Universiteit Leiden
The Netherlands

Modulation of genes involved in inflammation and cell death in atherosclerosis-susceptible mice

Zadelaar, Anna Susanne Maria

Citation
Zadelaar, A. S. M. (2006, March 23). *Modulation of genes involved in inflammation and cell death in atherosclerosis-susceptible mice*. Retrieved from https://hdl.handle.net/1887/4401

Version:	Corrected Publisher's Version
License:	Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden
Downloaded from:	https://hdl.handle.net/1887/4401

Note: To cite this publication please use the final published version (if applicable).

Chapter 6

The Dual PPARα/γ Agonist Tesaglitazar Reduces Atherosclerosis Development Beyond its Plasma Cholesterol-Lowering Effects in APOE*3Leiden Transgenic Mice

A Susanne M Zadelaar[1,2]; Lianne SM Boesten[2,3]; J Wouter Jukema[1]; Bart JM van Vlijmen[4]; Teake Kooistra[2]; Jef J Emeis[2]; Erik Lundholm[5]; German Camejo[5]; Louis M Havekes[1,2,3]

[1]Dept. of Cardiology, Leiden University Medical Center,
[2]TNO-Biomedical Research Unit, Leiden, The Netherlands,
[3]Dept. of General Iinternal Medicines, Leiden University Medical Center,
[4]Hemostasis and Thrombosis Research Center, Leiden University Medical Center, Leiden, The Netherlands,
[5]Astra Zeneca, Mölndal, Sweden.

Manuscript submitted

Abstract

Background – We investigated whether the dual PPARα/γ agonist tesaglitazar has anti-atherogenic effects in APOE*3Leiden mice with normal and reduced insulin sensitivity.

Methods & Results – ApoE*3Leiden 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 μmol/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 κB activity in the vessel wall.

Conclusions – 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.

Introduction

Agonists of the peroxisome proliferator-activated receptor (PPAR) α have positive effects on lipid metabolism both in animal models and in clinical practice[1-3]. Agonists of PPARγ – the thiazolidinediones rosiglitazone and pioglitazone – improve insulin resistance in type 2 diabetes, and pioglitazone improves the dyslipidemia associated with insulin resistance[4-6]. In addition to these effects, both PPARα and γ agonists have anti-inflammatory properties[7,8], and, therefore, have the potential to provide additional cardiovascular benefit[9].

PPARα and -γ 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[10]. 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) α and interferon (IFN) γ[11]; inhibiting interleukin (IL) 2 and TNFα secretion by monocytes[12]; and reducing IL-1-induced secretion of IL-6 via nuclear factor (NF) κB signaling pathways in SMCs[13,14].

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[15,16] and inhibit cytokine-induced expression of the adhesion molecules intercellular adhesion molecule-1 and vascular cell adhesion molecule-1[17]. In monocyte/macrophages, chemotaxis by monocyte chemotactic peptide-1 and proteolytic enzyme activity by matrix metalloproteinase-9 are inhibited[18-20], and the proliferation and migration of SMCs are inhibited[21]. Both PPARα and -γ stimulate ATP-binding cassette transporter A1 expression, thereby promoting cholesterol efflux from macrophages[22] and possibly cholesterol excretion into the gut.

In the clinical setting, PPARα agonists reduce cardiovascular disease (CVD) risk, especially in subjects with insulin resistance[23]. PPARγ agonists have been to shown reduce the progression of intima-media thickening in patients with coronary artery disease[24], and recent evidence suggests that pioglitazone reduces the incidence of myocardial infarction and stroke in patients with type 2 diabetes and pre-existing CVD. Dual PPARα/γ 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[25,26].

Tesaglitazar is a dual PPARα/γ agonist that has demonstrated positive effects on plasma glucose and lipid abnormalities in animal models of type 2 diabetes and metabolic syndrome[27]. Based on their effects in animal models, it has been proposed that dual PPARα/γ agonists may have additional benefits, beyond their cholesterol-lowering effect, in reducing components of insulin resistance that contribute to atherosclerosis and cardiovascular disease[27,28]. In this study, we examined whether tesaglitazar can confer additional cardiovascular benefit using APOE*3Leiden transgenic mice, an established model of human hyperlipidemia and atherosclerosis.

When fed a high-cholesterol diet, APOE*3Leiden 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-density 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*3Leiden mice develop atherosclerotic lesions that have comparable morphological, histological and immunohistochemical characteristics to human lesions[29-31]. Since plasma cholesterol levels in APOE*3Leiden 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[30], 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[30].

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 μmol/kg diet), equaling 20 μg/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.

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.

Table 1. Diets used during the study

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

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)[30].

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 (μU/mL) and glucose (mmol/L) concentrations divided by 22.5[32]. Plasma fibrinogen (home-made mouse kit)[33] 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[30]. Serial 5-μm 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[30]. Cross-sectional lesion areas were quantified using Leica Qwin morphometric software[34]. Four sections of each specimen were analyzed at 40-μm intervals to determine the average lesion number, type, and area[35]. In addition, descending aortas were isolated and snap frozen for 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 NFκB (a major regulatory component of inflammatory reactions) in the plaque. NFκB was detected using mouse anti-human p65-NFκB (F-6, 1:100, Santa Cruz Biotechnology). The level of NFκB-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 Methods

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 ± 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).

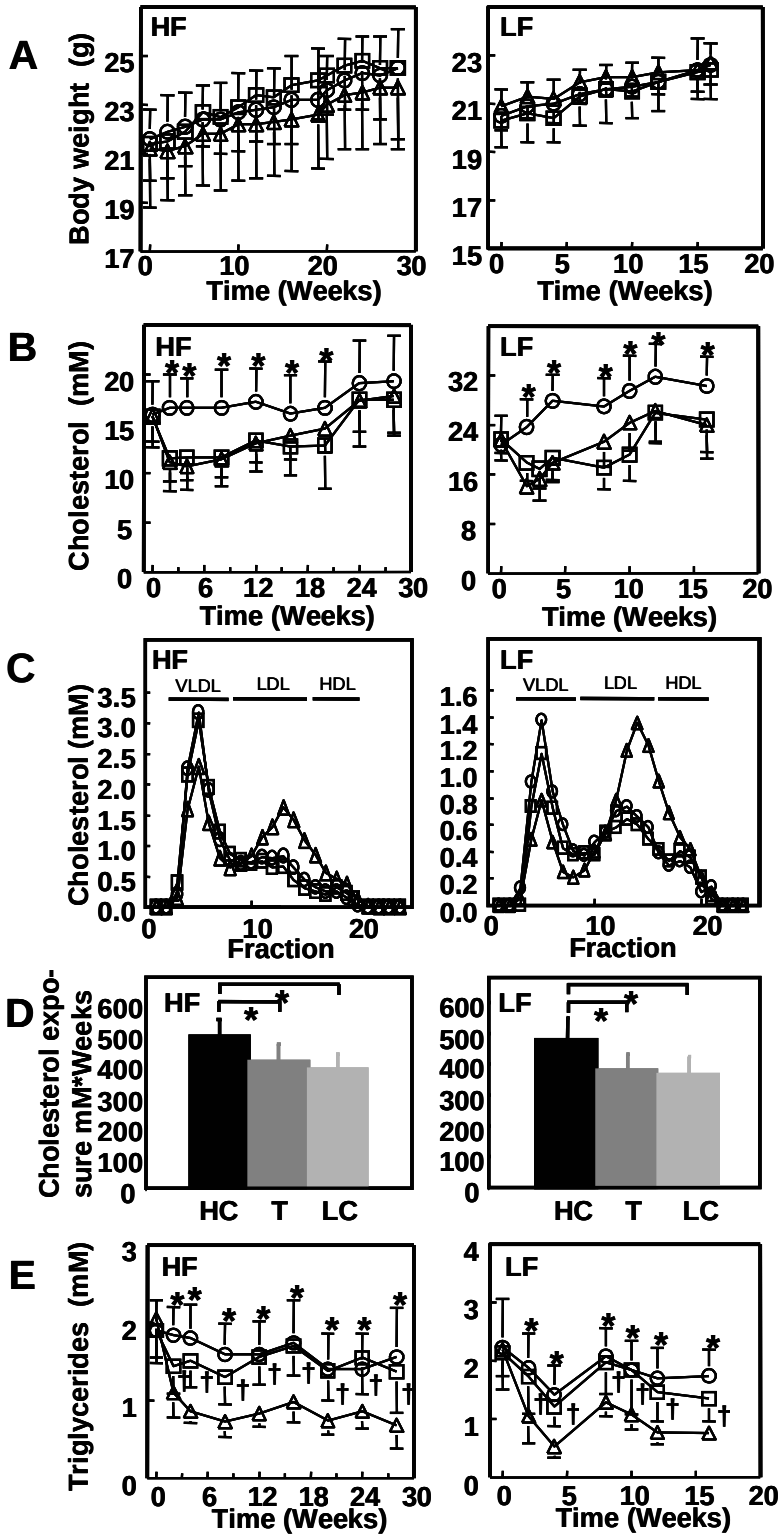

Figure 1. Effect of tesaglitazar on plasma lipids in ApoE3*Leiden mice with (left panels) and without (right panels) insulin resistance. A, body weight over time. B, plasma cholesterol over time. C, lipoprotein profiles. D, total cholesterol exposure. E, plasma triglycerides over time. To increase plasma 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

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±11.4 nmol/L for the HF groups and 41.4±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 ($P<0.05$).

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 (µg/L)	HOMA-IR
High fat					
	HC		6.8±0.8	1.3±0.8	11.1±6.8
	T	**28**	5.7±0.5*	0.6±0.4*	4.3±3.2*
	LC		5.5±0.6*	0.5±0.4*	3.4±2.7*
Low fat					
	HC		5.6±0.6	0.7±0.4	5.0±3.4
	T	**16**	5.1±0.4*	0.5±0.4	2.9±2.4*
	LC		5.5±0.5	0.5±0.5	3.7±3.2

HOMA-IR = Insulin (µU/mL) x (Glucose (mmol/L)/22.5)

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

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

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

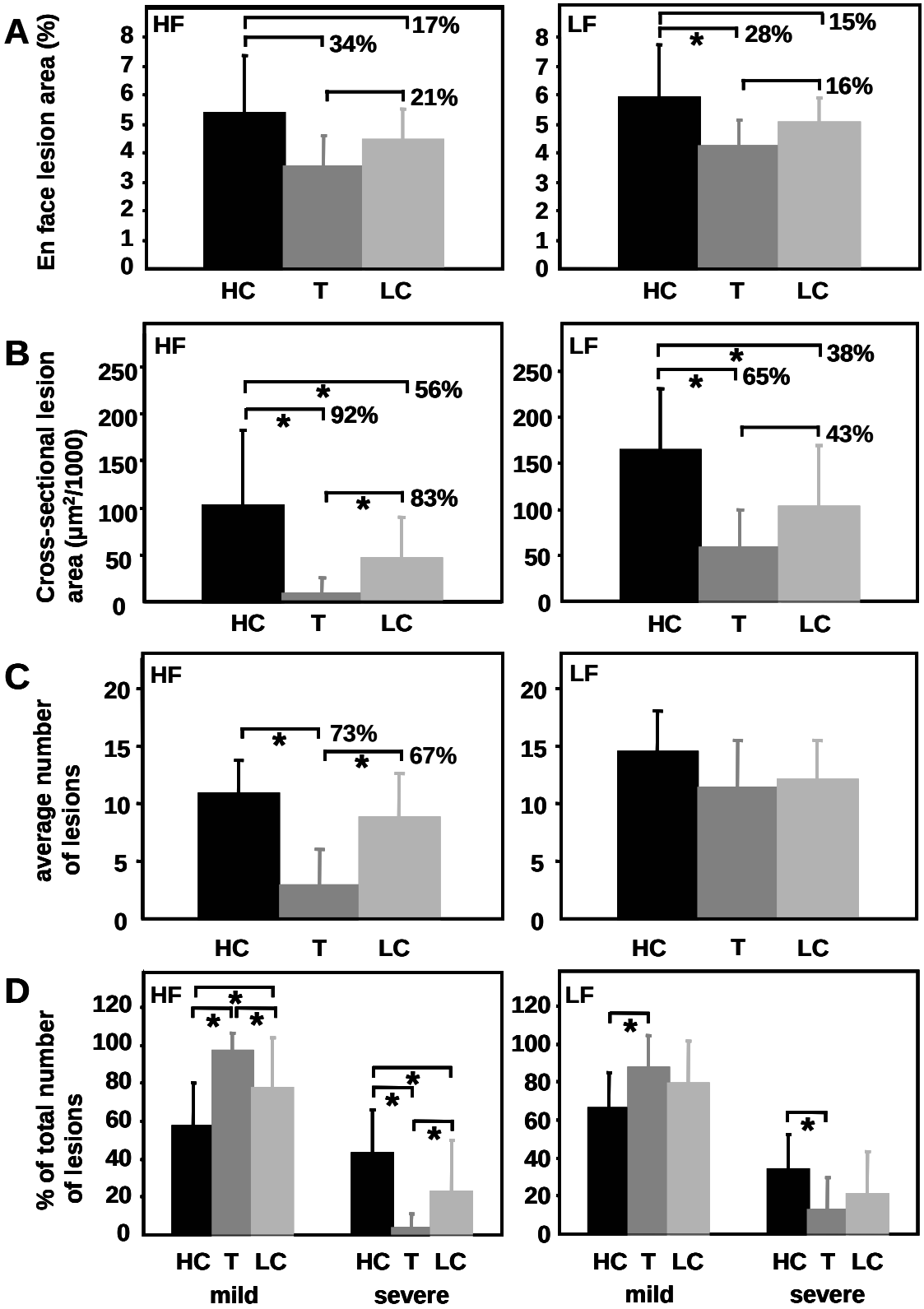

Figure 2. Effect of tesaglitazar on atherosclerosis in the aorta of ApoE3*Leiden mice with (left) and without (right) insulin resistance. Shown are A, the aortic "en face" atherosclerotic lesion area, B, the cross-sectional lesion area in the aortic valve area, C, total number of lesions and D, lesion severity. *P<0.05

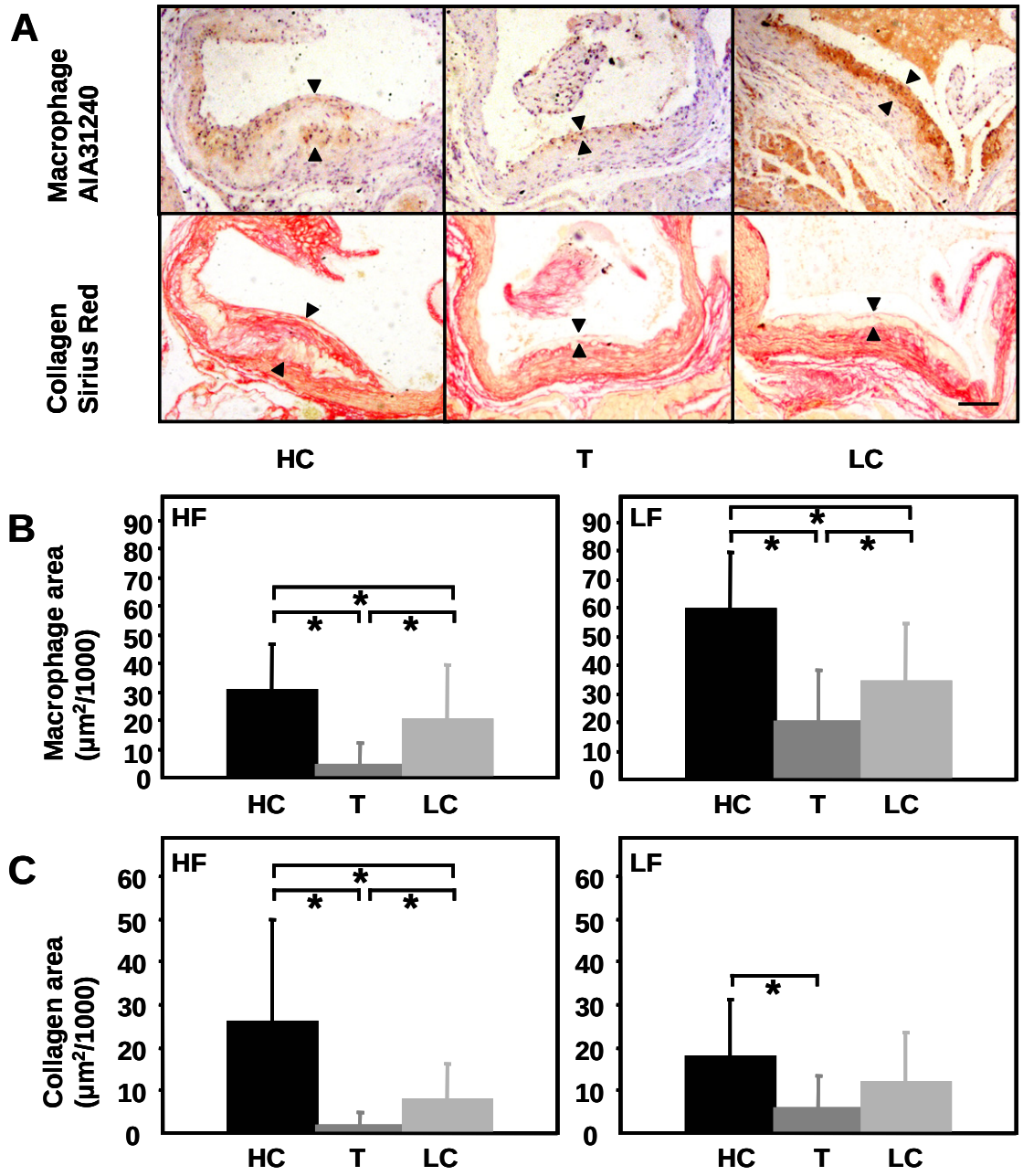

Figure 3. Cross-sectional lesion characteristics. A, Representative microscopic images of macrophage and collagen staining of HF-fed mice. B, macrophage area. C, collagen area. *P<0.05

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 (-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.

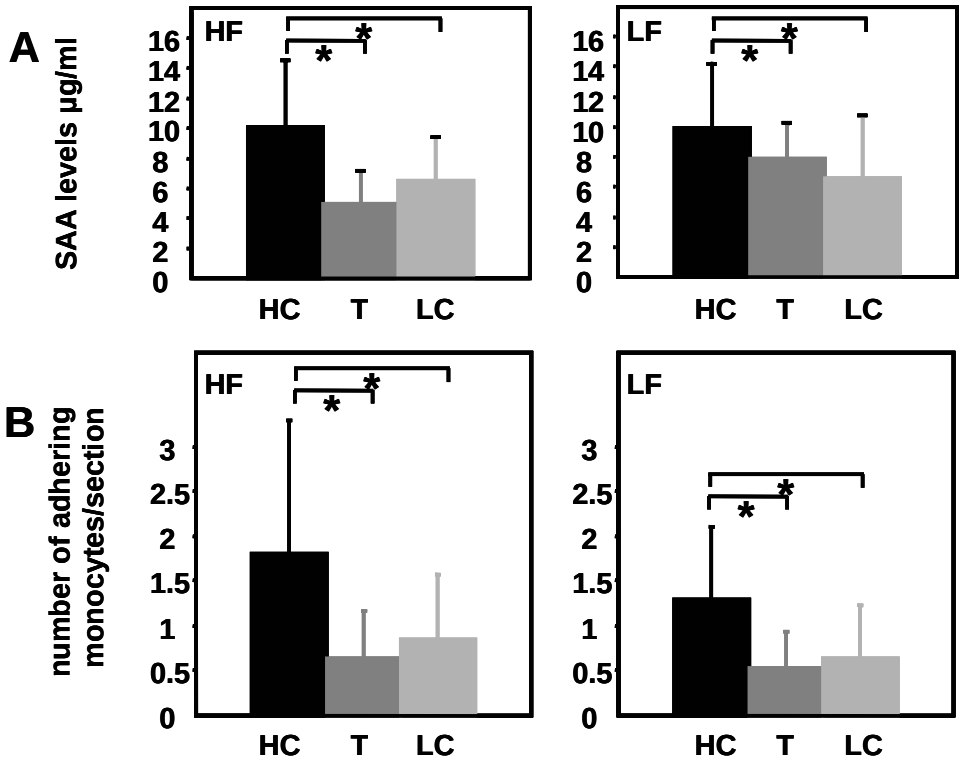

Figure 4. Effect of tesaglitazar on inflammatory parameters in ApoE3*Leiden mice with (left) or without (right) insulin resistance. A, plasma SAA level, B, monocyte adherence. *P<0.05

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).

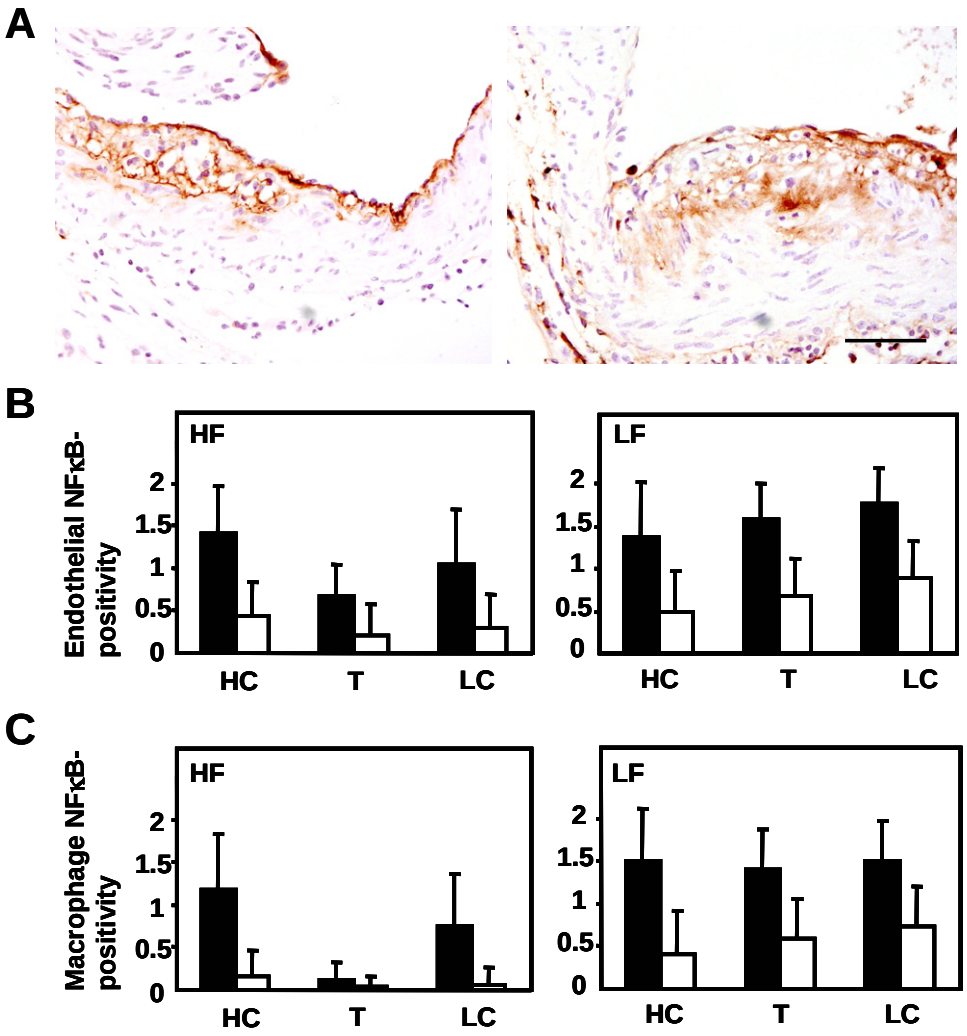

Figure 5. Effect of tesaglitazar on the inflammatory marker NFκB in atherosclerotic plaques of ApoE3*Leiden mice with (left) or without (right) insulin resistance. A, representative microscopic pictures of p65-NFκB-positive staining of atherosclerotic plaques (scale bar=100 μm). B, scoring of endothelial NFκB positivity in the cytosol (black bars) and nucleus (white bars). C, scoring of macrophage NFκB-positivity in the cytosol (black bars) and nucleus (white bars). *P<0.05

Discussion

This study showed that tesaglitazar has atherosclerosis reducing capacities in ApoE*3Leiden 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 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*3Leiden 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*3Leiden mice[29,30,36], 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[37,38] and treatment with a PPARα agonist reduces both plasma cholesterol and triglyceride levels (unpublished data). In addition, we showed in an earlier dose-finding study that ApoE*3Leiden mice respond to the dual PPARα/γ 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 μmol /kg diet (or 20 μg/kg body weight /day), a mild decrease in plasma cholesterol of approximately 20% was achieved. This dose is close to the tesaglitazar dose being tested in humans (1 mg per day).

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*3Leiden mice with the PPARα agonist fenofibrate (unpublished data). Since cholesteryl ester transfer protein is not expressed in mice, these particles could represent large apoE-rich HDL[39]. 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 NFκB 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 NFκB expression at the same location. Previous studies have shown evidence for anti-inflammatory activities of PPARα and -γ agonists, due to upregulation of IκB, leading to decreased NFκB/C-EBPβ complexes and suppression of C-reactive protein synthesis[40]. 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 PPARα/γ 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.

Acknowledgments

This study was supported by the Netherlands Organization of Scientific Research (NWO/ZonMw grant no. 902-26-242), the Netherlands Heart Foundation (grant no. 2000.051) and AstraZeneca, Mölndal, Sweden. J.W.J. is a clinical established investigator of the Netherlands Heart Foundation (2001D032). We thank the technicians of TNO-Biomedical Research and AstraZeneca-Sweden for their excellent technical assistance.

References

1. Frick MH, Elo O, Haapa K et al. Helsinki Heart Study: primary-prevention trial with gemfibrozil in middle-aged men with dyslipidemia. Safety of treatment, changes in risk factors, and incidence of coronary heart disease. *N Engl J Med*. 1987;317:1237-1245.
2. Rubins HB, Robins SJ, Collins D et al. Gemfibrozil for the secondary prevention of coronary heart disease in men with low levels of high-density lipoprotein cholesterol. Veterans Affairs High-Density Lipoprotein Cholesterol Intervention Trial Study Group. *N Engl J Med*. 1999;341:410-418.
3. Tailleux A, Torpier G, Mezdour H et al. Murine models to investigate pharmacological compounds acting as ligands of PPARs in dyslipidemia and atherosclerosis. *Trends Pharmacol Sci*. 2003;24:530-534.
4. Lebovitz HE, Dole JF, Patwardhan R et al. Rosiglitazone monotherapy is effective in patients with type 2 diabetes. *J Clin Endocrinol Metab*. 2001;86:280-288.

5. Rosenblatt S, Miskin B, Glazer NB et al. The impact of pioglitazone on glycemic control and atherogenic dyslipidemia in patients with type 2 diabetes mellitus. *Coron Artery Dis*. 2001;12:413-423.
6. Winkler K, Konrad T, Fullert S et al. Pioglitazone reduces atherogenic dense LDL particles in nondiabetic patients with arterial hypertension: a double-blind, placebo-controlled study. *Diabetes Care*. 2003;26:2588-2594.
7. Xu J, Storer PD, Chavis JA et al. Agonists for the peroxisome proliferator-activated receptor-alpha and the retinoid X receptor inhibit inflammatory responses of microglia. *J Neurosci Res*. 2005.
8. Zingarelli B, Cook JA. Peroxisome proliferator-activated receptor-gamma is a new therapeutic target in sepsis and inflammation. *Shock*. 2005;23:393-399.
9. Hansson GK. Inflammation, atherosclerosis, and coronary artery disease. *N Engl J Med*. 2005;352:1685-1695.
10. Duez H, Fruchart JC, Staels B. PPARS in inflammation, atherosclerosis and thrombosis. *J Cardiovasc Risk*. 2001;8:187-194.
11. Jiang C, Ting AT, Seed B. PPAR-gamma agonists inhibit production of monocyte inflammatory cytokines. *Nature*. 1998;391:82-86.
12. Marx N, Duez H, Fruchart JC et al. Peroxisome proliferator-activated receptors and atherogenesis: regulators of gene expression in vascular cells. *Circ Res*. 2004;94:1168-1178.
13. Poynter ME, Daynes RA. Peroxisome proliferator-activated receptor alpha activation modulates cellular redox status, represses nuclear factor-kappaB signaling, and reduces inflammatory cytokine production in aging. *J Biol Chem*. 1998;273:32833-32841.
14. Delerive P, De Bosscher K, Besnard S et al. Peroxisome proliferator-activated receptor alpha negatively regulates the vascular inflammatory gene response by negative cross-talk with transcription factors NF-kappaB and AP-1. *J Biol Chem*. 1999;274:32048-32054.
15. Delerive P, Martin-Nizard F, Chinetti G et al. Peroxisome proliferator-activated receptor activators inhibit thrombin-induced endothelin-1 production in human vascular endothelial cells by inhibiting the activator protein-1 signaling pathway. *Circ Res*. 1999;85:394-402.
16. Martin-Nizard F, Furman C, Delerive P et al. Peroxisome proliferator-activated receptor activators inhibit oxidized low-density lipoprotein-induced endothelin-1 secretion in endothelial cells. *J Cardiovasc Pharmacol*. 2002;40:822-831.
17. Pasceri V, Wu HD, Willerson JT et al. Modulation of vascular inflammation in vitro and in vivo by peroxisome proliferator-activated receptor-gamma activators. *Circulation*. 2000;101:235-238.
18. Jackson SM, Parhami F, Xi XP et al. Peroxisome proliferator-activated receptor activators target human endothelial cells to inhibit leukocyte-endothelial cell interaction. *Arterioscler Thromb Vasc Biol*. 1999;19:2094-2104.
19. Lee H, Shi W, Tontonoz P et al. Role for peroxisome proliferator-activated receptor alpha in oxidized phospholipid-induced synthesis of monocyte chemotactic protein-1 and interleukin-8 by endothelial cells. *Circ Res*. 2000;87:516-521.

20. Shu H, Wong B, Zhou G et al. Activation of PPARalpha or gamma reduces secretion of matrix metalloproteinase 9 but not interleukin 8 from human monocytic THP-1 cells. *Biochem Biophys Res Commun*. 2000;267:345-349.
21. Goetze S, Xi XP, Kawano H et al. PPAR gamma-ligands inhibit migration mediated by multiple chemoattractants in vascular smooth muscle cells. *J Cardiovasc Pharmacol*. 1999;33:798-806.
22. Chinetti G, Lestavel S, Bocher V et al. PPAR-alpha and PPAR-gamma activators induce cholesterol removal from human macrophage foam cells through stimulation of the ABCA1 pathway. *Nat Med*. 2001;7:53-58.
23. Fazio S, Linton MF. The role of fibrates in managing hyperlipidemia: mechanisms of action and clinical efficacy. *Curr Atheroscler Rep*. 2004;6:148-157.
24. Sidhu JS, Kaposzta Z, Markus HS et al. Effect of rosiglitazone on common carotid intima-media thickness progression in coronary artery disease patients without diabetes mellitus. *Arterioscler Thromb Vasc Biol*. 2004;24:930-934.
25. Fagerberg B, Edwards S, Halmos T et al. Tesaglitazar, a novel dual peroxisome proliferator-activated receptor alpha/gamma agonist, dose-dependently improves the metabolic abnormalities associated with insulin resistance in a non-diabetic population. *Diabetologia*. 2005;48:1716-1725.
26. Saad MF, Greco S, Osei K et al. Ragaglitazar improves glycemic control and lipid profile in type 2 diabetic subjects: a 12-week, double-blind, placebo-controlled dose-ranging study with an open pioglitazone arm. *Diabetes Care*. 2004;27:1324-1329.
27. Ljung B, Bamberg K, Dahllof B et al. AZ 242, a novel PPARalpha/gamma agonist with beneficial effects on insulin resistance and carbohydrate and lipid metabolism in ob/ob mice and obese Zucker rats. *J Lipid Res*. 2002;43:1855-1863.
28. Zuckerman SH, Kauffman RF, Evans GF. Peroxisome proliferator-activated receptor alpha,gamma coagonist LY465608 inhibits macrophage activation and atherosclerosis in apolipoprotein E knockout mice. *Lipids*. 2002;37:487-494.
29. Groot PH, van Vlijmen BJ, Benson GM et al. Quantitative assessment of aortic atherosclerosis in APOE*3 Leiden transgenic mice and its relationship to serum cholesterol exposure. *Arterioscler Thromb Vasc Biol*. 1996;16:926-933.
30. van Vlijmen BJ, van den Maagdenberg AM, Gijbels MJ et al. Diet-induced hyperlipoproteinemia and atherosclerosis in apolipoprotein E3-Leiden transgenic mice. *J Clin Invest*. 1994;93:1403-1410.
31. van Vlijmen BJ, Pearce NJ, Bergo M et al. Apolipoprotein E*3-Leiden transgenic mice as a test model for hypolipidaemic drugs. *Arzneimittelforschung*. 1998;48:396-402.
32. Matthews DR, Hosker JP, Rudenski AS et al. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia*. 1985;28:412-419.
33. Kockx M, Gervois PP, Poulain P et al. Fibrates suppress fibrinogen gene expression in rodents via activation of the peroxisome proliferator-activated receptor-alpha. *Blood*. 1999;93:2991-2998.

34. Paigen B, Morrow A, Holmes PA et al. Quantitative assessment of atherosclerotic lesions in mice. *Atherosclerosis*. 1987;68:231-240.
35. Volger OL, Mensink RP, Plat J et al. Dietary vegetable oil and wood derived plant stanol esters reduce atherosclerotic lesion size and severity in apoE*3-Leiden transgenic mice. *Atherosclerosis*. 2001;157:375-381.
36. Kleemann R, Princen HM, Emeis JJ et al. Rosuvastatin reduces atherosclerosis development beyond and independent of its plasma cholesterol-lowering effect in APOE*3-Leiden transgenic mice: evidence for antiinflammatory effects of rosuvastatin. *Circulation*. 2003;108:1368-1374.
37. Delsing DJ, Post SM, Groenendijk M et al. Rosuvastatin reduces plasma lipids by inhibiting VLDL production and enhancing hepatobiliary lipid excretion in ApoE*3-leiden mice. *J Cardiovasc Pharmacol*. 2005;45:53-60.
38. Verschuren L, Kleemann R, Offerman EH et al. Effect of low dose atorvastatin versus diet-induced cholesterol lowering on atherosclerotic lesion progression and inflammation in apolipoprotein E*3-Leiden transgenic mice. *Arterioscler Thromb Vasc Biol*. 2005;25:161-167.
39. Pitas RE, Innerarity TL, Arnold KS et al. Rate and equilibrium constants for binding of apo-E HDLc (a cholesterol-induced lipoprotein) and low density lipoproteins to human fibroblasts: evidence for multiple receptor binding of apo-E HDLc. *Proc Natl Acad Sci U S A*. 1979;76:2311-2315.
40. Kleemann R, Verschuren L, de Rooij BJ et al. Evidence for anti-inflammatory activity of statins and PPARalpha activators in human C-reactive protein transgenic mice in vivo and in cultured human hepatocytes in vitro. *Blood*. 2004;103:4188-4194.