Adiponectin abates diabetes-induced endothelial dysfunction by suppressing oxidative stress, adhesion molecules, and inflammation in type 2 diabetic mice

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Lee S, Zhang H, Chen J, Dellsperger KC, Hill MA, Zhang C. Adiponectin abates diabetes-induced endothelial dysfunction by suppressing oxidative stress, adhesion molecules, and inflammation in type 2 diabetic mice. Am J Physiol Heart Circ Physiol 303:H106–H115, 2012; First published May 4, 2012; doi:10.1152/ajpheart.00110.2012.—Adiponectin (APN), TNF-α/H9251, and leptin (42). APN is a 30-kDa circulating protein exclusively secreted by adipose tissue that plays a protective role against the development of diabetes and atherosclerosis (17). APN may inhibit both the inflammatory process and atherogenesis by suppressing the migration of monocytes/macrophages and their transformation into macrophage foam cells within the vascular wall (29, 36, 37). In animal models, APN deficiency is associated with increased inflammatory responses under conditions of stress and pathological conditions such as seen in diabetic patients as well as in rodent models of diabetes (18, 23, 31, 44). In addition, numerous epidemiological studies have shown that circulating APN levels are inversely correlated with CVD, and clinical observations have demonstrated that reduced APN is associated with impaired endothelial-dependent vasodilation (17, 38, 45, 46).

In obesity, increased macrophage infiltration into white adipose tissue and elevated chemoattractant gene expression contribute to the development of atherosclerosis (2). Since the progression of atherosclerosis partially depends on the interaction between the endothelium and monocytes, it is important to understand that several adhesion molecules, such as ICAM-1, play a key role in the recruitment of leukocytes and subsequent transmigration into the intima (21). ICAM-1 is constitutively expressed in endothelial cells and is upregulated in human atherosclerotic lesions (1, 41).

Atherosclerosis is considered a chronic inflammatory disease involving a complex interplay between vascular cells and infiltrating immune cells (e.g., macrophages) initiating endothelial dysfunction followed by inflammatory cell infiltration and lipid accumulation (16, 29, 30). Macrophages infiltrating into endothelial and smooth muscle cells secrete several pro-inflammatory cytokines, such as TNF-α, IL-6, and interferon (IFN)-γ, which can exacerbate the risk of atherosclerotic vascular diseases (22, 30).

APN plays an important role in glycemic control, hypertension, and atherosclerosis (31, 34). However, the role of APN in diabetes-induced endothelial dysfunction and its relationship to the inflammatory state have not been elucidated. Therefore, in this study, we tested the hypothesis that exogenous adenoviral delivery of APN will restore endothelial function in the aorta from db/db mice, a mouse model of type 2 diabetes.

METHODS

Materials. Adenovirus vector containing the gene for full-length mouse APN (Ad-APN) was generously gifted by Dr. Shinji Kihara (Department of Internal Medicine and Molecular Science, University of Osaka, Osaka, Japan). Human embryonic kidney (HEK)-293 AD cells were provided by Dr. Christopher P. Baines (Department of Biomedical Sciences, University of Missouri, Columbia, MO) and Dr. Maike Krenz (Department of Medical Pharmacology and Physiology, University of Missouri, Columbia, MO).
Mouse model of type 2 diabetes. All experimental protocols were approved by the Animal Care Committee of the University of Missouri (Columbia, MO). Fourteen- to fifteen-week-old male heterozygous control (CON) mice (m Lepr°; background strain: C57BLKS/J) and homozygous type 2 diabetic (db/db) mice (Lepr°; background strain: C57BLKS/J) were purchased from Jackson Laboratory (Bar Harbor, ME) and housed in an animal facility equipped with 12:12-h light-dark cycles and allowed free access to normal chow and water. In addition, APN knockout (APNKO) mice (background strain: C57BL/6J) and control age-matched wild-type (WT) mice (C57BL/6J) were purchased from Jackson Laboratory. At 6 wk of age, WT and APNKO mice were placed on either a normal chow diet with 17% of calories from fat or a high-fat (HF) diet with 60% calories from fat to induce obesity and insulin resistance (D12492, Research Diets, New Brunswick, NJ) for 10 wk. Only male mice were used for experiments. Body weights and nonfasting blood glucose levels, determined using a commercial One Touch UltraSmart glucometer (Lifespan, Milpitas, CA), were recorded before euthanization.

Adenovirus-mediated gene transfer. Adenoviruses were amplified using HEK-293 AD cells and purified using commercial adenovirus kits. HEK-293 AD cells were passaged two to three times before infection and cultured until the floating monolayer was 90–100% confluent. Cells were replated with 15 ml new growth media containing 10% FBS per 75-cm² flask with either Ad-AP or adenovirus containing β-galactosidase (Ad-β-gal) added to the culture. After 24 h, 10 ml growth media was added to the culture flask, and the viruses allowed to grow for another 24 h. When all cells were floating, the culture flask was gently shaken, and all media, including the cells, were transferred to a 50-ml sterile tube, which was centrifuged at 1,000 g for 5 min. The cell pellet was then resuspended in 0.8 ml culture medium. The adenoviruses were released from the cells by repeated freeze-thaw cycles using liquid nitrogen. After centrifugation at 10,000 g for 10 min, the supernatant was collected for immediate purification of adenoviruses using a commercial purification kit (Cell Biolabs, San Diego, CA) as specified by the company’s protocols. The concentration of purified adenovirus was measured using the commercial QuickTiter Adenovirus Titer Immunoassy Kit (Cell Biolabs) according to the manufacturer’s instructions. Either Ad-AP or Ad-β-gal at 1 × 10⁹ plaque-forming units was injected intravenously into the tail vein of CON and db/db mice. All mice were euthanized (7 days after virus injection). Mice that did not show an increase in serum APN levels (as measured by ELISA or Western blot analysis) were excluded from both the functional assessment and molecular analyses.

Functional assessment of the aorta. Endothelial function was assessed as previously described in detail (23). In brief, under anesthesia, aortas were rapidly excised and rinsed in cold physiological saline solution (PSS) containing (in mM) 118.99 NaCl, 4.69 KCl, 1.18 KH₂PO₄, 1.17 MgSO₄·7H₂O, 2.50 CaCl₂·2H₂O, 14.9 NaHCO₃, 5.5 d-glucose, and 0.03 EDTA. Fat and connective tissue surrounding the aorta were carefully removed using small forceps and spring scissors. Aortas were maintained in PSS bubbled with 95% O₂-5% CO₂ at 37°C for the entire experiment. Aortic rings, at 2 mm in length, were isometrically mounted in a multi channel myograph (model 610M, DMT). After a 15-min equilibration period, an optimal passive tension (80 ± 10 mN) was applied and followed by 30 min of equilibration. Aortic rings were precontracted with 1 µmol/l phenylephrine. Concentration-response curves were obtained by a cumulative addition of either the endothelial-dependent vasodilator ACh (1 µmol/l–10 µmol/l) or the endothelial-independent vasodilator sodium nitroprusside (SNP; 1 µmol/l–10 µmol/l). Relaxation at each concentration was measured and expressed as the percentage of force generated in response to phenylephrine. The contribution of nitric oxide (NO) to vasodilation was assessed by incubating aortic rings with the NO synthase inhibitor N²-nitro-l-arginine methyl ester (l-NAME; 100 µmol/l, 20 min).

Measurement of blood parameters. After animals were anesthetized with pentobarbital sodium (50 mg/kg ip), blood samples were obtained from the vena cava. A whole blood sample was held for 30 min at room temperature to allow clotting. The sample was centrifuged at 2,000–3,000 g for 10 min at 4°C; the serum was transferred in separate tubes without disturbing blood clots and stored at −80°C until analysis. Serum insulin and APN levels were measured with commercial ELISA kits (Millipore, Billerica, MA). Similarly, total cholesterol and triglyceride levels were assessed using spectrophotometric assays (Biovision, Milpitas, CA) according to the manufacturer’s instructions.

Determinations of mRNA levels. A quantitative PCR technique was used to analyze levels of mRNA expression for TNF-α, IL-6, ICAM-1, APN, endothelial NO synthase (eNOS), and VCAM-1. In brief, total RNA was isolated from aortas of CON and db/db mice using the fibrous tissue minikit (Qiagen, Valencia, CA) according to the manufacturer’s instructions. The quality and quantity of total RNA were determined using a Nano Drop ND-1000 Spectrophotometer (Nano Drop Technologies, Wilmington, DE). RNA was processed directly to cDNA using SuperScript III First-Strand Synthesis SuperMix (Invitrogen, Grand Island, NY). Quantitative real-time PCR analyses were performed using an i-Cycler (I-Q5, Bio-Rad Laboratories, Hercules, CA). Reactions were carried out in triplicate in a total volume of 25 µl using SYBR green qPCR Master Mix (Invitrogen). The 2−ΔΔCT method (ΔΔCT = CTtarget gene − CTβ-actin) was used to analyze the change of target gene expression. The housekeeping gene β-actin was used for internal normalization. Mean CT values for both the target and internal control genes were determined. Data are presented as fold changes of transcripts for the target gene normalized to β-actin compared with CON mice. Primers were designed to amplify mouse TNF-α (forward primer: 5′-GTCCTCCAAAGGATAGAGA-3′ and reverse primer: 5′-CATTCTGTTGGTGTCTAGCA-3′), mouse IL-6 (forward primer: 5′-CCGGAGAGAGACTTCACAG-3′ and reverse primer: 5′-TCCACCAGATTTCGCCAGAAC-3′), mouse ICAM-1 (forward primer: 5′-CTCCGTGGGGAGGAGATACT-3′ and reverse primer: 5′-TGCGCCCTCCGAGACATTAG-3′), mouse APN (forward primer: 5′-AGGTGGTAGTCGACCCG-3′ and reverse primer: 5′-GTTCCTACCTTGACTAAGCAAAGA-3′), mouse eNOS (forward primer: 5′-TGCCTTGAGAGATGTGATG-3′ and reverse primer: 5′-CTGAGAACTCTGTCAGGGGGGAC-3′), and mouse VCAM-1 (forward primer: 5′-GGCTGACCTGACTCCTGGTG-3′ and reverse primer: 5′-TCTCAGCTTCGGA-3′). All primers were obtained from Invitrogen.

Immunofluorescence staining. Immunohistochemistry was used to identify protein localization in sections of aortas. Aortas were embedded and sectioned at 5 µm thick. Slides were observed and analyzed using a microscope (model IX81, Olympus) equipped for fluorescence and a 40 objective (numerical aperture: 0.95).

Measurement of protein expression by Western blot analysis. For Western blot analyses, aortas were separately homogenized and sonicated in 200 µl lysis buffer (Cellytic MT Mammalian Tissue Lysis/ Extraction Reagent, Sigma-Aldrich, St. Louis, MO) with protease and phosphatase inhibitors (Sigma-Aldrich) at 1:100 ratios, respectively. Protein concentrations were assessed using the BCA protein assay kit (ThermoScientific, Rockford, IL). Equal amounts of protein (5 or 10 µg) were separated by 10% or 7.5% SDS-PAGE and transferred to polyvinylidene difluoride or nitrocellulose membranes (Bio-Rad). Membranes were blocked in 5% nonfat milk in PBS with 0.1% Tween 20.
20 or chemiluminescent blocker (Millipore) at room temperature for 1 h. Expression of SOD-1, SOD-3, APN, IFN-γ (Millipore, 1:500), gp91phox (BD Transduction Laboratories, San Jose, CA, 1:1,000), p47phox (Santa Cruz Biotechnology, Santa Cruz, CA, 1:100), nitrotyrosine (Abcam, 1:500), and β-actin protein was detected by Western blot analysis with the following primary antibodies at the given dilutions: SOD-1 (Calbiochem, La Jolla, CA, 1:1,000), SOD-3 (Stressgen, Ann Arbor, MI, 1:1,000), APN (R&D, 1:1,000), IFN-γ (Millipore, 1:500), gp91phox (BD Transduction Laboratories, San Jose, CA, 1:1,000), p47phox (Santa Cruz Biotechnology, Santa Cruz, CA, 1:100), nitrotyrosine (Abcam, 1:500), and β-actin (Abcam, 1:2,000). Horseradish peroxidase (HRP)-conjugated secondary antibodies (1:2,000) were used, and signals were visualized by ECL (Bio-Rad) or Luminata Forte Western HRP Substrate (Millipore). Blots were then scanned with a Fuji LAS 3000 densitometer. Relative amounts of protein expression were quantified and normalized to those of the corresponding internal reference, β-actin, and then subsequently normalized to corresponding CON mice, which were set to a value of 1.0. The specificity of all antibodies has been demonstrated in our previous studies (4, 23, 40, 51).

Statistical analysis. All values are presented as means ± SE. Statistical comparisons of vasomotor responses, with various treatments, were analyzed using ANOVA with repeated measures. Statistical significance of differences among groups, for relative mRNA and protein content, was determined by ANOVA for multiple comparisons. Post hoc analysis was performed using a Fisher’s least-significant-difference analysis. Statistical differences were considered significant at the P < 0.05 probability level.

Fig. 1. Basic characteristics and serum parameters of mice. A–E: body weight (A; n = 7–9), nonfasting blood glucose (B; n = 4–5), insulin (C; n = 4–5), homeostasis assessment model-insulin resistance (HOMA-IR; D; n = 4–5), and total cholesterol (E; n = 6) in control (CON) and diabetic (db/db) mice treated with either control adenovirus containing β-galactosidase (Ad-βgal; CON+βgal and db/db+βgal) or adenovirus containing adiponectin (Ad-APN; CON+APN and db/db+APN). Ad-βgal mice were higher in body weight and showed higher nonfasting blood glucose, insulin, and HOMA-IR compared with CON mice. Ad-APN did not alter these parameters in both CON and db/db mice. F: serum triglyceride (n = 7–11) levels were significantly increased in the db/db+βgal group compared with CON groups (CON+βgal and CON+APN). Ad-APN significantly reduced triglyceride levels in db/db mice. Data are means ± SE. *P < 0.05 vs. the CON+βgal group; +P < 0.05 vs. the CON+APN group; #P < 0.05 vs. the db/db+βgal group.

Fig. 2. Ad-APN increased APN in serum and the aorta. A: ELISA was performed in CON and db/db mice treated with either Ad-βgal or Ad-APN using serum. Sera were collected 7 days after adenovirus treatment. The results show that APN in serum was increased four- to fivefold in CON and db/db mice. n = 8–9 mice/group. B: protein levels of APN in the aorta were significantly decreased in db/db mice, and Ad-APN treatment increased protein levels in both CON and db/db aortas. n = 4–5 mice/group. Data are means ± SE. *P < 0.05 vs. the CON+βgal group; +P < 0.05 vs. the CON+APN group; #P < 0.05 vs. the db/db+βgal group.
RESULTS

Effects of APN on body weight, hyperglycemia, insulin resistance, and hyperlipidemia. To test whether APN is involved in the maintenance of body weight and glucose and lipid metabolism, we systemically delivered Ad-APN or Ad-βgal as a control into db/db and CON mice. Diabetic mice exhibited severe obesity, hyperglycemia, insulin resistance, and hyperlipidemia (Fig. 1, A–F). Ad-APN did not alter body weight (Fig. 1A), hyperglycemia (Fig. 1B), insulin resistance (Fig. 1D), or total cholesterol (Fig. 1E) in both CON and db/db mice. Ad-APN did not change serum triglyceride levels in CON mice but did decrease serum triglyceride levels in db/db mice (Fig. 1F).

APN levels. One week after vector injection, serum APN levels were 10.40 ± 0.38 μg/ml in Ad-βgal-treated CON mice,
muscle cells within the aorta (Fig. 3, cells from all groups (data not shown). In addition, APNKO endothelial cells (Fig. 3, cell marker). We confirmed that APN was localized within aortas (Figs. 2 and decreased APN protein levels compared with CON aortas showed impaired endothelial-dependent vasodilation As we have previously reported (23, 52), muscle cells within the aorta was visualized by staining APN the circulating bloodstream generated form, not by, endogenous production of APN within the aorta.

Colocalization of APN in endothelial cells of the mouse aorta. Colocalization of APN with endothelial or smooth muscle cells within the aorta was visualized by staining APN with vWF (endothelial cell marker) or α-actin (smooth muscle cell marker). We confirmed that APN was localized within endothelial cells (Fig. 3, A–L) and not aorta smooth muscle cells from all groups (data not shown). In addition, APNKO mice did not express APN in either endothelial or smooth muscle cells within the aorta (Fig. 3, M–S).

Delivery of APN abates diabetes-induced endothelial dysfunction. As we have previously reported (23, 52), db/db mouse aortas showed impaired endothelial-dependent vasodilation and decreased APN protein levels compared with CON aortas (Figs. 2B and 4A). Treatment with Ad-βgal did not alter endothelial function or serum APN levels in either CON or db/db mice. However, treatment with Ad-APN significantly improved endothelial function in the db/db aorta to the levels that were seen in the CON aorta (Fig. 4A). SNP-induced endothelial-independent vasodilation was identical among all groups (Fig. 4B). Incubation with the NO synthase inhibitor l-NAME completely abolished vasodilation of the aorta in all groups, suggesting that dilation of the aorta is dependent on NO (Fig. 4C). Thus, exogenous supplementation of APN improves endothelial function in db/db mice, and overproduction of APN did not affect endothelial function in CON mice.

Effects of APN on protein expression of molecules related to oxidative stress and inflammation. To elucidate the mechanism for the protective action of APN on endothelial dysfunction under diabetic conditions, we performed Western blot analysis for protein levels of the oxidative stress marker nitrotyrosine and the NADPH oxidase subunit gp91phox in CON and db/db mice. We (23) have previously demonstrated that diabetic mice showed higher protein expression of nitrotyrosine, gp91phox and IFN-γ, which may contribute to endothelial dysfunction in the aorta. Protein expression of nitrotyrosine and gp91phox was not altered in CON mice with Ad-APN, but APN significantly decreased these proteins in db/db mice (Fig. 5, A–C). However, p47phox protein expression was identical among groups (Fig. 5F). To test whether increased APN levels also increased antioxidant enzymes, protein expression of SOD-1 and SOD-3 was measured in CON and db/db mice treated with Ad-APN or Ad-βgal. Protein expression of SOD-1 was comparable among groups. Protein expression of SOD-3 was increased in db/db mice compared with CON mice, and Ad-APN administration decreased SOD-3 (Fig. 5, D and E).

Effects of APN on mRNA expression of molecules related to inflammation and adhesion. To test the anti-inflammatory and antiadhesion effects of APN, we examined mRNA expression of TNF-α and IL-6 as well as ICAM-1 and VCAM-1, respectively. In contrast to CON mice, expression of TNF-α, IL-6, and ICAM-1 in the aorta was significantly higher in Ad-βgal-treated db/db mice. The incremental increase of APN in db/db mice treated with Ad-APN resulted in a significant decrease in mRNA expression (Fig. 6A) compared with Ad-βgal-treated db/db mice. In addition, levels of mRNA expression for VCAM-1, APN, and eNOS were comparable among groups (Fig. 6B).

APN deficiency induces endothelial dysfunction independent of obesity and insulin resistance. A HF diet was fed to WT and APNKO mice for 10 wk to cause obesity and insulin resistance. HF feeding induced obesity, hyperinsulinemia, and insulin resistance without hyperglycemia in both WT and APNKO mice (Fig. 7). To test whether obesity and insulin resistance induced endothelial dysfunction in the aorta, we assessed endothelial function using a myograph. APN deficiency induced endothelial dysfunction, but 10 wk of a HF diet did not further impair endothelial function in either WT or APNKO mice (Fig. 8).
Fig. 5. Exogenous APN reduces diabetes-induced oxidative stress and inflammation. A and B: adenovirus-mediated supplementation of APN reduced protein expression of nitrotyrosine (A; n = 4–6) and gp91phox (B; n = 9–10) in the aorta of db/db mice. C: expression of interferon (IFN)-γ increased in the aorta of db/db mice. APN supplementation decreased protein expression of IFN-γ in db/db mice (n = 5). D: protein expression of SOD-1 was comparable between CON and db/db mice. Exogenous APN did not change protein expression of SOD-1 in the aorta in both CON and db/db mice (n = 4). E: protein expression of SOD-3 was upregulated in db/db mice compared with CON mice. APN decreased SOD-3 in diabetic mice (n = 4). F: protein expression of p47phox [NAD(P)H oxidase subunit] was identical among groups (n = 3). Data are means ± SE. *P < 0.05 vs. the CON + βgal group; #P < 0.05 vs. the CON + APN group; +P < 0.05 vs. the db/db + βgal group.
reducing serum triglyceride levels in nouse supplementation of APN normalizes dyslipidemia by APN serves as an effective antioxidant and shows promise for macrovascular function. Therefore, exogenous delivery of APN deficiency leads to increased oxidative stress and inflammation under HF diet conditions in animal models (3, 23). However, the functional significance of suppression of oxidative stress and inflammatory processes by APN in obesity/diabetes-induced endothelial dysfunction has not been addressed in the type 2 diabetic mouse model. Importantly, obesity/diabetes-induced endothelial dysfunction has been increasingly implicated in the pathogenesis of atherosclerosis, which is known to involve oxidative stress and inflammation. Our major new findings from this study are 1) exogenous supplementation of the serum protein APN using a viral vector ameliorates diabetes-induced endothelial dysfunction in a type 2 diabetic mouse model; 2) this improvement is due, at least in part, to downregulation of oxidative stress, adhesion molecules, and inflammatory cytokines in the aorta; and 3) exogenous supplementation of APN normalizes dyslipidemia by reducing serum triglyceride levels in db/db mice while, in this study, not affecting levels of glycerina or insulin sensitivity. Thus, in our study, exogenous APN supplementation led to an amelioration of endothelial dysfunction in obesity/diabetes by decreasing triglyceride levels as well as by suppressing oxidative stress, inflammation, and adhesion molecules in the macrovasculature, suggesting that APN acts as a regulator of macrovascular function. Therefore, exogenous delivery of APN serves as an effective antioxidant and shows promise for use in anti-inflammation strategies to improve endothelial function in type 2 diabetes mellitus.

**APN normalizes dyslipidemia.** Atherosclerosis is an abnormality in lipid metabolism that is characterized by high triglycerides, oxidized LDL, and low HDL-cholesterol levels (29, 30). The atherogenic process is initiated by the formation of oxidized LDL-cholesterol in the arterial wall, resulting in the recruitment of circulating monocytes and differentiation into macrophages (30, 47). A study (26) in humans showed that endothelial-dependent vasodilation induced by ACh was impaired in patients with both hypertriglyceridemia and hypercholesterolemia. Collectively, these findings provide strong support for dyslipidemia contributing to the development of endothelial dysfunction (26). Therefore, prevention of lipid accumulation may result in protection of the arterial wall from atherogenesis. Recently, Luo et al. (29) showed that APN, when overexpressed in macrophages of mice, resulted in decreased plasma cholesterol and triglyceride levels compared with control mice. However, in our study, exogenous APN supplementation significantly reduced serum triglyceride levels without changing total cholesterol levels.

**Exogenous APN delivery improves endothelial function by suppressing oxidative stress and increasing NO bioavailability.** Excessive oxidative stress seen in obesity/type 2 diabetes is generated through multiple mechanisms such as NAD(P)H oxidase, xanthine oxidase, mitochondrial respiratory chain, arachidonic acid metabolites, and uncoupled eNOS and can be inhibited by interventions such as exercise and pharmacological agents (5, 7, 10, 19, 23, 24). Prior work (6, 11) has shown that membrane-bound NAD(P)H oxidase is a major source of ROS in the vascular tissues. Hyperglycemia-induced superoxide production increases expression of NAD(P)H oxidase, which, in turn, generates more superoxide (39). Superoxide interactions with NO result in the formation of a highly reactive species, peroxynitrite, which contributes to vascular dysfunction through various mechanisms (28, 33, 39). Earlier, we and others (20, 23) have shown that diabetic and hypertensive mice exhibited increased oxidative stress via gp91phox-dependent NAD(P)H oxidase activation in the aorta. In addition, we and others (4, 49) have found that APN treatment decreased aortic ROS production in apolipoprotein E (ApoE) knockout mice and augmented NO bioavailability through AMP-activated protein kinase in diabetic mice, respectively. Our present study shows that exogenous APN delivery significantly ameliorated diabetes-induced endothelial dysfunction in the aorta without affecting SNP-induced endothelial-independent vasodilation. The expression of both gp91phox and nitrotyrosine protein (a biomarker of peroxynitrite production) in the aorta were increased in db/db mice, but these proteins were decreased with exogenous APN supplementation. Although delivery of APN improved endothelial-dependent vasodilation of the aorta in db/db mice, the improvement was completely abrogated by an incubation with the NOS inhibitor l-NAME, suggesting that exogenous APN improves endothelial function by increasing NO bioavailability.

**Anti-inflammatory and antiadhesive effects of APN.** Increased levels of inflammation and adhesion molecules in type 2 diabetes are major pathological markers in the progression of atherosclerotic CVD (15). Previously, ApoE knockout mice crossed with db/db mice exhibited increased rates of atherosclerosis, suggesting that diabetes accelerates atherosclerosis in
the mouse model (48). In the progression of atherosclerosis, endothelial dysfunction is an initiating factor followed by inflammatory cell infiltration and lipid accumulation in the vascular wall (25). Excessive ROS in diabetes appears to play a critical role in initiating the expression of proinflammatory molecules such as TNF-α, IFN-γ, IL-6, ICAM-1, VCAM-1, and monocyte chemotractant protein-1, which signal early steps in the development of atherosclerosis (27, 32). When the endothelium is activated, leukocyte molecules and chemokines promote the recruitment of monocytes and T cells (13, 14). Previously, we (8) showed that TNF-α production in the vasculature contributes to endothelial dysfunction. TNF-α, a key proinflammatory cytokine, is strongly associated with the pathology of diabetes-induced endothelial dysfunction (8, 9, 50). In addition, previous studies (1, 12, 21) have shown that ApoE knockout mice lacking IFN-γ and inhibition of ICAM-1 can delay the development of atherosclerosis, suggesting that inhibition of inflammation or adhesion molecules contributes to the prevention of atherosclerosis. Furthermore, Ouchi et al. (35) showed that APN attenuates TNF-α-stimulated expression of adhesion molecules, including ICAM-1, in human aortic endothelial cells.

In conclusion, the present study demonstrates that APN-mediated suppression of oxidative stress, adhesion molecules, inflammation, and lipid production in obesity/diabetes contributes to the restoration of endothelial function. Furthermore, exogenous Ad-APN supplementation reduces triglycerides and improves endothelial function in type 2 diabetic mice. Importantly, adenoviral delivery of APN also showed that APN functions to reduce inflammation and expression of adhesion molecules in the aorta. Collectively, these findings provide insights into potential therapeutic targets that ultimately could be directed to reducing cardiovascular morbidity and mortality.
EFFECT OF ADIPOONECTIN ON ENDOTHELIAL FUNCTION

GRANTS
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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

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