditions by FPLC revealed the accumulation of VLDL and LDL in the APOE2 mice (Fig. 1E and F). Western blot analysis confirmed the accumulation of apoB100- and apoB48-containing lipoproteins with excess apoE in APOE2 mice compared with APOE3 mice (Fig. 1E and F). These results illustrated that APOE2 mice recapitulated the abnormal lipoprotein metabolism in e2 human subjects, and thus can be used to assess the influence of apoE2 on metabolic diseases.

**Elevated postprandial inflammation in APOE2 mice.** Triglyceride-rich lipoproteins and postprandial lipidemia are major risk factors for metabolic and vascular diseases because of their direct influence on leukocyte activation, with increased neutrophil cell count and activation to exacerbate inflammation (19,20,30,31). Therefore, we also examined the role of the apoE2-associated delay in postprandial triglyceride-rich lipoprotein clearance on inflammation. Blood from chow-fed APOE2 and APOE3 mice was collected after an overnight fast and 2 h after a bolus olive oil meal for flow cytometry analysis. Whereas no differences in the number of granulocytes, monocytes, and lymphocytes were observed between APOE2 and APOE3 mice during the fasting state, the APOE2 mice had a significantly higher number of granulocytes compared with APOE3 mice during the postprandial period (Fig. 2A–C). Both APOE2 and APOE3 mice also exhibited reduced levels of monocytes in circulation postprandially, but no statistically significant differences were observed between APOE2 and APOE3 mice during the postprandial period (Fig. 2A–C). Both APOE2 and APOE3 mice also exhibited reduced levels of monocytes in circulation postprandially, but no statistically significant differences were observed between APOE2 and APOE3 mice during the postprandial period (Fig. 2A–C). Both APOE2 and APOE3 mice also exhibited reduced levels of monocytes in circulation postprandially, but no statistically significant differences were observed between APOE2 and APOE3 mice during the postprandial period (Fig. 2A–C). Both APOE2 and APOE3 mice also exhibited reduced levels of monocytes in circulation postprandially, but no statistically significant differences were observed between APOE2 and APOE3 mice during the postprandial period (Fig. 2A–C). Both APOE2 and APOE3 mice also exhibited reduced levels of monocytes in circulation postprandially, but no statistically significant differences were observed between APOE2 and APOE3 mice during the postprandial period (Fig. 2A–C). Both APOE2 and APOE3 mice also exhibited reduced levels of monocytes in circulation postprandially, but no statistically significant differences were observed between APOE2 and APOE3 mice during the postprandial period (Fig. 2A–C).
APOE2 PROMOTES INFLAMMATION AND HYPERINSULINEMIA

FIG. 2. Flow cytometry analysis of blood leukocytes in APOE2 and APOE3 mice. A and B: Representative plots of blood cells showing forward and side scattering of lymphocytes (L), monocytes (M), and granulocytes (G) from APOE2 and APOE3 mice, respectively. C: Bar graph showing the average number of each cell type ± SD in four APOE2 (open bars) and six APOE3 (filled bars) mice after overnight fast and 2 h after feeding a lipid-rich meal. *P < 0.05, difference from fasted mice of the same genotype; #P < 0.05, difference from APOE3 mice under same treatment conditions (n = 6 in each group). D and E: Representative histogram of granulocytes after staining with the fluorescent neutral lipid stain LipidTox. F: Bar graph showing mean neutral lipid staining intensity ± SD from four APOE2 (open bars) and eight APOE3 (filled bars) mice before (fasted) and 2 h after lipid meal feeding (postprandial). *P < 0.05, difference from the APOE3 mice. G: Inducible NOS (iNOS)–positive neutrophils. H: CD11b and Ly6G double-positive cells as the mean percentage of total leukocytes ± SD in the blood of four APOE2 (open bars) and six APOE3 (filled bars) mice under same feeding conditions (n = 6).

ApoE2 promotes diet-induced obesity and insulin resistance. The delay in postprandial triglyceride-rich lipoprotein clearance may also increase the partitioning of dietary fat to adipose tissues for storage. To test this possibility, we compared the body weights of APOE2 and APOE3 mice maintained on basal chow diet and then monitored their body weights in response to Western diet feeding. Results showed that 10-week-old male APOE2 mice were slightly heavier than APOE3 mice on chow diet (Fig. 3A). After Western diet feeding for 4 weeks, the APOE2 mice gained significantly more weight compared with the APOE3 mice (Fig. 3A). Analysis of body composition by nuclear magnetic resonance revealed that the differences in body weight gain between APOE2 and APOE3 mice were due to significantly higher body fat content in APOE2 mice (Fig. 3B).

Fasting glucose levels were similar between APOE2 and APOE3 mice under basal dietary conditions. Both groups of mice showed a slight but statistically insignificant increase in fasting glucose levels, but their fasting insulin levels were significantly elevated after 4 weeks on Western-type diet (Fig. 4A and B). Interestingly, fasting insulin levels were higher in APOE2 mice compared with APOE3 mice under both dietary conditions. In fact, fasting insulin levels in chow-fed APOE2 mice were similar to those observed in APOE3 mice fed the Western-type diet for 4 weeks (Fig. 4B), and the Western diet further elevated fasting insulin levels in APOE2 mice to levels approximately three times higher than those observed in Western diet-fed APOE3 mice (Fig. 4B). Calculations of homeostasis model assessment of insulin resistance index suggested that APOE2 mice are prediabetic with hyperinsulinemia even under basal dietary conditions (Fig. 4C). This hypothesis was confirmed by data showing a small but statistically significant increase of glucose intolerance in chow-fed APOE2 mice after an oral glucose load (Fig. 4D), despite their elevated plasma insulin levels compared with APOE3 mice (Fig. 4B). The insulin resistance phenotype of APOE2 mice was further confirmed by insulin tolerance tests, showing their delayed glucose disposal after insulin injection compared with that observed in APOE3 mice (Fig. 4E).

ApoE2 promotes adipocyte dysfunction and inflammation. The robust diet-induced obesity and hyperinsulinemia along with elevated postprandial systemic inflammation observed in the APOE2 mice suggests their potential increased susceptibility to diet-induced adipocyte dysfunction and inflammation compared with APOE3 mice. Therefore, subcutaneous (inguinal) and visceral (gonadal) fat depots
were harvested from APOE2 and APOE3 mice fed either basal or Western diet for 4 weeks for comparison. Histological examination of the adipose tissues revealed larger adipocytes in subcutaneous depots of APOE2 mice compared with APOE3 mice under chow-fed conditions (Fig. 5A and B). Significant enlargement of the adipocytes in both depots of APOE2 and APOE3 mice was observed after Western diet feeding, but the differences between APOE2 and APOE3 mice were no longer noticeable (Fig. 5A and B). The most striking difference between APOE2 and APOE3 mice was the prevalence of crown-like structures, indicative of dead adipocytes surrounded by macrophages (29), in the adipose tissues of APOE2 mice (Fig. 5C). Although APOE3 mice displayed a minimal, if any, number of crown-like structures in both subcutaneous and visceral adipose tissues under chow-fed conditions, and their presence was detectable only sporadically after 4 weeks of feeding with the Western-type diet, the crown-like structures were detectable consistently in both subcutaneous and visceral adipose depots of APOE2 mice even when maintained under chow-fed conditions (Fig. 5C). The number of crown-like structures was further increased in both adipose depots of APOE2 mice after Western diet feeding (Fig. 5C). The presence of crown-like structures, and their subsequent increase with Western diet feeding in

FIG. 3. Body weights and adiposity of APOE2 and APOE3 mice. Bar graphs showing mean body weights ± SD (A) and percent body fat ± SD (B) in 10-week-old male APOE2 and APOE3 mice fed either chow diet (filled bars) or 4 weeks after feeding the Western-type diet (open bars). *P < 0.05, difference from chow-fed mice (n = 6).

FIG. 4. Blood glucose and insulin levels. A: Mean fasting blood glucose ± SD. B: Fasting plasma insulin levels ± SD. C: Calculations of homeostasis model assessment of insulin resistance (HOMA) index in chow- (filled bars) and Western diet-fed (open bars) APOE2 and APOE3 mice. Bars with different letters denote significant differences at P < 0.05, whereas bars with similar letters were not statistically different (n = 6). Glucose tolerance tests with inset showing area under the curve (AUC) analysis (D) and insulin sensitivity tests (E) in APOE2 (open symbols) and APOE3 (filled symbols) mice. The data represent the mean from six mice in each group. *P < 0.05, difference from the APOE3 mice. A.U., arbitrary units.
adipose tissues of APOE2 mice, is consistent with the interpretation that apoE2 promotes adipocyte dysfunction and increases susceptibility to diet-induced inflammation.

The analysis of adipokine expression revealed significantly elevated leptin expression in both subcutaneous and visceral adipose tissues after Western diet feeding (Fig. 6A). Consistent with results showing exaggerated diet-induced adiposity in APOE2 mice, diet-induced leptin expression was also higher in the APOE2 mice compared with APOE3 mice (Fig. 6A). Interestingly, adiponectin expression levels were similar between chow- and Western diet–fed APOE2 mice, whereas adiponectin expression levels in the subcutaneous tissue of APOE3 mice were higher after Western diet feeding (Fig. 6B). As a result, adiponectin-to-leptin ratios in subcutaneous and visceral fat depots were lower in APOE2 mice, and the differences were further exaggerated upon Western diet feeding. These data provided additional support for the elevated basal inflammation (dysfunction) of both subcutaneous and visceral fat depots of APOE2 mice, and the condition was further exaggerated when fed the Western-type diet.

**Elevated adipose tissue macrophage content and inflammation in APOE2 mice.** To further test the hypothesis that adipose tissues in APOE2 mice are dysfunctional (more inflamed), macrophage recruitment and retention marker gene expression in both subcutaneous (inguinal) and visceral (gonadal) adipose depots were compared between basal and Western diet–fed APOE2 and APOE3 mice. In subcutaneous adipose tissues, no significant differences in expression of monocyte chemoattractant protein-1 (MCP-1) and MIP-1α were observed between APOE2 and APOE3 mice under basal dietary conditions, with elevated MIP-1α expression observed in APOE2 mice after feeding the Western-type diet for 4 weeks (Fig. 6C and D). Interestingly, the number of macrophages, as measured by F4/80 mRNA, was significantly higher (by approximately twofold) in subcutaneous adipose of APOE2 mice compared with APOE3 mice (Fig.
indicating elevated basal inflammation even in subcutaneous adipose of APOE2 mice. The influence of APOE2 on diet-induced adipose tissue inflammation was readily observed in visceral adipose tissues, with MCP-1, MIP-1α, and F4/80 gene expression elevated three- to fourfold in APOE2 mice, whereas expression of these genes was not significantly different in APOE3 mice after 4 weeks on Western diet (Fig. 6C–E). The elevated expression of F4/80 genes in adipose tissues of APOE2 mice compared with APOE3 mice was consistent with a similar
increase of F4/80+ cells in the stromal-vascular cell fractions of APOE2 adipose tissues observed by flow cytometry (APOE2 mice, 47.54% macrophages; APOE3 mice, 33.68% macrophages; \( P < 0.05 \)).

The expression of proinflammatory M1 macrophage marker genes, such as tumor necrosis factor-\( \alpha \) (TNF-\( \alpha \)) and NO2 (32), was also significantly elevated in subcutaneous adipose tissues of APOE2 mice compared with APOE3 mice under both basal and Western diet conditions. In fact, the expression of both TNF-\( \alpha \) and NO2 in chow-fed APOE2 mice was similar to that observed in Western diet-fed APOE3 mice (Fig. 6F and G). Western diet feeding further exaggerated the elevated expression of NO2 in subcutaneous adipose tissues of APOE2 mice (Fig. 6G). Likewise, in the more inflammatory visceral adipose tissues, TNF-\( \alpha \) expression was similar between basal diet-fed APOE2 mice and APOE3 mice fed either diet. The expression of TNF-\( \alpha \) in visceral fat depots of APOE2 mice, but not APOE3 mice, was additionally increased after feeding the Western diet for 4 weeks (Fig. 6F). The expression of NO2 in visceral depots was similar and diet responsive in both APOE2 and APOE3 mice (Fig. 6G). Surprisingly, the expression levels of alternative macrophage activation genes, such as the type 2 mannose receptor (MR2) (31), were also significantly higher in subcutaneous adipose tissues of basal diet-fed APOE2 mice, to a level similar to that observed in APOE3 mice after Western diet feeding. Western diet further exaggerated alternative macrophage activation in subcutaneous adipose tissues of APOE2 mice (Fig. 6F). In the typically more inflamed visceral adipose depots, no significant differences in M2C2 expression were observed between basal chow-fed APOE2 and APOE3 mice, and both strains responded to Western diet feeding with similar induction of M2C2 (Fig. 6F). Similar results were obtained using arginase-1 mRNA as a marker of M2 macrophages (data not shown). Taken together, these results indicate that macrophages in subcutaneous adipose tissues of APOE2 mice are more activated compared with APOE3 mice through both classical and alternative activation pathways, whereas both strains are sensitive to diet-induced activation of visceral adipose tissue macrophages.

**Elevated plasma cytokine levels in APOE2 mice.** In addition to differences in gene expression between adipose tissues isolated from APOE2 and APOE3 mice, we also measured plasma levels of adipokines and inflammatory cytokines in these animals. Consistent with the gene expression data, no difference in plasma adiponectin levels was observed between APOE2 and APOE3 mice (Fig. 7A). In contrast, significantly higher levels of leptin, interleukin-6, and plasminogen activator inhibitor-1 were observed in the plasma of APOE2 mice (Fig. 7B–D).

**DISCUSSION**

Human population studies have suggested that the apoE2-encoding \( \varepsilon 2 \) allele may be a genetic modifier for increased risk of developing obesity and type 2 diabetes (4,9,10). The current study used human APOE2 and APOE3 gene replacement mice and showed that the underlying mechanism is the impaired clearance of apoE2-containing triglyceride-rich lipoproteins from circulation, leading to increased postprandial lipid uptake by leukocytes to promote inflammation, and chronic lipid deposition in adipose tissues to increase adiposity and susceptibility to diet-induced obesity. The combination of elevated adiposity and inflammation accelerates adipose tissue dysfunction, with increased macrophage infiltration and inflammation observed in adipose tissues of APOE2 mice after a short 4-week dietary regimen. The consequence of these metabolic changes is the accelerated hyperinsulinemia observed in Western diet-fed APOE2 mice compared with APOE3 mice.

The mechanism by which apoE2 promotes postprandial lipid uptake by leukocytes and chronic lipid deposition in adipose tissues has not been established. Typically, lipids from triglyceride-rich lipoproteins are taken up by leukocytes and adipocytes through two distinct mechanisms, one involving lipoprotein lipase-mediated hydrolysis of the triglycerides followed by the uptake of the liberated fatty acids (33,34) and another via whole lipoprotein particle endocytosis after binding to LDL receptor–related protein-1 (LRP1), VLDL receptor, and/or heparin sulfate proteoglycans (HSPGs) (27,35,36). Previous studies have shown that apoE2-containing lipoproteins are poor substrates for lipoprotein lipase in comparison with apoE3 lipoproteins (37). Moreover, lipoprotein lipase expression was reported to be similar between APOE2 and APOE3 mice, but the APOE2 adipocytes are defective in the uptake of lipase-derived fatty acids (38). Importantly, despite the defective binding of apoE2 lipoproteins to the LDL receptor, apoE2 lipoproteins bind with similar affinity as apoE3 lipoproteins to LRP1, VLDL receptor, and HSPG (39–43). Previous studies have shown the importance of LRP1, VLDL receptor, and HSPG in mediating lipid deposition in adipocytes (27,35,36). The receptor-associated protein, which binds to both LRP1 and VLDL receptor in inhibiting their interaction with apoE-containing lipoproteins, has also been reported to suppress triglyceride-rich lipoprotein-induced leukocyte activation (19). Thus, it appears more likely that the elevated postprandial leukocyte lipid uptake and inflammation as well as the chronic

![FIG. 7. Plasma cytokine levels. Plasma was obtained from APOE2 (open bars) and APOE3 (filled bars) to determine the levels of adiponectin (A), leptin (B), interleukin-6 (C), and plasminogen activator inhibitor-1 (PAI-1) (D). The data represent mean ± SD from six mice in each group. *P < 0.05, significant difference from APOE3 mice.](image-url)
dietary lipid deposition in APOE2 mice are mediated through apoE2-containing triglyceride-rich lipoprotein endocytosis via one of the cell surface receptors. Nevertheless, additional studies are necessary to test this hypothesis.

It is interesting to note that APOE2 adipocytes were previously reported to have an increased rate of stored triglyceride hydrolysis as well as impaired de novo lipogenesis compared with APOE3 adipocytes (38). Despite these abnormalities, we observed elevated obesity with increased adipocyte cell size in APOE2 mice compared with APOE3 mice upon chronic feeding of the Western-type diet. These observations illustrated that the impaired hepatic clearance of apoE2-containing triglyceride-rich lipoproteins leads to redistribution of dietary fat to adipocytes in APOE2 mice to promote obesity. Moreover, the elevated hydrolysis and turnover of stored triglycerides is also expected to promote adipocyte lipotoxicity to exacerbate inflammation (44). The observation of an increased number of crown-like structures, representing macrophage aggregates surrounding dead adipocytes (29), in APOE2 adipose tissues compared with APOE3 adipose tissues is consistent with this interpretation.

In summary, our data showed increased sensitivity to postprandial inflammation and diet-induced obesity in mice expressing human apoE2 compared with apoE3. It is important to note that, in contrast to human e2 carriers, the APOE2 mice displayed hyperlipidemia even under low-fat, chow-fed conditions. There are also significant differences in triglyceride-rich lipoprotein metabolism between mice and humans. Determining whether human e2 carriers are also more sensitive to postprandial inflammation and diet-induced obesity compared with e3 subjects will require additional studies.

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D.G.K. conducted the experiments, analyzed the data, and wrote the manuscript. E.S.K., J.E.B., C.M., and C.T.G. conducted the experiments and analyzed the data. T.K.C. and N.L.W. analyzed the data, provided critical insights, and contributed to writing, reviewing, and editing the manuscript. D.Y.H. planned the project, designed the experiments, analyzed the data, and wrote and reviewed the manuscript. D.Y.H. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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