Nutritional regulation of adipose tissue apolipoprotein E expression

Zhi Hua Huang,1 Raul M. Luque,1,3 Rhonda D. Kineman,1,3 and Theodore Mazzone1,2

1Department of Medicine, University of Illinois at Chicago, 2Department of Pharmacology, University of Illinois at Chicago, and 3Jesse Brown Veterans Administration Medical Center, Chicago, Illinois

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Huang ZH, Luque RM, Kineman RD, Mazzone T. Nutritional regulation of adipose tissue apolipoprotein E expression. Am J Physiol Endocrinol Metab 293: E203–E209, 2007.—Apolipoprotein E (apoE) is a multifunctional protein that is highly expressed in human and murine adipose tissue. Endogenous adipocyte apoE expression influences adipocyte triglyceride turnover and modulates the expression of genes involved in lipid synthesis and oxidation. We now demonstrate the regulation of adipose tissue apoE expression by nutritional status in lean and obese mice. Obesity induced by high-fat diet, or by hyperphagia in ob/ob mice, produces significant reduction of adipose tissue apoE expression at the protein and messenger RNA level. Fasting in C57BL/6J mice for 24 h significantly increased apoE protein and messenger RNA levels. In ob/ob mice, transplantation of adipose tissue from lean littermate controls to restore circulating leptin levels produced significant weight loss over 12 wk and also produced an increase in adipose tissue apoE expression. The increase in adipose tissue apoE expression in this model, however, did not require leptin. Adipose tissue apoE was also significantly increased in ob/ob mice after a 48-h fast or after 7 days of caloric restriction. In summary, obesity suppresses adipose tissue apoE expression, whereas fasting or weight loss increases it. From our previous observations, these changes in adipose tissue apoE expression will have significant impact on adipose tissue lipid flux and lipoprotein metabolism. Furthermore, these results suggest adipose tissue apoE participates in defending adipose tissue and organismal energy homeostasis in response to nutritional perturbation.

Apolipoprotein E (apoE) is a multifunctional protein with an established role in cardiovascular and neurological health (4, 17). In line with this, the apoE gene is highly expressed in cells of the nervous system and in cells important for maintaining vascular wall homeostasis, i.e., macrophages (8, 18). In these cells, apoE has been shown to play an important role in sterol flux, antioxidant defense, and cellular response to injury (4, 8, 9, 11, 13, 17, 18). It has been previously shown that apoE is highly expressed in human adipose tissue (23); however, after the initial observation, there has been little subsequent information regarding regulation of its expression or a potential physiological role for apoE in this tissue. More recently, our laboratory (22) has shown that apoE expression in adipose tissue is subject to significant regulation by peroxisome proliferator-activated receptor (PPAR)-γ agonists and TNF-α in vitro and in vivo. For example, treatment of humans with impaired glucose tolerance with PPAR-γ agonists (pioglitazone) or incubation of isolated adipocytes with ciglitazone significantly increases adipocyte apoE expression. TNF-α treatment of adipocytes reduces apoE expression by almost 90%. More recently, our group (10) has shown that apoE expression in murine adipose tissue, although present in adipose tissue macrophages, is largely accounted for by expression in adipocytes. By comparing lipid metabolism and gene expression in freshly isolated adipose tissue and cultured adipocytes from apoE−/− mice with results from wild-type controls and by using apoE adenoviruses to restore apoE expression in adipocytes from apoE−/− mice, we have established an important role for endogenous adipocyte apoE expression in adipocyte-differentiated function (10). Compared with wild-type adipocytes, adipocytes from apoE−/− mice are smaller, have less triglyceride (TG) mass, have lower free fatty acid mass, and demonstrate increased expression of genes in the fatty acid oxidation pathway. The absence of apoE expression also leads to reduced TG synthesis and increased TG hydrolysis in isolated adipocytes. Furthermore, the absence of endogenous apoE expression in adipocytes reduces the accumulation of adipocyte TG in response to incubations with apoE-containing very-low-density lipoprotein (VLDL) (10). The important role of endogenous apoE expression in adipocyte TG balance is further demonstrated by experiments that examined the role of apoE in adipocytes stimulated with PPAR-γ agonists. PPAR-γ treatment of adipocytes leads to increased apoE expression and increased TG accumulation. In the absence of apoE expression, PPAR-γ stimulation produces a smaller increase in TG synthesis and significantly less adipocyte TG accumulation (10).

Although we recognize that the total absence of adipocyte apoE expression may produce downstream effects different from those produced by more graded changes in its expression level, the above results demonstrate that endogenous adipocyte apoE plays a major role in adipocyte TG turnover and energy flux. Evaluating factors that regulate endogenous apoE expression, particularly in vivo, would be important to further understand the role of adipose tissue apoE within an integrated model of adipose tissue lipid metabolism and systemic energy balance. As noted above, it is already established that adipose apoE expression is regulated by factors with established roles in organismal and adipocyte energy flux (PPAR-γ agonists and TNF-α) (22). In the present study, we provide results of experiments designed to evaluate changes in the expression of adipose tissue apoE that accompany changes in organismal nutritional status. We used diet-induced and leptin-deficient obesity and examined the effects of acute and chronic caloric deprivation to evaluate nutritional regulation of adipose tissue apoE expression.

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MATERIALS AND METHODS

Animals. All experimental procedures were approved by the Institutional Animal Care and Use Committees of the University of Illinois at Chicago and the Jesse Brown Medical Center. C57BL/6J mice were purchased from Jackson Laboratories (Bar Harbor, ME). Diet-induced obesity was generated by feeding 4-wk-old mice with a high-fat diet (fat = 60 kcal%, carbohydrate = 20 kcal%, protein = 20 kcal%; no. D12492, Research Diets, Brunswick, NJ) for 16 wk. These mice were compared with mice maintained on a lower-fat diet (fat = 10 kcal%, carbohydrate = 70 kcal%, protein = 20 kcal%; no. D12450B, Research Diets). At 20 wk, mice were weighed and killed for collection of intra-abdominal fat pads, which were weighed and used for Western blot or quantitative RT-PCR (qRT-PCR). Blood was also collected for measurement of insulin, glucose, leptin, free fatty acid, and lipid levels. ob/ob mice on a C57BL/6J background and their lean littermate controls were also purchased from Jackson Laboratories. Mice were killed at 10 wk for harvest of fat pads and blood as described above. For fasting protocols, 10-wk-old C57BL/6J were fasted for 12 or 24 h and compared with mice maintained on a standard chow diet. Ten-week-old ob/ob mice were fasted for 48 h and compared with ob/ob mice maintained on a standard chow diet for that same period. At the end of each fasting period, mice were killed as described above. To evaluate the effect of chronic caloric deprivation in this model, ob/ob mice were pair-fed with ob/ob mice undergoing subcutaneous leptin infusion [as part of another series of experiments (15)] for 7 days. This resulted in an approximately two-thirds reduction in food intake vs. ob/ob mice fed ad libitum. After 7 days of reduced caloric intake, mice were killed as described above. For adipose tissue transplantation experiments, 6-wk-old ob/ob mice were subcutaneously implanted with white adipose tissue (WAT) from 12-wk-old lean littermates as previously described (7). Approximately 1 g of adipose tissue was implanted subcutaneously through several small incisions on the shaved skin of the back. A control group of ob/ob mice were sham operated for comparison. Twelve weeks after the procedure, mice were killed as described above.

Immunoblot. WAT was minced and lysed in radioimmununoprecipitation assay buffer supplemented with protease inhibitor cocktail for 30 min at 4°C (22). The lysate was centrifuged at 14,000 rpm for 15 min, and the supernatants were collected. Protein (15 μg) was separated on 10% SDS-PAGE gels and transferred to nitrocellulose membranes and blotted with rabbit anti-rat apoE antisemur. After incubation with primary antibody, horseradish peroxidase-conjugated secondary antibody (Pierce, Rockford, IL) immune complexes were detected by enhanced chemiluminescence (Amersham Biosciences, Piscataway, NJ) and quantitated by ZeroD scan software (Scanalytics, Fairfax, VA). A similar method was used to detect actin or tubulin signals, which were used as internal controls. Similar results for all immunoblots were obtained with actin or tubulin as internal controls or by using no correction for the apoE signal. Therefore, the actin-corrected results are presented.

mRNA quantitation by real-time PCR. WAT was flash frozen at the time of harvest. At time of measurement, tissue was minced and total RNA was extracted with an RNeasy lipid tissue mini kit (Qiagen, Valencia, CA). First-strand cDNA was synthesized from 1 μg of total RNA using random hexamer primers according to the manufacture’s instructions (first-strand cDNA synthesis kit; Fermentas, Hanover, MD). Real-time PCR was performed on each sample in triplicate with a Stratagene MX 3000P in 25 μl of total volume with the use of Brilliant SYBR green qRT-PCR master mix (Stratagene, La Jolla, CA). PCR primers (forward, reverse) utilized were as follows: murein (m)-β-actin, CTGGGACGACATGGAGAGA, AGAG-GCATACTTGGGACACA; mAP, AGTGCCCAGACGAACACACC, CTTCCGCATAGTGCTCCCA; mF4/80, CTGGACAACGTATGTGGAGGA, AGGCAGACATACAGGAGAGA; and mCD68, ACT-TCCGGCCATGTTCCTC, GGCTGGTATTGTTGTCGT. Data were normalized for the expression of β-actin and analyzed by using comparative critical threshold (10).

Other assays. Cellular protein, circulating free fatty acids, and glucose levels were measured as previously described. Insulin and leptin levels were measured with Linco ELISA kits (St. Charles, MO) as previously described. We used a kit from Wako (Richmond, VA) to measure total circulating plasma TG and a kit from Invitrogen (Carlsbad, CA) to measure total cholesterol (TC). Results of some of these measurements have been previously reported (15, 16) but are included here to provide context for changes in adipose tissue apoE expression.

Statistical analysis. Results are expressed as means ± SD for immunoblot and blood measurements. For qRT-PCR, results are presented as mean values with individual values for each mouse (representing the average of 3 measurements) shown. Statistical difference of values was analyzed by Student’s t-test or ANOVA with the use of SPSS (Chicago, IL). P < 0.05 was considered significant.

RESULTS

High-fat feeding suppresses adipose tissue apoE expression. C57BL/6J mice were maintained for 16 wk on a high-fat or lower-fat diet starting at 4 wk of age. At time of death, the mice on the high-fat diet weighed ~39% more, and fat pads weighed over twice as much, compared with mice on the lower-fat diet (Table 1). Consistent with this increased overall body and adipose tissue weight, insulin, glucose, and leptin levels were significantly higher in mice maintained on the high-fat diet. Free fatty acid levels tended to be higher in mice on the high-fat diet, but this did not reach statistical significance. TG and TC levels were higher in high-fat diet mice, but only TC reached statistical significance. apoE expression in adipose tissue was significantly suppressed in mice on the high-fat diet (Fig. 1A). Consistent with the results of the immunoblot, apoE mRNA levels were also significantly reduced in adipose tissue obtained from mice on the high-fat diet (Fig. 1B). CD68 and F4/80 mRNA levels were significantly increased in high-fat diet mice, consistent with increased infiltration of macrophages into adipose tissue in these mice (5, 21).

Fasting increases apoE expression in adipose tissue. C57BL/6J mice maintained on a chow diet were fasted for 24 h and compared with mice maintained on ad libitum access to a standard chow diet. As shown in Table 2, at the time of death, fasted mice tended to weigh less; however, this did not reach statistical significance. There was no difference in the weight of fat pads between the two groups of mice. Insulin levels, glucose levels, and leptin levels significantly fell in response to

Table 1. Circulating hormone and metabolite levels in C57BL/6J male mice fed a LF or a HFD for 16 wk

<table>
<thead>
<tr>
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<th>LFD</th>
<th>HFD</th>
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<tbody>
<tr>
<td>Body weight, g</td>
<td>30.69 ± 1.21 (7)</td>
<td>42.76 ± 1.48† (6)</td>
</tr>
<tr>
<td>Fat pads, g</td>
<td>1.25 ± 0.12 (7)</td>
<td>2.73 ± 0.14† (6)</td>
</tr>
<tr>
<td>Insulin, mg/ml</td>
<td>2.30 ± 0.30 (7)</td>
<td>13.40 ± 5.60† (6)</td>
</tr>
<tr>
<td>Glucose, mg/dl</td>
<td>139.00 ± 9.00 (7)</td>
<td>170.00 ± 10.80† (6)</td>
</tr>
<tr>
<td>Leptin, mg/ml</td>
<td>1.25 ± 0.10 (7)</td>
<td>2.53 ± 0.30† (6)</td>
</tr>
<tr>
<td>FFA, nmol/ml</td>
<td>0.63 ± 0.03 (7)</td>
<td>0.73 ± 0.06 (6)</td>
</tr>
<tr>
<td>TG, mg/dl</td>
<td>118.2 ± 4.53 (7)</td>
<td>174.5 ± 68.4 (7)</td>
</tr>
<tr>
<td>TC, mg/dl</td>
<td>103.1 ± 8.8 (5)</td>
<td>139.3 ± 18.5† (5)</td>
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</table>

Values are means ± SD; nos. in parentheses indicate no. of mice analyzed for each hormone or metabolite. FFA, free fatty acids; TG, triglycerides; TC, total cholesterol. *P < 0.05 and †P < 0.01, indicating values that differ between high-fat diet (HFD) and low-fat diet (LFD) conditions.
the fast; however, free fatty acid levels significantly increased in response to the 24-h fast. TG and TC levels did not change with fasting (not shown). Twelve hours of fasting did not significantly change adipose tissue apoE levels (Fig. 2A). Twenty-four hours of fasting, however, did significantly increase apoE expression. Consistent with the results of the immunoblot, apoE mRNA levels in adipose tissue were also significantly increased by the 24-h fast. CD68 and F4/80 expression also increased, indicating increased infiltration of macrophages into adipose tissue after the acute fasting period.

Adipose tissue apoE expression in ob/ob mice. We next examined apoE expression in adipose tissue obtained from ob/ob mice or lean littermate controls. This comparison addressed whether a high-fat diet is needed to suppress apoE expression in adipose tissue or whether the same suppression would be observed in obesity secondary to hyperphagia in mice maintained on a standard chow diet. The ob/ob mice weighed significantly more and had significantly larger fat pads than lean littermate controls (Table 3). Insulin and glucose levels

Table 2. Circulating hormone and metabolite levels in C57BL/6J male mice under fed and fasting (24 h) conditions

<table>
<thead>
<tr>
<th></th>
<th>Fed</th>
<th>Fasted for 24 h</th>
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<tbody>
<tr>
<td>Body weight, g</td>
<td>26.70±1.27 (8)</td>
<td>24.40±1.45 (7)</td>
</tr>
<tr>
<td>Fat pads, g</td>
<td>0.40±0.20 (8)</td>
<td>0.40±0.28 (7)</td>
</tr>
<tr>
<td>Insulin, ng/ml</td>
<td>1.28±0.24 (8)</td>
<td>0.19±0.02† (7)</td>
</tr>
<tr>
<td>Glucose, mg/dl</td>
<td>150.30±4.40 (8)</td>
<td>84.00±3.41† (7)</td>
</tr>
<tr>
<td>Leptin, ng/ml</td>
<td>0.27±0.04 (4)</td>
<td>0.08±0.02* (7)</td>
</tr>
<tr>
<td>FFA, nmol/ml</td>
<td>0.91±0.09 (8)</td>
<td>1.51±0.06† (7)</td>
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Values are means ± SD; nos. in parentheses indicate no. of mice analyzed for each hormone or metabolite. *P < 0.05 and †P < 0.01, indicating values that differ between fed and fasting groups.
apoE expression in the adipose tissue of C57BL/6J mice, fasting produced a significant increase in mice. TG and TC levels did not change significantly in fasted acid levels were not different between fasted and fed fat pads did not change. Insulin and glucose levels fell appro-
significant reduction in body weight (Table 4). The weight of 
tolerated this duration of fast. Forty-eight hours of fast led to a 

Table 3. Circulating hormone and metabolite levels in ob/ob mice and lean littermates in fed conditions

<table>
<thead>
<tr>
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<th>Lean</th>
<th>ob/ob</th>
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<tbody>
<tr>
<td>Body weight, g</td>
<td>23.70±0.83 (5)</td>
<td>52.85±2.07 (5)</td>
</tr>
<tr>
<td>Fat pads, g</td>
<td>0.43±0.05 (5)</td>
<td>3.51±0.19 (5)</td>
</tr>
<tr>
<td>Insulin, ng/ml</td>
<td>1.90±0.40 (5)</td>
<td>48.50±8.80 (6)</td>
</tr>
<tr>
<td>Glucose, mg/dl</td>
<td>148.00±10.00 (6)</td>
<td>288.44±24.02 (6)</td>
</tr>
<tr>
<td>FFA, nmol/µl</td>
<td>1.40±0.10 (6)</td>
<td>1.40±0.00 (6)</td>
</tr>
<tr>
<td>TG, mg/dl</td>
<td>94.9±20.2 (3)</td>
<td>189.7±10.6 (3)</td>
</tr>
<tr>
<td>TC, mg/dl</td>
<td>89.7±6.5 (3)</td>
<td>140.2±5.9 (3)</td>
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Values are means ± SD; nos. in parentheses indicate no. of mice analyzed for each hormone or metabolite. *P < 0.01, indicating values that differ between lean and ob/ob mice.

were also significantly higher, but free fatty acid levels were 
not different between the two groups of mice. Both TG and TC 
levels were significantly elevated in ob/ob mice. The ob/ob 
mice expressed significantly less apoE in freshly isolated 
adipose tissue (Fig. 3A). Consistent with the immunoblot results, 
apoE mRNA levels were also significantly lower (Fig. 3B). CD68 
and F4/80 levels were also significantly higher in adipose tissue 
from ob/ob mice than from lean littermate controls.

To gain insight into any potential role of leptin for regulating 
apoE expression in adipose tissue, we performed several addi-
tional experiments in ob/ob mice. We first undertook trans-
plantation of WAT from lean littermate controls to restore 
circulating leptin in ob/ob mice. As shown in Table 4, leptin 
levels were 0.60±0.10 ng/ml in WAT-transplanted mice and 
nondetectable in sham-operated controls. After transplantation of wild-type WAT, ob/ob mice lost ~40% of body weight over 
12 wk, had significantly smaller fat pads, and had a significant 
reduction in circulating glucose level. The ob/ob mice that 
received adipose tissue transplant from lean littermate controls 
demonstrated a significant increase in adipose tissue apoE 
expression compared with sham-operated controls (Fig. 4A). 
Recipients of adipose tissue also had a significant increase in 
apoE mRNA levels in adipose tissue and significant increases 
in CD68 and F4/80 expression in adipose tissue (Fig. 4B).

The results of the WAT transplantation experiments are 
consistent with the conclusion that leptin, or weight loss, 
influenced adipose tissue apoE in ob/ob mice. Therefore, we 
next fasted ob/ob mice, or maintained them on a calorie-
restricted diet for 7 days, to determine the effect on adipose 
tissue apoE expression. Fasted ob/ob mice were compared with 
nonfasted ob/ob mice maintained on Chow diet. Fasting in this 
model was prolonged for up to 48 h because these obese mice 
tolerated this duration of fasting. Forty-eight hours of fast led to a 
significant reduction in body weight (Table 4). The weight of 
fat pads did not change. Insulin and glucose levels fell appropri-
ately and significantly during the fast; however, free fatty 
acid levels were not different between fasted and fed ob/ob 
mice. TG and TC levels did not change significantly in fasted 
ob/ob mice (not shown). Similar to that shown in fasting 
C57BL/6J mice, fasting produced a significant increase in 
apoE expression in the adipose tissue of ob/ob mice (Fig. 5A). 
apoE mRNA levels also increased (Fig. 5B). CD68 and F4/80 
levels fell in adipose tissue obtained from fasted ob/ob mice 
(Fig. 5B).

To gain insight into any differences between acute vs. 
chronic negative energy balance, we evaluated ob/ob mice 
maintained on two-thirds of the chow consumed by ob/ob mice 
allowed to feed ad libitum to evaluate the effect of chronic 
weight loss in the absence of leptin. Mice fed in this manner 
over 7 days lost 23% of body weight vs. that shown in fed 
ob/ob mice (Table 4). There was no significant change in the 
weight of fat pads, but insulin and glucose levels fell signifi-
cantly. Adipose tissue apoE expression significantly increased 
after 7 days of reduced caloric intake (Fig. 6).

DISCUSSION

Our group (22) has previously shown that apoE expression 
in 3T3-L1 cells is increased by treatment with PPAR-γ ago-
nists and decreased by treatment with TNF-α (22). In murine 
adipose tissue, we subsequently showed that >80% of apoE 
expression is accounted for by adipocyte expression (10). We 
have also demonstrated that endogenous adipocyte apoE 
expression has important effects on adipocyte TG turnover 
and the expression of adipocyte genes related to fatty acid 
oxidation (10). The regulation of adipocyte apoE by PPAR-γ ago-
Table 4. Circulating hormones and metabolite levels in leptin-deficient ob/ob mice under fed, fasted (48 h), and restricted diet (7 days) conditions or with WAT transplantation

<table>
<thead>
<tr>
<th></th>
<th>WAT Transplant</th>
<th>Sham Transplant</th>
<th>Fed</th>
<th>Fasted for 48 h</th>
<th>Restricted Diet (7 days)</th>
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<tbody>
<tr>
<td>Body weight, g</td>
<td>31.10±1.23† (5)</td>
<td>52.85±2.07 (5)</td>
<td>51.10±3.36 (5)</td>
<td>43.00±2.05† (5)</td>
<td>41.30±1.01† (5)</td>
</tr>
<tr>
<td>Fat pads, g</td>
<td>1.94±0.09* (5)</td>
<td>3.39±0.34 (5)</td>
<td>3.84±0.84 (5)</td>
<td>3.89±0.25 (5)</td>
<td>3.63±0.21 (5)</td>
</tr>
<tr>
<td>Insulin, ng/ml</td>
<td>ND</td>
<td>ND</td>
<td>37.00±12.00 (5)</td>
<td>3.40±1.26† (5)</td>
<td>5.00±3.30† (5)</td>
</tr>
<tr>
<td>Glucose, mg/dl</td>
<td>129.5±4.27* (5)</td>
<td>287.5±42.16 (5)</td>
<td>375.00±58.02 (5)</td>
<td>187.00±55.00* (5)</td>
<td>135.00±11.00* (5)</td>
</tr>
<tr>
<td>Leptin, ng/ml</td>
<td>0.60±0.01 (5)</td>
<td>0.00±0.00 (5)</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
</tr>
<tr>
<td>FFA, nmol/ml</td>
<td>ND</td>
<td>ND</td>
<td>1.57±0.3 (5)</td>
<td>1.36±0.10 (5)</td>
<td>1.00±0.10 (5)</td>
</tr>
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</table>

Values are means ± SD; nos. in parentheses indicate no. of mice analyzed for each hormone or metabolite. WAT, white adipose tissue; ND, not done. *P < 0.05, †P < 0.01, and ‡P < 0.001 for the difference between the sham-transplanted and recipient mice (ob/ob) vs. WAT-transplanted mice.

Fig. 4. Adipose tissue transplantation from lean controls increases adipose tissue apoE expression in ob/ob mice. White adipose tissue (WAT) from lean littermates was transplanted into ob/ob recipient mice (n = 4) as described in MATERIALS AND METHODS. Native ob/ob adipose tissue from the recipient ob/ob mice was harvested after 12 wk for immunoblot of apoE (A) or qRT-PCR for apoE, F4/80, and CD68 mRNA levels (B). Results are presented as means ± SD of 4 determinations (A) or means with individual values shown (B). #P < 0.05 and ##P < 0.01 for the difference between the sham-transplanted and adipose-transplanted mice.

NUTRITIONAL REGULATION OF ADIPOSE TISSUE apoE

There are several pathways by which obesity and weight loss could regulate adipose tissue apoE expression. The apoE gene has been shown to respond to PPAR-γ agonists, TNF-α, and sterol content in adipocytes and macrophages (6, 19, 22, 23).
Macrophage infiltration and polarization state could contribute to changes in adipocyte apoE expression in response to obesity (14). Macrophages that accumulate in adipose tissue during induction of obesity display a “classically activated” phenotype with increased levels of TNF-α expression and could be the proximate mediator of the decreased adipocyte apoE expression in the obese state that we have observed (14, 22). Our nutritional interventions also produced perturbations in the level of several hormones or metabolites known to strongly influence adipocyte gene expression. In C57BL/6J mice, diet-induced obesity increased glucose, leptin, and insulin levels and decreased adipose tissue apoE expression. Conversely, fasting in C57BL/6J mice decreased glucose, leptin, and insulin levels and increased apoE expression in adipose tissue. Given the important information indicating that adipocytes can be direct targets of leptin, glucose, and insulin (1, 12, 20), the observations in Tables 1 and 2 and Figs. 1 and 2 suggest leptin, glucose, and insulin could play roles in the modulation of adipose tissue apoE expression in response to nutritional perturbation. Although the role of insulin and glucose will require comprehensive evaluation in other in vivo models, our results address and definitively rule out a need for leptin in the modulation of adipose tissue apoE expression. Chronic weight loss after adipose tissue transplant to partially restore circulating leptin levels increased apoE expression in adipose tissue, but caloric restriction of ob/ob mice over 7 days did as well. Furthermore, adipose tissue apoE expression responded briskly to an acute fast in ob/ob mice.

Important aspects of the regulation of adipose tissue apoE and the elucidation of an important role of endogenous adipocyte apoE in adipocyte lipid turnover are very recent observations. The extent to which exogenous circulating apoE can substitute for endogenous adipocyte apoE function also remains to be more fully explored. With respect to this question, however, we have previously shown that, even after incubation in apoE-containing serum for 10–14 days, TG content remains lower in apoE−/− adipocytes than in wild-type adipocytes (10). We have also shown that, in freshly isolated adipose tissue, the
absence of endogenous apoE expression leads to a marked defect in TG uptake from exogenous VLDL that contains apoE (10). With information now available, the changes in adipose tissue apoE expression in response to nutritional intervention can be rationalized in the following way. In adipocytes, apoE expression leads to increased TG mass, increased TG synthesis, and decreased TG hydrolysis. In view of these observations, the increased apoE expression that accompanies fasting could be viewed as a homeostatic response to limit further loss of adipocyte TG. Conversely, suppression of apoE expression in adipocytes in the obese state could represent a mechanism for minimizing further growth of adipose tissue TG stores. Although changes in adipose tissue apoE with both fasting and weight loss are statistically significant at the protein and mRNA levels, the suppression of expression in obesity is larger in magnitude than the increased expression observed with weight loss or fasting. On the basis of our previous observation (10), suppression of adipose tissue apoE will reduce adipocyte acquisition of TG from VLDL. This could contribute to altered VLDL composition and, therefore potentially, to disordered systemic VLDL metabolism that accompanies obesity.

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