

# Chapter 7

PPAR $\alpha/\gamma$  agonism and  
atherosclerosis



# The dual PPAR $\alpha/\gamma$ agonist tesaglitazar reduces atherosclerosis development beyond its plasma cholesterol-lowering effects in APOE\*3-Leiden transgenic mice

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## ABSTRACT

We investigated whether the dual PPAR $\alpha/\gamma$  agonist tesaglitazar has anti-atherogenic effects in APOE\*3-Leiden mice with normal and reduced insulin sensitivity.

APOE\*3-Leiden transgenic mice were fed either a low-fat (LF) diet or a high-fat (HF), insulin-resistance-inducing diet. In both LF and HF-fed mice, one group received a high-cholesterol supplement (1% wt/wt; HC group). A second group received the same HC supplement along with tesaglitazar 0.5  $\mu$ mol/kg diet (T group). A third (control) group received a low cholesterol supplement (0.1% wt/wt; LC group), which resulted in plasma cholesterol levels similar to those of the T group. In both HF- and LF-fed mice, tesaglitazar decreased plasma cholesterol by 20% compared with the respective HC groups; cholesterol levels were similar in the T and LC groups. In LF-fed mice, tesaglitazar reduced atherosclerosis in the aortic root up to 65%, whereas the cholesterol-matched LC group had a reduction of 38%. In HF-fed mice, tesaglitazar produced a 92% reduction in atherosclerosis, while a 56% reduction was seen in the cholesterol-matched LC group. Furthermore, tesaglitazar treatment significantly reduced lesion number beyond that expected from cholesterol lowering, and induced a shift to less severe lesions. Concomitantly, tesaglitazar reduced macrophage-rich and collagen areas in both HF- and LF-fed mice. In addition, tesaglitazar treatment reduced inflammatory markers, including plasma serum amyloid A levels, the number of adhering monocytes, and nuclear factor  $\kappa$ B activity in the vessel wall.

Tesaglitazar has anti-atherosclerotic effects that go beyond plasma cholesterol lowering. These effects were more pronounced in HF-fed mice. Tesaglitazar may exert these actions via anti-inflammatory effects.

**A**gonists of the peroxisome proliferator-activated receptor (PPAR)  $\alpha$  have positive effects on lipid metabolism both in animal models and in clinical practice.<sup>1-3</sup> Agonists of PPAR  $\gamma$ - the thiazolidinediones rosiglitazone and pioglitazone - improve insulin resistance in type 2 diabetes, and pioglitazone improves the dyslipidemia associated with insulin resistance.<sup>4,6</sup> In addition to these effects, both PPAR $\alpha$  and  $\gamma$  agonists have anti-inflammatory properties,<sup>7,8</sup> and, therefore, have the potential to provide additional cardiovascular benefit.<sup>9</sup>

PPAR $\alpha$  and  $\gamma$  agonists appear to act at two different levels to counteract atherosclerosis. Systemically, they ameliorate the atherogenic lipid profile by reducing plasma free fatty acids and triglycerides, and increasing high-density lipoprotein (HDL) cholesterol levels.<sup>10</sup> At the cellular level, PPAR agonists act on most cell types involved in atherosclerosis, including endothelial cells, smooth muscle cells (SMCs), macrophages and lymphocytes, reducing their involvement in the tissue response associated with plaque development. These agonists dampen the systemic response to inflammation by reducing levels of plasma proteins such as C-reactive protein (CRP), tumor necrosis factor (TNF)  $\alpha$  and interferon (IFN)  $\gamma$ ;<sup>11</sup> inhibiting interleukin (IL) 2 and TNF $\alpha$  secretion by monocytes;<sup>12</sup> and reducing IL-1-induced secretion of IL-6 via nuclear factor (NF)  $\kappa$ B signaling pathways in SMCs.<sup>13,14</sup>

PPAR agonists have a number of other actions that positively modulate vascular effects. In the endothelium, for example, they inhibit production of the vasoconstrictor endothelin-1<sup>15,16</sup> and inhibit cytokine-induced expression of the adhesion molecules intercellular adhesion molecule-1 and vascular cell adhesion molecule-1.<sup>17</sup> In monocyte/macrophages, chemotaxis by monocyte chemoattractant peptide-1 and proteolytic enzyme activity by matrix metalloproteinase-9 are inhibited,<sup>18-20</sup> and the proliferation and migration of SMCs are inhibited.<sup>21</sup> Both PPAR $\alpha$  and  $\gamma$  stimulate ATP-binding cassette transporter A1 expression, thereby promoting cholesterol efflux from macrophages<sup>22</sup> and possibly cholesterol excretion into the gut.

In the clinical setting, PPAR $\alpha$  agonists reduce cardiovascular disease (CVD) risk, especially in subjects with insulin resistance.<sup>23</sup> PPAR $\gamma$  agonists have been shown to reduce the progression of intima-media thickening in patients with coronary artery disease,<sup>24</sup> and recent evidence suggests that pioglitazone reduces the incidence of myocardial infarction and stroke in patients with type 2 diabetes and pre-existing CVD.<sup>25</sup> Dual PPAR $\alpha/\gamma$  agonists, which are at an earlier stage of clinical development, have been shown to improve both glucose and lipid abnormalities in patients with insulin resistance and type 2 diabetes.<sup>26,27</sup>

Tesaglitazar is a dual PPAR $\alpha/\gamma$  agonist that has demonstrated positive effects on plasma glucose and lipid abnormalities in animal models of type 2 diabetes and metabolic syndrome.<sup>28</sup> Based on their effects in animal models, it has been proposed that dual PPAR $\alpha/\gamma$  agonists may have additional benefits, beyond their cholesterol-lowering effect, in reducing components of insulin resistance that contribute to atherosclerosis and cardiovascular disease.<sup>28,29</sup> In this study, we examined whether tesaglitazar can confer additional cardiovascular benefit using APOE\*3-Leiden transgenic mice, an established model of human hyperlipidemia and atherosclerosis.

When fed a high-cholesterol diet, APOE\*3-Leiden transgenic mice develop a human-like lipoprotein profile, which includes elevated plasma levels of very-low density lipoprotein (VLDL), intermediate density lipoprotein (IDL) and low-densi-

ty lipoprotein (LDL) and leads to the development of atherosclerosis. In addition, when fed a high-calorie, high-fat, high-cholesterol diet, these mice develop insulin resistance. Depending on their plasma cholesterol levels, APOE\*3-Leiden mice develop atherosclerotic lesions that have comparable morphological, histological and immunohistochemical characteristics to human lesions.<sup>30-32</sup> Since plasma cholesterol levels in APOE\*3-Leiden transgenic mice can be titrated to any level by adjusting dietary cholesterol intake, we were able to study the effects of tesaglitazar on atherogenesis, independent of its total plasma cholesterol lowering effect. In addition, we were able to examine these effects under both normal and mild insulin-resistant conditions.

## METHODS

### Animals

Female heterozygous APOE\*3-Leiden transgenic mice (3–4 months of age), characterized by an ELISA for human apoE,<sup>31</sup> were used. Animal experiments were approved by the Institutional Animal Care and Use Committee of The Netherlands Organization for Applied Scientific Research (TNO). Animals were provided by TNO-Biomedical Research.

### Diets

During a run-in period of 3 weeks, animals received either a high-fat/high-cholesterol (HF/HC) diet, containing 23% (wt/wt) bovine lard, or a low-fat/high-cholesterol (LF/HC) Western-type diet, containing 15% (wt/wt) cocoa butter.<sup>31</sup>

After the run-in period, the HF/HC mice were matched for age and cholesterol level into 3 groups of 17 mice each (Table 1). The mice maintained the HF diet in addition to one of the following three treatments. The high-cholesterol (HF/HC) group received a diet containing 1% (wt/wt) cholesterol. The tesaglitazar-treated group (HF/T) received the same diet as the HC group, but the diet was supplemented with tesaglitazar (0.5  $\mu$ mol/kg diet), equaling 20  $\mu$ g/kg body weight per day. Tesaglitazar [(S)-2-Ethoxy-3-[4-[2-(4-methylsulphonyloxyphenyl) ethoxy]phenyl propanoic acid] was provided by AstraZeneca R&D, Mölndal, Sweden. The low-cholesterol (HF/LC) group received a diet containing 0.1% (wt/wt) cholesterol to titrate the plasma cholesterol level to that of the T group, as deduced from previous experiments in our lab. The LC group served as the cholesterol-matched control. The three groups of HF-fed mice were treated for 28 weeks.

**Table 1.** Diets used during the study

Diet	Treatment	Duration
High cholesterol (1% wt/wt)	High cholesterol and tesaglitazar (1% wt/wt; 0.5 $\mu$ mol/kg)	Matched low cholesterol (0.1% wt/wt)
High Fat	HF/HC	HF/T
Low Fat	LF/HC	LF/T
		HF/LC
		LF/LC
		28 weeks
		16 weeks

Animals that received the LF/HC diet during the run-in period were similarly randomized into three groups containing 17 mice. In addition to their LF diet, the three groups received treatment as described above, except that treatment lasted 16 weeks. Thus, the three treatment groups were LF/HC, LF/T and LF/LC. All animals had free access to food and water. Body weight and food intake were monitored throughout the study.

### Analysis of plasma

After a 4-hour fast, commercially available kits were used to measure total plasma cholesterol (No. 1489437; Roche Diagnostics) and triglyceride levels (No. 337-B; Sigma Diagnostics). Cholesterol exposure was calculated as the area under the curve of cholesterol levels versus time in weeks. Lipoprotein distribution was determined by fast performance liquid chromatographic (FPLC) size fractionation (Pharmacia).<sup>31</sup>

Glucose and insulin levels were determined following sacrifice at week 28 for HF-fed animals and week 16 for LF-fed animals. Plasma glucose was measured using commercial reagents (No. 2319 and 2320; Instruchemie) and plasma insulin was measured using a mouse specific ELISA (10-1150-01, AlpcO). Homeostasis model assessment-insulin resistance (HOMA-IR), a surrogate measure of insulin resistance, was calculated as the product of fasting insulin ( $\mu\text{U}/\text{mL}$ ) and glucose ( $\text{mmol}/\text{L}$ ) concentrations divided by 22.5.<sup>33</sup> Plasma fibrinogen (home-made mouse kit)<sup>34</sup> and serum amyloid A (SAA; Biosource) were measured using specific ELISAs.

### Analysis of atherosclerosis

After 28 weeks (for HF-fed mice) and 16 weeks (for LF-fed mice), animals were sacrificed and the hearts were harvested, fixed and embedded in paraffin.<sup>31</sup> Serial 5- $\mu\text{m}$  cross-sections of the entire aortic valve area were prepared and stained with hematoxylin-phloxin-saffron (HPS) for histological analysis, and with Sirius Red to quantify the collagen area. Atherosclerotic lesions were categorized into types I-V, as described previously.<sup>31</sup> Cross-sectional lesion areas were quantified using Leica Qwin morphometric software.<sup>35</sup> Four sections of each specimen were analyzed at 40- $\mu\text{m}$  intervals to determine the average lesion number, type, and area.<sup>36</sup> In addition, descending aortas were isolated and snap frozen until further analysis. During later analysis, vessels were cleaned of adherent fat, and then stained for lipids using Oil red O for “en face” morphometry of the atherosclerotic lesion area (Leica Qwin morphometric software). All analyses were performed blind, without prior knowledge of feeding regime or treatment.

The number of monocytes adhering to atherosclerotic plaques may give an indication of endothelial activation, and thereby of the inflammatory status of the plaque. Macrophages were detected using AIA31240 antiserum (1:3000, Accurate Chemical and Scientific). The inflammatory status of plaques was further examined by estimating the local presence of NF $\kappa$ B (a major regulatory component of inflammatory reactions) in the plaque. NF $\kappa$ B was detected using mouse anti-human p65-NF $\kappa$ B (F-6, 1:100, Santa Cruz Biotechnology). The level of NF $\kappa$ B-positive staining was scored in the cytoplasm and nucleus for both macrophages and endothelial cells 0-2 (0=no positivity, 1=1 to 5 positive cells, 2=above 5 positive cells).

## Statistical analysis

Non-parametric Mann-Whitney U-tests were used to analyze treatment differences, unless stated otherwise. Probability values of  $P < 0.05$  (two-sided) were considered significant. Frequency data for lesion categorization were compared using the Fisher's exact test. All data are presented as mean  $\pm$  SD.

## RESULTS

### Plasma lipids and lipoprotein profiles

In both the HF and LF-fed mice, body weight (Figure 1A) and food intake (data not shown) did not differ between the three treatment groups during the study periods. In the HF-fed mice, plasma cholesterol levels were 22% lower in the tesaglitazar-treated group than in the HF/HC group (Figure 1B). A similar pattern was seen in the LF-fed mice, with plasma cholesterol levels 21% lower in the tesaglitazar-treated group than in the LF/HC group (Figure 1B). As required by the experimental design (Table 1), the total plasma cholesterol levels were similar in the HF/LC and HF/T groups and in the LF/LC and LF/T groups.

Lipoprotein profiles of the mice showed that tesaglitazar decreased VLDL cholesterol levels in both HF- and LF-fed mice (data not shown). Additionally, following tesaglitazar treatment, a lipoprotein fraction appeared with a size between LDL and HDL lipoproteins (Figure 1C). Western blot analysis revealed that this lipoprotein fraction was poor in apoAI and apoB, but rich in apoE (data not shown).

As derived from the area under the curve of Figure 1B, the HF/HC and LF/HC groups had significantly increased exposure to cholesterol compared with the respective tesaglitazar-treated and LC groups (Figure 1D). There was no significant difference in cholesterol exposure between tesaglitazar-treated and LC control groups. Triglyceride levels were significantly lower in tesaglitazar-treated groups compared with HC groups (Figure 1E) with both HF and LF diets ( $P < 0.05$ ).

Plasma tesaglitazar levels reached  $38.6 \pm 11.4$  nmol/L for the HF groups and  $41.4 \pm 11.7$  nmol/L for the LF groups (n.s.).

### Insulin sensitivity

Changes in glucose and insulin levels during the study are shown in Table 2. In HF-fed mice, the HOMA-IR index indicated insulin resistance in HF/HC mice at 28 weeks. HOMA-IR was significantly lower in both the HF/T and HF/LC groups compared with the HF/HC group. In LF-fed mice, only tesaglitazar treatment significantly reduced HOMA-IR compared with both LF/HC and LF/LC mice ( $P < 0.05$ ).

### Atherosclerosis

Tesaglitazar reduced atherosclerosis in treated mice compared with the respective HC groups and cholesterol-matched LC groups. In HF-fed mice, "en face" preparations of the descending aorta showed that lesion area was reduced by 34% in the tesaglitazar-treated group compared with the HF/HC group, and by 21% compared with the cholesterol-matched HF/LC control group (Figure 2A). These changes did not reach statistical significance. In the LF-fed mice, lesion area was significantly

**Table 2.** HOMA-IR calculations as a measure for insulin resistance in high-fat- and low-fat-fed mice.

Diet	Treatment	Weeks	Glucose (mmol/L)	Insulin ( $\mu\text{g/L}$ )	HOMA-IR
High fat	HC	28	6.8 $\pm$ 0.8	1.3 $\pm$ 0.8	11.1 $\pm$ 6.8
	T		5.7 $\pm$ 0.5*	0.6 $\pm$ 0.4*	4.3 $\pm$ 3.2*
	LC		5.5 $\pm$ 0.6*	0.5 $\pm$ 0.4*	3.4 $\pm$ 2.7*
Low fat	HC	16	5.6 $\pm$ 0.6	0.7 $\pm$ 0.4	5.0 $\pm$ 3.4
	T		5.1 $\pm$ 0.4*	0.5 $\pm$ 0.4	2.9 $\pm$ 2.4*
	LC		5.5 $\pm$ 0.5	0.5 $\pm$ 0.5	3.7 $\pm$ 3.2

HOMA-IR = Insulin ( $\mu\text{U/mL}$ ) x (Glucose (mmol/L)/22.5)

\* Significantly different from HC,  $P < 0.05$

HC: high cholesterol; T: tesaglitazar; LC: low cholesterol

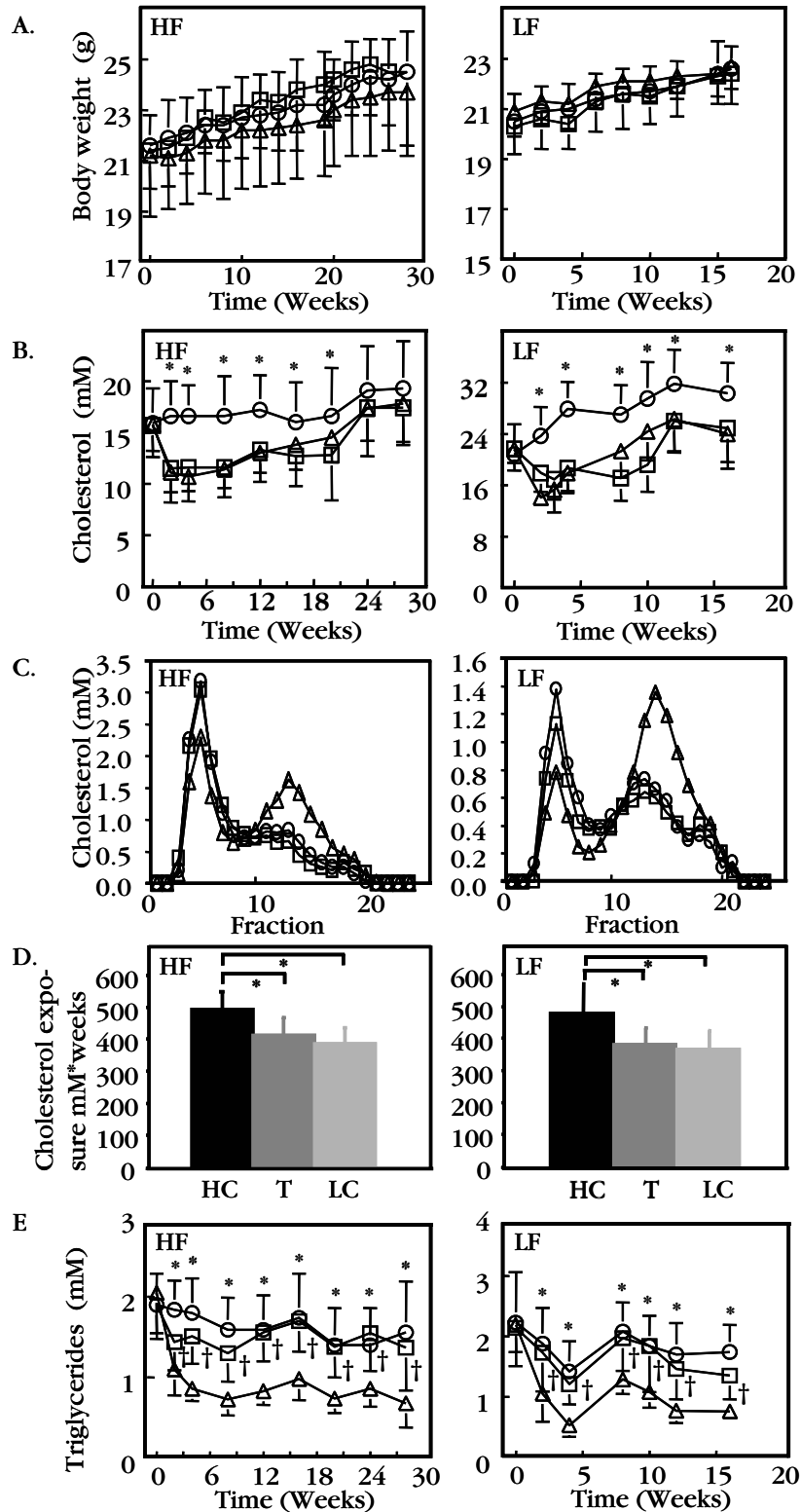
reduced by 28% ( $P < 0.05$ ) in the tesaglitazar-treated group compared with the LF/HC group, and by 16% (n.s.) compared with the cholesterol-matched LF/LC group (Figure 2B).

Consistent with the descending aorta data, cross-sections of the aortic valve area showed that tesaglitazar reduced atherosclerosis (Figure 2B). In HF-fed mice, treatment with tesaglitazar significantly reduced total lesion area by 92% compared with the HF/HC group, and by 83% compared to the cholesterol-matched HF/LC control group ( $P < 0.05$ ). In the LF-fed mice, tesaglitazar treatment resulted in a significant 65% reduction in total lesion area compared with the LF/HC group and a non-significant 43% reduction in total lesion area compared with the cholesterol-matched LF/LC control group.

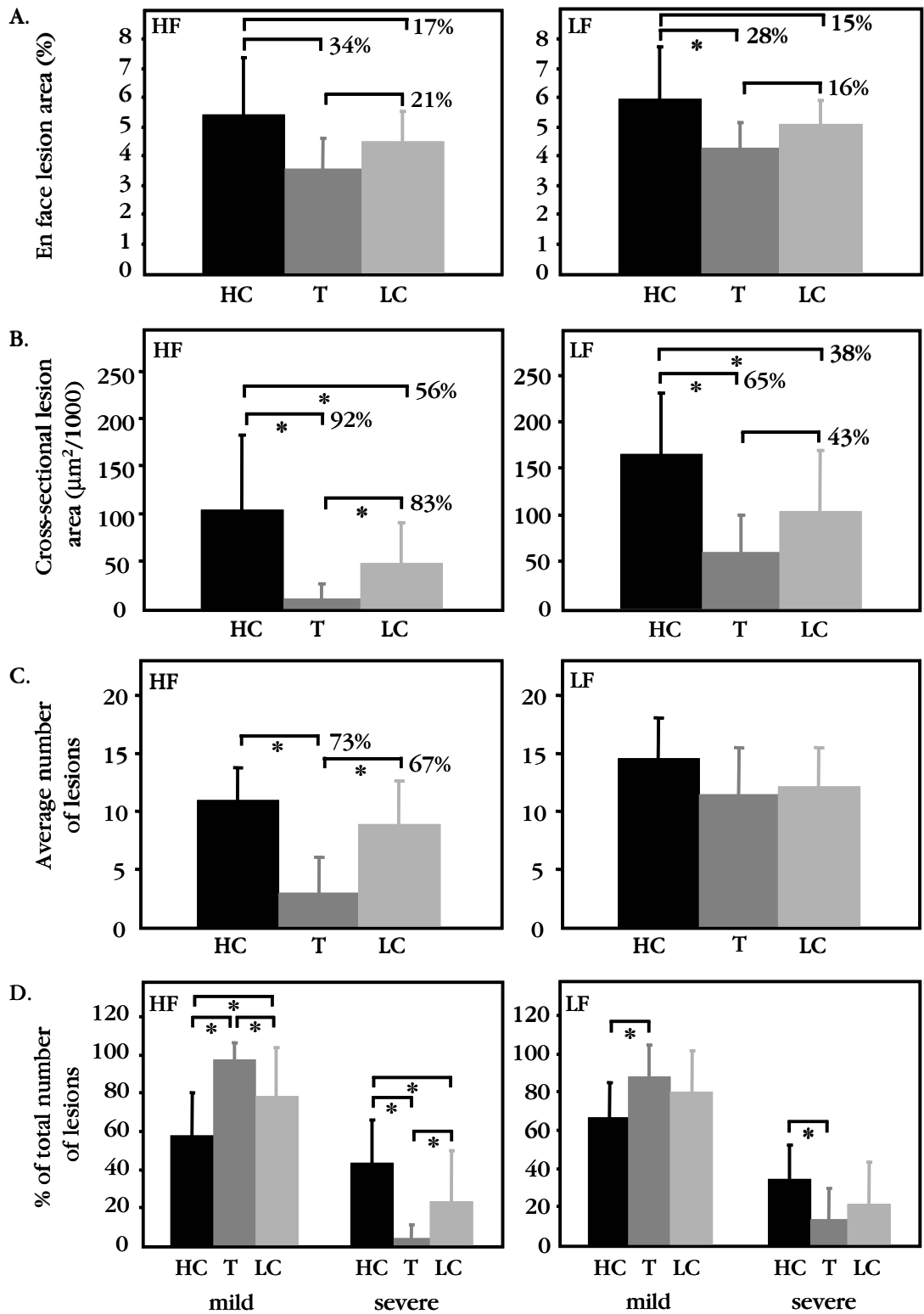
In the same cross-sections, the average number of lesions per animal did not differ significantly between the HC and LC control groups in HF-fed mice (Figure 2C). However, treatment with tesaglitazar significantly reduced the average number of lesions by 73% compared with the HF/HC group and by 67% compared with the cholesterol-matched HF/LC group ( $P < 0.05$ ). Treatment with tesaglitazar did not affect the average number of lesions in LF-fed mice (Figure 2C). When lesions were categorized as either mild or severe in HF-fed mice, there was a significant shift ( $P < 0.05$ ) from severe to mild lesions in tesaglitazar-treated animals (Figure 2D). Although there was a similar trend seen in LF-fed mice (Figure 2D), there was no difference in mild and severe lesion categorization between the LF/T and LF/LC groups.

To further characterize the atherosclerotic lesions, we measured macrophage and collagen areas in cross-sections serial to those used for morphometry (Figure 3). In HF-fed mice, the macrophage-positive area was larger in the HC group compared with the tesaglitazar and LC groups (Figure 3A,C). Moreover, the macrophage-positive area was smaller in the tesaglitazar group than in the cholesterol-matched LC group. The collagen-positive areas followed a similar trend (Figure 3A,C). Since the total cross-sectional lesion area was larger in LF-fed mice than in HF-fed mice, macrophage and collagen areas were also larger and the absolute areas followed the lesion area trend (data not shown). When expressed as a percentage of the total

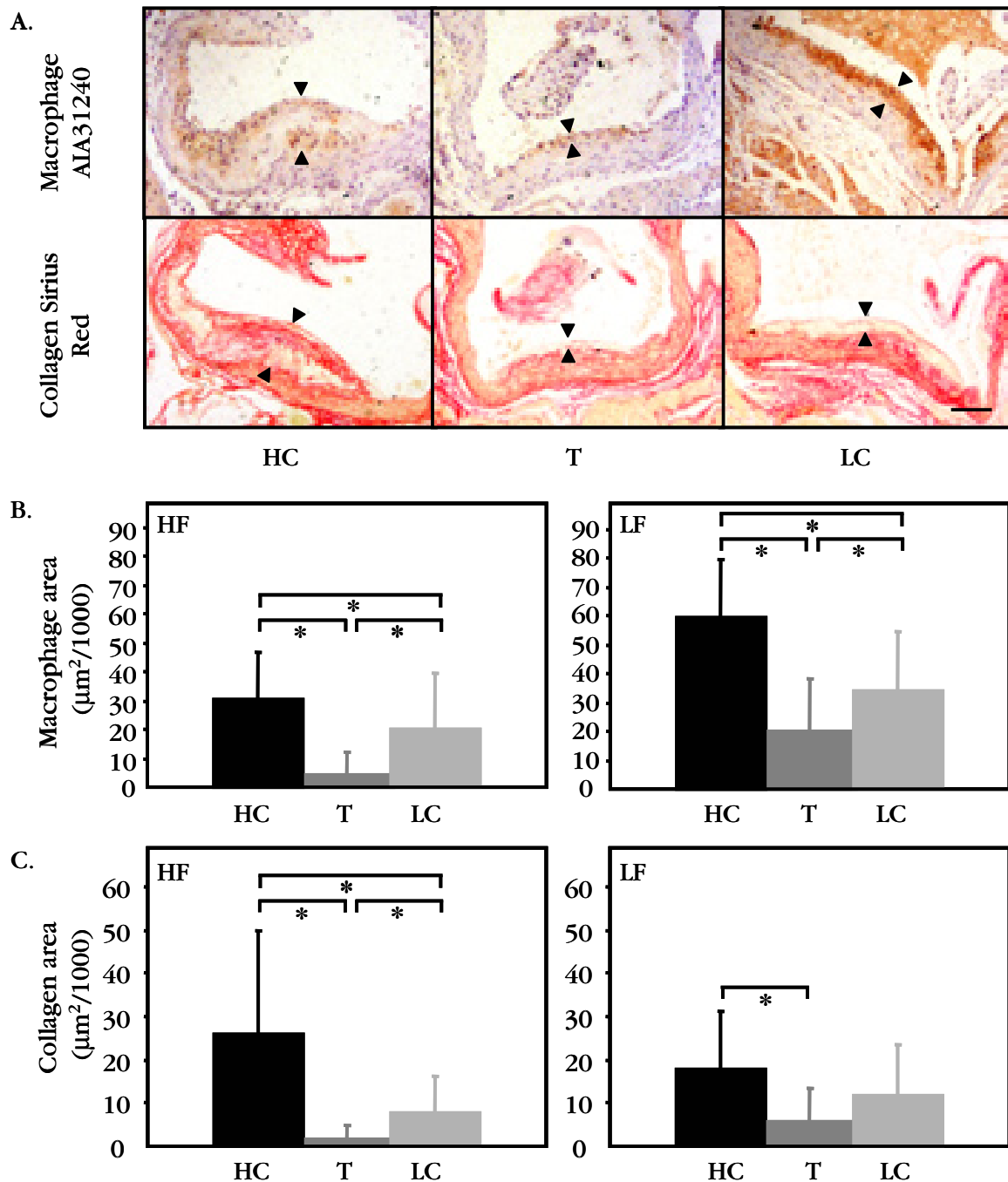




**Figure 1.** Effect of tesaglitazar on plasma lipids in APOE $^3$ Leiden mice with (left panels) and without (right panels) insulin resistance. (A.) body weight over time (B.) plasma cholesterol over time (C.) lipoprotein profiles (D.) total cholesterol exposure (E.) plasma triglycerides over time. To increase plasma cholesterol levels at 20 weeks, the HF/LC dietary cholesterol was increased from 0.1% to 0.5% cholesterol (wt/wt). Plasma cholesterol levels in the HF/LC group returned to levels comparable to the T-group by 24 weeks. Circles = HF/HC or LF/HC; triangles = HF/T or LF/T; squares = HF/LC or LF/LC; \*P<0.05



**Figure 2.** Effect of tesaglitazar on atherosclerosis in the aorta of APOE3\*Leiden mice with (left) and without (right) insulin resistance. Shown are (A.) the aortic “en face” atherosclerotic lesion area (B.) the cross-sectional lesion area in the aortic valve area (C.) total number of lesions and (D.) lesion severity. \*P<0.05

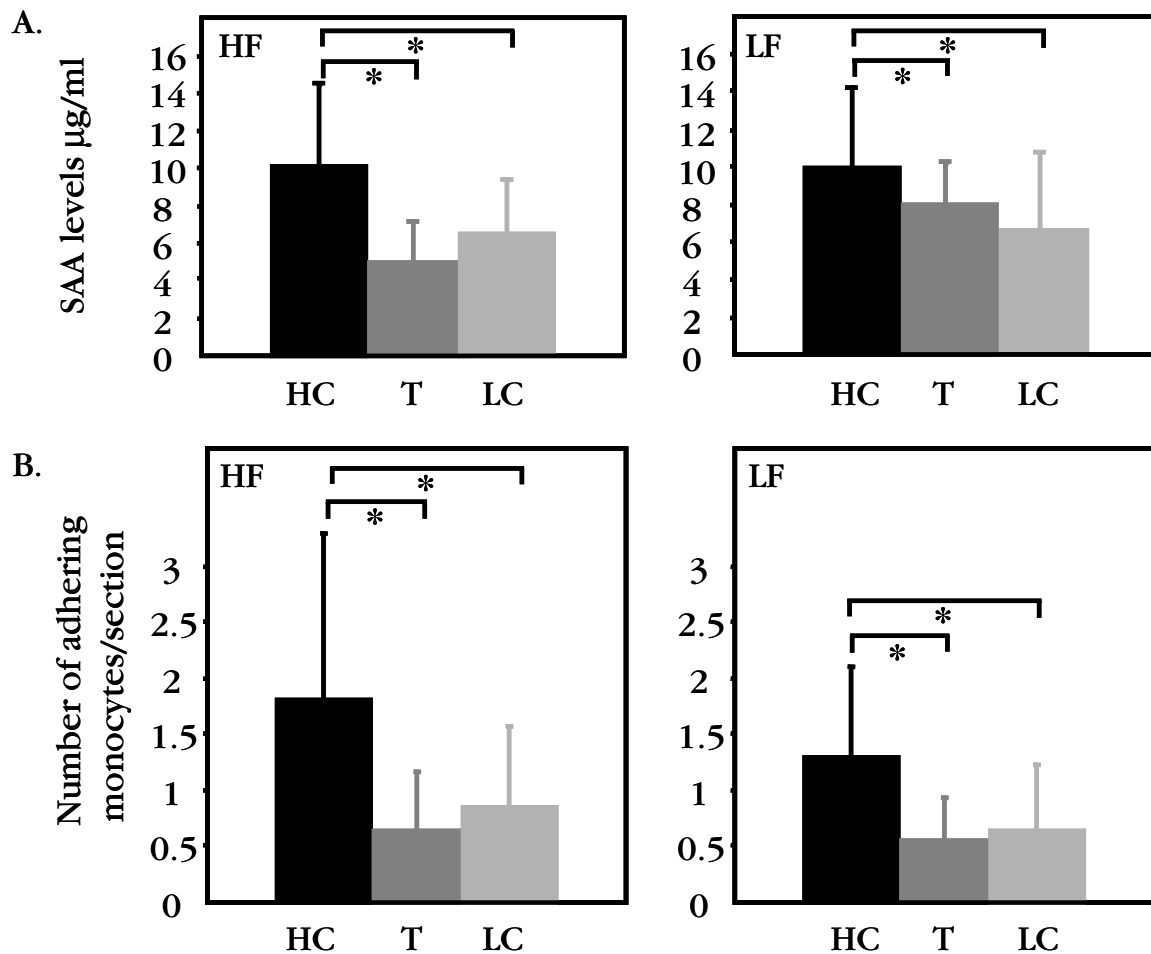


**Figure 3.** Cross-sectional lesion characteristics. (A.) Representative microscopic images of macrophage and collagen staining of HF-fed mice. (B.) macrophage area (C.) collagen area. \* $P < 0.05$

lesion area, collagen decreased and macrophages increased in accordance with the shift to less severe lesions.

### Inflammatory markers

SAA levels were significantly reduced ( $P < 0.05$ ) in tesaglitazar-treated groups compared with HC groups in both HF-fed (-50.5%) and LF-fed mice (-20.9%) (Figure 4A). In HF-fed mice, tesaglitazar treatment reduced SAA levels further than LC treatment



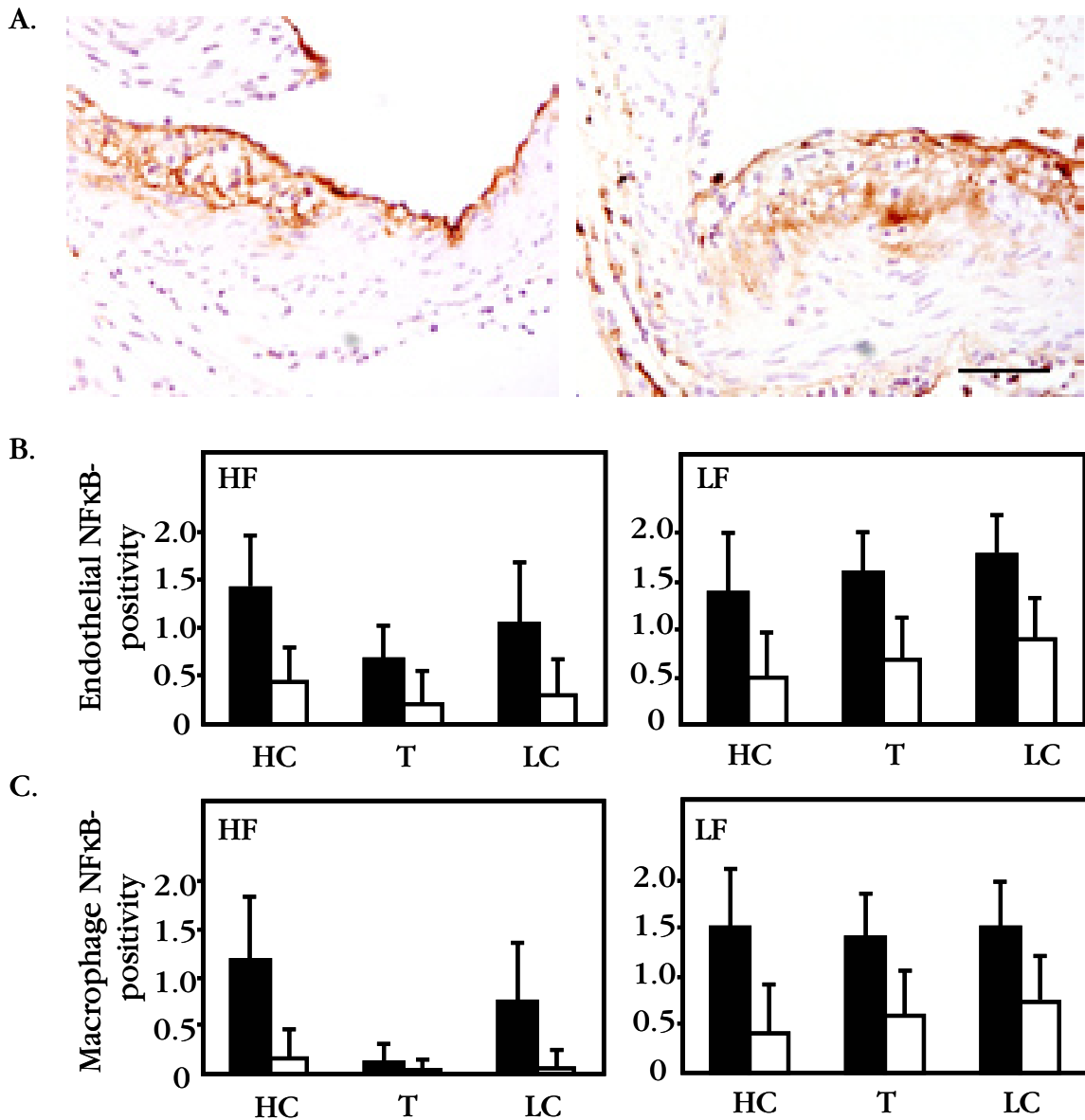
**Figure 4.** Effect of tesaglitazar on inflammatory parameters in APOE3\*Leiden mice with (left) or without (right) insulin resistance. (A.) plasma SAA level (B.) monocyte adherence. P<0.05

(-23%). Fibrinogen levels were unaffected (data not shown). In both HF-fed and LF-fed mice there were fewer adhering monocytes in tesaglitazar-treated groups compared with HC groups (Figure 4B). There were no differences between the tesaglitazar-treated groups and the cholesterol matched LC control groups.

P65-NFκB staining was found in the cytosol and nuclei of both endothelial cells and macrophages (Figure 5A). SMCs remained unstained. Positively stained endothelial cells were observed on plaques. When observed on normal vessel walls, the positively stained cells were in close proximity to the shoulder regions of plaques. In both HF-fed and LF-fed mice, P65-NFκB expression (Figure 5B,C) followed the same pattern as total lesion numbers (Figure 2C).

## DISCUSSION

This study showed that tesaglitazar has atherosclerosis reducing capacities in APOE3\*Leiden transgenic mice that cannot be attributed solely to its reduction of plasma total cholesterol. This anti-atherosclerotic effect was more notable when the animals were placed on a diet that generated insulin resistance, obesity and moderate hypertriglyceridemia, conditions that contribute to metabolic syndrome in



**Figure 5.** Effect of tesaglitazar on the inflammatory marker NF $\kappa$ B in atherosclerotic plaques of APOE $^{3*}$ Leiden mice with (left) or without (right) insulin resistance. (A.) Representative microscopic pictures of p65-NF $\kappa$ B-positive staining of atherosclerotic plaques (scale bar=100  $\mu$ m). (B.) Scoring of endothelial NF $\kappa$ B positivity in the cytosol (black bars) and nucleus (white bars). (C.) Scoring of macrophage NF $\kappa$ B-positivity in the cytosol (black bars) and nucleus (white bars). \*P<0.05

humans. The mechanism by which tesaglitazar reduced atherogenesis in these mice involved direct actions on the pro-inflammatory tissue response of vascular cells.

The hyperlipidemic APOE $^{3*}$ Leiden mice used here have a lipoprotein profile that is more similar to the human profile than those of either apoE $^{-/-}$  or LDLr $^{-/-}$  mice. In agreement with previous studies with APOE $^{3*}$ Leiden mice,<sup>30,37</sup> we were able to titrate plasma cholesterol levels by adjusting dietary cholesterol intake. Previous studies have also shown that these mice respond to hypolipidemic drugs; treatment with statins reduces plasma cholesterol<sup>38,39</sup> and treatment with a PPAR $\alpha$  agonist reduces both plasma cholesterol and triglyceride levels (unpublished data). In addi-

tion, we showed in an earlier dose-finding study that APOE\*3-Leiden mice respond to the dual PPAR $\alpha/\gamma$  agonist tesaglitazar. In the present study, we aimed for mild cholesterol lowering with tesaglitazar, in order to investigate direct anti-atherosclerotic effects on the vascular wall. At a dose of tesaglitazar 0.5  $\mu\text{mol/kg}$  diet (or 20  $\mu\text{g/kg}$  body weight/day), a mild decrease in plasma cholesterol of approximately 20% was achieved.

The lipoprotein profiles of the mice suggested that treatment with tesaglitazar resulted in the formation of an additional particle, sized between the LDL and HDL fractions. Western blotting characterized the particle as poor in apoAI and apoB, but rich in apoE (data not shown). Similar lipoprotein profiles have been observed following treatment of APOE\*3-Leiden mice with the PPAR $\alpha$  agonist fenofibrate (unpublished data). Since cholesteryl ester transfer protein is not expressed in mice, these particles could represent large apoE-rich HDL.<sup>40</sup> Furthermore, the appearance of these large apoE-rich particles during tesaglitazar treatment was associated with a decrease in atherosclerosis, suggesting that they may have favorable anti-atherosclerotic properties. However, it remains unclear whether the accumulation of these particles is clinically relevant, or a mouse-specific effect.

To examine the pleiotropic effects of tesaglitazar that might contribute to a reduction in atherosclerosis beyond that provided by lipoprotein changes, we analyzed the levels of anti-inflammatory markers SAA and fibrinogen in plasma, and examined adhering monocytes and vascular NF $\kappa$ B expression in atherosclerotic plaques. We found a decrease in plasma SAA levels for tesaglitazar-treated mice, but no change in plasma fibrinogen levels. In tesaglitazar-treated mice, fewer monocytes adhered to the endothelium over plaques, coinciding with decreased NF $\kappa$ B expression at the same location. Previous studies have shown evidence for anti-inflammatory activities of PPAR  $\alpha$  and  $\gamma$  agonists, due to upregulation of I $\kappa$ B, leading to decreased NF $\kappa$ B/C-EBP $\beta$  complexes and suppression of C-reactive protein synthesis.<sup>41</sup> Our model provides further evidence of the anti-inflammatory effects of tesaglitazar, including reduced total lesion area as a result of decreased relative macrophage and collagen areas. Both effects contributed to the observed decrease in plaque severity in drug-treated animals. These anti-inflammatory effects were more pronounced in the tesaglitazar groups than in the LC groups, and were thus not due to cholesterol lowering per se.

Although the anti-inflammatory effect of tesaglitazar was observed in both HF- and LF-fed mice, the effect of tesaglitazar was greater under HF-fed conditions. This might be ascribed to differences in the level of insulin resistance between the two groups. However, we cannot exclude the possibility that the difference might be due to the relative length of the treatment periods (28 vs. 16 weeks), or to a difference in plasma cholesterol levels.

In summary, the dual PPAR $\alpha/\gamma$  agonist tesaglitazar showed significant anti-atherogenic effects in this mouse model, especially in animals with moderate insulin resistance. These positive results did not solely result from tesaglitazar-induced reductions in total cholesterol levels. In addition to the beneficial effects on lipid and glucose abnormalities previously shown in animal models of type-2 diabetes and the metabolic syndrome, tesaglitazar also demonstrated anti-inflammatory and anti-atherosclerotic effects in the vascular wall. The results from this study show that

the beneficial effects of tesaglitazar on atherosclerosis involve a number of different pathways.

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