Absence of Peroxisome Proliferator-Activated Receptor-α Abolishes Hypertension and Attenuates Atherosclerosis in the Tsukuba Hypertensive Mouse

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Abstract—Peroxisome proliferator-activated receptor-α is widely distributed in the vasculature where it is believed to exert pleiotropic antiatherogenic effects. Its role in the regulation of blood pressure is still unresolved; however, some evidence suggests that it may affect the renin-angiotensin system. We investigated its role in angiotensin II–induced hypertension in the Tsukuba hypertensive mouse (THM). This is a model of hypertension and atherosclerosis because of high angiotensin II and aldosterone levels as a result of the transgenic expression of the entire human renin-angiotensin system. Making the THM animals deficient in Peroxisome proliferator-activated receptor-α (THM/PPARKO) totally abolished hypertension and myocardial hypertrophy. This was accompanied by a reduction in plasma human active renin in THM/PPARKO mice compared with THM animals from 3525±128 mU/L to 1910±750 mU/L (P<0.05) and by a normalization of serum aldosterone (1.6±0.29 nmol/L versus 3.4±0.69 nmol/L; P=0.003). In the THM/PPARKO mice, the extent of atherosclerosis at the aortic sinus after a 12-week period on an atherogenic diet was decreased by >80%. In addition, the spontaneous formation of foam cells from peritoneal macrophages, a blood pressure–independent event, was reduced by 92% in the THM/PPARKO mice, suggesting protection from the usual oxidative stress in these animals, possibly because of lower prevailing angiotensin II levels. Finally, chronic fenofibrate treatment further elevated blood pressure in THM animals but not in THM/PPARKO animals. Taken together, these data indicate that peroxisome proliferator-activated receptor-α may regulate the renin-angiotensin system. They raise the possibility that its activation may aggravate hypertension and hasten atherosclerosis in the context of an activated renin-angiotensin system. (Hypertension. 2007;50:1-2.)

Key Words: PPARα ■ renin ■ angiotensin ■ hypertension ■ atherosclerosis ■ transgenic mice

First identified and cloned from the liver as the nuclear receptor that mediates peroxisomal proliferation, proliferator-activated receptor (PPAR)-α has been found to be widely expressed in all of the cellular components of the vascular wall, where it is shown to exert pleiotropic antiatherogenic effects. Thus, in addition to its lipid-lowering effect, which mostly takes place in the liver, PPARα is believed to impart direct protection in the vessel wall by intervening at essentially every level of the atherogenic process: inflammation, monocyte recruitment and adhesion, cholesterol transport, plaque formation, and thrombosis, mostly through downregulation of nuclear factor κB and activator protein-1 (AP-1). In contrast to the plethora of data regarding the atherogenic process, the role of PPARα in the regulation of vascular tone and blood pressure is still unclear. Several studies conducted in different rat models of hypertension yielded inconsistent results. In a previous study, we reported that the atherosclerosis-prone apolipoprotein E (apoE)–null mice were protected from diet-induced atherosclerosis when made deficient in PPARα. Quite unexpectedly, the absence of PPARα protected these animals from atherosclerosis despite a worse diet-induced lipoprotein profile compared with apoE–null mice with a normal PPARα gene. In this study, we had also noted a lower blood pressure in the double knockout mice fed the atherogenic diet. Because the mice were not frankly hypertensive, the effect on blood pressure in itself could not explain the protection from atherosclerosis. However, a sustained reduction of the aortic expression of monocyte chemotactic protein-1, an important target gene of angiotensin II (Ang II), in the apoE/PPARα–null mice, suggested that PPARα could have an impact on the local expression of this proatherogenic cytokine, possibly through an effect on systemic or locally expressed renin-angiotensin system (RAS).
The Tsukuba hypertensive mouse (THM) is an established model of Ang II-mediated hypertension and atherosclerosis. This animal is obtained for each mating by crossing males from the transgenic line of mice that are homozygous for the human renin gene (hRen<sup>+/−</sup>) with females from the line that are homozygous for the human angiotensinogen transgene (hAGT<sup>+/−</sup>). Because the system is species specific, the parental lines are normotensive. However, all of the offspring are doubly heterozygous (hRen<sup>+/−</sup>/hAGT<sup>+/−</sup>), and having the full human RAS complement, they generate high levels of Ang II, which, in turn, are responsible for the elevation of aldosterone and the development of hypertension and salt sensitivity.\(^7\) In the present study we sought to investigate the role of PPARα in the generation of hypertension and the extent of atherosclerosis in this transgenic model of human RAS overexpression by moving the PPARα-null genotype into the original THM model.

**Methods**

**Animals, Breeding Scheme, and Genotyping**

PPARα-null THM mice (THM/PPARKO) were obtained by moving the PPARα-null genotype in the respective transgenic line (hRen<sup>+/−</sup> and hAGT<sup>+/−</sup>) by cross-breeding. After identification of the F2 PPARα-null animals carrying the respective human transgenes, reconstitution of the THM genotype on the PPARα-null background was carried out by appropriate mating; THM/PPARKO pups were then identified by genotyping as detailed in the online supplemental data (available at http://hyper.ahajournals.org).

**Treatments**

For experiments aimed at assessing atherosclerosis, THM animals were placed on an atherogenic, high-fat “Western” diet (Harlan Teklad diet 88137) at the age of 8 weeks until sacrifice 12 weeks later.

As an experimental mirror image experiment to knocking out PPARα, we also sought to further activate the receptor by administering the PPARα agonist fenofibrate (Sigma-Aldrich). In this set of experiments, the drug was dissolved in ethanol and mixed with the drinking water (1% vol/vol) for 6 weeks. The average fenofibrate dose ingested by a mouse was 100 mg kg<sup>−1</sup> d<sup>−1</sup>.

**Blood Pressure Measurement**

Starting at 8 weeks, after acclimatization of the animals, repeated long-term blood pressure assessment in awake mice was achieved taking a noninvasive approach using 3-channel computerized tail-cuff IITC system model 3M229 BP, attached to the 31BP software (IITC Life Science Inc). Further information regarding the rationale for electing noninvasive measurement and the details of the method can be found in the online supplement.

**Atherosclerosis and Hormonal Determinations**

Atherosclerosis being a major end point of the study, biochemical determinations related to lipid profile and glucose metabolism under the high-fat diet. Hormonal investigations related to assessing levels of RAS components: human renin and aldosterone. The methods used to assess those analytes are given in the online supplemental data.

**Atherosclerosis Assessment and Heart Pathology**

For atherosclerosis assessment, hearts were flushed with heparin containing PBS, sectioned through the ventricles to include the aortic sinus, and embedded in OCT (Sakura Finetek). Analysis of atherosclerotic lesions was carried out as described previously.\(^8\)

Hearts for pathological studies were harvested and kept overnight in freshly prepared 4% paraformaldehyde in PBS (pH 7.4). Specimens were subsequently paraffin embedded, cut, mounted, and stained with hematoxylin/eosin.

**Peritoneal Foam Cell Formation**

In vivo foam cell formation was assessed in peritoneal macrophages. After being fed a high-fat diet for 8 weeks, THM and THM/PPARKO mice received an intraperitoneal injection of thioglycollate (2 mL per animal), and thioglycollate-elicited peritoneal macrophages were harvested 4 days later. Cells were plated in DMEM with 10% FCS at a density of 2.10<sup>6</sup> cells/cm<sup>2</sup>. Two days after plating, adhering macrophages were stained with oil-red-O for 30 minutes and counterstained with hematoxylin (Gill2 solution) for 30 seconds. The percentage of oil-red-O stainable foam cells for each animal was then quantitated from 5 different fields viewed at ×40 magnification.

**Statistical Analysis**

Data are expressed as mean±SE. Comparisons between groups were done using 2-tailed Student’s t test, paired t test, or the χ<sup>2</sup> test, as indicated, using the Instat software (GraphPad). Statistical significance was assumed for P<0.05.

**Results**

**Reconstitution of the THM Mouse With the PPARα-Null Genotype**

We first generated and identified the F2 PPARα<sup>−/−</sup> animals that carried either the hRen or the hAGT gene. These animals then served to reconstitute the THM genotype. As shown in Figure 1, desired animals carrying both transgenes were ultimately identified by PCR. In this litter of 11, all obligatorily PPARα<sup>−/−</sup> pups, only 3 had both transgenes and were, thus, THM/PPARKO mice. All breeding cages and weaned animals until the age of 6 weeks were given low-sodium (9 mg/L) mineral water, because survival of the THM pups, but not that of the THM/PPARKO mice, depended on elimination of salt from the drinking water.

**Metabolic and Hormonal Impact of PPARα Deficiency in THM Mice**

**Weight**

At the age of 8 weeks, all of the mice were placed on a high-fat atherogenic diet, which promoted significant weight...
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in Hypertensive Transgenic Mice

Hypertension and Myocardial Hypertrophy

THM/PPARKO Animals Are Protected From Hypertension

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Lipid Profile and Glucose Metabolism

Because PPARα has a central role in hepatic lipoprotein metabolism, we examined whether its absence affected the lipid profile in THM mice fed a high-fat diet. By and large, no differences in lipid profile were seen at baseline or during the 3 months of high-fat feeding between THM and THM/PPARKO animals (Table S1, available in the online supplement). Notably, none of the models had significant hyperlipidemia as a potential contributor for accelerated atherosclerosis.

Despite their heavier weight, THM/PPARKO mice appeared to enjoy protection from diet-induced glucose elevation (Table S1). This protection encompassed not only fasting glucose, as THM/PPARKO animals subjected to an intraperitoneal glucose tolerance test demonstrated far better glucose tolerance than the THM mice (Figure S2).

Human Active Renin And Aldosterone Concentrations

Plasma human circulating active renin concentration was decreased almost by half in THM/PPARKO mice compared with THM mice (1910 ± 750 mU/L versus 3525 ± 128 mU/L; P<0.05). These concentrations are still ~50 times higher than the highest concentration measured in humans in the upright position. However, because the high tap water sodium content had seemed to be directly responsible for the perinatal attrition in the THM animals, sparing the THM/PPARKO pups, we suspected the reduction in human renin translated into a physiologically relevant decrease in aldosterone. Indeed, aldosterone concentration in the adult THM/PPARKO animals was markedly lower (1.6 ± 0.29 nmol/L; n = 26) than in the THM animals (3.4 ± 0.69 nmol/L; n = 18; P = 0.003) and essentially identical to what we commonly measure in C57/Bl6 mice.

THM/PPARKO Animals Are Protected From Hypertension and Myocardial Hypertrophy

In spite of having the entire complement of the human genes, like the original THM mice, THM/PPARKO mice were entirely protected from hypertension. At all of the time points, THM/PPARKO remained normotensive, whereas THM animals were hypertensive right from the beginning of the experiment (differences between groups were highly significant; P<0.0001 at all of the time points for both systolic and diastolic blood pressures; Figure 2). This also translated into protection from heart hypertrophy. Heart/body ratio was 7.37 ± 0.28 mg·g−1 in THM mice but was only 5.0 ± 0.23 mg·g−1 in THM/PPARKO mice (P<0.0001). Moreover, this was accompanied by clear differences in the histological appearance of the myocardium between the groups. THM hearts displayed thickened myofibers with disrupted architecture, whereas THM/PPARKO hearts retained normal histological characteristics (Figure 3).

The Extent of Atherosclerosis Is Decreased in THM/PPARKO Mice

Under a high-fat diet, THM mice develop atherosclerotic lesions at the aortic sinus. The extent of atherosclerotic lesions was, thus, quantitated after 12 weeks on a high-fat diet. As illustrated in Figure 4A and 4B, atherosclerotic lesions occupied 11.8 ± 2.3% of the aortic root in THM mice. However, the extent of the lesions was decreased by >80% to take up only 1.9 ± 0.5% of the vessel area in THM/PPARKO animals (P<0.001; Figure 4C).

Because the high levels of Ang II exert a potent oxidative stress, we evaluated the degree of spontaneous peritoneal foam cell formation in these high-fat–fed animals as a blood pressure–independent marker for the atherogenic process. No additional in vitro oxidative stress or lipid loading of macrophages was necessary to demonstrate the generation of typical foam cells in THM animals. As a mirror image to what we observed in the vessel wall, the number of peritoneal foam macrophages was dramatically suppressed in THM/PPARKO compared with THM mice (Figure 4D and 4E). In THM mice, 12.4 ± 3.8% of all of the peritoneal macrophages
appeared as lipid-loaded foam cells compared with only 0.96±0.2% in THM/PPARKO mice (\(P<0.002\); Figure 4F).

**Fenofibrate Treatment Worsens Hypertension in THM Mice**

Because the abolition of hypertension appeared to be a direct result of the lack of PPAR\(\alpha\) in THM/PPARKO mice, we reasoned that chronic activation of the receptor by an exogenous ligand might worsen the hypertension in THM animals. To this end, 8- to 10-week-old mice were treated with fenofibrate. The results of a typical experiment are shown in Figure 5. Both systolic and diastolic blood pressure increased significantly with fenofibrate in these animals. However, this effect of fenofibrate was not seen in THM/PPARKO mice (data not shown), indicating that it depended on the expression of the nuclear receptor.

**Discussion**

In this study, we chose the THM as our paradigm to evaluate the role of PPAR\(\alpha\) in Ang II-associated hypertension and atherosclerosis. The THM mouse is a very well-characterized model of hypertension because of an extremely active RAS and elevated Ang II. In addition to displaying severe pathological changes in the kidneys, the heart, and the arteries, this mouse is also a model of atherosclerosis, which develops predominantly at the aortic sinus.7,8,12 Because our previous studies in the nonhypertensive apoE-null model indicated that

![Figure 4](image)

**Figure 4.** Absence of the PPAR\(\alpha\) gene confers protection from diet-induced atherosclerosis in THM mice and reduces formation of peritoneal foam cells. Representative cross-sections of OCT-embedded hearts at the level of the aortic sinus and stained with oil-red-O at ×40 magnification showing intense subintimal plaque formation in THM mice (A) but more discreet lesions in the THM/PPARKO animals (B). Comparison of computerized morphometry and quantitation of the extent of lesions at the sinus expressed as percentage of the vessel area is given for 9 THM and 15 THM/PPARKO mice; \(P<0.001\) (C). Spontaneous foam cell formation after 12 weeks of high-fat feeding in THM peritoneal macrophages detected on oil-red-O staining at ×20 magnification (D) is significantly reduced in THM/PPARKO (E). Quantitation of the average percentage of foam cells in 12 THM and 13 THM/PPARKO animals demonstrates a 92% reduction in THM/PPARKO mice; \(P=0.02\) (F).

![Figure 5](image)

**Figure 5.** The PPAR\(\alpha\) ligand fenofibrate elevates blood pressure in THM mice. Eleven THM mice (■) received fenofibrate, whereas 10 control mice received the vehicle (□). By 6 weeks of treatment, fenofibrate had increased systolic blood pressure by an average of 10 mm Hg (\(P<0.05\)) compared with baseline and diastolic blood pressure by ~7 mm Hg (\(P<0.01\)) compared with baseline. **\(P<0.001\) between groups. Similar results were obtained in 2 subsequent experiments.
the absence of PPARα was linked to lower blood pressure and reduced atherosclerosis, we anticipated that deficient expression of PPARα might lead to amelioration of both hypertension and arterial injury in the THM mice as well. The most striking finding of this study was, indeed, the complete protection from hypertension in THM/PPARKO mice, which harbored exactly the same genetic complement leading to expression of the transgenic human RAS in THM animals. This marked favorable effect on blood pressure in PPARα-null mice was associated, as reported previously, with better glucose tolerance and took place despite increased gain in weight in high-fat–fed THM/PPARKO compared with the PPARα wild-type original THM animals.6,13–16

Further support for the role of PPARα in the regulation of blood pressure in the THM mouse is provided by the significant rise in blood pressure under chronic fenofibrate treatment and the lack of effect in the THM/PPARKO mouse. These observations are in line with an earlier report that restoration of hepatic PPARα expression in low-density lipoprotein (LDL) receptor/PPARα-null mice through injection of a recombinant PPARα adenovirus abolished the protection from dexamethasone-induced hypertension in these animals.17

The attainment of normal blood pressure in such a clearly renin-dependent model as the THM mouse through ablation of the PPARα gene would, in itself, imply that the absence of PPARα interferes with renin-related mechanism(s) of hypertension. The observed 50% reduction in circulating human active renin in THM/PPARKO mice apparently reflects one such mechanism. Additional effects must be still assumed, because plasma active renin remained high even in the THM/PPARKO mice. Indeed, there is previous evidence that more than one element of the RAS-aldosterone system may be regulated by PPARα. In line with the reduction in circulating active renin in this study, PPARα-deficient mice, re-expression of hepatic PPARα in LDL receptor/PPARα-null mice was accompanied by more than doubling of the plasma renin activity. In addition, a PPARα response element has been identified in the renin gene suggesting potential regulation at the transcriptional level.18,19 Furthermore, a PPARα binding element in the promoter of the human angiotensinogen gene has been described recently. Because bezafibrate binding competed with that of the hepatic nuclear factor-4 in a reporter assay, it was suggested that binding of PPARα ligands to the angiotensinogen promoter might be particularly significant in extrahepatic tissues where hepatic nuclear factor-4 is not expressed.20 Because THM mice express the human angiotensinogen transcript in the kidneys at levels comparable to those measured in liver,21 the elimination of a putative PPARα-angiotensinogen interaction at this site is composed of an attractive candidate mechanism through which the RAS activity might be modulated in THM/PPARKO animals.

Although not directly assessed in this study, the reduction in renin in THM/PPARKO mice is expected to lead to a decrease in Ang II production and, consequently, to attenuation of RAS effects downstream to Ang II. The completely normal aldosterone levels measured in the THM/PPARKO mice are in line with the notion that some major Ang II-dependent effects are greatly downregulated in the absence of PPARα.

It is of interest that, whereas PPARγ agonists generally decrease blood pressure in humans,22,23 reported effects of PPARα ligands on blood pressure have been, at best, conflicting. None of the large primary or secondary coronary artery disease prevention studies involving fibrates demonstrated any trend toward blood pressure reduction. In a recent study, treatment with fenofibrate for 3 weeks in normal volunteers increased systolic blood pressure, as assessed by ambulatory blood pressure monitoring.24 Treatment with rosiglitazone, a PPARγ agonist, decreases blood pressure in virtually all types of rodent genetic or experimental hypertension, including in a doubly transgenic mouse model similar to the THM mouse.2,25,26 In contrast, the use of PPARα ligands in experimental models of hypertension has yielded more modest results. Nevertheless, some of the published evidence suggests a beneficial effect of PPARα activation on Ang II-induced hypertension in rodents.4,27,28 In these studies, high levels of Ang II were achieved for relatively short periods of time (7 to 12 days) via a subcutaneous infusion of the peptide. Although this strategy resulted in frank hypertension, partially ameliorated by the concomitant administration of a PPARα ligand, the differences between the models should be emphasized. Exogenous administration of Ang II suppresses plasma renin activity and typically downregulates the expression of the renin gene, whereas the THM mouse is a paradigm for persistent and chronic activation of the entire RAS. Interestingly, in such a study that reported amelioration of Ang II-induced hypertension by PPARα activation in rats, both fenofibrate and Ang II alone caused a modest and comparable rise in aldosterone; however, their combined effect on aldosterone levels was more than additive.4 It should also be noted that, in contrast to rosiglitazone, fenofibrate had no effect on blood pressure in deoxycorticosterone acetate–salt rats.29,30 It is tempting to speculate that this lack of effect could be because of continued stimulation of the kidney renin gene expression, which is typically shut down in this model.31

The other major outcome of this study was the very significant protection from diet-induced atherosclerosis that PPARα deficiency conferred to THM mice. Although the total protection from hypertension in THM/PPARKO mice has likely played an important role in averting atherosclerosis, our results suggest that pressure-independent mechanisms also participated in the observed antiatherosclerotic effect. Ang II is a very potent atherogenic proinflammatory cytokine, and the atherosclerosis seen in these animals is probably not only because of shear stress. Indeed, infusion of Ang II at doses that did not alter blood pressure accelerated atherosclerosis in apoE-deficient mice with the appearance of lipid-laden macrophages in the lesions.32 It is noteworthy that the reduced foam cell formation that we observed in THM/PPARKO-derived peritoneal macrophages was seen under blood pressure–unrelated conditions. This supports the notion that, independent of hemodynamic alterations, THM/PPARKO mice were spared some of the oxidative stress generated by high levels of Ang II in their THM counterparts. Most of the oxidative stress exerted by Ang II is because of
superoxide production by the activated reduced nicotinamideadenine dinucleotide phosphate oxidase complex. In addition to generating reactive oxygen species, which act themselves as signaling molecules, activation of reduced nicotinamideadenine dinucleotide phosphate oxidase by Ang II increases LDL peroxidation by macrophages and uptake of oxidized LDL via scavenger receptors, such as lectin-like oxidized LDL receptor-1.33,34 Both of these processes are central to the pathogenesis of atherosclerosis. Nevertheless, it would be useful to assess whether blood pressure normalization, per se, by a treatment that does not affect the RAS can modulate transformation of peritoneal macrophage to foam cells.

When discussing the putative mechanism by which lack of PPARα protects THM mice from atherosclerosis, one cannot ignore the large body of literature pointing to a beneficial effect of PPARα activation. Ever since the identification of PPARα in all of the cellular components of the vessel wall, numerous studies have added credence to the notion that, in addition to its effect on lipoprotein metabolism in the liver, PPARα has pleiotropic antiatherogenic vascular effects (reviewed in Reference 35). Several discordant studies, however, have suggested that, under certain conditions, PPARα activation may have paradoxical proatherogenic effects. For instance, in a recent study, PPARα activation induced reduced nicotinamide-adenine dinucleotide phosphate oxidase activity in human and mouse macrophages, thereby increasing LDL oxidation. It was further shown that oxidized LDL is, in turn, capable of binding PPARα, thus potentially perpetuating this oxidative and proinflammatory cycle.36 This sequence of events is highly reminiscent of the action of Ang II on macrophages and suggests the existence of a common pathway. Finally, it should be pointed out that, although many in vitro studies have suggested a beneficial effect of PPARα activation on an array of factors typically involved in the atherogenic process, clinical and animal studies have had much less convincing results. Suffice it to say that despite being introduced into clinical practice 40 years ago, fibrates have not had nearly the impact on atherosclerotic cardiovascular morbidity and mortality that statins have demonstrated.

Perspectives
Using the THM mouse as a model of an activated RAS and high Ang II-associated hypertension and atherosclerosis, we have shown that PPARα is necessary for the development of its full phenotype. Absence of PPARα abolished the hypertension and greatly reduced the extent of diet-induced atherosclerosis. Conversely, activation of PPARα worsened hypertension in this model. Although the mechanisms behind these observations have not been fully elucidated, we have provided evidence that PPARα regulates the expression of the RAS starting with its rate-limiting step, renin. Based on the findings of the current study and on some published data, we suggest that, in the setting of an activated RAS and high endogenous levels of Ang II, PPARα activation could be detrimental. In this context, PPARα activation is likely to promote even higher levels of Ang II, increase oxidative stress, raise blood pressure, and ultimately hasten atherosclerosis. Further investigations aimed at delineating the relationship

ships between the transcription factor PPARα and the components of the RAS and their respective molecular effectors are needed.

Acknowledgments
We thank Shulamit Feuchtwanzer and Lea Markevich-Wasser for their expert technical assistance with the aldosterone and human renin determinations.

Source of Funding
This study was supported by a US-Israel Binational Science Foundation grant 2000190.

Disclosures
None.

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