Macrophage Adiponectin Expression Improves Insulin Sensitivity and Protects Against Inflammation and Atherosclerosis

Nanlan Luo, Jian Liu, B. Hong Chung, Qinglin Yang, Richard L. Klein, W. Timothy Garvey, and Yuchang Fu

OBJECTIVE—Adiponectin is one of several important metabolically active cytokines secreted from adipose tissue. Epidemiologic studies have associated low-circulating levels of this adipokine with multiple metabolic disorders including obesity, insulin resistance, type 2 diabetes, and cardiovascular disease. To investigate adiponectin-mediated changes in metabolism in vivo, we generated transgenic mice that specifically express the human scavenger receptor A-I gene enhancer/promoter.

METHODS AND RESULTS—Using this transgenic mouse model, we found that adiponectin expression was associated with reduced whole-animal body and fat-pad weight and an improved lipid accumulation in macrophages when these transgenic mice were fed with a high-fat diet. Moreover, these macrophage Ad-TG mice exhibit enhanced whole-body glucose tolerance and insulin sensitivity with reduced proinflammatory cytokines, MCP-1 and TNF-α (both in the serum and in the metabolic active macrophage), adipose tissue, and skeletal muscle under the high-fat diet condition. Additional studies demonstrated that these macrophage adiponectin transgenic animals exhibit reduced macrophage foam cell formation in the arterial wall when these transgenic mice were crossed with an LDL receptor–deficient mouse model and were fed a high-fat diet.

CONCLUSIONS—These results suggest that adiponectin expressed in macrophages can physiologically modulate metabolic activities in vivo by improving metabolism in distal tissues. The use of macrophages as carriers for adiponectin, a molecule with ant diabetic, anti-inflammatory, and antiatherogenic properties, provides a novel and unique strategy for studying the mechanisms of adiponectin-mediated alterations in body metabolism in vivo. Diabetes 59:791–799, 2010

The metabolic syndrome is an associated cluster of traits that include hyperinsulinemia, abnormal glucose tolerance, obesity, hypertension, and a dyslipidemia characterized by high triglycerides, low HDL cholesterol, and small-dense LDL particles leading to atherosclerosis. It is considered one of the main causes of mortality in the U.S. and in developing countries (1–3). Insulin resistance and hyperlipidemia have been linked to each of the traits, suggesting that these metabolic disorders and insulin resistance as well as hyperlipidemia are intimately related to one another. The diagnosis of the metabolic syndrome is a powerful risk factor for future development of type 2 diabetes and accelerated atherosclerosis resulting in heart attacks, stroke, and peripheral vascular disease.

Atherosclerosis is considered a chronic inflammatory disease and a disorder of lipid metabolism (4). The complex physiopathologic process is initiated by the formation of cholesterol-rich lesions in the arterial wall. The accumulation of cholesterol-rich lipoproteins in the artery wall results in the recruitment of circulating monocytes, their adhesion to the endothelium, and their differentiation into macrophage foam cells. Macrophages play a crucial role in this process because they accumulate large amounts of lipid to form the foam cells that initiate the formation of the lesion and participate actively in the development of the atherosclerotic lesion. Because the transformation of macrophages into foam cells is a critical component of atherosclerotic lesion formation (5), the prevention or reversal of cholesterol accumulation or the production of inflammatory mediators in macrophage foam cells could result in protection from multiple pathological effects of atherosclerosis and other abnormal metabolic disorders.

Macrophages are a heterogeneous population of phagocytic cells found throughout the body that originate from the mononuclear phagocytic system (6). These are highly plastic cells that arise from circulating myeloid-derived blood monocytes that have entered target tissues and gained the phenotypic and functional attributes of their tissue of residence. Recent attention has focused on the potential role of macrophages in the process of metabolic diseases (7,8). It has been shown that in obesity, adipose tissue contains an increased number of resident macrophages and that, in some circumstances, macrophages can constitute up to 40% of the cell population within an adipose tissue depot (9,10); and macrophages are obviously a potential source of secreted proinflammatory factors to other tissues for insulin resistance. This correlative evidence has led to the concept that macrophages can directly influence other insulin target tissues and systemic insulin resistance. Furthermore, animal models have also been reported to evidence the causal role of the macrophage in leading to insulin resistance (11). When these animals were fed with a high-fat diet, the macrophage-specific inflammatory pathway knockout mice were relatively protected from glucose intolerance and hyperinsulinemia, and these results showed a global improvement in insulin sensitivity in all insulin-target receptors.
tissues. These studies are consistent with the interpretation that the macrophage is an important, and potentially initiating, cell type in the process of inflammation-induced insulin resistance (12). To prevent macrophage infiltration into these insulin-target tissues will have beneficial effects on the inflammatory response and the abnormal metabolic state.

Adiponectin (also known as apM1, AdipoQ, Gbp28, and Acrp30) is an adipocytokine identified by screening adipose-specific genes in the human cDNA projects (13). Epidemiological evidence has indicated that circulating adiponectin levels are reduced in patients with insulin resistance, type 2 diabetes, obesity, or cardiovascular disease (14–16). When examined, low plasma adiponectin levels in these disease states are accompanied by reduced adiponectin gene expression in adipose tissue (17,18). There is also evidence that adiponectin gene polymorphisms may be associated with hypoadiponectinemia together with insulin resistance and type 2 diabetes (19). Therefore, low levels of adiponectin and adiponectin gene variation have been associated with obesity and insulin resistance. The metabolic effects and action mechanisms for adiponectin are less clear.

Adiponectin has been shown to inhibit macrophage foam cell formation by downregulating scavenger receptor A (SR-A) expression and acyl-CoA: cholesterol-acyltransferase one (ACAT1) expression (20,21). It has been also reported that adiponectin may inhibit both the inflammatory process and atherosclerosis by suppressing the migration of monocytes/macrophages and their transformation into macrophage foam cells in the vascular wall (20,22). These results suggest adiponectin’s inhibitory role in macrophage foam cell formation from human monocyte-derived macrophages. Our recently published data have demonstrated that expression of adiponectin in human THP-1 macrophage cells can modulate multiple pathways of lipid metabolism and reduce macrophage foam cell formation (23). All of these data point to the anti-inflammatory and antiatherogenic role of adiponectin during atherosclerosis.

In current studies, we have generated an adiponectin macrophage-specific transgenic mouse model, and our results have shown that expression of adiponectin in mouse macrophages can significantly reduce cholesterol and triglyceride accumulation in these mouse macrophages and contribute to the reduction of macrophage foam cell formation in the atherosclerotic lesions when these macrophage-modified transgenic animals are crossed with the LDL receptor (Ldlr)-deficient mouse model under a high-fat diet condition. Furthermore, the adiponectin-modified mouse macrophages also alter systemic metabolism in other metabolic active tissues with cell interactions either through infiltration or circulation by bloodstream, which influences the levels of inflammatory cytokines, monocyte chemoattractant protein (MCP)-1 and tumor necrosis factor (TNF)-α, and thus physiologically improves whole-body glucose tolerance and insulin sensitivity.

These observations suggest that adiponectin can alter the important metabolic activities in other tissues through macrophage infiltration and circulation in addition to elevated adiponectin levels. Therefore, our studies provide new insights for investigating the mechanisms of metabolism and inflammatory response in vivo.

**RESEARCH DESIGN AND METHODS**

To generate the macrophage adiponectin transgenic (Ad-TG) mice, full-length cDNA encoding the human adiponectin gene with a V5 epitope tag fused at the 3' end was subcloned into a plasmid vector containing the human SR-AI gene enhancer/promoter (5.0 kb) (kindly provided by Dr. Chris Glass, Department of Cellular and Molecular Medicine, University of California at San Diego). DNA fragments (8.5 kb) starting from the enhancer/promoter to human growth hormone tail were purified before they were microinjected into mouse embryos.

Transgenic animals were created by injections of the appropriate DNA fragments into the pronucleus of inbred C57BL/6 single-cell embryos by our Transgenic Animal/Embryonic Stem Cell Core at the University of Alabama at Birmingham (UAB). All of the transgenic mice were backcrossed more than 10 generations into the C57BL/6 genetic background before being used for an experiment. To investigate the atherosclerotic lesion formation, the Ad-TG mice (Ad-TG) were crossed with the Ldlr-deficient mice (Ldlr<sup>−/−</sup>); J, category no. 002207; The Jackson Laboratories), a common model for cardiovascular research. The Ad-TG/Ldlr and control (wild type [WT/Ldlr]) mice were fed with a high-fat diet (60% kcal % fat) from the Research Diets Company (New Brunswick, NJ) for 6 months before the atherosclerotic lesion regions were measured and analyzed.

Wild-type and transgenic mice at 20 weeks of age but fed with the high-fat diet for 16 weeks were then killed and bled by cardiac puncture. Plasma was isolated by centrifugation at 12,000g for 10 min and stored at −80°C until analyses were performed. All of these animals were housed in a specific pathogen-free facility with 12-h light/dark cycles and received a standard laboratory chow diet except for those undergoing the high-fat diet experiments. Only male mice were used for the experiments. All animal procedures were approved by the Institutional Animal Care and Use Committee of the Animal Resources Program at UAB.

**Glucose and insulin tolerance testing.** To determine the expression level of genes coding for inflammatory cytokines, total RNA was extracted from mouse hearts and transgenic macrophages and plasma using a commercially available TRIzol reagent from Invitrogen (Carlsbad, CA) according to the manufacturer’s instructions. The quantitative real-time PCR analysis was performed using an Mx3000P Real-Time PCR System (Stratagene). Reactions were carried out in triplicate in a total volume of 20 µl using a Brilliant SYBR Green QPCR Master Mix (Stratagene). Quantification was calculated using the starting quantity of the cDNA of interest relative to that of 18S ribosomal cDNA in the same sample.

The plasma levels of MCP-1 and TNF-α were measured by ELISA Strips (Signosig, Sunnyvale, CA) according to the manufacturer’s instructions. The insulin levels in mouse plasma were measured using an ultra-sensitive mouse insulin ELISA kit from Crystal Chem (Downers Grove, IL) according to the protocols from the manufacturer.

**Glucone and insulin tolerance testing.** Glucose tolerance testing and insulin tolerance testing in adiponectin transgenic and control mice were performed as previously described (24). Mice were injected with glucose or insulin at 20 weeks of age after consuming the high-fat diet for 16 weeks. To determine glucose tolerance, animals were first fasted overnight and then given an intraperitoneal injection of glucose solution (100 mg glucose/10 g body wt). Mouse tail blood drops were taken, and glucose concentration was determined at baseline (prior to injection) and at 30, 60, 90, 120, and 150 min postinjection using a HemoCue glucose 201 glucometer. To determine insulin tolerance, mice were fasted for 6 h the morning of the determination and then administered an intraperitoneal injection of insulin solution (1.5 units insulin/kg body wt). Glucose levels were measured in blood samples collected as above described for the glucose tolerance test.
Measurements of glucose metabolism in skeletal muscle. Insulin-stimulated glucose uptake assays in skeletal muscle strips were performed as previously described (25). For measurement of glucose transport activity, the muscle strips were dissected from control wild-type and transgenic mice at 20 weeks of age, treated with insulin (100 nmol/l) for 30 min at 37°C, and then incubated with 1H-2-deoxyglucose for 3 min at 37°C before the reaction was stopped by incubation with 1 N NaOH at 70°C for 5 min. Aliquots of the supernatant were centrifuged and added to the scintillation mixtures and then counted for the isotope activities. In these glucose uptake experiments, the distribution space of radiolabeled L-glucose was used to correct for nonspecific carryover of radioactivity with the cells and uptake of hexose by simple diffusion as previously described (26).

Oil red O staining of tissue and tissue sections. Mice were killed after being fed the high-fat diet for 6 months. The aortas were dissected and fixed in phosphate-buffered formaldehyde (10%), and lipid accumulating in the tissue was detected by staining with Oil red O (27). The percentage of Oil red O–positive tissue was quantitated in the excised aorta between the arch and the common iliac artery in stained en face preparations.

To quantify lipid accumulation in tissue sections, the entire aorta was harvested and prepared with frozen tissue matrix (OCT) and frozen tissue was sectioned at a thickness of 5 μm with a Cryostat for a total of 300 sections. These tissue sections were then incubated with a working solution of Oil red O for 3 h before the examinations were started (27).

Western blot analysis. Mouse tissue was homogenized in tissue lysis buffer containing freshly added protease inhibitor cocktail (Sigma). Cell lysate proteins (25 μg protein) were separated by SDS-PAGE and then electrophoretically transferred onto nitrocellulose membranes and incubated overnight at 4°C with blocking solution (5% nonfat milk in Tris-buffered saline (TBS)). The blocked membranes were separately incubated with the specific antibodies (1:5,000 dilutions with 1% nonfat milk in TBS) for 1 h at room temperature and washed three times with TBS buffer containing 0.1% Tween 20 for 15 min at room temperature with shaking. The secondary antibody conjugated to horseradish peroxidase (Santa Cruz Biotechnology) against the primary antibody was added, incubated, and washed as described above for the first antibody. Immunoreactive protein bands were detected using the Enhance Chemiluminescence Kit (New England Nuclear Life Science Products, Boston, MA).

Statistics. Experimental results are reported as means ± SE. Statistical analyses were conducted using the unpaired Student’s t test assuming unequal variance unless otherwise indicated. Significance was defined as P < 0.05.

RESULTS

Generation of the transgenic mouse model with adiponectin specifically expressed in macrophages. To investigate the functional roles of adiponectin in vivo, we generated adiponectin macrophage-specific transgenic mice expressing the human adiponectin gene (Ad-TG mice). Full-length cDNA encoding the human adiponectin gene with a V5 epitope tag fused at the 3’ end was
subcloned into the plasmid DNA containing the human scavenger receptor A-I (SR-AI) gene enhancer/promoter (5.0 kb). DNA fragments (8.5 kb) starting from the enhancer/promoter to human growth hormone tail were purified before they were microinjected into mouse embryos (Fig. 1A). Transgenic animals were created by injections of the appropriate DNA fragments into the pronucleus of inbred C57BL/6 single-cell embryos. We identified the adiponectin transgene expression in mouse macrophages only from the transgenic animals by using a primer pair that amplified the junction sequence between the adiponectin gene and the V5 epitope tag (Fig. 1B). Adiponectin gene expression was not detected in other tissues isolated from the transgenic animals (data not shown). The levels of adiponectin protein in mouse plasma were also examined in control wild-type and transgenic mice at 6 weeks of age using an adiponectin antibody (Fig. 1C). Mice transgenic for the adiponectin gene had 2- to 4-fold \( P < 0.01 \) elevated circulating adiponectin levels compared with those of control wild-type animals.

**Reduction of body weight and fat in Ad-TG mice fed the high-fat diet.** We first assessed whether growth and development were affected in Ad-TG mice compared with that in wild-type mice. There were no significant differences in reproduction, food consumption, or development between control and transgenic mice when these animals were fed chow or the high-fat diet (data not shown). However, the total body weights and visceral fat tissue masses in Ad-TG mice fed with the high-fat diet were significantly lower \( P < 0.01 \) compared with those of control wild-type animals (Fig. 2A–C).

To confirm these data, we conducted mouse whole-body dual-energy X-ray absorptiometry analyses (Fig. 2D) in control wild-type and Ad-TG mice fed with the high-fat diet for 16 weeks (20 weeks of age). As shown by the data presented in Fig. 2D, whole-body fat accumulation relative to the whole-body weight was significantly reduced (45 vs. 48%; \( P < 0.01 \)) in transgenic mice compared with control mice—in agreement with the data observed in Fig. 2A–C.

**Decreased macrophage lipid accumulation, macrophage infiltration, and proinflammatory cytokine expression/secretion in Ad-TG mice.** To investigate the effect of tissue-specific adiponectin expression on macrophage metabolism, mouse peritoneal macrophages were isolated from 20-week-old control and transgenic mice and the cellular contents of cholesterol and triglyceride were determined (Fig. 3A). Both cholesterol and triglyceride levels were significantly reduced \( P < 0.01 \) in macrophages from Ad-TG mice compared with those from the control mouse macrophages. Similarly, plasma cholesterol and triglyceride levels were significantly reduced in transgenic compared with control mice \( P < 0.01 \) (Fig. 3B).

We also examined the impact of adiponectin expression in macrophages from transgenic mice on MCP-1 and TNF-α gene expression in macrophage, adipose tissue, and skeletal muscle, as well as plasma levels of these proinflammatory cytokines in animals fed the high-fat diet. The expression of the adiponectin gene in Ad-TG mouse macrophages led to a reduced expression of both the MCP-1 and TNF-α genes in macrophages and adipose and skeletal muscle tissues compared with those cells or tissues from the control animals (Fig. 4A–C). Similarly, the plasma concentrations of MCP-1 and TNF-α were significantly reduced in transgenic compared with those in control mice (Fig. 4D). Thus, the expression and secretion of adiponectin in mouse macrophages resulted in favorable
changes not only in macrophage lipid metabolism but also in the expression of proinflammatory cytokines in macrophage, adipose tissue, and skeletal muscle in vivo.

To further investigate whether these reduced proinflammatory cytokines from the metabolic active tissues of Ad-TG mice was due to the decreased macrophage filtrations in these tissues, we next examined the macrophage accumulation by detecting macrophage-specific F4/80, macrophage galactose N-acetyl-galactosamine–specific lectin 1 (MglI, a marker for an alternatively activated macrophage [M2a] phenotype), and chemokine receptor 7 (CCR7, a marker for the classically activated macrophage [M1] phenotype) gene expression in adipose, skeletal muscle, and liver tissues (Fig. 5A–C). The percentage of these macrophage-specific gene-expressing cells in these active metabolic tissues was markedly lower than that of control wild-type animals. Especially, the F4/80 gene, a marker specific for mature macrophages associated with “crown-like structure” in mice fed a high-fat diet, was significantly decreased in the three active metabolic tissues from Ad-TG mice.

**Improved glucose tolerance and insulin sensitivity in Ad-TG mice.** To investigate systemic insulin sensitivity in vivo, we performed glucose tolerance and insulin tolerance tests in the control and transgenic mice fed the high-fat diet for 16 weeks. Plasma glucose levels were consistently higher (P < 0.01) in control wild-type mice when insulin was injected for the insulin tolerance tests compared with levels in transgenic mice. Plasma insulin levels at baseline in transgenic mice fed either regular chow or the high-fat diet were significantly lower (P < 0.05) than the insulin levels in wild-type mice (Fig. 6C). Thus, selective, specific expression of adiponectin in mouse macrophages influences systemic metabolism in distal tissues as demonstrated by significantly reduced hyperglycemia and improved insulin sensitivity.

**Glucose uptake and insulin-signaling pathway in mouse skeletal muscle.** To further investigate the role of adiponectin in glucose homeostasis, we assessed insulin-stimulated glucose transport activity in skeletal muscle tissues from both control wild-type and Ad-TG mice fed the high-fat diet for 16 weeks (Fig. 7A). Insulin-augmented glucose uptake in skeletal muscle from Ad-TG mice was significantly increased, by 47% (P < 0.05), compared with the levels in wild-type mice. These results indicated that macrophage adiponectin expression can significantly enhance insulin-stimulated glucose transport capacity in skeletal muscle, suggesting a novel role of macrophage adiponectin in improving insulin sensitivity in this target tissue. Thus, the expression and secretion of adiponectin from macrophages transgenic for the adiponectin gene resulted in favorable changes in vivo in insulin-stimulated glucose transport in skeletal muscle—further demonstrating the beneficial effects of macrophage-produced adiponectin.
ponectin on glucose metabolism in distal tissues of this transgenic mouse model.

We next asked whether macrophage adiponectin expression would also affect insulin signaling in the skeletal muscle. Akt phosphorylation was assessed under insulin-stimulated conditions in skeletal muscle tissues from control and Ad-TG mice fed the high-fat diet for 16 weeks (Fig. 7B). Serine phosphorylation of Akt (serine 473) was enhanced with insulin stimulation in the Ad-TG mouse skeletal muscles compared with those from the wild-type animals.

FIG. 5. Reduced macrophage accumulation in Ad-TG mice. Control wild-type (WT) and Ad-TG mice were fed with the high-fat diet for 20 weeks; and adipose tissue (A), skeletal muscle (B), and liver (C) were dissected. Total RNA was isolated from these tissues, cDNAs were synthesized, and quantitative PCR was performed with F4/80, MglI, and CCR7 gene primers. Means ± SE from three separate experiments with triplicate samples (n = 9 for each group) are presented. *P < 0.05; **P < 0.01.

Decrease of macrophage foam cell formation in atherosclerotic lesions of Ad-TG mice. Since macrophages play a crucial role in atherosclerosis as a result of their potential to accumulate large amounts of lipid and to form the foam cells that are the hallmark of the atherosclerotic lesion, we determined whether adiponectin expression by macrophages could modify macrophage lipid homeostasis and reduce the lipid accumulation in this cell type that is characteristic of foam cell formation and of mouse athero-
sclerotic lesions in vivo. We first crossed mice transgenic for the human adiponectin gene with Ldlr-deficient mice, which is a common mouse model for cardiovascular research. We then fed the Ad-TG/Ldlr and the control WT/Ldlr mice with the high-fat diet for 16 weeks to enable the development of atherosclerotic lesions in arterial vessels.

We first quantitated the extent of atherosclerosis in this mouse model by measuring the thickness of the innominate artery wall in atherosclerotic lesions of the control and double-crossed Ad-TG mice using an in vivo imaging system (Vevo 770 μ-ultrasound system; VisualSonics company) (Fig. 8A). Quantification of these real-time physiological data showed reduced atherooclerosis (reduced artery wall thickness) in Ad-TG/Ldlr mice compared with that in control animals (Fig. 8B).

To confirm the results of the in vivo imaging analysis, the control and Ag-TG animals were also killed to enable examination of the arteries using histological analysis and en face artery dissection. Serial 5-μm-thickness cross-sections were then generated from the aortic origin beginning at the base of the aortic valve. The tissue sections and en face artery dissection were stained with the Oil red O solution (Fig. 8C–F) as previously described (27). Tissue morphological analysis of the artery sections revealed significantly increased Oil red O staining material in the arterial vessels from control mice (Fig. 8C and D) compared with arterial vessels from Ad-TG/Ldlr mice (Fig. 8E and F). These data suggest that fewer foam cells were formed in the arterial vessels from Ad-TG/Ldlr mice than those from the control WT-Ldlr animals.

DISCUSSION

Adiponectin is naturally expressed and secreted exclusively from adipocytes, and adiponectin expression is undetectable in macrophages. To investigate the mechanisms of adiponectin-mediated alterations of whole-body metabolism in vivo, we developed a mouse model in which the adiponectin gene was specifically expressed in macrophages using a human SR-AI enhancer/promoter. Our studies have shown that adiponectin expression in vivo by macrophages made transgenic for the human adiponectin gene can significantly decrease cholesterol and triglyceride accumulation in the macrophages. This altered macrophage metabolism presumably results either from reduced lipid uptake into the cells or from enhanced HDL cholesterol–mediated efflux, as we described previously in human THP-1 macrophages (23). Furthermore, the adiponectin-modified macrophages infiltrate or circulate into other metabolically active tissues such as adipose and skeletal muscle tissues, and there they can influence favorable changes in multiple metabolic pathways in these tissues. As demonstrated by our present data, macrophage-produced adiponectin can effect favorable alternations in proinflammatory cytokine release, glucose uptake, and the insulin-signaling pathway. Because macrophages in the arterial wall may be converted to the cholesterol-laden foam cells during atherogenesis, we crossed our macrophage Ad-TG mouse with the Ldlr-deficient mouse, a standard animal model for atherosclerosis. Our data clearly showed that macrophage foam cell formation in atherosclerotic lesions was significantly reduced in this double-crossed mouse model. These results suggest that adiponectin plays an important role in whole-body metabolism by modulating inflammatory and metabolic signaling pathways, by improving glucose tolerance and insulin resistance, and by reducing atherogenesis, which are typical characteristics of the metabolic syndrome.

We demonstrated previously that when adiponectin was overexpressed in mouse 3T3-L1 adipocytes, insulin-stimulated glucose uptake was increased in these fat cells (28). Similar results were also reported when adiponectin was overexpressed in vivo in adipose tissue of ob/ob mice, which are leptin deficient and have abnormal glucose and insulin levels. These ob/ob Ad-TG mice showed normalized glucose and insulin levels, with an improvement in insulin sensitivity even though they were morbidly obese with significantly higher levels of adipose tissue than their ob/ob littermates. Therefore, modestly increasing the levels of circulating adiponectin in these transgenic mice completely rescued the diabetic phenotype in ob/ob mice (29). Interestingly, our transgenic mouse model with macrophages expressing adiponectin not only showed improved insulin sensitivity and inflammation in skeletal muscle and adipose tissues but fat mass, macrophage and plasma lipid accumulation, and foam cell formation was also favorably reduced resulting from the adiponectin expressed in mouse macrophage cells. Thus, using macrophages as the carriers for in vivo delivery of adiponectin, which is an antidiabetic, anti-inflammatory and antiatherogenic cytokine, may provide a novel, unique stra-
egy to develop a new therapeutic application for the treat-
ment of human metabolic syndrome.

It has recently been reported that in an Ad-TG mouse
model, adiponectin specifically expressed in liver and
macrophage infiltration in adipose tissue was reduced
(30). Interestingly, in our macrophage Ad-TG animals, we
did not observe a significant change for macrophage
subtypes (e.g., from M1 to M2) in adipose, skeletal muscle,
or liver tissues even though all of the macrophage infiltra-
tion in these tissues was reduced. This may result in
altered metabolism in these active metabolic tissues since
the infiltrated macrophages also exhibit reduced inflam-
matory cytokine secretion. Similar to the results reported
from studies of liver Ad-TG mice, the expression levels of
adiponectin receptors in skeletal muscle and adipose
tissues were not significantly altered (data not shown).

Although adiponectin is a specific adipokine expressed
and secreted physiologically only from the adipocytes, our
results show that adiponectin from the modified macro-
phages can also exert powerful anti-inflammatory and
antiatherogenic effects on whole-body metabolism. Thia-
zolidenediones, which are antidiabetic drugs, have been
shown to increase adiponectin expression and plasma
concentrations in obese type 2 diabetic patients (31,32);
consistent with these reports, our current data have
further demonstrated that the increased adiponectin
concentrations from macrophages link to the decreased
proinflammatory cytokines in the metabolic active cells/
tissues as well as plasma.

The molecular basis of the cell/tissue interactions among
adiponectin-expressing macrophages and other metabolic
active tissues is still not fully understood. It is not clear how

FIG. 8. Analysis of mouse arterial vessels and atherosclerotic lesions. A: the image indicates how to measure the top wall, lumen, and bottom wall
of an innominate artery using a Vevo 770 µ-ultrasound in vivo imaging system for the atherosclerotic lesion area. B: The thickness of the
innominate artery top walls in the atherosclerotic lesion areas from control WT-Ldlr (n = 11) and Ad-TG/Ldlr (n = 10) mice fed with the high-fat
diet for 6 months were measured and analyzed with an S-Mode Protocol with a Vevo 770 µ-ultrasound imaging system. Error bars represent
±SEM. *P < 0.05. C and D: Control WT/Ldlr mice fed with the high-fat diet for 6 months were analyzed using Oil red O staining for 24 h for
lipid-loaded foam cells (one arrow) in tissue longitudinal section (C) and fatty strips (two arrows) in en face artery dissection (D). E and F: tissue
longitudinal section (E) and en face artery dissection (F) of arterial vessels from Ad-TG/Ldlr mice fed with the high-fat diet for 6 months. (A
high-quality digital representation of this figure is available in the online issue.)
the macrophage-produced adiponectin directly influences productions of proinflammatory cytokines in macrophages or adipose and skeletal muscle tissues. It is not clear whether these favorable changes result from the secretion of other anti-inflammatory molecules from the adiponectin-modified macrophages or the reduced lipid accumulation and inflammatory response in these macrophages result in reduced negative impact when the macrophages reside in other tissues or circulate inside other tissues via circulation system. Clearly, additional studies are required, and the potential for other cellular or molecular physiological effects among these metabolically active tissues should be further pursued in the future. Our macrophage Ad-TG mouse model provides an excellent research system for investigating and evaluating the potential role of these physiological mechanisms in vivo. Our current results from these unique mouse models also provide new insight into the prevention and therapy of metabolic syndrome in humans.

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