Peroxisome Proliferator–Activated Receptor δ Agonist GW1516 Attenuates Diet-Induced Aortic Inflammation, Insulin Resistance, and Atherosclerosis in Low-Density Lipoprotein Receptor Knockout Mice

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Objective—The peroxisome proliferator–activated receptor (PPAR) δ regulates systemic lipid homeostasis and inflammation. However, the ability of PPARδ agonists to improve the pathology of pre-established lesions and whether PPARδ activation is atheroprotective in the setting of insulin resistance have not been reported. Here, we examine whether intervention with a selective PPARδ agonist corrects metabolic dysregulation and attenuates aortic inflammation and atherosclerosis.

Approach and Results—Low-density lipoprotein receptor knockout mice were fed a chow or a high-fat, high-cholesterol (HFHC) diet (42% fat, 0.2% cholesterol) for 4 weeks. For a further 8 weeks, the HFHC group was fed either HFHC or HFHC plus GW1516 (3 mg/kg per day). GW1516 significantly attenuated pre-established fasting hyperlipidemia, hyperglycemia, and hyperinsulinemia, as well as glucose and insulin intolerance. GW1516 intervention markedly reduced aortic sinus lesions and lesion macrophages, whereas smooth muscle α-actin was unchanged and collagen deposition enhanced. In aortae, GW1516 increased the expression of the PPARδ-specific gene Adfp but not PPARα- or γ-specific genes. GW1516 intervention decreased the expression of aortic proinflammatory M1 cytokines, increased the expression of the anti-inflammatory M2 cytokine Arg1, and attenuated the iNos/Arg1 ratio. Enhanced mitogen-activated protein kinase signaling, known to induce inflammatory cytokine expression in vitro, was enhanced in aortae of HFHC-fed mice. Furthermore, the HFHC diet impaired aortic insulin signaling through Akt and forkhead box O1, which was associated with elevated endoplasmic reticulum stress markers CCAAT-enhancer-binding protein homologous protein and 78kDa glucose regulated protein. GW1516 intervention normalized mitogen-activated protein kinase activation, insulin signaling, and endoplasmic reticulum stress.

Conclusions—Intervention with a PPARδ agonist inhibits aortic inflammation and attenuates the progression of pre-established atherosclerosis. (Arterioscler Thromb Vasc Biol. 2014;34:52-60.)

Key Words: atherosclerosis ▪ inflammation ▪ insulin resistance ▪ lipids

The principal cause of mortality in patients with type 2 diabetes mellitus is atherosclerosis, a chronic inflammatory disease that is the primary precursor underlying most cardiovascular events. Although the molecular and pathophysiological links between type 2 diabetes mellitus and atherosclerosis are not fully understood, a crucial factor is likely insulin resistance. This is, in part, because of the promotion of multiple independent risk factors associated with cardiovascular disease, including obesity, hypertension, and dyslipidemia. Dyslipidemia associated with insulin resistance is characterized by increased plasma triglyceride (TG)-rich very-low-density lipoprotein (VLDL) and cholesteryl ester–rich low-density lipoprotein (LDL), both of which can permeate a compromised endothelium and initiate atherogenesis. Therapeutic strategies to reduce plasma LDL have proven effective in reducing cardiovascular events. However, a significant unmet medical need persists, making VLDL-lowering strategies an attractive therapeutic target.

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Subendothelial retention of atherogenic lipoproteins leads to a series of maladaptive immune responses culminating in the development of macrophage foam cells. Foam cells play a critical role in the progression of fatty streaks toward more advanced lesions. In particular, M1 macrophages secrete inflammatory effector cytokines such as interleukin (IL)-1β and tumor necrosis factor-α, driven predominantly by mitogen-activated protein kinase (MAPK) and nuclear factor

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(NF)-κB signaling. However, insulin signaling, namely the Akt/forkhead box O1 (FoxO1) pathway, may also contribute to arterial inflammation. In vitro, II/β is a FoxO1 target gene in macrophages, with fatty acid–induced insulin resistance. Despite 1 study reporting the contrary, a growing body of evidence suggests that in vivo, arterial insulin resistance directly promotes atherosclerosis. Global deletion of Akt1 in apolipoprotein E–deficient mice accelerated coronary artery disease and aortic atherosclerosis, concomitant with significant aortic inflammation. Hematopoietic deletion of the insulin receptor in LDL receptor knockout (Ldlr−/−) mice amplified atherogenesis, an effect attributed to impaired macrophage Akt signaling. Furthermore, increased areas of apoptotic macrophages and necrotic core have been visualized in atherosclerotic lesions from patients with type 2 diabetes mellitus. Collectively, these studies support the concept that arterial insulin resistance promotes inflammation and atherogenesis.

Peroxisome proliferator–activated receptors (PPARs) are a class of ligand-dependent transcription factors involved in the regulation of metabolic and inflammatory signaling. Three isofoms exist (α, γ, δ), which exhibit overlapping but distinct patterns of tissue distribution and function. Although PPARδ has been considered the most enigmatic PPAR, this receptor has emerged as an important regulator of cellular lipid homeostasis. Peroxisome proliferator–activated receptor δ (PPARδ) activation inhibits both macrophage lipid accumulation and pro-inflammatory cytokine expression in response to human VLDL. Furthermore, TG accumulation was decreased via angiotensin-II–stimulated fatty acid β-oxidation. Attenuated cytokine expression was mediated through both inhibition of extracellular signal–regulated kinase 1/2 and activation of Akt/FoxO1 signaling. In vivo, Lee et al demonstrated that macrophage deletion of Pparδ in Ldlr−/− mice paradoxically decreased atherogenesis. This effect was attributed to the suppression of atherogenic inflammation by liberation of the inflammatory repressor protein B-cell lymphoma-6 because this protein is normally sequestered by the PPARδ-retinoid X receptor corepressor complex. These studies highlight that deletion of Pparδ mimics the liganded state of the receptor, suggesting that ligand activation may be atheroprotective. However, studies examining the effects of synthetic PPARδ agonists using prevention protocols in mice have produced a spectrum of results. In a second study, GW0742 reduced lesion development in female Ldlr−/− mice, although the doses used yielded serum drug levels 2-fold higher than the EC50 values for murine PPARδ and PPARγ, raising the possibility that atheroprotection by GW0742 was not PPARδ specific. In Ldlr−/− mice fed a high-fat diet, low doses of GW0742 prevented the development of angiotensin II–accelerated atherosclerosis. The next-generation PPARδ agonist (GW1516) at PPARδ–specific doses prevented the development of atherosclerosis in apolipoprotein E–deficient mice fed a high-fat diet, concomitant with reduced aortic inflammatory cytokine expression. Although on balance these studies indicate that PPARδ-specific agonists prevent the development of atherosclerosis and arterial inflammation, it is unknown whether PPARδ agonists are atheroprotective in an intervention model with pre-established insulin resistance and atherosclerosis. Furthermore, the effect of PPARδ activation on lesion pathology, as well as aortic inflammatory signaling cascades, insulin resistance, and endoplasmic reticulum (ER) stress, has not been examined.

In the present study, we use C57BL/6 J Ldlr−/− mice fed a high-fat, high-cholesterol (HFHC) diet, a model of diet-induced dyslipidemia and insulin resistance. After a diet induction phase, intervention with the addition of the PPARδ–specific agonist GW1516 to the HFHC diet resulted in reversal of metabolic dysregulation, including reduced plasma lipids, glucose, and insulin and improved glucose and insulin tolerance. Intervention with GW1516 inhibited aortic MAPK and NF-κB signaling, attenuated aortic inflammation, improved indices of aortic insufficiency, and decreased aortic ER stress, and collectively attenuated the progression of pre-established atherosclerosis.

Materials and Methods

Materials and Methods are available in the online-only Supplement.

Results

GW1516 Improves HFHC-Induced Metabolic Dysregulation in Ldlr−/− Mice

Male C57BL/6 J Ldlr−/− mice were administered an HFHC diet for 4 weeks. The metabolic effects of intervention with the PPARδ agonist GW1516 were evaluated after an additional 8 weeks (Figure 1A). GW1516 at 3 mg/kg per day resulted in GW nonfasting serum concentrations at the end of the dark and light cycles of 604±72 and 369±26 nmol/L, respectively, for a mean concentration of 487±50 nmol/L. This plasma concentration is below the EC50 for murine PPARγ (1 μmol/L) and well below the EC50 for murine PPARα (2.5 μmol/L). GW1516 intervention significantly attenuated HFHC-induced weight gain without affecting caloric intake (Figure IA and IB in the online-only Data Supplement). GW1516 decreased fasting plasma cholesterol, TG, and nonesterified fatty acids compared with 4-week baseline levels, whereas dyslipidemia in mice remaining on the HFHC diet alone continued to progress (Figure 1B). Fast protein liquid chromatography analyses demonstrated that reduced plasma cholesterol in GW1516-treated mice was because of a significant reduction in VLDL cholesterol and a modest but not statistically significant reduction in LDL cholesterol (Figure 1C). GW1516 increased high-density lipoprotein cholesterol by 35% (Figure 1C). The GW1516-mediated
GW1516 intervention significantly reduced cleaved caspase-3 expression vs HFHC (12 weeks; *P<0.05), indicating a reduction in percent lesion SM cell content (Figure 2E; Figure III in the online-only Data Supplement). However, SM α-actin occupied 40% of lesion area in HFHC-fed mice at 12 weeks, which was similar to that of GW1516 intervention mice. As assessed by trichrome staining, the HFHC diet at 12 weeks increased collagen deposition to 25% of lesion area, which was further increased (to 35%) in GW1516 intervention mice (data not shown), despite no further effect on percent lesion SM cell content (Figure 2E; Figure III in the online-only Data Supplement).

To further elucidate the effect of GW1516 intervention on lesion collagen, picrosirius red–stained fibrous cap existed in lesions in both circumstances (Figure 2F). This technique also revealed that a collagen-containing fibrous cap existed in lesions in both circumstances (Figure 2B). To determine whether collagen fiber integrity in the cap was affected by GW1516 intervention, collagen birefringence was quantified (Figure 2B and 2G). This revealed a significant 36% increase in the retardation of polarized light by lesion cap collagen in mice subjected to GW1516 intervention (Figure 2G), denoting more densely packed and aligned collagen fibrils (Figure 2B, arrows). Lesion apoptosis, assessed by cleaved caspase-3 staining, was detected in 1.7% of cells within lesions from 12-week HFHC-fed mice (Figure 2H; Figure IV in the online-only Data Supplement). GW1516 intervention significantly reduced cleaved caspase-3 staining to 1.0% of cells (*P<0.05), indicating a reduction in

GW1516 Attenuates Aortic Sinus Atherosclerosis and Aortic Inflammation in HFHC-Fed Ldlr−/− mice

Examination of aortic sinus atherosclerosis revealed that oil red-O–stained lesion area of HFHC-fed mice at 4 weeks progressed significantly (>6-fold) by week 12 (Figure 2A and 2C). In contrast, although lesion area continued to increase, the area was significantly attenuated in GW1516 intervention mice by ≈33% compared with animals remaining on HFHC alone (Figure 2C). GW1516 intervention influenced lesion composition. As a percent of total area, lesions of HFHC-fed animals at either 4 or 12 weeks displayed accumulation of monocyte and macrophage antibody–2–positive macrophages, which was significantly attenuated by intervention with GW1516 (representative images are shown in Figure III in the online-only Data Supplement, and quantification is shown in Figure 2D). No appreciable smooth muscle (SM) α-actin or collagen deposition was observed in lesions of HFHC-fed mice at 4 weeks, and values were low in 12-week Chow-fed mice (Figure 2E; Figure III in the online-only Data Supplement). However, SM α-actin occupied 40% of lesion area in HFHC-fed mice at 12 weeks, which was similar to that of GW1516 intervention mice. As assessed by trichrome staining, the HFHC diet at 12 weeks increased collagen deposition to 25% of lesion area, which was further increased (to 35%) in GW1516 intervention mice (data not shown), despite no further effect on percent lesion SM cell content (Figure 2E; Figure III in the online-only Data Supplement).
apoptotic cells within lesions. Caspase-3act–positive cells were undetectable in sections from 4-week HFHC-fed mice and low in chow-fed mice at 12 weeks.

Lipid analyses of full-length aortae from HFHC-fed mice at 12 weeks revealed that TG and total cholesterol mass increased 1.4-fold and 1.6-fold compared with HFHC-fed mice at 4 weeks (Figure 3A and 3B). GW1516 supplementation decreased aortic TG by 60% compared with the 12-week HFHC cohort and by 40% compared with the 4-week HFHC-fed mice, but the latter was not significant. GW1516 decreased aortic total cholesterol by 27% compared with HFHC-fed mice at 12 weeks, but values remained elevated (30%) compared with HFHC-fed mice at 4 weeks. Collectively, these analyses indicate that GW1516 intervention attenuates lesion progression and results in development of smaller, lipid-depleted, more stable lesions.

A panel of cytokines known to modulate atherogenesis was examined in full-length aortae. After 4 weeks of HFHC feeding, only chemokine (C-C motif) ligand 3 (CCL3) and intercellular adhesion molecule-1 (ICAM1) expressions were increased compared with chow-fed controls, which is indicative of monocyte recruitment without overt inflammation (Figure 3C). However, the expression of proinflammatory M1 cytokines CCL3, IL1b, ICAM1, tumor necrosis factor (TNF), IL6, and chemokine (C-C motif) ligand 2 (CCL2) was markedly induced (2- to 25-fold) in the aortae of HFHC-fed mice at 12 weeks. In contrast, although all cytokines were elevated in GW1516-treated mice compared with HFHC-fed mice at 4 weeks, cytokine expression was significantly lower (−25% to −85%; mean, −60%) compared with HFHC-fed mice at 12 weeks (Figure 3C). Although monocyte and macrophage antibody–2–stained macrophages within lesions decreased ≈25% in GW1516-treated mice, the greater reduction in cytokine expression suggests that macrophages remaining in lesions of GW1516-treated mice were less inflammatory. Furthermore, compared with 4 weeks, 12-week HFHC feeding significantly increased the aortic expression of the M1 marker inducible nitric oxide synthase (iNos) (3-fold) and suppressed the expression of the anti-inflammatory M2 marker arginase 1 (Arg1) (−53%), resulting in an exacerbated iNos/Arg1 ratio (20-fold; Figure 3D). GW1516 intervention completely reversed this expression pattern. Together, these data indicate that in PPARδ agonist–treated mice, lesion macrophage content is lower (Figure 2D) and there is a shift from M1 to M2 cytokine expression, suggesting an increase in the proportion of macrophages with M2 polarization.

We examined cell signaling cascades known to regulate the macrophage inflammatory response.5 Compared with HFHC-fed mice at 4 weeks, activation of extracellular signal–regulated kinase 1/2 and p38 was observed in full-length aortae of HFHC-fed mice at 12 weeks (Figure 4A). Phosphorylated extracellular signal–regulated kinase 1/2 and phosphorylated p38 were significantly lower in GW1516-treated mice, indicating that GW intervention inhibits the development of activated MAPK signaling. We observed a marked induction of aortic}

![Figure 2. GW1516 attenuates high-fat, high-cholesterol (HFHC)–induced atherosclerosis. A, Representative photomicrographs of aortic sinus sections stained with oil red-O and counterstained with hematoxylin. Scale bar, 500 μm. B, Representative photomicrographs of aortic sinus sections stained with picrosirius red and imaged using circularly polarizing light and liquid crystal compensation. Scale bar, 250 μm. Arrows depict birefringent collagen fibers on the surface of atherosclerotic lesions, consistent with fibrous caps of varying organization. Color encoding of light retardation (nm) is depicted in the gradient map (blue: low; red: high). C, Quantification of neutral lipid area (oil red-O). D, MOMA-2 (macrophages) and E smooth muscle (SM) α-actin (smooth muscle cells) expressed as lesion area (oil red-O) or % lesion area (MOMA-2, SM α-actin; n=6–9/group). Representative photomicrographs are available in Figure III in the online-only Data Supplement for MOMA-2 and SM α-actin. F and G, Quantification of collagen expressed as % lesion area (F) and mean collagen fibril light retardation (G), determined from picrosirius red–stained sections (B) and visualization using circularly polarized light (n=8–12/group). H, Quantification of lesion apoptosis determined by % caspase-3 act–positive cells relative to total cells in aortic sinus lesions (n=5–11/group). Representative photomicrographs are available in Figure IV in the online-only Data Supplement. Staining for collagen, SM α-actin, and caspase-3 act–positive cells was undetectable in lesions from HFHC 4-week sections. Values from individual mice are represented by symbols, and the mean is indicated by a single horizontal line. Different letters indicate significant differences (P<0.05).]

![A panel of cytokines known to modulate atherogenesis was examined in full-length aortae. After 4 weeks of HFHC feeding, only chemokine (C-C motif) ligand 3 (CCL3) and intercellular adhesion molecule-1 (ICAM1) expressions were increased compared with chow-fed controls, which is indicative of monocyte recruitment without overt inflammation (Figure 3C). However, the expression of proinflammatory M1 cytokines CCL3, IL1b, ICAM1, tumor necrosis factor (TNF), IL6, and chemokine (C-C motif) ligand 2 (CCL2) was markedly induced (2- to 25-fold) in the aortae of HFHC-fed mice at 12 weeks. In contrast, although all cytokines were elevated in GW1516-treated mice compared with HFHC-fed mice at 4 weeks, cytokine expression was significantly lower (−25% to −85%; mean, −60%) compared with HFHC-fed mice at 12 weeks (Figure 3C). Although monocyte and macrophage antibody–2–stained macrophages within lesions decreased ≈25% in GW1516-treated mice, the greater reduction in cytokine expression suggests that macrophages remaining in lesions of GW1516-treated mice were less inflammatory. Furthermore, compared with 4 weeks, 12-week HFHC feeding significantly increased the aortic expression of the M1 marker inducible nitric oxide synthase (iNos) (3-fold) and suppressed the expression of the anti-inflammatory M2 marker arginase 1 (Arg1) (−53%), resulting in an exacerbated iNos/Arg1 ratio (20-fold; Figure 3D). GW1516 intervention completely reversed this expression pattern. Together, these data indicate that in PPARδ agonist–treated mice, lesion macrophage content is lower (Figure 2D) and there is a shift from M1 to M2 cytokine expression, suggesting an increase in the proportion of macrophages with M2 polarization. We examined cell signaling cascades known to regulate the macrophage inflammatory response.5 Compared with HFHC-fed mice at 4 weeks, activation of extracellular signal–regulated kinase 1/2 and p38 was observed in full-length aortae of HFHC-fed mice at 12 weeks (Figure 4A). Phosphorylated extracellular signal–regulated kinase 1/2 and phosphorylated p38 were significantly lower in GW1516-treated mice, indicating that GW intervention inhibits the development of activated MAPK signaling. We observed a marked induction of aortic}
NF-κB signaling in HFHC-fed mice at 12 weeks, as demonstrated by increased phosphorylated inhibitor of nuclear factor-κ-B kinase and phosphorylated inhibitor of nuclear factor of κ light chain gene enhancer in B-cells, α (Figure 4B). In contrast, GW1516 intervention attenuates NF-κB activation (Figure 4B). This suggests that GW1516 diminishes aortic inflammation, in part, by attenuating diet-induced activation of inflammatory signaling cascades.

GW1516 Intervention Corrects Diet-Induced Aortic Insulin Signaling and ER Stress and Exerts PPARδ-Specific Vessel Wall Effects

Genetic manipulations resulting in impaired insulin signaling in hematopoietic cells exacerbate atherosclerosis, partly because of increased aortic inflammation and ER stress.4,9,10 We, therefore, hypothesized that impaired aortic insulin signaling contributed to the proinflammatory phenotype of aortae in HFHC-fed mice. Aortic phosphorylated Akt and phosphorylated FoxO1 in fasted and acutely refed mice were examined after the 8-week intervention phase. Compared with chow-fed mice, phosphorylated Akt and phosphorylated FoxO1 were higher in aortae from fasted HFHC-fed mice, but in contrast to chow-fed mice were not further increased by refeeding (Figure 5A). GW1516 intervention completely restored the fasting-to-feeding dynamic regulation of phosphorylated Akt and phosphorylated FoxO1 to patterns observed in chow-fed controls (Figure 5A). The Src homology 2 domain–containing tyrosine phosphatase-1 is primarily expressed by guest on August 30, 2017 http://atvb.ahajournals.org/ Downloaded from
by hematopoietic cells and is a known negative regulator of hepatic insulin signaling. Aortae from HFHC-fed mice at 12 weeks were significantly enriched for the Src homology 2 domain–containing tyrosine phosphatase-1 transcript (Ptpn6; 5-fold) and Src homology 2 domain–containing tyrosine phosphatase-1 protein (30-fold), both of which were strongly attenuated by GW1516 intervention (Figure 5B and 5C).

Concomitant with dysregulated aortic insulin signaling was a significant increase in ER stress markers GRP78 and CHOP in aortae of HFHC-fed mice at 12 weeks (Figure 5D). The CHOP target gene and negative regulator of insulin signaling, tribbles homolog 3 (Trib3), was also elevated (Figure 5E). GW1516 intervention restored GRP78, CHOP, and Trib3 to levels observed in chow-fed controls (Figure 5D and 5E).

To determine whether GW1516 exerted effects directly within the aorta, we examined the expression of known PPARδ target genes. Expression of adipose differentiation related protein (Adfp), angiopoietin-like 4 (Angptl4), and carnitine palmitoyltransferase (Cpt1α) was significantly increased in aortae of GW1516-treated mice compared with HFHC-fed mice or chow-fed controls at 12 weeks (Figure 6A). Expression of target genes specific for PPARα (acyl-CoA oxidase [Acox]) and PPARγ (lipoprotein lipase [Lpl] and fatty acid binding protein 4 [Fabp4]) was unaffected by GW1516 intervention (Figure 6B). Similar results were observed in liver (data not shown). This indicates that GW1516 exerts a direct effect on the arterial wall, which likely contributes to attenuation of aortic inflammation, insulin resistance, and ER stress, as well as
lesion progression. These data further indicate that with respect to PPARs, the aortic effects of GW1516 are PPARδ specific.

Discussion

The risk of atherosclerosis is elevated approximately 4-fold in adults with type 2 diabetes mellitus. Despite this, therapeutic strategies to alleviate atherosclerosis associated with insulin-resistant syndromes remain sparse. Here, we demonstrate in mice that intervention with a synthetic PPARδ agonist, in the context of diet-induced dyslipidemia and insulin resistance, attenuates the progression of early stage lesions to larger plaques. PPARδ activation was associated with beneficial changes in lesion composition, including fewer macrophages, increased expression of M2 markers, less lipid, increased collagen, decreased ER stress, and fewer apoptotic cells, characteristic of lesions with a more stable phenotype. Furthermore, we show that in HFHC-fed mice, the aberrant inflammatory response and impaired insulin signaling within the aorta are reversed by PPARδ activation.

Dyslipidemia associated with insulin resistance is characterized by elevated plasma VLDL and LDL, concomitant with reduced plasma high-density lipoprotein. Although statins effectively lower plasma LDL, they do not fully correct other features of atherosclerosis risk, namely elevated plasma VLDL, decreased high-density lipoprotein, insulin resistance, and body fat composition. The present study demonstrates that intervention by a PPARδ agonist corrects previously established metabolic disturbances. Although plasma LDL cholesterol was modestly reduced, PPARδ activation primarily targeted plasma VLDL and high-density lipoprotein. This is consistent with human studies demonstrating that PPARδ agonists correct mixed dyslipidemia in patients with metabolic syndrome. The current study contributes to the plausibility of PPARδ agonists as therapeutic agents for metabolic dysregulation associated with insulin resistance. Whether PPARδ agonists will have an effect in a setting where elevated LDL is a primary determinant of atherosclerosis remains to be determined.

We recently demonstrated in cultured macrophages that PPARδ activation attenuates VLDL-induced TG accumulation and proinflammatory cytokine expression. We extend these in vitro findings, demonstrating that GW1516 intervention prevents further increase in aortic TG, in concert with significant induction of the PPARδ target genes Angptl4 and Cpt1a. This suggests that GW1516 may stimulate aortic fatty acid β-oxidation and inhibit aortic lipoprotein lipase activity, thus contributing to reduced atherogenesis. We provide evidence that inflammatory cells within the aorta of HFHC-fed animals were polarized to the proinflammatory M1 phenotype. Furthermore, GW1516 intervention increased the anti-inflammatory M2 state, consistent with reports demonstrating that alternative M2 activation of adipose tissue macrophages and hepatic Kupffer cells is, in part, mediated by PPARδ. M2 macrophages are thought to contribute to tissue remodeling and repair and are increased in lesions undergoing regression. Although GW1516 did not induce size regression of early lesions, the M2 phenotype was associated with significant slowing of lesion progression. Longer-term studies are required to assess the effect of PPARδ agonists on more advanced stage lesions. Nevertheless, the present study demonstrates that PPARδ activation alleviates aortic lipid accumulation and inflammation, thus contributing to attenuated lesion development.

That GW1516 increased lesion collagen deposition without altering percent SM cell content is possibly as a result of PPARδ activation of the synthesis and deposition of extracellular matrix by lesion SM cells. This hypothesis is consistent with a report that PPARδ activation in cultured vascular SM cells inhibits IL-1β–induced matrix metalloproteinase-2 and matrix metalloproteinase-9 expression. Reduced lipid deposition in vascular SM cells restores their capacity to elaborate extracellular matrix. Thus, the ability of PPARδ agonists to improve the function of lipid-loaded vascular SM cells merits further attention.

The MAPK and NF-κB signaling pathways regulate inflammatory cytokine expression. In the aortae of HFHC-fed animals, both signaling cascades were significantly activated. GW1516 intervention substantially blunted these changes. In cultured cardiomyocytes, the PPARδ agonist GW0742 attenuated lipopolysaccharide-induced NF-κB activation through increased IκB expression, thereby inhibiting nuclear translocation of NF-κB. We did not observe appreciable changes in total aortic IκB protein. Thus, the mechanism by which PPARδ inhibits NF-κB activation in the context of aortic inflammation remains to be determined. With respect to MAPK activation, GW0742 inhibited angiotensin II–induced phosphorylation of extracellular signal–regulated kinase 1/2 and p38 in cultured mouse macrophages via upregulation of regulator of G-protein signaling (Rgs)-4 and Rgs-5. Consistent with this report, we observed a significant upregulation of both Rgs4 and Rgs5 in aortae of GW1516-treated mice compared with aortae of HFHC-fed mice (Figure V in the online-only Data Supplement). Collectively, our intervention studies suggest that PPARδ activation within the aorta dampens inflammatory signaling, leading to attenuation of inflammatory cytokine expression.

Impaired insulin signaling in the vasculature has emerged as a major contributor to lesion progression. In mice, macrophage deletion of the insulin receptor accelerated the development of advanced lesions, and loss of Akt1 led to severe atherosclerosis. Endothelial cell–specific deletion of 3 FoxO isoforms resulted in atheroprotection, attributed, in part, to an anti-inflammatory effect. Although these gene deletion models highlight the significance of vascular insulin signaling in atherogenesis, these studies do not identify whether insulin signaling becomes dysregulated during lesion development. Here, we demonstrate that mice with diet-induced atherosclerosis exhibit impaired aortic insulin signaling, as evidenced by loss of dynamic fasting-to-refeeding regulation of both Akt and FoxO1 phosphorylation, coupled to an induction of negative regulators of insulin signaling, Src homology 2 domain–containing tyrosine phosphatase-1 and Trib3. This suggests that the loss of insulin regulation of both Akt and FoxO1 results in FoxO1 target genes such as Il6 being chronically transcribed rather than dynamically regulated. This mechanism may contribute to the accumulation of proinflammatory mediators within the vessel wall, inducing a state of chronic low-grade inflammation. Impaired aortic insulin...
signaling was also correlated with elevations in ER stress markers GRP78 and CHOP. Furthermore, activation of aortic PPARδ restored dynamic insulin signaling and attenuated ER stress. It is important to note that the presence of arterial insulin resistance did not impair the ability of GW1516 to attenuate pre-established lesion progression. Thus, although difficult to quantify, it remains possible that improved insulin signaling within GW1516-treated aortae contributes to atheroprotection. In this study, a major factor in the attenuation of lesion development by intervention with GW1516 is reduction of plasma lipids, particularly VLDL/intermediate-density lipoprotein, thereby reducing the atherogenic stimulus. However, we demonstrate that in the aorta, GW1516 stimulates PPARδ-specific target genes, which are known to improve macrophage lipid homeostasis and attenuate the inflammatory response. Although these effects likely contribute to the observed reduction in atherosclerosis, further studies are required to elucidate the extent to which improved metabolic parameters versus direct vessel wall effects contribute to PPARδ-mediated atheroprotection. Nevertheless, the current study provides strong evidence that intervention to an HHF diet with a PPARδ agonist suppresses and favorably modifies the HHFC diet-induced progression of early lesions. It will be important to determine whether intervention by PPARδ activation improves the pathology of more advanced lesions and whether extended treatment achieves regression. We conclude that PPARδ activation remains a viable therapeutic target for atherosclerosis prevention and treatment.

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Disclosures
None.

References
Vascular insulin resistance has been postulated to accelerate atherogenesis by increasing inflammation and endoplasmic reticulum stress. The peroxisome proliferator–activated receptor (PPAR) \(\delta\) is a ligand-dependent transcription factor that regulates insulin sensitivity, lipid homeostasis, and inflammation. We demonstrate for the first time that intervention with a PPAR\(\delta\) agonist, in the context of diet-induced dyslipidemia and insulin resistance, attenuates the progression of early stage lesions to larger plaques. PPAR\(\delta\) activation was associated with beneficial changes in lesion composition, including fewer macrophages, increased M2 cytokine expression, less lipid, increased collagen, decreased endoplasmic reticulum stress, and fewer apoptotic cells, characteristic of more stable lesions. Furthermore, we show that in high-fat, high-cholesterol–fed mice, the inflammatory response and insulin signaling within the aorta are impaired, but these abnormalities are reversed by PPAR\(\delta\) activation. Collectively, these findings highlight a role for PPAR\(\delta\) agonists in the prevention and treatment of atherosclerosis, even in a setting of pre-established insulin resistance and atherosclerosis.

### Significance

Vascular insulin resistance has been postulated to accelerate atherogenesis by increasing inflammation and endoplasmic reticulum stress. The peroxisome proliferator–activated receptor (PPAR) \(\delta\) is a ligand-dependent transcription factor that regulates insulin sensitivity, lipid homeostasis, and inflammation. We demonstrate for the first time that intervention with a PPAR\(\delta\) agonist, in the context of diet-induced dyslipidemia and insulin resistance, attenuates the progression of early stage lesions to larger plaques. PPAR\(\delta\) activation was associated with beneficial changes in lesion composition, including fewer macrophages, increased M2 cytokine expression, less lipid, increased collagen, decreased endoplasmic reticulum stress, and fewer apoptotic cells, characteristic of more stable lesions. Furthermore, we show that in high-fat, high-cholesterol–fed mice, the inflammatory response and insulin signaling within the aorta are impaired, but these abnormalities are reversed by PPAR\(\delta\) activation. Collectively, these findings highlight a role for PPAR\(\delta\) agonists in the prevention and treatment of atherosclerosis, even in a setting of pre-established insulin resistance and atherosclerosis.
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SUPPLEMENTAL FIGURES

PPARδ agonist GW1516 attenuates diet-induced aortic inflammation, insulin resistance and atherosclerosis in Ldlr−/− mice

Lazar A. Bojic, Amy C. Burke, Sanjiv S. Chhoker, Dawn E. Telford, Brian G. Sutherland, Jane Y. Edwards, Cynthia G. Sawyez, Rommel G. Tirona, Hao Yin, J. Geoffrey Pickering and Murray W. Huff
Supplemental Figure I: GW1516-treatment attenuates rate of body weight gain and epididymal fat mass. *Ldlr*−/− mice were fed a standard lab chow, or a high-fat, high-cholesterol diet (HFHC) for 4 weeks. For a subsequent 8 weeks, chow-fed mice remained on chow; the HFHC-fed mice either remained on HFHC alone or HFHC supplemented with GW501516 (GW1516) (3mg/kg/day). A, Body weight. Arrow indicates time of GW1516 intervention. B, Caloric intake expressed as kcal per gram body weight per day of study. C, Epididymal fat mass in grams. Data is presented as mean +/- SEM (n=8-12/group). Different letters indicate significant differences, (P<0.05).
Supplemental Figure II

A  Fasting Blood Glucose

B  Fasting Plasma Insulin

C  Glucose Tolerance Test

D  Insulin Tolerance Test

Supplemental Figure II: GW1516 improves diet-induced dysregulation of metabolic indices.

Ldlr−/− mice were fed standard chow, or a high-fat, high-cholesterol diet (HFHC) for 4 weeks. For a subsequent 8 weeks, chow-fed mice remained on chow; the HFHC-fed mice either remained on HFHC alone or HFHC supplemented with GW501516 (GW1516) (3mg/kg/day). **A**, Fasting blood glucose levels. **B**, Fasting plasma insulin concentrations. **C**, Intraperitoneal glucose tolerance test at 12 weeks. **Inset graph**, absolute area under the curve (AUC), (glucose mmol/Lx120min). **D**, Intraperitoneal insulin tolerance test at 12 weeks. **Inset graph**, absolute AUC (glucose mmol/Lx60min). Data is presented as mean +/- SEM (n=8-12/group). Different letters indicate significant differences, (P<0.05).
Supplemental Figure III: GW1516 improves atherosclerotic lesion morphology. Ldlr<sup>−/−</sup> mice were fed a standard lab chow, or a high-fat, high-cholesterol diet (HFHC) for 4 weeks. For a subsequent 8 weeks, chow-fed mice remained on chow; the HFHC-fed mice either remained on HFHC alone or HFHC supplemented with GW501516 (GW1516) (3mg/kg/day). Mice were fasted for 4h prior to sacrifice. A, and B, Photomicrographs of serial sections of the aortic sinus stained with MOMA-2 (monocyte/macrophage antibody-2), antibody to smooth muscle (SM) α-actin, or trichrome. The bar indicates 500 μm (A) or 250 μm (B). Arrows indicate positive staining for macrophages (MOMA-2), smooth muscle cells (SM α-actin) or collagen (trichrome).
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PPARδ agonist GW1516 attenuates diet-induced aortic inflammation, insulin resistance and atherosclerosis in Ldlr"/" mice

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References


10. Burnett JR, Wilcox LJ, Telford DE, Kleinstiver SJ, Barrett PH, Newton RS, Huff MW. Inhibition of HMG-CoA reductase by atorvastatin decreases both VLDL and LDL


SUPPLEMENTAL FIGURES

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**Supplemental Figure I**

**A** Body Weight

![Graph showing body weight over days on diet](image)

- **Chow**
- **HFHC (12wks)**
- **HFHC (4wks) → HFHC + GW1516 (8wks)**

**B** Caloric Intake

![Bar graph showing caloric intake](image)

**C** Epididymal Fat Mass

![Bar graph showing epididymal fat mass](image)

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**Supplemental Figure I: GW1516-treatment attenuates rate of body weight gain and epididymal fat mass.** *Ldlr<sup>-/-</sup>* mice were fed a standard lab chow, or a high-fat, high-cholesterol diet (HFHC) for 4 weeks. For a subsequent 8 weeks, chow-fed mice remained on chow; the HFHC-fed mice either remained on HFHC alone or HFHC supplemented with GW501516 (GW1516) (3mg/kg/day). **A**, Body weight. Arrow indicates time of GW1516 intervention. **B**, Caloric intake expressed as kcal per gram body weight per day of study. **C**, Epididymal fat mass in grams. Data is presented as mean +/- SEM (n=8-12/group). Different letters indicate significant differences, (P<0.05).
Supplemental Figure II

A Fasting Blood Glucose

B Fasting Plasma Insulin

C Glucose Tolerance Test

D Insulin Tolerance Test

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