

Defective Lipid Delivery Modulates Glucose Tolerance and Metabolic Response to Diet in Apolipoprotein E-Deficient Mice

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OBJECTIVE—Apolipoprotein E (ApoE) regulates plasma lipid levels via modulation of lipolysis and serving as ligand for receptor-mediated clearance of triglyceride (TG)-rich lipoproteins. This study tested the impact of modulating lipid delivery to tissues on insulin responsiveness and diet-induced obesity.

RESEARCH DESIGN AND METHODS—ApoE^{+/+} and apoE^{-/-} mice were placed on high-fat-high-sucrose diabetogenic diet or control diet for 24 weeks. Plasma TG clearance, glucose tolerance, and tissue uptake of dietary fat and glucose were assessed.

RESULTS—Plasma TG clearance and lipid uptake by adipose tissue were impaired, whereas glucose tolerance was improved in control diet-fed apoE^{-/-} mice compared with apoE^{+/+} mice after an oral lipid load. Fat mass was reduced in apoE^{-/-} mice compared with apoE^{+/+} mice under both dietary conditions. The apoE^{-/-} mice exhibited lower body weight and insulin levels than apoE^{+/+} mice when fed the diabetogenic diet. Glucose tolerance and uptake by muscle and brown adipose tissue (BAT) was also improved in mice lacking apoE when fed the diabetogenic diet. Indirect calorimetry studies detected no difference in energy expenditure and respiratory quotient between apoE^{+/+} and apoE^{-/-} mice on control diet. Energy expenditure and uncoupling protein-1 expression in BAT were slightly but not significantly increased in apoE^{-/-} mice on diabetogenic diet.

CONCLUSIONS—These results demonstrated that decreased lipid delivery to insulin-sensitive tissues improves insulin sensitivity and ameliorates diet-induced obesity. *Diabetes* 57:5–12, 2008

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Received for publication 22 March 2007 and accepted in revised form 30 September 2007.

Published ahead of print at <http://diabetes.diabetesjournals.org> on October 2007. DOI: 10.2337/db07-0403.

Additional information for this article can be found in an online appendix at <http://dx.doi.org/10.2337/db07-0403>.

apo, apolipoprotein; BAT, brown adipose tissue; FPLC, fast-performance liquid chromatography; ipGTT, intraperitoneal glucose tolerance test; LpL, lipoprotein lipase; oFTT, oral fat tolerance test; SAT, subcutaneous white adipose tissue; TG, triglyceride; TGRL, triglyceride-rich lipoprotein; UCP-1, uncoupling protein-1.

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Insulin resistance, characterized by reduced responsiveness of peripheral tissues to insulin, plays a major role in the development of type 2 diabetes (1,2). Although glucose homeostasis still takes the center stage in the definition of insulin resistance, more emphasis has been attributed recently to aberrant lipid metabolism as a contributing factor of this metabolic disorder. The concept of "lipotoxicity" describes how abnormalities in fatty acid metabolism, resulting in inappropriate storage of lipids in muscle and liver, ultimately lead to the pathogenesis of insulin resistance (3,4). Ectopic lipid accumulation in skeletal muscle and liver may be a result of increased delivery of fatty acid when energy intake exceeds adipose tissue storage capacity (4). On reaching insulin-sensitive tissues, excessive intracellular fatty acid decreased insulin-mediated glucose transport (5–7) through modulation of the insulin-signaling cascade, leading to decreased GLUT-4 translocation to the plasma membrane and reduced intracellular glucose concentrations (8). In contrast, decreasing fatty acid uptake into skeletal muscle with deletion of fatty acid transporters improved insulin sensitivity and protected mice from developing diet-induced insulin resistance (9,10).

Dietary and endogenously synthesized lipid nutrients are also delivered to peripheral tissues via triglyceride (TG)-rich lipoproteins (TGRLs) such as chylomicrons, VLDL, and their remnant lipoproteins. The lipids associated with TGRLs are taken up by cells and tissues via two mechanisms: one requiring lipoprotein lipase (LpL)-catalyzed hydrolysis of the TGs to fatty acid at the cell surface followed by the subsequent cellular uptake of nonesterified fatty acid and the second involving whole-lipoprotein particle uptake via receptor-mediated pathway(s). Perturbations in one or both of these pathways have been shown to significantly influence sensitivity to diet-induced obesity and insulin resistance in several genetically modified mouse models. For example, the absence of apolipoprotein (apo) CIII, a natural inhibitor of LpL, enhances LpL-dependent uptake of TGRL-derived fatty acids by adipose tissue, resulting in elevated susceptibility to diet-induced obesity and insulin resistance (11). In contrast, mice overexpressing apoCII are protected from obesity and insulin resistance (12), apparently through a direct interaction of apoCII with nonesterified fatty acids to prevent their cellular uptake (13). Although apoCII also inhibits TRGL binding to hepatic lipoprotein receptors (14,15), whether receptor-mediated TRGL uptake by insulin-sensitive high-energy-metabolism tissue plays a role in modulating susceptibility to obesity and diabetes is less clear from these studies. The VLDL receptor-deficient mice

TABLE 1
Metabolic characteristics of *apoE*^{-/-} and *apoE*^{+/-} mice

	Control diet		Diabetogenic diet	
	<i>ApoE</i> ^{-/-}	<i>ApoE</i> ^{+/-}	<i>ApoE</i> ^{-/-}	<i>ApoE</i> ^{+/-}
Fasted state (6 h)				
TG (mg/dl)	73.2 ± 21*†	44 ± 18*‡	101 ± 24*‡	60 ± 11*‡
Cholesterol (mg/dl)	550 ± 137*	77 ± 39*†	743 ± 224*	235 ± 50*†
Glucose (mg/dl)	132 ± 17*‡	155 ± 16*‡	188 ± 40†	211 ± 35†
Insulin (ng/ml)	0.7 ± 0.2†‡	0.8 ± 0.2‡	4.8 ± 2.9*†	7.6 ± 1.8*
Fed state				
TG (mg/dl)	101 ± 32	102 ± 31‡	138 ± 27*‡	108 ± 20*‡
Cholesterol (mg/dl)	564 ± 138*†	104 ± 30*†	813 ± 217*†	237 ± 49*†
Glucose (mg/dl)	150 ± 12*†‡	182 ± 28*‡	195 ± 25†	186 ± 19
Insulin (ng/ml)	1.1 ± 0.2*†‡	1.5 ± 0.2*†‡	5.9 ± 3.3*†	10.5 ± 4.3*†

Data are means ± SD from blood samples collected from eight mice per group at week 12 of the study. **P* < 0.05 *apoE*^{-/-} vs. *apoE*^{+/-} mice; †*P* < 0.05 control vs. diabetogenic diet within genotype; ‡*P* < 0.05 fasted vs. fed state within genotype.

have also been shown to be less susceptible to diet-induced obesity and insulin resistance (16). Defective tissue lipid uptake in this latter model was attributed to reduced LpL activity (17).

The current study took advantage of the *apoE*-deficient (*apoE*^{-/-}) mice, with well-established defects in receptor-mediated lipoprotein uptake (18,19), to investigate whether the suppression of whole particle lipoprotein uptake by high energy metabolism tissues affects insulin responsiveness and diet-induced obesity.

RESEARCH DESIGN AND METHODS

Male *apoE*^{-/-} and *apoE*^{+/-} C57BL/6 mice (The Jackson Laboratories, Bar Harbor, ME) were housed in a pathogen-free environment with all animal care and experimental procedures conforming to institutional guidelines for animal experiments. Mice were initially fed the D12328 control diet from Research Diets (New Brunswick, NJ) containing 16 kcal% protein, 73 kcal% corn starch, and 11 kcal% fat. Subsequently, *apoE*^{-/-} and *apoE*^{+/-} mice at 3 months of age were randomly divided into four groups (*n* = 8) and placed on either the control diet or the D12331 diabetogenic diet from Research Diets composed of 16.4 kcal% protein, 26 kcal% sucrose, and 58 kcal% fat (20). Whole-body fat and lean mass were measured in conscious mice using ¹H magnetic resonance spectroscopy (EchoMRI-100; Echomedical Systems, Houston, TX).

Biochemical assays. Intraperitoneal glucose tolerance test (ipGTT) was performed by injection of glucose (2 g/kg) after 6 h or overnight fast. Blood samples were collected immediately before and 15, 30, 60, and 120 min after injection. Plasma TG clearance was determined by gastric gavage of olive oil (5 µl/g body wt) after a 16-h overnight fast. Blood samples were collected immediately before and at 1, 2, and 3 h after oral bolus load. Blood glucose was determined with an Accu Check Active Glucometer (Roche Applied Science, Penzberg, Germany). Plasma insulin levels were measured with the Ultra Sensitive Rat Insulin ELISA kit (Crystal Chem, Chicago, IL) using rat insulin as the standard. Plasma TGs and cholesterol levels were measured by enzymatic assay kits (Thermo Electron, Waltham, MA). Samples were analyzed individually except for lipoprotein separation in which pooled samples (0.25 ml) from five animals per group were subjected to fast-performance liquid chromatography (FPLC) gel filtration on two Superose 6 columns connected in series.

Measurement of 2-deoxy-D-[1,2-³H]glucose uptake by target tissues.

Mice were fasted overnight and then gavaged with olive oil (5 µl/g body wt). After 45 min, 2-deoxy-D-[1,2-³H]glucose (NEN Life Science Products, Boston, MA) was administered as an intravenous bolus of 0.5 µCi/g body wt. Plasma samples were collected to measure glucose levels and radioactivity determinations for calculation of specific ³H radioactivity per unit of glucose in plasma. Animals were killed after 15 min and extensively perfused with ice-cold saline, and tissues were excised, weighed, and solubilized with NCSR-II Tissue Solubilizer (Amersham, Arlington Heights, IL) for radioactivity determinations in a liquid scintillation counter. Data are reported as total amount of glucose, based on the amount of radioactivity, taken up per milligram tissue. The phosphorylation of 2-deoxy[³H]glucose was not assessed in this study because the contribution of glucose kinase is expected to be minimal during the 15-min measurement period.

Determination of energy balance. *ApoE*^{-/-} and *apoE*^{+/-} mice were acclimated to respiratory chambers for 4 days before measurements. Energy

expenditure, respiratory quotient, food intake, fluid intake, and locomotor activity were measured simultaneously over a 24-h period using a customized 32-cage, Indirect Calorimetry System combined with Drinking and Feeding Monitor and TSE ActiMot system (TSE-Systems, Germany). The volume of oxygen consumed (Vo₂) and carbon dioxide produced (Vco₂) each hour was measured using a paramagnetic oxygen sensor and a spectrophotometric CO₂ sensor over a 24-h period. Respiratory quotient was calculated as the ratio of Vco₂ produced to Vo₂ consumed. Energy expenditure was calculated as the product of calorific value of O₂ and Vo₂ per kilogram body weight, where the calorific value of O₂ = 3.815 + (1.232) (respiratory quotient). Total calories expended were calculated to determine daily fuel use. The proportion of protein, fat, and carbohydrate that was used during that 24-h period was calculated under the assumption that protein use is equivalent to protein intake for adult animals (21,22). The percent of daily fuel use derived from carbohydrate and fat was determined using formulae and constants derived by Elia and Livesey (23). Daily caloric intake was calculated by multiplying the mass of daily food intake in grams by the physiological fuel value of the diet in kilocalories per gram. Complete energy balance measurements were taken at the beginning and at 4 and 16 weeks after initiation of the dietary treatment.

Immunoblot analysis. An aliquot of 25 µl from each FPLC fraction was separated by SDS-PAGE, transferred to polyvinylidene fluoride paper, and blotted with antibodies against apoAI (Novus Biologicals, Littleton, CO) and apoE (Santa Cruz Biotechnology, Santa Cruz, CA). Expression of uncoupling protein-1 (UCP-1) in brown adipose tissue (BAT) was analyzed by immunoblotting tissue homogenates in radioimmunoprecipitation assay buffer with UCP-1-specific antibodies (Calbiochem, San Diego, CA). Immunoreactive proteins were detected by incubating the blots with fluorescently labeled species-specific secondary antibodies (Molecular Probes, Eugene, OR) and visualized by the Infrared Imaging System Odyssey (Li-Cor Biosciences, Lincoln, NE).

Statistical analysis. Numerical data are expressed as means ± SE. An unpaired two-tailed Student's *t* or one-way ANOVA test with Bonferroni's multiple comparison test was used for statistical analysis of one-group variables. *P* values < 0.05 were considered significant.

RESULTS

The *apoE*^{-/-} mice had markedly increased fasting plasma cholesterol levels compared with *apoE*^{+/-} mice when fed either control or diabetogenic diets (Table 1). Fasting plasma TG levels were markedly higher in *apoE*^{-/-} mice compared with *apoE*^{+/-} mice, regardless of the diet (Table 1). Diabetogenic diet increased fasting TG levels significantly in both groups of mice but to a larger extent in *apoE*^{-/-} mice. Although we found similar TG levels in nonfasted *apoE*^{+/-} and *apoE*^{-/-} mice under control dietary conditions, the nonfasting TG levels were higher in *apoE*^{-/-} mice compared with *apoE*^{+/-} mice when they were fed diabetogenic diet. Fractionation of plasma lipoproteins by FPLC showed markedly increased cholesterol levels in the VLDL and IDL/LDL fractions of *apoE*^{-/-} mice compared with *apoE*^{+/-} mice (Fig. 1A and B) as described previously (24). Feeding the diabetogenic diet

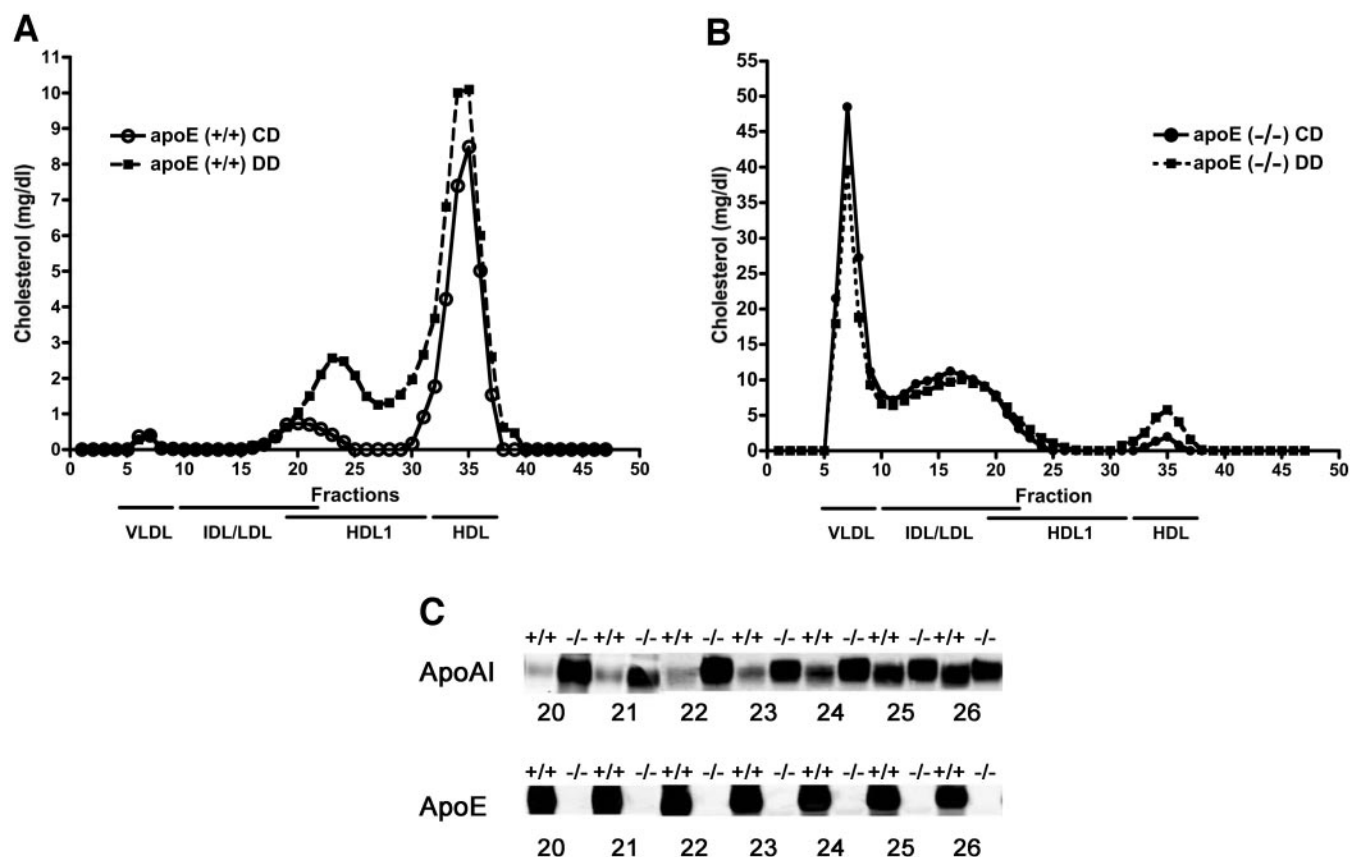


FIG. 1. Analysis of plasma lipoproteins in *apoE*^{-/-} and *apoE*^{+/+} mice. Pooled plasma (0.25 ml) from five *apoE*^{+/+} (A) and five *apoE*^{-/-} (B) mice maintained on control diet (○) or diabetogenic diet (■) was applied to two FPLC Superose columns, and 0.5-ml fractions were collected for cholesterol determinations. According to calibration with standard lipoproteins, the peak fractions 7–9 represent VLDL, fractions 15–22 are mainly IDL/LDL particles, and fractions 20–26 contain mainly large HDL₁. The second peak (fractions 30–37) is mainly HDL. C: Immunoblot analysis of apoAI and apoE in fractions 20–26 from *apoE*^{+/+} and *apoE*^{-/-} mice.

resulted in a marked increase in HDL cholesterol in both *apoE*^{-/-} and *apoE*^{+/+} mice compared with control diet-fed groups (Fig. 1A and B). In addition, significant increase in cholesterol level was observed in fractions 20–26 of diabetogenic diet-fed *apoE*^{+/+} mice (Fig. 1A). Because of the expected overlaps in particle sizes between IDL/LDL and HDL₁ lipoproteins, immunoblot analysis was performed with antibodies against apoAI and apoE, two proteins present in HDL₁ but not in LDL. Results confirmed that the cholesterol increase in these fractions was due primarily to increase levels of HDL₁ (Fig. 1C). The diabetogenic diet-induced elevation of HDL₁ level was not apparent in *apoE*^{-/-} mice nor in mice fed control diet (Fig. 1B and C). The increased VLDL and decreased HDL₁ concentrations in *apoE*^{-/-} mice are consistent with the well-established defects in receptor-mediated lipid transport pathways associated with apoE deficiency. These results also established that *apoE*^{-/-} mice are appropriate models for investigating whether suppression of receptor-mediated lipid transport to high-energy metabolism tissues affects insulin responsiveness and diet-induced obesity.

The control diet-fed *apoE*^{-/-} mice exhibited lower fasting glucose but not insulin levels compared with *apoE*^{+/+} mice (Table 1). Importantly, both glucose and insulin levels were significantly lower in *apoE*^{-/-} mice than in *apoE*^{+/+} mice during the fed state when the animals were maintained on the control diet (Table 1), suggesting that *apoE*^{-/-} mice on control diet were more

insulin sensitive than *apoE*^{+/+} mice. Diabetogenic diet feeding increased insulin levels in both groups of mice under both fasting and fed states, but the increases were significantly less in *apoE*^{-/-} mice compared with *apoE*^{+/+} mice (6.86- vs. 9.5-fold in fasted state and 5.36- vs. 7-fold in fed state, respectively). Although glucose levels were similar in both groups of diabetogenic diet-fed mice, the markedly lower fasting and fed insulin levels in *apoE*^{-/-} mice are indicative of their reduced sensitivity to diet-induced insulin resistance. These latter findings were confirmed by ipGTT after a 6-h fasting period. In these experiments, the response to glucose was found to be significantly improved in *apoE*^{-/-} mice compared with *apoE*^{+/+} mice regardless of the diet (Fig. 2A).

The highly efficient glucose tolerance mechanism in the lipid transport-defective *apoE*^{-/-} mice suggested that the delayed uptake of lipids into tissues during the postprandial state may reduce intracellular lipid accumulation and thereby lead to increased insulin sensitivity. This hypothesis was examined directly by performing ipGTT with or without a simultaneously administered oral fat tolerance test (oFTT) in mice on control diet after an overnight fast. This experimental design allowed us to compare glucose disposal in a truly fasted state with or without an acute influx of lipids. We found that whereas plasma TG clearance after an oral lipid load was significantly impaired in *apoE*^{-/-} mice (Fig. 2B), glucose tolerance was notably improved in *apoE*^{-/-} mice compared with *apoE*^{+/+} mice when an oFTT was conducted simultaneously (Fig. 2C). In

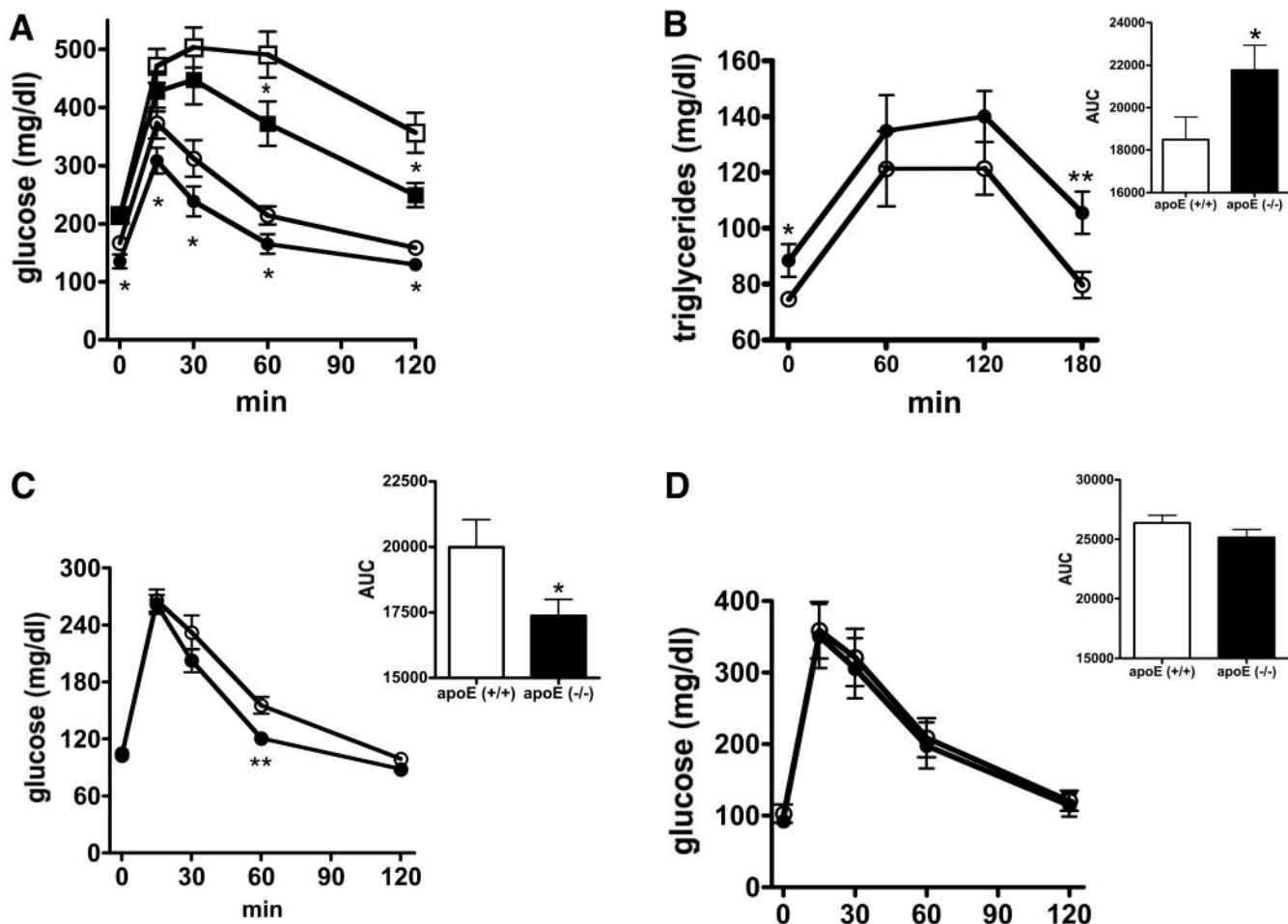


FIG. 2. Glucose and lipid tolerance tests in *apoE*^{-/-} and *apoE*^{+/+} mice. The *apoE*^{-/-} (filled symbols) and *apoE*^{+/+} (open symbols) mice were maintained on control (*n* = 20; circles) or diabetogenic (*n* = 8; squares) diets. ipGTT was performed after a 6-h fasting period (A). Alternatively, the mice were fasted overnight (B–D) before determination of plasma TG levels in response to oral fat load (B) or ipGTT in the presence (C) or absence (D) of an oral lipid load. Calculations of area under curve (AUC) of the data presented in B–D are included in the insets to each figure. Data are expressed as means $\pm$ SE. **P* < 0.05; ***P* < 0.005.

contrast, no difference in glucose response between *apoE*^{-/-} and *apoE*^{+/+} mice was observed when ipGTT was performed after an overnight fast without concomitant influx of dietary lipids (Fig. 2D). The latter results contrasted with results obtained after a 6-h fasting period (Fig. 2A). The difference is likely due to ongoing lipid uptake and metabolism in *apoE*^{+/+} mice 6 h after feeding, which was not apparent in *apoE*^{-/-} mice with defective receptor-mediated lipid uptake.

The impact of defective lipid uptake on glucose disposal by various tissues was investigated by intravenous GTT using deoxy[³H]glucose as a tracer after its infusion with lipids into *apoE*^{+/+} and *apoE*^{-/-} mice. Based on our previous observations that circulating glucose levels peaked at 2 min and were eliminated thereafter by first-order kinetics after intravenous administration of glucose with plasma glucose levels reducing to 50% levels after 15 min (25), we examined the disposition of the deoxy[³H]glucose in various tissues 15 min after intravenous injection as an indication of the rate of glucose uptake by each tissue. We observed a significant increase in glucose uptake by BATs and skeletal muscle but not the heart of *apoE*^{-/-} mice compared with *apoE*^{+/+} mice on diabetogenic diet (Fig. 3A).

Additional experiments were also conducted to assess the impact of apoE-mediated lipid uptake on glucose

metabolism in various tissues by analyzing tissue distribution of deoxy[³H]glucose with and without a concomitant oral lipid load in *apoE*^{+/+} and *apoE*^{-/-} mice maintained on control low-fat diet. When deoxy[³H]glucose was administered in the presence of an oral fat load, a significant increase in deoxy[³H]glucose uptake by the subcutaneous white adipose tissue (SAT) was observed in control diet-fed *apoE*^{-/-} mice compared with that in *apoE*^{+/+} mice (Fig. 3B). The uptake of deoxy[³H]glucose by other tissues was similar between both groups under these conditions. The adipose-specific difference in deoxyglucose uptake between *apoE*^{+/+} and *apoE*^{-/-} mice with a fatty meal may explain the slight but significant improvement of glucose tolerance in the *apoE*^{-/-} mice when glucose was administered together with a fat load. Interestingly, whereas the difference in SAT uptake of deoxy[³H]glucose between control diet-fed *apoE*^{+/+} and *apoE*^{-/-} mice was sustained in the absence of an oral lipid load, significantly less deoxyglucose uptake was observed in the skeletal muscle of *apoE*^{-/-} mice compared with that in *apoE*^{+/+} mice in the absence of an oral fat load (Fig. 3C). The latter observation is consistent with data showing no difference in glucose tolerance between chow-fed *apoE*^{+/+} and *apoE*^{-/-} mice in the absence of an oral lipid load.

The decrease in postprandial fat clearance in *apoE*^{-/-} mice compared with *apoE*^{+/+} mice suggested that the

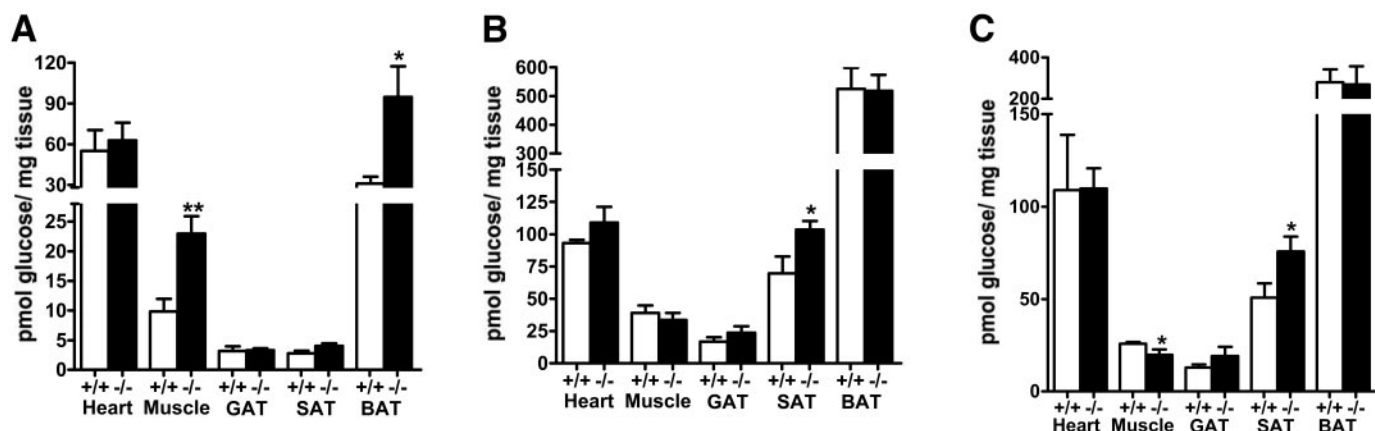


FIG. 3. Tissue uptake of deoxy-[³H]glucose by *apoE*^{-/-} and *apoE*^{+/+} mice. A: 2-deoxy-[³H]glucose uptake by heart, skeletal muscle, gonadal (GAT), SAT, and BAT of diabetic diet-fed *apoE*^{+/+} (□) and *apoE*^{-/-} (■) mice 15 min after intraperitoneal injection of the radiolabeled tracer. B and C: Tissue uptake of deoxy-[³H]glucose by control diet-fed *apoE*^{+/+} (□) and *apoE*^{-/-} (■) mice with or without an oral fat load, respectively. Data are expressed as means ± SE for *n* = 5 mice per group. **P* < 0.05; ***P* < 0.005.

apoE^{-/-} mice may also be resistant to diet-induced adiposity. Therefore, body weight and fat mass of *apoE*^{-/-} and *apoE*^{+/+} mice were analyzed after feeding them with either control or diabetic diet. Despite similar body weights between the two groups of animals during regular control diet feeding, the *apoE*^{-/-} mice had significantly lower whole-body fat mass compared with that observed in *apoE*^{+/+} mice (Fig. 4A). The *apoE*^{-/-} mice also gained significantly less whole-body fat mass after diabetic diet feeding compared with *apoE*^{+/+} mice, resulting in significantly reduced total body weights after 16 weeks (Fig. 4A and B). The differences in fat mass and body weight gain between *apoE*^{+/+} and *apoE*^{-/-} mice were not related to major differences in energy use, because both groups of mice showed similar energy expenditure (base on lean body mass) when fed either the basal or diabetic diet (Fig. 5A and B). Caloric intake and respiratory quotient were also similar between *apoE*^{+/+} and *apoE*^{-/-} mice on both diets (Fig. 5C and D; Supplemental Figure S1, which is detailed in the online appendix [available at <http://dx.doi.org/10.2337/db07-0403>]). Interestingly, a slight increase in UCP-1 expression in BAT of *apoE*^{-/-} mice was consistently observed, but the increase did not reach statistical significance (Fig. 5E).

DISCUSSION

Defective receptor-mediated plasma lipoprotein clearance is a well-established hallmark of apoE deficiency in mice (26). In addition, the *apoE*^{-/-} mice have also been reported to have altered immune functions (27) with an exaggerated inflammatory response to infection (28) and an age-dependent neurodegeneration of the central nervous system (29). The present study reveals that *apoE*^{-/-} mice are more insulin sensitive in skeletal muscle and BAT and have reduced adiposity and insulin resistance compared with *apoE*^{+/+} mice when they are challenged with a diet rich in fat and sucrose. The increased insulin sensitivity and resistance to diet-induced obesity in the *apoE*^{-/-} mice is probably unrelated to its altered immune functions because increased inflammatory response is generally associated with diet-induced obesity and insulin resistance (30,31). The resistance to diet-induced obesity and improved insulin sensitivity of *apoE*^{-/-} mice are also unlikely due to the altered functions in the central nervous system, because 3-month-old mice were used in the cur-

rent study and neurological functions were reported to be

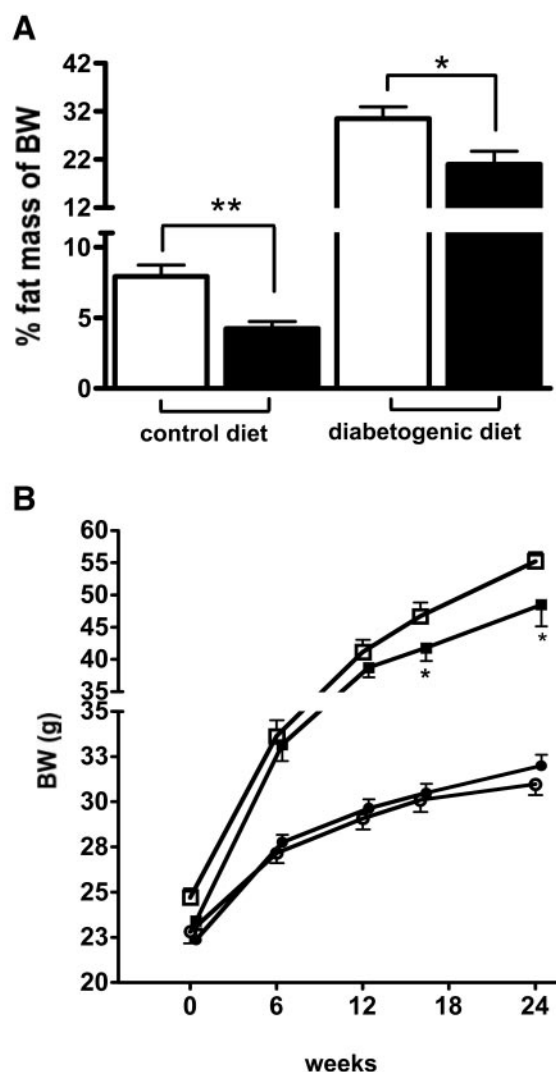


FIG. 4. Whole-body fat, as percentage of whole-body weight (BW) at week 16 of the study (A) and body weight distribution (B) in *apoE*^{-/-} (filled bars) and *apoE*^{+/+} (open bars) mice on control (circles) and diabetic diet (squares). Data are expressed as means ± SE for *n* = 8 mice per group. **P* < 0.05; ***P* < 0.005.

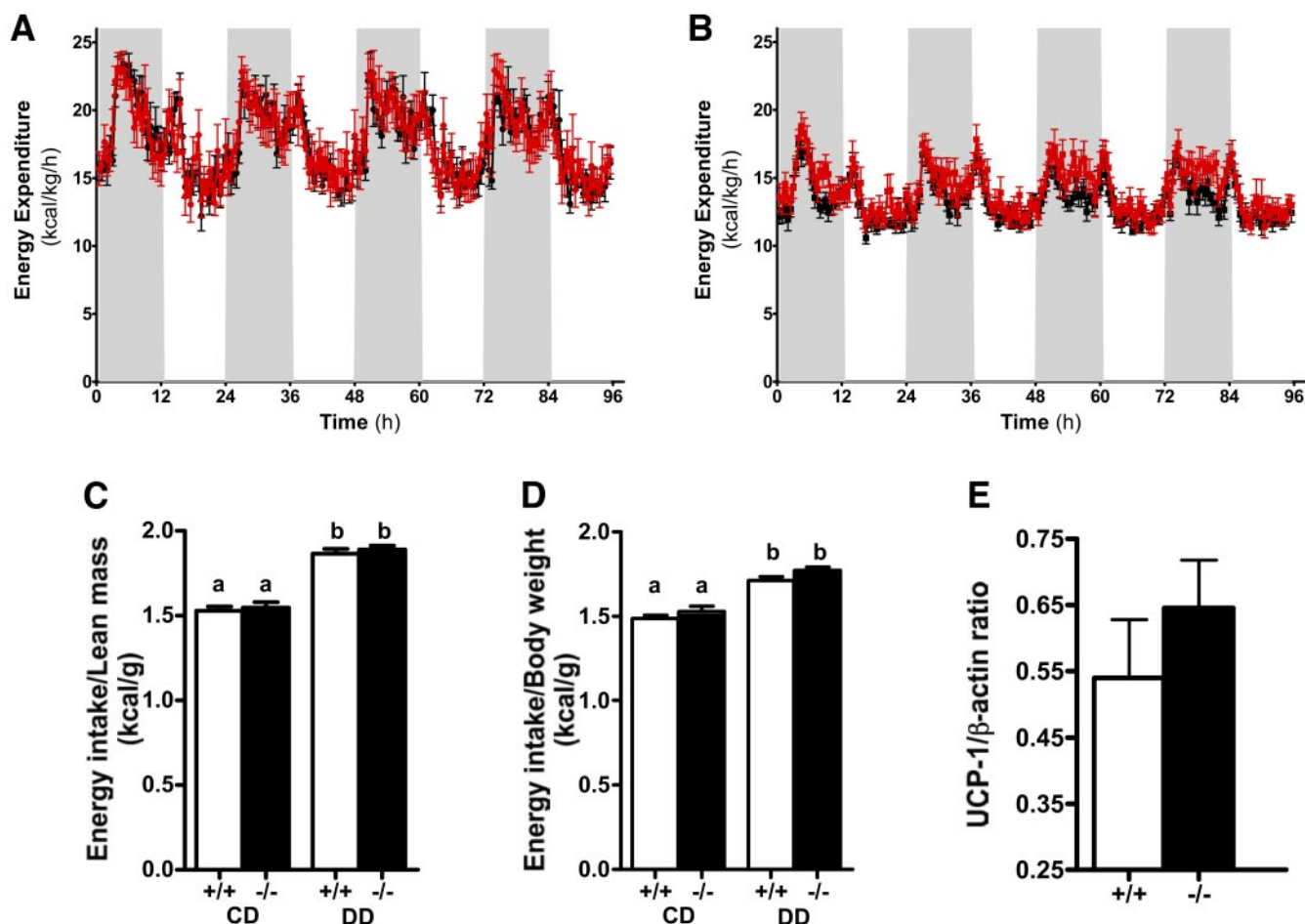


FIG. 5. Energy expenditure in *apoE*^{-/-} (red tracings) and *apoE*^{+/+} (black tracings) mice on control diet (CD) (A) and diabetic diet (DD) (B) per kilogram lean body mass during dark (gray shaded areas) and light (white shaded areas) phases at week 16 of the study. C and D: Energy intake per gram lean body mass and gram body weight, respectively, of *apoE*^{+/+} and *apoE*^{-/-} mice fed control diet or diabetic diet. E: UCP-1 protein levels normalized to β-actin levels in BATs of *apoE*^{+/+} and *apoE*^{-/-} mice maintained on the diabetic diet at week 16 of the study. a and b denote differences between the bars at $P < 0.05$.

normal in 8-month-old *apoE*^{-/-} mice (32) with neurodegeneration observed only in older *apoE*^{-/-} mice (29). The normal food intake and locomotive activities observed in our *apoE*^{-/-} mice are consistent with this interpretation. Therefore, the obesity resistance and improved insulin sensitivity in *apoE*^{-/-} mice reported in the current study is most likely due to reduced lipid transport to insulin-sensitive tissues, ameliorating diet-induced obesity and insulin resistance in these animals.

Two independent research groups have previously shown that *apoE*^{-/-} mice have lower body fat and reduced adipocyte size compared with *apoE*^{+/+} mice (33,34). However, their results in diet-induced obesity experiments were inconsistent. Chiba et al. (34) found that *apoE*^{-/-} mice in the *ob/ob* background failed to gain weight compared with *apoE*^{+/+} *ob/ob* mice on a high-fat diet, with most of the difference in body weight attributed to the difference in fat mass. These investigators used food intake restriction analysis to show that food intake did not play a role in the observed difference in body weight. In contrast, Schreyer et al. (33) reported no difference in body weight gain between *apoE*^{-/-} and *apoE*^{+/+} mice fed a high-fat-high-sucrose diet, but their study showed that food intake was significantly increased in *apoE*^{-/-} mice compared with *apoE*^{+/+} mice. Of note, they also detected

a substantial decrease in adiposity despite the increased food intake in *apoE*^{-/-} mice compared with *apoE*^{+/+} mice fed a high-fat-high-sucrose diet. Our study confirmed earlier findings that *apoE*^{-/-} mice have a significant lower body fat percentage compared with *apoE*^{+/+} mice. In contrast to the results published by Schreyer et al. (33), we detected significant lower body weights in *apoE*^{-/-} compared with *apoE*^{+/+} mice after 16 weeks of feeding diabetic diet with no differences in caloric intake between the two groups. Our findings indicate that the observed difference in body weight was largely due to a difference in fat mass (Fig. 4A).

The reduced body fat percentage in *apoE*^{-/-} mice may be explained by impaired TG clearance from circulation and decreased lipid uptake by adipose tissue after an oral lipid load (Figs. 2B and 3). Three distinct mechanisms may play a role in the observed impaired lipid clearance from circulation and delivery to target tissues. First, the absence of apoE impaired receptor-mediated clearance of TGRLs from circulation (26). Second, *apoE*^{-/-} mice were reported to be resistant to heparin-induced lipolysis (35), and LpL activity in these animals was moderately reduced (35). Our data showing small but statistically significant delay in postprandial TG clearance and elevated plasma TG levels in *apoE*^{-/-} mice compared with those observed

in *apoE*^{+/+} mice are consistent with the earlier reports. The moderately decreased TGRL lipolysis and clearance mechanism may decrease lipid uptake by the fat tissues. Third, the impaired hepatic VLDL secretion in *apoE*^{-/-} mice (36,37) may also result in reduced amount of lipids transported from the liver to target tissues. Data presented in the online appendix (Supplemental Fig. S2) confirming previously reported results of TG accumulation in liver of *apoE*^{-/-} mice (38,39) are supportive of this possibility. All three of these mechanisms may be working together in conferring the resistance to diet-induced obesity in the *apoE*^{-/-} mice. Finally, fat absorption has also been reported to be reduced in *apoE*^{-/-} mice (40), although this observation was not consistently observed in other studies (33,41). Regardless of whether fat absorption is impaired in *apoE*^{-/-} mice, their elevated plasma TG and lower glucose and insulin levels in both fasted and fed states, even under basal dietary conditions, indicated that abnormalities in plasma lipid transport are independent variables in modulating sensitivity to diet-induced obesity and insulin resistance.

Recent evidence suggested that resistance to insulin-stimulated glucose metabolism is not a primary event in obesity but is secondary to lipid accumulation in target tissues resulting from full responsiveness to insulin-stimulated lipogenic activity (3,4). Our results demonstrating similar glucose tolerance between overnight-fasted *apoE*^{+/+} and *apoE*^{-/-} mice despite their difference in adiposity, lower glucose and insulin levels in *apoE*^{-/-} mice compared with *apoE*^{+/+} mice during the fed state, and better glucose tolerance in *apoE*^{-/-} mice than in *apoE*^{+/+} mice after an oral lipid load are consistent with this hypothesis. Based on a novel concept that resistance to insulin-stimulated glucose metabolism might be a protective mechanism that reduces lipid-induced damage to tissue by excluding glucose from cells, and therefore decreasing glucose-derived lipogenesis (42–45), we interpreted the observed deterioration of glucose tolerance after an oral lipid load in the *apoE*^{+/+} mice as an acute measure to prevent excessive intracellular fat storage. In contrast, in the absence of apoE, lipid uptake may be impaired after an oral fat load, resulting in reduced lipid accumulation in tissues, and consequently, changes in insulin-stimulated glucose metabolism may not be necessary to prevent lipid-induced damage. The same decrease of lipid delivery in the absence of apoE may well be the mechanism responsible for the observed reduction in diet-induced adiposity and insulin resistance of *apoE*^{-/-} mice.

Results reported in the current study also showed no detectable difference in food intake between *apoE*^{-/-} and *apoE*^{+/+} mice fed control or diabetogenic diet. Nevertheless, total body fat was reduced in *apoE*^{-/-} mice, and body weight gain was lower in *apoE*^{-/-} mice fed a diabetogenic diet. These findings suggested that a potential increase in energy expenditure may account for the surplus in energy intake that is not stored in adipose tissue of *apoE*^{-/-} mice. However, indirect calorimetry analysis of *apoE*^{-/-} and *apoE*^{+/+} mice showed that energy expenditure was similar between *apoE*^{-/-} and *apoE*^{+/+} mice on control diet throughout the study. Whereas the *apoE*^{-/-} mice exhibited a slight increase in energy expenditure during the dark phase compared with *apoE*^{+/+} mice after 16 weeks on diabetogenic diet, this difference did not reach statistical significance. We hypothesize that a difference in energy metabolism that is below the detection limit of the gener-

ally insensitive 24-h indirect calorimetry, but nevertheless relevant for chronic body composition development, is responsible for the differences in energy balance, which are leading to different levels of adiposity. A trend toward increased UCP-1 expression in BAT of *apoE*^{-/-} mice compared with that in *apoE*^{+/+} mice is consistent with this possibility. Additionally, the prolonged retention of dietary lipids in circulation may also contribute significantly to the decreased adiposity in *apoE*^{-/-} mice compared with that observed in *apoE*^{+/+} mice.

Overall, our findings demonstrated that lipid delivery to adipose tissue is impaired in the absence of apoE, and as a consequence, diet-induced adiposity and insulin resistance is reduced. Although we cannot rule out a potential role of intracellular apoE in modulating adiposity and tissue insulin sensitivity, nevertheless these results implicated that modulation of TGRL clearance can be a potential treatment strategy to ameliorate diet-induced obesity and insulin resistance. Such an approach must be taken cautiously because impaired TGRL clearance would lead to hyperlipidemia and increased risk of premature coronary artery disease. The selective inhibition of lipid partitioning to insulin-sensitive tissues without incurring hyperlipidemia is necessary for alleviation of diet-induced metabolic disorders. In this regard, the current study revealed that deficiency of the ligand for TGRL transport, namely apoE, is effective in reducing lipid-induced obesity and diabetes but incurring hyperlipidemia. Identifying the receptors on each tissue responsible for mediating apoE-dependent lipid uptake may be valuable in identifying specific targets for reducing diet-induced obesity and diabetes without the adverse effects of hyperlipidemia and premature atherosclerosis.

ACKNOWLEDGMENTS

D.Y.H. has received National Institutes of Health Grant R01-DK-74932. M.H.T. has received National Institutes of Health Grant R01-DK-69987. S.H. has received Scientist Development Award 0635079N from the American Heart Association.

We thank Jessica Nickel, Joe Drennan, and Lauren Wancata for performing the metabolic assays.

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