Oxidative Stress Regulates Adipocyte Apolipoprotein E and Suppresses Its Expression in Obesity

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OBJECTIVE—Endogenous expression of apolipoprotein E (apoE) has a significant impact on adipocyte lipid metabolism and is markedly suppressed in obesity. Adipose tissue oxidant stress is emerging as an important mediator of adipocyte dysfunction. These studies were undertaken to evaluate the role of oxidant stress for regulation of adipocyte apoE.

RESEARCH DESIGN AND METHODS—ApoE gene and protein expression in 3T3-L1 adipocytes or mature adipocytes and adipose tissue from C57/BL6 mice was evaluated after induction of oxidant stress. The response of adipose tissue and adipocytes from obese compared with lean mice to antioxidants was also assessed.

RESULTS—Oxidant stress in 3T3-L1 cells or adipocytes and adipose tissue from lean mice significantly reduced apoE mRNA and protein level. Inclusion of an antioxidant eliminated this reduction. Oxidant stress was accompanied by activation of the nuclear factor-κB (NF-κB) transcription complex, and its effect on apoE was eliminated by an NF-κB activation inhibitor. Treatment of freshly isolated adipose tissue or mature adipocytes from obese mice with antioxidant increased apoE expression but had no effect on cells or tissue from lean mice. Incubation of freshly isolated adipocytes from lean mice with stromovascular cells from obese mice significantly suppressed adipocyte apoE compared with incubation with stromovascular cells from lean mice, but this suppression was reversed by inclusion of antioxidant or a neutralizing antibody to tumor necrosis factor-α.

CONCLUSIONS—Oxidant stress significantly modulates adipose tissue and adipocyte apoE expression. Furthermore, oxidant stress contributes to suppression of adipocyte apoE in obesity. This suppression depends on interaction between adipose tissue stromovascular cells and adipocytes. ***Diabetes* 57: 2992–2998, 2008**

Obesity is widely recognized as an increasingly prevalent cause of metabolic and cardiovascular disease (1,2). It has also been recently appreciated that obesity is associated with a chronic inflammatory reaction in adipose tissue and that this inflammation is closely associated with metabolic and cardiovascular risk (1,3,4). Adipose tissue from obese animals or humans is characterized by the influx of inflammatory cells, primarily macrophages, into its stromovascular compartment with increased local production of proinflammatory cytokines (5–8). There is also a concomitant increase in the production of reactive oxygen species (ROS) in adipose tissue (9). The localized inflammation with oxidative stress in adipose tissue leads to important changes in adipocyte gene expression with downstream effects on adipocyte lipid metabolism and triglyceride content. Adipose tissue inflammation and oxidant stress also produce a systemic increase in circulating inflammatory cytokines and ROS with adverse effects on systemic insulin action and substrate metabolism (1,9,10).

Mature adipocytes and macrophages express a number of proteins in common, and one of these is apolipoprotein E (apoE). In macrophages, the endogenous expression of apoE functions primarily to facilitate lipid flux (11,12). However, macrophage-derived apoE in the arterial wall has also been associated with local anti-inflammatory and antioxidant effects (13–15). ApoE is also highly expressed in hepatocytes and steroidogenic cells (16–18). Like macrophages, these two cell types experience high lipid flux related to their differentiated functions of lipoprotein metabolism and steroid hormone secretion, respectively. Adipocytes also experience high lipid flux as part of their differentiated function, and high-level expression of apoE was first noted by Zechner et al. (19). More recently, an important role for endogenously expressed adipocyte apoE for modulating adipocyte lipid and lipoprotein metabolism has been established (20).

Comparing freshly isolated or cultured adipocytes from apoE$^{-/-}$ mice with those from wild-type mice demonstrated that adipocytes from the former were smaller, contained less lipid, synthesized less triglyceride, and had increased rates of triglyceride hydrolysis (20). Furthermore, these differences were maintained after differentiation of preadipocytes to adipocytes followed by long-term culture in apoE-containing serum. Adipose tissue from apoE$^{-/-}$ mice also accumulated less triglyceride after incubation with apoE-rich VLDL compared with that from wild-type mice and expressed higher levels of genes involved in fatty acid oxidation. These observations support a role for endogenously expressed apoE in overall adipocyte lipid metabolism. This notion is further supported by physiologically relevant regulation of adipocyte apoE. Adipocyte apoE is increased by the systemic administration of peroxisome proliferator–activated receptor-γ (PPARγ) agonists and decreased by the systemic administration of the proinflammatory peptide angiotensin II (21,22). Furthermore, diet-induced or leptin-deficient obesity leads to marked suppression of adipocyte apoE (23). In addressing potential mechanisms for this suppression in obesity, we have previously shown that tumor necrosis factor-α (TNF-α), a proinflammatory cytokine produced by inflammatory adipose tissue macrophages in obesity, suppresses adipocyte apoE expression (21). Emerging evidence has identified adipose tissue ROS as important

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effectors producing adipocyte dysfunction in obesity (9). The current studies were undertaken to evaluate a role for oxidative stress in regulating adipocyte apoE expression and its role in mediating the reduced adipocyte apoE expression observed in obesity.

RESEARCH DESIGN AND METHODS

Cell culture media, fetal bovine serum (FBS), and antibiotics were purchased from Invitrogen (Carlsbad, CA). Goat-derived apoE antiserum was from International Immunology (Murrieta, CA). Phospho-inhibitor of κBα (phospho-IκBα) and IκBα antibodies were from Cell Signaling Technology (Danvers, MA). TNF-α neutralizing antibody was from Biovision (Mountain View, CA). The nuclear factor-κB (NF-κB) activation inhibitor, 6-amino-4-(4a-phenoxyphenylethylamino) quinazoline (QNZ), was purchased from Calbiochem. Liberase blendzyme 3 was from Roche. Insulin, dexamethasone, 3-isobutyl-1-methylxanthine (IBMX), hydrogen peroxide, *N*-acetyl-L-cysteine (NAC), xanthine oxidase, hypoxanthine, glucose oxidase, and BSA were obtained from Sigma (St. Louis, MO).

Cell culture and isolation of primary adipocytes. 3T3-L1 cells were obtained from American Type Culture Collection (Rockville, MD). Cells were maintained in 10% FBS-supplemented Dulbecco's modified Eagle's medium (DMEM) with penicillin and streptomycin in a 5% CO_2 incubator at 37°C. Two days after confluence, cells were differentiated by incubating in differentiation medium containing 0.5 mmol/l IBMX, 0.2 μmol/l dexamethasone, and 10 μg/ml insulin. Three days after addition of this differentiation cocktail, cells were placed in DMEM containing 10 μg/ml insulin and 10% FBS. All experiments were performed 10 days after differentiation.

Male C57BL/6J (10- to 12-week-old) mice, male *ob/ob* (10- to 12-week-old) mice, or their lean littermates were from The Jackson Laboratories (Bar Harbor, ME). All animal protocols were approved by the Institutional Animal Care and Use Committee of the University of Illinois, Chicago. Intra-abdominal fat pads were harvested, washed with sterile PBS, and separated into two parts. One part was used for tissue incubation, and the other part was used for isolation of mature adipocytes and the stromovascular fraction. For tissue incubation, fat pads were minced into 1- to 2-mm pieces under sterile conditions and incubated in DMEM containing 0.5% BSA and treated as described in the figure legends. For isolation of mature adipocytes and the stromovascular fraction, fat pads were cut into 1- to 2-mm pieces and digested using 0.5 mg/ml Liberase Blendzyme 3 in DMEM for 1 h at 37°C in a shaking water bath. After digestion, cells were centrifuged at 300 × *g* for 5 min, and the suspended mature adipocytes were separated from the pelleted stromovascular fraction for experiments. For some experiments, mature adipocytes from lean mice were incubated in serum-free DMEM containing 0.5% BSA with 1.0×10^6 stromovascular cells from either lean or *ob/ob* mice. After 4 h, stromovascular cells were pelleted, and floating adipocytes were collected for measurement of apoE mRNA. NAC (20 mmol/l), 4 μg/ml anti-TNF antibody, or both were added during some incubations as indicated in the figure legends. For some experiments, the stromovascular fraction from adipose tissue harvested from the subcutaneous space of *ob/ob* mice was isolated as described above.

ApoE mRNA quantitation. Total RNA was isolated from adipose tissue, floating adipocytes, stromovascular cells, or 3T3-L1 adipocytes using RNeasy mini kit (Qiagen, Valencia, CA). First-strand cDNA was synthesized from 1 μg total RNA using Thermoscript RT-PCR System (Invitrogen) according to the manufacturer's instructions. Real-time PCR was performed on each sample using the Mx3000p Quantitative PCR system (Stratagene, La Jolla, CA) using iTaq SYBR Green Supermix with ROX. The relative quantity of apoE mRNA was calculated after correction for β-actin mRNA abundance and was expressed for each experiment as fold change compared with the experimental control (20). The primer pairs used for amplification of apoE and β-actin genes were 5′-AGGATCTACGCAACCGACTC-3′, 5′-GGCGATGCATGTTC CACTA-3′ and 5′-GGCCCAGAGCAAGAGAGGTA-3′, 5′-GGACTCATCG TACTCCTGCT-3′, respectively. Reaction product purity was confirmed by examination of melting curves for a singe peak.

Western blotting. Total protein was extracted from cells or tissue using radioimmunoprecipitation assay buffer (0.5% sodium deoxycholate, 0.1% SDS, 1% Triton X, 20 mmol/l Trisma base, 150 mmol/l NaCl, and 5 mmol/l EDTA), supplemented with protease inhibitor cocktail. Samples were centrifuged for 5 min, the top fat layer was discarded, and the middle clear layer of solubilized protein was collected. Total protein concentration was analyzed using Bio-Rad DC protein assay. Fifty micrograms of protein for each sample was subjected to SDS-PAGE analysis, transferred to nitrocellulose, and probed with antibodies for apoE, IκBα phosphorylated IκBα, or β-actin. Western blot images were quantitated using ImageQuant TL software (GE Healthcare, Piscataway, NJ) using β-actin as an internal loading control.

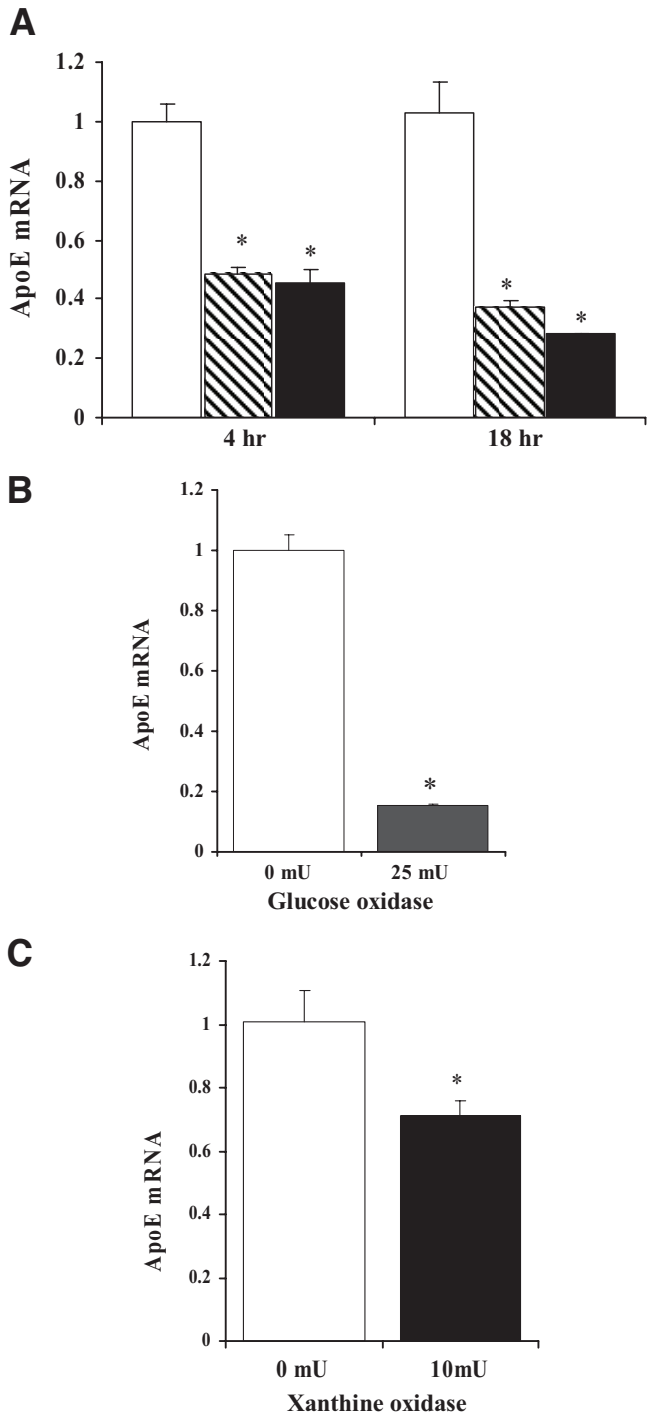

FIG. 1. Oxidative stress reduces apoE mRNA level in adipocytes. *A*: 3T3-L1 adipocytes were preincubated for 6 h in serum-free medium and then treated with 0, 0.5, or 1 mmol/l H_2O_2 for 10 min. Cells were then incubated in serum-free medium for an additional 4 or 18 h before being harvested for measurement of apoE mRNA. □, 0.0 mmol/l; ▨, 0.5 mmol/l; ■, 1.0 mmol/l. Adipocytes were treated with or without 25 mU/ml glucose oxidase (*B*) or 10 mU/ml xanthine oxidase (*C*) with 0.6 mmol/l hypoxanthine for 18 h in serum-free medium. Total RNA was extracted and apoE mRNA level was measured using RT-PCR. Each experiment was performed using triplicate samples and was repeated three times with similar results. Results shown are from a representative experiment as means ± SD. *$P < 0.001$ for the difference compared with untreated control.

NF-κB pathway involvement. NF-κB pathway activation was first assessed by detecting the level of IκBα phosphorylation. After treatment of 3T3-L1 adipocytes with 1 mmol/l H_2O_2 for 0, 1, 5, or 10 min, cells were lysed in the presence of phosphatase inhibitor cocktail. Fifty micrograms of total cell

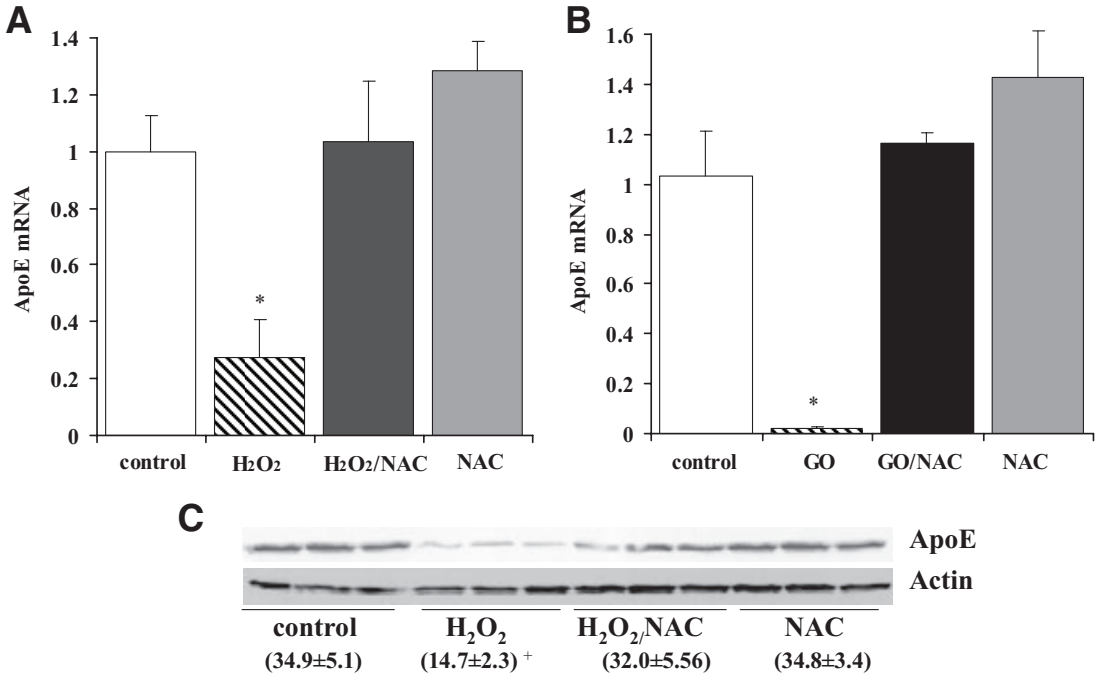

FIG. 2. NAC abrogates the effect oxidative stress on apoE expression in adipocytes. 3T3-L1 adipocytes in serum-free medium were incubated with 20 mmol/l NAC for 1 h before incubation with the indicated oxidative stress agents. *A*: Cells were treated with 1 mmol/l H_2O_2 for 10 min in the presence or absence of NAC and then harvested 4 h later for measurement of apoE mRNA. *B*: Cells were treated with 25 mU/ml glucose oxidase (GO) for 18 h in the presence or absence of NAC and then harvested for measurement of apoE mRNA. *C*: Western blot showing apoE protein expression level in adipocytes treated with 1 mmol/l H_2O_2 for 10 min in the presence or absence of NAC. Cells were then harvested 18 h after treatment with H_2O_2. Signal quantitation is shown in parentheses. Experiments were performed using triplicate samples and repeated three times with similar results. Results are shown as means ± SD, *$P < 0.002$, +$P < 0.02$ for the difference compared with untreated control.

extract was subjected to Western blot analysis using an antibody specific for IκBα phosphorylated at serine 32/36. Blots were stripped and reprobed with an antibody to total IκBα protein. The involvement of the NF-κB pathway was also assessed by adding QNZ (an inhibitor of NF-κB complex activation) to cells in the presence or absence of 1 mmol/l H_2O_2.

Measurement of cellular H_2O_2. Cellular ROS was measured using fluorescent dye 5(-and-6)-chloromethyl-2′7′-dichlorohydroflorescein deacetate acetyl ester (CM-H_2DCFDA; Molecular Probes) as previously described with minor modifications (24). After treatment with glucose oxidase or xanthine oxidase, differentiated adipocytes were washed with phenol-free DMEM and incubated with 2 μmol/l CM-H_2DCFDA for 45 min at 37°. Fluorescence was analyzed using BIOTEK Synergy H Fluorescent plate reader at an excitation wavelength of 485 nm and emission at 530 nm. Cellular ROS concentration was quantitated using a H_2O_2 standard curve.

Statistical analysis. Statistical differences between experimental groups were evaluated using Student's two-sample *t* test. *P* values <0.05 were considered significant. All data are expressed as means ± SD of the sample replicate number indicated in the figure legends.

RESULTS

In the first series of experiments, we evaluated the impact of oxidative stress on adipocyte apoE gene expression in 3T3-L1 cells. Oxidative stress was first modeled by the exogenous addition of hydrogen peroxide (9,25). Incubation of 3T3-L1 cells with this agent for only 10 min reduced apoE mRNA level by ~50% in cells harvested 4 and 18 h after treatment (Fig. 1*A*). Endogenous cellular production of ROS was induced by incubating cells for 18 h with glucose oxidase or xanthine oxidase (26,27). These incubations increased the production of endogenous cellular ROS to 21.5 ± 1.9 and 14.9 ± 1.5 μmol/l/well, respectively, compared with 6.0 ± 0.8 μmol/l/well in control cells ($P <$ 0.05 for control compared with glucose oxidase or xanthine oxidase). Incubation with either agent also significantly reduced apoE mRNA level in adipocytes (Fig. 1*B* and *C*). NAC is an antioxidant compound previously shown to mitigate the effect of ROS on adipocyte gene expression in vitro and in vivo (9,28). Treatment of cells with NAC alone had no effect on apoE mRNA or protein level in 3T3-L1 cells (Fig. 2*A*–*C*). However, inclusion of NAC with either hydrogen peroxide or glucose oxidase eliminated significant downregulation of apoE expression.

The NF-κB pathway has been identified as an important mediator of oxidant stress on adipocyte gene expression. We next evaluated the participation of this pathway for the effect of oxidant stress on the adipocyte apoE gene using two complementary approaches. Activation of the NF-κB transcription complex requires phosphorylation of the inhibitory IκBα subunit at Ser 32/36. This phosphorylation triggers IκBα proteosomal degradation with subsequent activation of the NF-κB transcription complex. Treatment of 3T3-L1 cells with hydrogen peroxide for 5 min increased the cellular level of phosphorylated IκBα (designated IκBα-P in Fig. 3*A*, *top panel*). After 10 min, there was a significant decrease in total cellular IκBα consistent with its increased degradation. These results indicate that treatment of 3T3-L1 adipocytes with hydrogen peroxide at the dose and time used for our experiments was sufficient for activation of the NF-κB transcription complex. To further evaluate the importance of the NF-κB pathway for the apoE gene response, we evaluated the effect of QNZ, an inhibitor of NF-κB activation (29). Addition of QNZ alone had no effect on apoE mRNA level in 3T3-L1 adipocytes (Fig. 3*B*). Inclusion of QNZ with hydrogen peroxide, however, completely eliminated the suppression of apoE gene expression produced by the latter agent.

We next extended our observations to examine the impact of oxidative stress on apoE expression in freshly isolated adipose tissue and mature adipocytes from C57BL/6J mice (Fig. 4*A*). Measurement of apoE mRNA level in whole-murine adipose tissue, mature adipocytes,

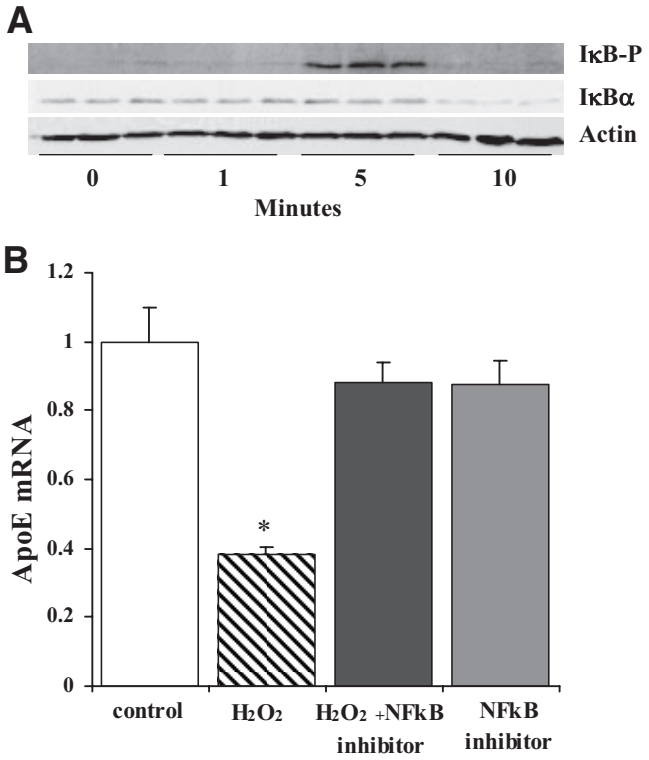

FIG. 3. NF-κB pathway mediates the effect of oxidative stress on apoE expression in adipocytes. *A*: 3T3-L1 adipocytes in serum-free medium for 6 h were treated with 1 mmol/l H_2O_2 for 0, 1, 5, and 10 min. Cells were then immediately lysed in the presence of phosphatase inhibitor cocktail. Fifty micrograms of protein were resolved on SDS-PAGE and probed with phospho-IκBα (Ser 32/36) antibody. The nitrocellulose membrane was stripped and reprobed with anti-IκBα antibody. The blots shown are representative of three separate experiments. *B*: 3T3-L1 adipocytes were placed in serum-free medium for 6 h and then preincubated with 100 nmol/l NF-κB activation inhibitor QNZ for 1 h before treatment with 1 mmol/l H_2O_2 for 10 min. Cells were washed and incubated for an additional 4 h without additions. The results shown are from a representative experiment performed using triplicate samples and repeated two times with similar results. Results are shown as means ± SD. *$P < 0.001$ for the difference compared with untreated control.

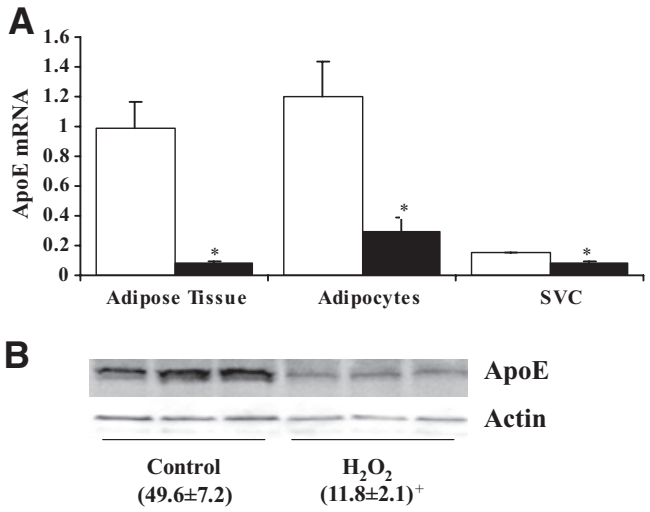

FIG. 4. Effect of oxidative stress on apoE mRNA level in freshly isolated adipose tissue, mature adipocytes, and stromovascular cells from C57Bl/6J mice. *A*: Intra-abdominal fat pads were isolated and adipose tissue, mature adipocytes, and the stromovascular fraction (SVC) were separated and treated with 1 mmol/l H_2O_2 for 10 min. Cells and tissue were washed and incubated in serum-free medium for an additional 4 h before harvest. All results are expressed as fold change compared with apoE mRNA level in untreated adipose tissue. Results shown are means ± SD of cells and adipose tissue from six mice, *$P < 0.005$ for the difference compared with untreated control. *B*: Western blot for the effect of 1 mmol/l H_2O_2 (10 min) on apoE protein expression in isolated adipose tissue harvested 18 h after treatment with H_2O_2. Signal quantitation is shown in parentheses. $^+P < 0.02$ for the difference compared with control. □, no addition; ■, H_2O_2.

and the stromovascular fraction showed that mature adipocytes accounted for the preponderance of apoE message level in adipose tissue, as we have previously reported (20). Treatment of these fractions with hydrogen peroxide for 10 min produced significant reduction of apoE mRNA level in whole adipose tissue and mature adipocytes, completely consistent with results obtained in 3T3-L1 cells. ApoE expression in the stromovascular cell fraction was also reduced by treatment with hydrogen peroxide; however, its overall contribution to adipose tissue apoE mRNA level and its level of expression compared with mature adipocyte expression was small. We next evaluated the effect of oxidant stress on apoE protein expression in freshly isolated adipose tissue. Adipose tissue was incubated with or without H_2O_2 for 10 min and then harvested 18 h later for Western blot. H_2O_2 treatment reduced apoE protein level by >70% (Fig. 4*B*).

Obesity leads to increased production of ROS in adipose tissue, and we have previously shown that adipose tissue and mature adipocyte apoE expression are reduced in obese mice compared with lean mice. Therefore, we next evaluated a potential role for ROS in contributing to reduced adipose tissue and adipocyte apoE expression in obesity. We approached this question by comparing the impact of treating freshly isolated adipose tissue or mature adipocytes from obese and lean mice with the antioxidant NAC (Fig. 5). As we have previously reported, apoE expression is lower in adipose tissue and adipocytes isolated from *ob/ob* mice compared with lean littermate controls (23). Treatment of adipose tissue or adipocytes isolated from lean mice with NAC did not significantly impact apoE expression level. However, treatment of adipose tissue or mature adipocytes isolated from obese mice with NAC increased adipose tissue mRNA level by five- and fourfold, respectively. These results indicate that adipose tissue oxidative stress is an important factor contributing to the suppression of adipose tissue and adipocyte apoE in obesity.

Obesity is characterized by an influx of inflammatory cells into the stromovascular compartment of adipose tissue. Signaling between inflammatory cells in the stromovascular compartment and adipocytes has been identified as an important contributor to adipocyte dysfunction in obesity (5–7,30). We next addressed the question of whether the adipose tissue stromovascular fraction from obese mice could modulate the expression of apoE in adipocytes obtained from lean mice (Fig. 6*A*). Freshly isolated mature adipocytes from lean mice were incubated alone or with the stromovascular cell fraction from lean mice or from obese mice. After 4 h of incubation, apoE mRNA levels were suppressed in the adipocytes incubated with the stromovascular cell fraction from obese mice by >80%. We next evaluated whether the suppressive effect of the stromovascular fraction was different for stromovascular fractions isolated from visceral or subcutaneous adipose depot of obese mice (Fig. 6*B*). Addition of the obese stromovascular fraction from either fat depot significantly suppressed adipocyte apoE mRNA level, but the suppression produced by the visceral stromovascular fraction was significantly greater than that produced by the subcutaneous stromovascular fraction.

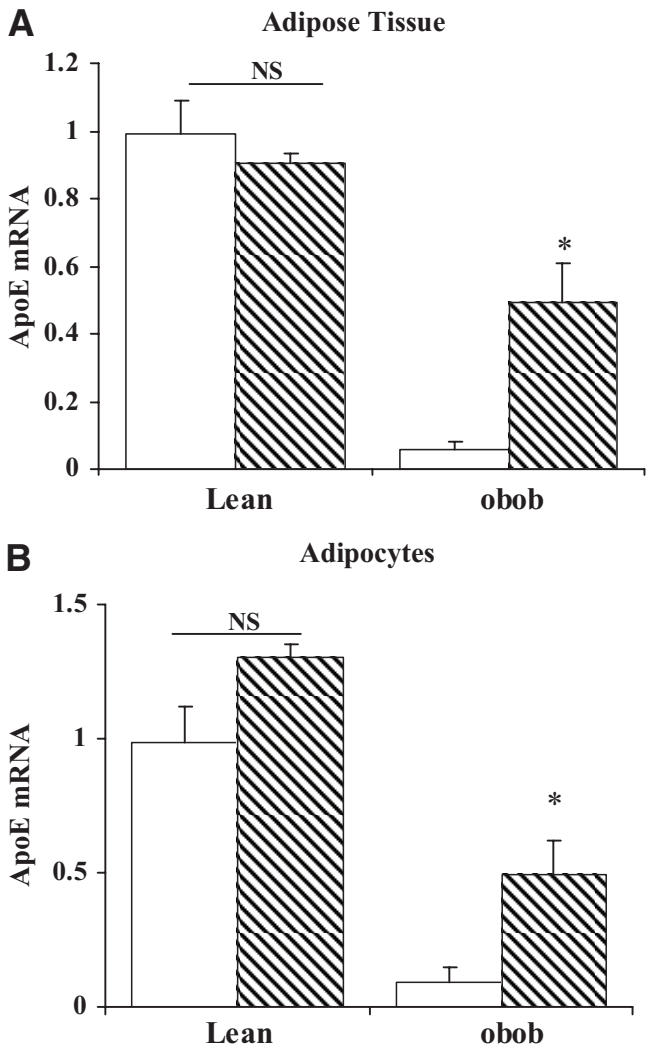

FIG. 5. NAC increases apoE mRNA level in freshly isolated adipose tissue and mature adipocytes from *ob/ob* mice. Freshly isolated adipose tissue (*A*) or freshly isolated mature adipocytes (*B*) from fat pads of *ob/ob* mice or lean littermate controls were isolated as described in RESEARCH DESIGN AND METHODS and incubated alone or with 20 mmol/l NAC for 4 h. Total RNA was extracted, and apoE mRNA was measured. Results are expressed as fold change compared with apoE level in untreated lean mice and are means ± SD of cells and adipose tissue from six mice. □, no addition; ▨, NAC. *$P < 0.0005$ for comparison of untreated vs. NAC treated.

We performed experiments to determine whether the effect of the stromovascular fraction from obese mice was related to the induction of oxidant stress in adipocytes (Fig. 7*)*. Adipocytes from lean mice were incubated alone or with the visceral stromovascular fraction from obese mice plus the additions indicated in Fig. 7. Consistent with the results in Fig. 6*A*, the obese stromovascular fraction alone produced a >80% suppression of apoE mRNA level. Inclusion of antioxidant NAC during the incubation significantly attenuated the effect of the obese stromovascular fraction on adipocyte apoE expression. Because we have previously demonstrated an important role for TNF-α in regulating adipocyte apoE (21,31), we also evaluated the effect of including a neutralizing antibody to TNF-α during these incubations. The inclusion of this antibody also opposed the suppression of adipocyte apoE by the obese stromovascular fraction. The inclusion of both the antioxidant and neutralizing antibody restored adipocyte apoE to control level. In separate experiments, we evaluated the effect of the NF-κB inhibitor QNZ on suppression of adipocyte apoE by the obese stromovascular fraction. QNZ alone had no effect on adipocyte apoE mRNA level (1.1 ± 0.2 compared with control). Addition of the obese stromovascular fraction reduced apoE mRNA level to 0.4 ± 0.1 compared with control ($P < 0.01$). Addition of QNZ with the obese stromovascular fraction prevented downregulation of apoE mRNA level (1.01 ± 0.1). The above results indicate that cross talk between adipocytes and stromovascular cells enhances oxidant stress-related reduction of adipocyte apoE in obesity.

DISCUSSION

The current studies were undertaken to evaluate the importance of oxidative stress for modulating adipocyte apoE expression. Using 3T3-L1 cells, we show that ROS reduce apoE mRNA and protein expression and that this reduction can be prevented by the general antioxidant NAC (Figs. 1 and 2). Analogous to other adipocyte genes regulated by oxidative stress, these effects on the apoE adipocyte gene are mediated by the NF-κB pathway (Fig. 3). We extend our observations from 3T3-L1 cells to primary cells by showing that ROS also suppress apoE mRNA and protein level in freshly isolated murine adipose tissue and adipocytes (Fig. 4). Using freshly isolated adipose tissue and adipocytes from *ob/ob* mice and lean littermate controls, we show that the suppression of apoE level that we have previously reported in *ob/ob* mice could be partially reversed by NAC (Fig. 5). We further show that interaction between the *ob/ob* stromovascular fraction and adipocytes is important for producing the suppression of adipocyte apoE and that this suppression is reversed by incubation with NAC and a neutralizing antibody to TNF-α (Fig. 6). In aggregate, our results establish that oxidant stress regulates adipocyte apoE gene expression, that oxidative stress contributes to the suppression of adipocyte apoE in obesity, and that this suppression in obesity depends on interaction between the adipose tissue stromovascular fraction and adipocytes. Both ROS and TNF-α play an important role in mediating the impact of the obese stromovascular fraction on adipocyte apoE, and both appear to use NF-κB activation as a final common pathway for suppressing its expression. Both ROS and TNF-α (21,31) act directly on adipocytes to regulate apoE gene expression. This is consistent with the interpretation that NAC and TNF-α neutralizing antibodies also act directly on adipocytes during coincubation with the obese stromovascular fraction to prevent reduction of apoE expression (Fig. 7). However, our experiments do not rule out an additional effect of NAC or TNF-α neutralizing antibodies on stromovascular fraction cells during the coincubation. Our observations also establish that the stromovascular fraction from the visceral fat depot of obese mice more effectively reduces adipocyte apoE expression compared with that from the subcutaneous depot. This is consistent with observations that in obesity, visceral fat may be the primary contributor to metabolic derangements (1,3).

Although high-level expression of apoE in human and murine adipose tissue has been known for some time, its physiological function has only recently been explored (19,20). Freshly isolated adipocytes from apoE$^{-/-}$ mice are smaller and contain less triglyceride compared with those from wild-type mice despite in vivo exposure to a hyperlipidemic environment. In the apoE$^{-/-}$ mouse

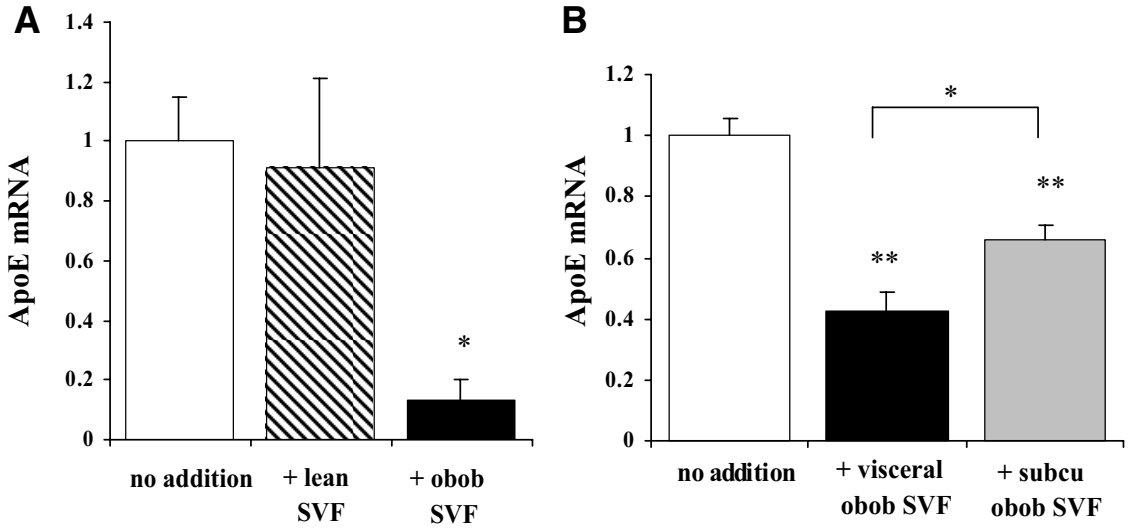

FIG. 6. Importance of stromovascular-adipocyte cross-talk for regulating adipocyte apoE expression in obesity. *A*: Mature adipocytes from lean mice were incubated alone or with 1.0 × 10^6 cells from the stromovascular fraction (SVF) of lean mice or *ob/ob* mice for 4 h as described in RESEARCH DESIGN AND METHODS. Adipocytes were harvested for measurement of apoE mRNA. *B*: Mature adipocytes from lean mice were incubated alone or with 1 × 10^6 cells isolated from the stromovascular fraction of visceral or subcutaneous fat from *ob/ob* mice. After 4 h, adipocytes were harvested for measurement of apoE mRNA. Results are means ± SD from five lean mice and are representative of two experiments with similar results. **P* < 0.01, ***P* < 0.001.

model, reduced triglyceride in freshly isolated adipocytes could be due to the absent expression of apoE in adipocytes or, alternatively, to lack of apoE on the surface of circulating lipoproteins. An important role for endogenous adipocyte apoE for modulating adipocyte triglyceride content and adipocyte lipoprotein metabolism is supported by the following observations: *1*) The difference in lipid content between apoE$^{-/-}$ adipocytes and wild-type adipocytes is maintained after long-term culture in apoE-containing serum; *2*) in apoE$^{-/-}$ adipocytes incubated with apoE-containing serum, the adenoviral-mediated expression of apoE significantly increases adipocyte triglyceride mass; and *3*) freshly isolated adipose tissue from apoE$^{-/-}$ mice accumulates significantly less triglyceride after incubation with apoE-rich VLDL compared with that from wild-type mice (20).

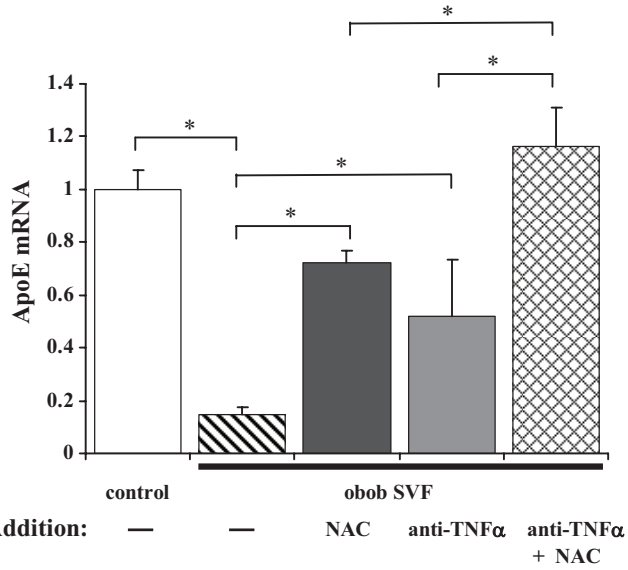

FIG. 7. Role of ROS and TNF-α in suppression of adipocyte apoE by the obese stromovascular fraction. Adipocytes from lean mice were incubated alone or with 1.0 × 10^6 stromovascular fraction (SVF) cells from *ob/ob* mice alone or with the indicated additions. After 4 h, adipocytes were harvested for measurement of apoE mRNA. Results are means ± SD of adipocytes from five lean mice and are representative of two experiments with similar results. **P* < 0.01.

The above observations indicate that endogenous apoE expression is important for adipocyte acquisition of triglyceride from extracellular triglyceride-rich lipoproteins (TGRLs). In this way, the level of apoE expression in adipocytes could influence the partitioning of lipid in circulating TGRLs between adipocytes and other tissues. For example, reduced TGRL lipid deposition in adipose tissue resulting from reduced adipocyte apoE expression (such as that observed in obesity) could favor lipid delivery to liver and muscle, where deposition of this lipid has been implicated in producing tissue-specific insulin resistance (32–35). We have previously shown that the lipogenic response to PPARγ agonists is defective in apoE$^{-/-}$ adipocytes even when incubated in the presence of apoE-containing serum. Increased adipocyte apoE expression could also, therefore, participate in the expansion of adipose tissue that is observed with administration of PPARγ agonists (36–38). These issues will require further study.

The above considerations underline the importance of integrating adipocyte apoE into an overall model of adipocyte lipid metabolism. As noted above, systemic administration or treatment of isolated cells with PPARγ agonists increases adipocyte apoE expression (21). Conversely, treatment with the proinflammatory peptide angiotensin II reduces adipocyte apoE (22). Diet-induced or leptin-deficient obesity leads to reduced apoE expression, and we have previously demonstrated that TNF-α reduces adipocyte apoE expression (21,23). The observations in the current manuscript indicate that ROS and oxidant stress present in obesity are an important pathway for suppressing adipocyte apoE expression. This pathway has high pathophysiological importance given the emerging evidence that oxidative stress in adipose tissue is increased in both obesity and diabetes (9,28), two diseases becoming increasingly prevalent worldwide.

Our observations also provide evidence for an important interaction between adipocytes and adipose tissue stromovascular cells for inducing oxidative stress with reduced apoE expression in adipocytes. This pro-oxidant and proinflammatory interaction suggests that interventions that reduce adipose tissue inflammation and oxidant stress will increase adipocyte apoE expression. PPARγ agonists suppress a proinflammatory phenotype in macrophages and produce apoptosis of adipose tissue macrophages (39). In addition, a specific anti-inflammatory effect of PPARγ agonists in adipose tissue has been demonstrated (40,41). PPARγ agonists could therefore increase adipocyte apoE both by a direct effect on the adipocyte apoE gene (21) and by suppressing adipose tissue inflammation.

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REFERENCES

1. Fantuzzi G, Mazzone T: Adipose tissue and atherosclerosis: exploring the connection. *Arterioscler Thromb Vasc Biol* 27:996–1003, 2007
2. Haffner SM: Relationship of metabolic risk factors and development of cardiovascular disease and diabetes. *Obesity* 14 (Suppl. 3):121S–127S, 2006
3. Berg AH, Scherer PE: Adipose tissue, inflammation, and cardiovascular disease. *Circ Res* 96:939–949, 2005
4. Wellen KE, Hotamisligil GS: Obesity-induced inflammatory changes in adipose tissue. *J Clin Invest* 112:1785–1788, 2003
5. Lumeng CN, DeYoung SM, Bodzin JL, et al.: Increased inflammatory properties of adipose tissue macrophages recruited during diet-induced obesity. *Diabetes* 56:16–23, 2007
6. Xu H, Barnes GT, Yang Q, et al.: Chronic inflammatory in fat plays a crucial role in the development of obesity-related insulin resistance. *J Clin Invest* 112:1821–1830, 2003
7. Weisberg SP, McCann D, Desai M, et al.: Obesity is associated with macrophage accumulation in adipose tissue. *J Clin Invest* 112:1796–1808, 2003
8. Neels JG, Olefsky JM: Inflamed fat: what starts the fire? *J Clin Invest* 116:33–35, 2006
9. Furukawa S, Fujita T, Shimabukuro M, et al.: Increased oxidative stress in obesity and its impact on metabolic syndrome. *J Clin Invest* 114:1752–1761, 2004
10. Kershaw EE, Flier JS: Adipose tissue as an endocrine organ. *J Clin Endocrinol Metab* 89:2548–2556, 2004
11. Lin C-Y, Duan H, Mazzone T: Apolipoprotein E-dependent cholesterol efflux from macrophages: kinetic analysis and divergent mechanism for endogenous versus exogenous apolipoprotein E. *J Lipid Res* 40:1618–1626, 1999
12. Mazzone T, Reardon C: Expression of heterologous human apolipoprotein E by J774 macrophages enhances cholesterol efflux to HDL_3. *J Lipid Res* 35:1345–1353, 1994
13. Stannard AK, Riddell DR, Sacre SM, et al.: Cell-derived apolipoprotein E (ApoE) particles inhibit vascular cell adhesion molecule-1 (VCAM-1) expression in human endothelial cells. *J Biol Chem* 276:46011–46016, 2001
14. Riddell DR, Graham A, Owen JS: Apolipoprotein E inhibits platelet aggregation through the L-arginine:nitric oxide pathway: implications for vascular disease. *J Biol Chem* 272:89–95, 1997
15. Miyata M, Smith JD: Apolipoprotein E allele-specific antioxidant activity and effects on cytotoxicity by oxidative insults and beta-amyloid peptides. *Nat Genet* 14:55–61, 1996
16. Teusink B, Mensenkamp AR, van der Boom H, et al.: Stimulation of the in vivo production of very low density lipoproteins by apolipoprotein E is independent of the presence of the low density lipoprotein receptor. *J Biol Chem* 276:40693–40697, 2001
17. Linton MF, Hasty AH, Babaev VR, et al.: Hepatic apo E expression is required for remnant lipoprotein clearance in the absence of the low density lipoprotein receptor. *J Clin Invest* 101:1726–1736, 1998
18. Thorngate FE, Strockbine PA, Erickson SK, et al.: Altered adrenal gland cholesterol metabolism in the apoE-deficient mouse. *J Lipid Res* 43:1920–1926, 2002
19. Zechner R, Moser R, Newman TC, et al.: Apolipoprotein E gene expression in mouse 3T3-L1 adipocytes and human adipose tissue and its regulation by differentiation and lipid content. *J Biol Chem* 266:10583–10588, 1991
20. Huang ZH, Reardon CA, Mazzone T: Endogenous apoE expression modulates adipocyte triglyceride content and turnover. *Diabetes* 55:3394–3402, 2006
21. Yue L, Rassouli N, Ranganathan G, et al.: Divergent effects of PPAR agonists and TNF on adipocyte apoE expression. *J Biol Chem* 279:47626–47632, 2004
22. Rao P, Huang ZH, Mazzone T: Angiotensin II regulates adipocyte apolipoprotein E expression. *J Clin Endocrinol Metab* 92:4366–4372, 2007
23. Huang ZH, Luque RM, Kineman RD, et al.: Nutritional regulation of adipose tissue apolipoprotein E expression. *Am J Physiol Endocrinol Metab* 293:E203–E209, 2007
24. Lei T, Xie W, Han J, et al.: Medium-chain fatty acids attenuate agonist-stimulated lipolysis, mimicking the effects of starvation. *Obes Res* 12:599–611, 2004
25. Rudich A, Tirosh A, Potashnik R, et al.: Prolonged oxidative stress impairs insulin-induced GLUT4 translocation in 3T3-L1 adipocytes. *Diabetes* 47:1562–1569, 1998
26. Sakamoto T, Woodcock-Mitchell J, Marutsuka K, et al.: TNF-alpha and insulin, alone and synergistically, induce plasminogen activator inhibitor-1 expression in adipocytes. *Am J Physiol* 276:C1391–C1397, 1999
27. Potashnik R, Bloch-Damti A, Bashan N, et al.: IRS1 degradation and increased serine phosphorylation cannot predict the degree of metabolic insulin resistance induced by oxidative stress. *Diabetologia* 46:639–648, 2003
28. Lin Y, Berg AH, Iyangar P, et al.: The hyperglycemia-induced inflammatory response in adipocytes: the role of reactive oxygen species. *J Biol Chem* 280:4617–4626, 2005
29. Tobe M, Isobe Y, Tomizawa H, et al.: Discovery of quinazolines as a novel structural class of potent inhibitors of NF-kappa B activation. *Bioorg Med Chem* 11:383–391, 2003
30. Suganami T, Nishida, J, Ogawa, Y: A paracrine loop between adipocytes and macrophages aggravates inflammatory changes: role of free fatty acids and tumor necrosis factor α. *Arterioscler Thromb Vasc Biol* 25:2062–2068, 2005
31. Yue L, Mazzone T: Tumor necrosis factor alpha-mediated suppression of adipocyte apolipoprotein E gene transcription: primary role for the nuclear factor (NF)-kappaB pathway and NFkappaB p50. *Endocrinology* 149:4051–4058, 2008
32. Kim JK, Fillmore JJ, Chen Y, et al.: Tissue-specific overexpression of lipoprotein lipase causes tissue-specific insulin resistance. *Proc Natl Acad Sci U S A* 98:7522–7527, 2001
33. Kim JY, van de Wall E, Laplante M, et al.: Obesity-associated improvements in metabolic profile through expansion of adipose tissue. *J Clin Invest* 117:2621–2637, 2007
34. Boden G, She P, Mozzoli M, et al.: Free fatty acids produce insulin resistance and activate the proinflammatory nuclear factor-κB pathway in rat liver. *Diabetes* 54:3458–3465, 2005
35. Lewis GF, Carpentier A, Adeli K, et al.: Disordered fat storage and mobilization in the pathogenesis of insulin resistance and type 2 diabetes. *Endocr Rev* 23:201–229, 2002
36. Rasouli N, Raue U, Miles LM, et al.: Pioglitazone improves insulin sensitivity through reduction in muscle lipid and redistribution of lipid into adipose tissue. *Am J Physiol Endocrinol Metab* 288:E930–E934, 2005
37. Smith SR, De Jonge L, Volaufova J, et al.: Effect of pioglitazone on body composition and energy expenditure: a randomized controlled trial. *Metabolism* 54:24–32, 2005
38. Mazzone T, Meyer PM, Feinstein SB, et al.: Effect of pioglitazone compared with glimepiride on carotid intima-media thickness in type 2 diabetes: a randomized trial. *JAMA* 296:2572–2581, 2006
39. Bodles AM, Varma V, Yao-Borengasser A, et al.: Pioglitazone induces apoptosis of macrophages in human adipose tissue. *J Lipid Res* 47:2080–2088, 2006
40. Varma V, Yao-Borengasser A, Bodles AM, et al.: Thrombospondin-1 is an adipokine associated with obesity, adipose inflammation, and insulin resistance. *Diabetes* 57:432–439, 2005
41. Di Gregorio GB, Yao-Borengasser A, Rasouli N, et al.: Expression of CD68 and macrophage chemoattractant protein-1 genes in human adipose and muscle tissues: association with cytokine expression, insulin resistance, and reduction by pioglitazone. *Diabetes* 54:2305–2313, 2005