Hyperglycemia and advanced glycosylation end products suppress adipocyte apoE expression: implications for adipocyte triglyceride metabolism

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Espiritu DJ, Huang ZH, Zhao Y, Mazzone T. Hyperglycemia and advanced glycosylation end products suppress adipocyte apoE expression: implications for adipocyte triglyceride metabolism. Am J Physiol Endocrinol Metab 299: E615–E623, 2010. First published July 20, 2010; doi:10.1152/ajpendo.00273.2010.—Endogenous adipocyte apolipoprotein E (apoE) plays an important role in adipocyte lipoprotein metabolism and lipid flux. A potential role for hyperglycemia in regulating adipocyte apoE expression and triglyceride metabolism was examined. Exposure of adipocytes to high glucose or advanced glycosylation end product-BSA significantly suppressed apoE mRNA and protein levels. This suppression was significantly attenuated by antioxidants or inhibitors of the NF-κB transcription pathway. Hyperglycemia in vivo led to adipose tissue oxidant stress and significant reduction in adipose tissue and adipocyte apoE mRNA level. Incubation with antioxidant in organ culture completely reversed this suppression. Hyperglycemia also reduced adipocyte triglyceride synthesis, and this could be completely reversed by adenosine-mediated increases in apoE. To more specifically evaluate an in vivo role for adipocyte apoE expression on organinal triglyceride distribution in vivo, WT or apoE knockout (EKO) adipose tissue was transplanted in EKO recipient mice. After 12 wk, WT adipocytes transplanted in EKO mice accumulated more triglyceride compared with transplanted EKO adipocytes. In addition, EKO recipients of WT adipose tissue had reduced hepatic triglyceride content compared with EKO recipients transplanted with EKO adipose tissue. Our results demonstrate that hyperglycemia and advanced glycosylation end products suppress the expression of adipocyte apoE in vitro and in vivo and thereby reduce adipocyte triglyceride synthesis. In vivo results using adipose tissue transplantation suggest that reduction of adipocyte apoE, and subsequent reduction of adipocyte triglyceride accumulation, could influence lipid accumulation in nonadipose tissue.

adipocytes; adipose tissue; adipose tissue transplantation; hyperglycemia; apolipoprotein E; oxidant stress

Apolipoprotein E (apoE) is a multifunctional protein that is widely expressed in mammalian tissues. Specifically, this protein is a major product of cell types that experience large lipid fluxes as part of their differentiated function, for example, macrophages, steroidogenic cells, and adipocytes. In each of these cell types, it has also been demonstrated that apoE plays an important role in modulating lipid flux (1, 9, 11–13, 15, 18, 21, 27).

Adipocytes from apoE knockout (EKO) mice are smaller and contain less lipid compared with adipocytes from wild-type (WT) controls (8). In experimental models in which adipocytes are analyzed immediately after harvest from the in vivo milieu, it cannot be determined if these differences relate to the absence of apoE on circulating lipoproteins or the absence of endogenous adipocyte apoE expression. Recently, this issue has been clarified in two ways. First, EKO adipocytes differentiated from preadipocytes in vitro, and maintained in culture for 2 wk in the presence of triglyceride-rich lipoproteins (TGRL) that are enriched with apoE, remain smaller and lipid-poor compared with WT controls (8). Second, EKO adipocytes harvested from EKO adipose tissue that was transplanted in WT hosts for >12 wk remain smaller compared with WT adipocytes harvested from similarly transplanted WT adipose tissue (5). Therefore, even when exposed to a WT in vivo environment with a normal level of circulating apoE, lack of endogenous adipocyte apoE leads to smaller and lipid-poor adipocytes. Results from the above in vitro and in vivo experimental models support the conclusion that endogenous adipocyte apoE expression has a critical role in supporting adipocyte lipoprotein metabolism and lipid accumulation.

Circulating TGRL can be an important source of adipocyte lipid. The above results, therefore, point to a defect in EKO adipocytes that impairs acquisition of lipid from TGRL. This conclusion has been confirmed, and the mechanisms for impaired lipid acquisition from TGRL by EKO adipocytes have been examined in detail (7). Freshly isolated and cultured EKO adipocytes incubated with apoE-containing TGRL synthesize less triglyceride compared with WT adipocytes, and two distinct mechanisms have been shown to contribute to this decrease. First, EKO adipocytes express fewer cell surface very low density lipoprotein (VLDL) receptors. This reduction leads to significantly reduced TGRL binding and internalization by EKO adipocytes. Second, the absence of endogenous adipocyte apoE significantly reduces fatty acid internalization by adipocytes, and this impaired free fatty acid internalization interferes with acquisition of free fatty acids that are liberated from extracellular TGRL by the action of extracellular lipoprotein lipase. Both of the above changes in EKO adipocytes lead to the reduced availability of intracellular fatty acid to serve as substrate for adipocyte triglyceride synthesis.

The important role endogenous adipocyte apoE plays in adipocyte lipoprotein and lipid metabolism makes it important to understand factors that regulate its expression. The regulation of adipocyte apoE expression has been examined in detail and is consistent with a physiological role for endogenous apoE in adipocyte differentiated function. Peroxisome proliferator-activated receptor (PPAR)γ agonists, inflammatory cytokines, and peptide hormones regulate adipocyte apoE expression in both in vitro and in vivo models (3, 6, 8, 17, 24, 25). Diet-induced obesity suppresses adipocyte apoE expression, and this suppression is mediated by increased local production of reactive oxygen species (ROS) and inflammatory cytokines.
in adipose tissue (3, 6). Conversely, suppression of adipocyte apoE expression in obesity can be reversed by caloric deprivation or weight loss (6).

Hyperglycemia has been established as a potent stimulus for adipose tissue inflammation and oxidant stress in vitro and in vivo (10). The current studies were undertaken to evaluate whether hyperglycemia regulates adipocyte apoE expression in vitro and in vivo and to evaluate the implications of this regulation for adipocyte triglyceride metabolism.

MATERIALS AND METHODS

Materials. Cell culture media, FBS, and antibiotics were purchased from Invitrogen (Carlsbad, CA). Rabbit-derived rat apoE antibody was a gift from Dr. Catherine Reardon, University of Chicago. Receptor for advanced glycosylation end product (RAGE) neutralizing antibody was obtained from Biovision (Mountain View, CA). NF-κB activation inhibitor 6-amino-4-(4a-phenoxyphenyl)ethylamino)quinazoline (QNZ) was purchased from Calbiochem. Insulin, dexamethasone, 3-isobutyl-1-methylxanthine (IBMX), hydrogen peroxide, N-acetyl-l-cysteine (NAC), streptozotocin (STZ), and BSA were obtained from Sigma (St. Louis, MO). Liberase Blendzyme 3 was from Roche.

Cell culture. 3T3-L1 cells were obtained from American Type Culture Collection (Rockville, MD). Cells were maintained in 10% FBS-supplemented DMEM supplemented with penicillin and streptomycin in a 5% CO2 incubator at 37°C. Postconfluence (2 days), cells were differentiated by incubating cells in differentiation medium containing 0.5 mM IBMX, 0.2 μM dexamethasone, and 10 μg/ml insulin for 3 days. Cells were then placed in 25 mM glucose in DMEM containing 10 μg/ml insulin and 10% FBS for two more days. For experiments evaluating the effect of high glucose, cells were then incubated in medium containing 10% FBS in DMEM with 5.5 mM glucose before the start of the experimental incubations described in legends for Figs. 1–7. For advanced glycosylation end product (AGE)-BSA treatment, after differentiation, adipocytes were maintained in DMEM with 5.5 mM glucose in the presence of 10% FBS until the start of experimental incubations. NF-κB pathway involvement was assessed by using the NF-κB pathway inhibitor QNZ (10 mM). Treatment with the general antioxidant NAC (20 mM) was added to assess a role for oxidant stress. apoE expression in cultured adipocytes was induced using adenoviral transduction as previously described (8).

Animals and isolation of adipose tissue and primary mature adipocytes. The animal protocol used was approved by the Institutional Animal Care and Use Committee of the University of Illinois at Chicago. Male C57Bl/6J aged 12 wk were from Jackson Laboratories (Bar Harbor, ME). Hyperglycemia was induced in mice by a single intraperitoneal injection of STZ (200 mg/kg body wt) freshly dissolved in citrate buffer (pH = 4.5). Control mice were injected with citrate buffer only. Blood glucose and body weight were measured at baseline and daily. After STZ treatment (48 h), blood glucose concentration exceeded 300 mg/dl in STZ-treated mice. At that time, the intra-abdominal fat pads were harvested from STZ- and control mice. Adipose tissue was divided for measurement of ROS, isolation of RNA, isolation of mature adipocytes, or use in organ culture. Isolation of mature adipocytes and adipose tissue organ culture was performed as previously described in detail (3). Male EKO mice and WT controls on the C57Bl/6J background were from previously described sources (5, 7). The transplantation of EKO or WT adipose tissue into EKO recipients was performed as previously described in detail (3). Mature adipocytes were isolated from transplanted adipose tissue 12 wk after transplantation as previously described (3, 5). Transplant recipients were fasted for 6 h before harvest of transplanted adipose tissue and liver. Sizing of mature adipocytes isolated from adipose tissue was performed as previously described (5). Hematoxylin–eosin-stained sections of paraffin-embedded adipose tissue (10 μm thickness) were prepared as previously described (5, 8).

AGE-BSA preparation. AGE-BSA was prepared by incubating 50 mg/ml BSA (fraction V, fatty acid-free endotoxin-free) with 0.5 mM α-glucose and 5 mM diethylenetriamine-pentaacetic acid in 0.2 phosphate buffer, pH 7.4, for 8 wk at 37°C. Unincorporated sugar was removed by dialysis against PBS. BSA incubated under similar conditions in the absence of α-glucose was used as control.

mRNA quantitation. Total RNA was isolated from adipose tissue or from adipocytes using the RNeasy mini kit (Qiagen, Valencia, CA). First-strand cDNA was synthesized from 1 μg of total RNA using the Thermoscript RT-PCR System (Invitrogen) according to the manufacturer’s instructions. Real-time PCR was performed on each sample in triplicate using the Mx3000p Quantitative PCR system (Stratagene, La Jolla, CA) using iTaq SYBR Green Supermix with ROX. Quantity of apoE mRNA was calculated after correction with β-actin mRNA and expressed as apoE mRNA quantity relative to control. The primer pairs used for amplification of apoE, CD68, and β-actin genes were 5'-AGGGTGGGAAGAAAGGTAAAGC-3', 5'-GGCGATGCATGTGTTCACACTA-3'; 5'-AGGGTGGGAAGAAAGGTAAAGC-3', 5'-AGGGTTGGAAGAAAGGTAAAGC-3'; and 5'-GGCCCAAGACGAGAGGTTA-3', 5'-GGACTCATCAGTCTCTGCT-3', respectively. Primer pairs for murine IL-6 are 5'-CTCCTTGGGAGCTATGCTGGT-3' and 5'-GCCATTGCGCAACTCTTTCCTC-3'.

Western blot and lipid measurements. For Western blotting, 50 μg of protein were subjected to SDS-PAGE, transferred to nitrocellulose membrane, and probed with antibodies for apoE as previously described in detail (8). Adipocyte total lipid content was estimated (based on adipocyte size), and measurement of the rate of adipocyte triglyceride synthesis using [14C]oleic acid precursor was performed as previously described (5, 7). Measurement of liver triglyceride mass as milligram triglyceride per milligram DNA was performed using an enzymatic assay kit from Wako Chemicals (5, 7).

Measurement of intracellular ROS generation. Tissue H2O2 was measured using the fluorescent dye 5-(and-6)-chloromethyl-2′,7′-dichlorofluorescein deacetate acetyl ester (CM-H2DCFDA; Molecular Probes) as previously described (3) with some modifications. Fat pads from hyperglycemic or control mice were cut into 1-mm pieces and incubated with 2 μM CM-H2DCFDA for 45 min at 37°C in phenol-free, low-glucose DMEM. Fluorescence was analyzed using a BIOTEC Synergy H Fluorescent plate reader at an excitation wavelength of 485 nm and emission at 530 nm. After ROS concentration was determined, total protein was extracted, quantitated, and used for fluorescence correction.

Statistical analysis. Statistical differences between experimental groups were evaluated using Student’s t-test. P values of <0.05 were considered significant. All data are expressed as means ± SD for triplicate samples as indicated in the legends for Figs. 1–7. The experiments shown are representative of two to three experiments with similar results unless otherwise indicated.

RESULTS

In the first series of experiments, we evaluated the impact of elevated glucose levels on apoE mRNA and protein expression in 3T3-L1 adipocytes. Cells maintained in 5.5 mM glucose for 6 days were incubated for an additional 2 days in fresh medium with the same glucose concentration or containing 25 mM glucose. At the end of this incubation, apoE mRNA levels were reduced by almost 80% in the cells incubated in high glucose. apoE protein expression was similarly reduced by 60% (Fig. 1).

Hyperglycemia has been shown to increase production of ROS by adipocytes, and we have previously shown that oxidative stress and ROS can reduce adipocyte apoE expression in vitro and in vivo (3, 10). In the next series of experiments, we evaluated a role for ROS in mediating the suppression of
adipocyte apoE expression during incubation in high glucose by inclusion of the antioxidant NAC. As shown in Fig. 2, inclusion of NAC abrogated the suppression of apoE mRNA (Fig. 2A) level and protein (Fig. 2B) expression by high glucose. One common pathway by which ROS regulate gene expression involves NF-κB-mediated transcriptional regulation (2, 4, 19). We have previously shown that adipocyte apoE gene transcription is regulated by the NF-κB complex, that activation of this pathway suppresses apoE gene transcription, and have identified the specific gene response element and NF-κB transcription complex subunit proteins involved (24).

In that same series of experiments, we also showed that suppression of apoE gene transcription mediated by NF-κB activation can be specifically prevented by the NF-κB pathway inhibitor QNZ. The results shown in Fig. 2, C and D, demonstrate that the suppression of adipocyte apoE expression by high glucose can be at least partially eliminated by inhibition of the NF-κB pathway with QNZ.

The results in Figs. 1 and 2 demonstrate regulation of adipocyte apoE by incubations in high glucose of relatively short duration. To evaluate a potential pathway by which chronic hyperglycemia might also impact adipocyte apoE gene expression, we evaluated a role for AGE. AGE-modified proteins accumulate in tissues during chronic hyperglycemia in vivo and have already been shown to produce proinflammatory and oxidant stress responses in monocyte/macrophages and endothelial cells (23). AGE-modified proteins have also been shown to promote carbonyl stress and production of ROS in adipocytes (20). Accordingly, AGE-BSA was prepared, and its effect on adipocyte apoE expression was compared with unmodified BSA. The results in Fig. 3 show that incubation of 3T3-L1 cells with AGE-BSA led to an approximate 60% reduction in apoE mRNA level (Fig. 3A) and protein level (Fig. 3B). AGE-BSA also stimulated IL-6 mRNA expression in adipocytes compared with control BSA by 16-fold (P < 0.03, not shown). The involvement of ROS and the NF-κB pathway was further investigated as done in the experiments presented in Fig. 2. Figure 4 shows that inclusion of the antioxidant NAC (Fig. 4A) or the NF-κB pathway inhibitor QNZ (Fig. 4B) eliminated the ability of AGE-BSA to reduce adipocyte apoE expression.

AGE-modified protein binds with high affinity to the RAGE, and RAGE expression has been reported in adipocytes (20). However, AGE proteins can also bind to other cell surface receptors, including some that are highly expressed in adipocytes such as CD36 (14). It, therefore, was of interest to determine which adipocyte receptor was involved in regulating adipocyte apoE gene expression. C57Bl/6 mice were made hyperglycemic by a single injection of STZ; control mice were injected with vehicle only. Glucose level was monitored daily, and mice were used within 24 h of glucose levels exceeding 300 μg/dl in the STZ-treated group. Table 1 shows the initial and final body weights for the control and STZ-treated mice, as well as blood glucose level at these times. At the time of adipose tissue isolation, glucose was significantly higher in STZ-treated mice, and body weight was significantly lower compared with control mice. Levels of intracellular ROS in freshly isolated adipose tissue from control and STZ mice are compared with control mice. Levels of intracellular ROS in freshly isolated adipose tissue from control and STZ mice are compared with control mice. Levels of intracellular ROS in freshly isolated adipose tissue from control and STZ mice are compared with control mice. Levels of intracellular ROS in freshly isolated adipose tissue from control and STZ mice are compared with control mice.
Freshly isolated adipose tissue was also used for measurement of apoE and CD68 mRNA level. The latter serves as a marker reflecting macrophage infiltration in adipose tissue. As shown in Fig. 5B, hyperglycemia produced a 70% reduction in adipose tissue apoE mRNA level and an approximate fourfold increase in adipose tissue abundance of the macrophage marker CD68. To determine if the reduction in the adipose tissue apoE mRNA level could be specifically ascribed to adipocytes vs. adipose tissue macrophages that also express apoE, we also measured apoE mRNA in mature adipocytes isolated from adipose tissue. Hyperglycemia in vivo produced a 80% reduction in mature adipocyte apoE mRNA level (Fig. 5C).

In view of the in vitro role of ROS for regulating adipocyte apoE expression, and the elevated levels of ROS we measured as a result of in vivo hyperglycemia, we next determined if incubation of adipose tissue with the antioxidant NAC could reverse the suppression of apoE. For the experiments shown in Fig. 5C, adipose tissue from five control and five diabetic mice was freshly isolated and maintained in organ culture over 6 h. Some cultures included the antioxidant NAC. As shown, inclusion of NAC had no effect on apoE mRNA abundance in adipose tissue isolated from normoglycemic control mice but approximately doubled apoE mRNA level in adipose tissue harvested from hyperglycemic mice.

We next performed a series of experiments to evaluate the implications of the hyperglycemia-mediated reduction of adipocyte apoE expression for adipocyte triglyceride synthesis. Figure 6A shows that 3T3-L1 adipocytes incubated in high glucose synthesize significantly less triglyceride compared with those incubated in lower glucose. This result is consistent with previous observations demonstrating that EKO adipocytes synthesize less triglyceride compared with WT adipocytes (8). However, because hyperglycemia may have additional effects on adipocytes (i.e., separate from suppression of apoE) that could influence triglyceride synthesis, we performed experiments to determine if the hyperglycemia-induced reduction in triglyceride synthesis could be reversed by restoring adipocyte apoE expression. Figure 6B shows the results of a Western blot
measuring apoE expression in adipocytes incubated in low or high glucose and transduced with an adenovirus expressing LacZ or apoE as indicated, and shows restoration of high physiological apoE expression by the apoE adenovirus in adipocytes incubated in high glucose. Figure 6C shows that this increase in apoE expression in adipocytes incubated in high glucose significantly increases adipocyte triglyceride synthesis, indicating that suppression of endogenous adipocyte apoE expression significantly contributes to the decreased triglyceride synthesis observed in adipocytes incubated in high glucose. Reduced endogenous adipocyte apoE expression has been shown to produce multiple abnormalities in adipocyte triglyceride metabolism (3, 5, 7, 8). In aggregate, the above results indicate that hyperglycemia produces adipose tissue oxidant stress, and thereby suppresses endogenous adipocyte apoE expression, which then reduces adipocyte triglyceride synthesis. Reduced adipocyte apoE expression leads to reduced adipocyte triglyceride accumulation because of defects in adipocyte internalization of fatty acid and TGRL (7, 8). Poorly controlled diabetes, along with other pathophysiological conditions associated with adipose tissue oxidant stress (e.g., obesity), are characterized by reduced apoE expression in adipocytes and the ectopic deposition of fat in liver and muscle. We, therefore, utilized an adipose tissue transplantation model to evaluate whether endogenous apoE expression in adipose tissue could alter the in vivo distribution of triglyceride between adipose and nonadipose tissue. To address this question, we transplanted WT or EKO adipose tissue in EKO recipients. After 12 wk, we evaluated triglyceride parameters in transplanted adipocytes and in the liver and muscle of transplant recipients. The EKO recipients of transplanted EKO or WT adipose tissue maintained similar levels of circulating triglyceride and cholesterol throughout the experimental period (data not shown). After transplantation (12 wk), mice were euthanized, and the transplanted adipose tissue, liver, and soleus muscle were collected for analysis. Freshly isolated transplanted WT adipocytes were larger, had higher total lipid content, and increased rates of triglyceride synthesis compared with similarly transplanted EKO adipocytes (Fig. 7, A–D). Figure 7E shows that transplantation of WT adipocytes in EKO mice had no impact on the content of triglyceride in the soleus muscle of recipient mice but led to a significant decrease in hepatic triglyceride content. The above results support the notion that the endogenous expression of adipocyte apoE favors accumulation of triglyceride in adipocytes and thereby reduces its deposition in the liver.

**DISCUSSION**

The results in this manuscript establish that hyperglycemia in vitro and in vivo, or the AGE-modified proteins that accumulate in tissues under conditions of chronic in vivo hyperglycemia, can suppress the expression of adipose tissue and adipocyte apoE. Hyperglycemia has been shown to increase the endogenous generation of ROS in adipose tissue both in vitro and in vivo and thereby promote a proinflammatory program of gene expression in adipose tissue and adipocytes by activating the NF-κB transcription complex (2–4, 10, 19). Our data show that antioxidants or inhibitors of the NF-κB pathway eliminate hyperglycemia-mediated or AGE-mediated suppression of adipocyte apoE. AGE proteins have been shown to bind to specific receptors, including RAGE, and to activate second messenger pathways involved in inflammatory gene regulation (16, 22). Adipocytes express a number of receptors that can bind AGE proteins, including RAGE and CD36 (20). The data in Fig. 4, C and D, show that 6 h incubation in AGE-BSA significantly suppresses adipocyte apoE mRNA expression but that inclusion of a neutralizing antibody for RAGE completely abrogates the suppression, establishing a primary role for RAGE.

Our data also indicate that hyperglycemia suppresses adipocyte triglyceride synthesis. Although such suppression could relate to multiple effects of elevated glucose on adipocyte metabolism, our data clearly establish a role for suppressed apoE for mediating this suppression, since the increased expression of adipocyte apoE using adenoviral transduction significantly reverses hyperglycemia-mediated suppression of adipocyte triglyceride synthesis. The data in this report also support the notion that factors that suppress endogenous adipocyte apoE expression, like hyperglycemia or obesity (6), will...
suppress adipocyte triglyceride accumulation in vivo and favor its distribution to nonadipose tissue. After transplantation of WT or EKO adipose tissue in EKO recipients, and an additional 12 wk in an identical in vivo environment, transplanted WT adipocytes accumulate more triglyceride than transplanted EKO adipocytes. Furthermore, transplant recipients receiving WT adipose tissue have significantly less triglyceride in their liver.

Because the major source of adipocyte triglyceride comes from circulating TGRL, results from both in vitro and in vivo experimental models indicate that EKO adipocytes are less efficient in acquiring lipid from these lipoproteins (5, 7, 13). There are two major pathways by which adipocytes acquire lipid from circulating TGRL. One pathway involves endocytic internalization and lysosomal processing of entire TGRL particles after they bind to cell surface receptors. The second, and likely more important pathway, involves hydrolysis of the triglyceride core of TGRL by extracellular lipoprotein lipase that is produced and secreted by adipocytes. This hydrolysis releases free fatty acids that can be internalized by adipocytes to drive intracellular triglyceride synthesis. Both of the above pathways are defective in EKO adipocytes (7). Detailed mechanistic studies have shown that EKO adipocytes express fewer cell surface VLDL receptors leading to a substantial reduction in the binding and internalization of TGRL. In addition, even though EKO adipocytes produce and secrete normal amounts of lipoprotein lipase, they display a defect in the internalization

Table 1. Body weight and blood glucose in control and STZ-treated mice

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<td>Control (n = 5)</td>
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Values are means ± SE; n, no. of mice. STZ, streptozotocin. *P < 0.01 for control vs. STZ mice.

Fig. 4. Pathways mediating regulation of adipocyte apoE gene expression by AGE. Control or AGE-BSA (0.3 mg/ml) was added to mature 3T3-L1 cells in the presence or absence of NAC (A) or QNZ (B) for 18 h before harvest of cells for measurement of apoE mRNA. C: AGE-modified BSA was added to mature 3T3-L1 cells for the times indicated before measurement of apoE mRNA. D: cells were incubated with control or AGE-BSA (0.3 mg/ml) for 6 h with or without inclusion of a neutralizing antibody for the receptor for advanced glycosylation end product (RAGE). Values shown are means ± SD of triplicate samples. *P < 0.05 for control compared with AGE-BSA. #P < 0.01 for the effect of NAC, QNZ, or the RAGE neutralizing antibody.
of extracellular fatty acids that is associated with a significant reduction in cell surface caveolae (7).

The demonstration that hyperglycemia and AGE-modified proteins regulate adipocyte apoE expression expands the list of physiologically relevant regulators of adipocyte apoE expression. The regulatory pathways by which hyperglycemia and AGE-BSA regulate adipocyte apoE expression are similar to those involved in its regulation by obesity (3, 6) and likely involve endogenous generation of ROS and activation of the NF-κB transcription pathway (3, 24); Lin et al. (10) have

Fig. 5. Hyperglycemia in vivo suppresses adipose tissue and adipocyte apoE expression. Freshly isolated adipose tissue (A–C) or adipocytes (B) from control or streptozotocin (STZ)-treated diabetic mice was used for measurement of reactive oxygen species (ROS; A) or of apoE and CD68 mRNA levels (B). C: freshly collected adipose tissue from control or STZ diabetic mice was maintained in organ culture in the presence or absence of 20 mM NAC for 6 h. At that time, the tissue was washed with PBS, and apoE mRNA levels were measured. Results shown are means ± SD of adipose tissue collected from five separate mice. *P < 0.01 for the difference between control and diabetic mice. #P < 0.01 for the effect of NAC.

Fig. 6. Impact of hyperglycemia-induced apoE suppression on adipocyte triglyceride (Tg) synthesis. A: 3T3-L1 adipocytes were incubated in high or low glucose as described in the legend to Fig. 1. At the end of this incubation, triglyceride synthesis was measured during a 2-h incubation in [14C]oleic acid as described in MATERIALS AND METHODS. B: 3T3-L1 cells were incubated in low glucose or high glucose and transduced with an adenovirus expressing LacZ or apoE as indicated. Western blot for cellular apoE was performed as described in MATERIALS AND METHODS. C: 3T3-L1 cells were incubated in high or low glucose with the indicated adenovirus exactly as done in B and then used for measurement of triglyceride synthesis. *P < 0.05 and **P < 0.01.

OA, oleic acid.
shown that hyperglycemia increases generation of ROS and IL-6 in adipocytes. We have previously identified the apoE gene regulatory element mediating the suppression of adipocyte apoE in response to inflammatory cytokines as an NF-κB binding site and have identified the protein subunits of the NF-κB transcription complex responsible for suppressing apoE gene transcription (24). In that same series of experiments, we demonstrated that QNZ acting at this site prevented tumor necrosis factor-α suppression of adipocyte apoE. Other physiologically relevant factors that regulate adipocyte apoE expression include caloric deprivation and weight loss, and PPARγ agonists, both of which substantially increase adipocyte apoE expression in vitro and in vivo (6, 25, 26).

Results in this and previous reports show that obesity or hyperglycemia suppresses adipocyte apoE expression and that this suppression results from the adipose tissue oxidative stress associated with these conditions. Endogenous apoE expression is important for efficient acquisition of triglyceride from extracellular TGRL, and reduced adipocyte apoE expression leads to reduced internalization of TGRL and free fatty acids by adipocytes. Because of this, the suppression of adipocyte apoE expression in obesity and hyperglycemia could play an...
important role favoring the partitioning of TGRL lipid away from adipocytes and adipose tissue and into other tissues, thereby contributing to the ectopic fat deposition that can accompany glycemic decompensation or obesity in either type 1 or type 2 diabetes. In this way, endogenous adipocyte apoE could function as an important molecular link mediating cross talk between organismal nutritional/metabolic state and lipid trafficking between adipose and nonadipose tissues.

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DISCLOSURES

The authors have nothing to disclose.

REFERENCES


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