Dexamethasone induction of hypertension and diabetes is PPAR-α dependent in LDL receptor–null mice

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Hypertension and diabetes are common side effects of glucocorticoid treatment. To determine whether peroxisome proliferator–activated receptor-α (PPAR-α) mediates these sequelae, mice deficient in low-density lipoprotein receptor (Ldlr−/−), with (Ppara+/−) or without (Ppara−/−) PPAR-α, were treated chronically with dexamethasone. Ppara+/−, but not Ppara−/−, mice developed hyperglycemia, hyperinsulinemia and hypertension. Similar effects on glucose metabolism were seen in a different model using C57BL/6 mice. Hepatic gluconeogenic gene expression was increased and insulin-mediated suppression of endogenous glucose production was less effective in dexamethasone-treated Ppara+/− mice. Adenoviral reconstitution of PPAR-α in the livers of nondiabetic, normotensive, dexamethasone-treated Ppara−/− mice induced hyperglycemia, hyperinsulinemia and increased gluconeogenic gene expression. It also increased blood pressure, renin activity, sympathetic nervous activity and renal sodium retention. Human hepatocytes treated with dexamethasone and the PPAR-α agonist Wy14,643 induced PPARα and gluconeogenic gene expression. These results identify hepatic activation of PPAR-α as a mechanism underlying glucocorticoid-induced insulin resistance.

Glucocorticoids are among the most commonly prescribed medications. Their anti-inflammatory properties are crucial for people with organ transplants, asthma, malignancies, rheumatologic syndromes, vision-threatening eye disease, skin disorders, glomerulopathies, pain syndromes and other conditions. Unfortunately, chronic glucocorticoid therapy has serious side effects. Older studies report diabetes in up to 25% and hypertension in 20% of patients treated with exogenous steroids1–3. These prevalence numbers are probably underestimated. Current diagnostic criteria for these disorders are more inclusive. Industrialized populations are increasingly obese and thereby predisposed to both type 2 diabetes and hypertension. Endogenous glucocorticoid excess, a rare condition known as Cushing syndrome, causes abnormal glucose metabolism and hypertension in 80–90% of patients4. The mechanisms responsible for steroid-induced diabetes and hypertension are unclear.

Hypertension and diabetes are components of insulin resistance, a poorly understood metabolic condition associated with obesity and atherosclerosis. Glucocorticoids may be involved in subsets of patients with insulin resistance. Obesity is associated with subtle abnormalities of the hypothalamic–pituitary–adrenal axis, suggesting glucocorticoid excess5–7. Phenotypic features of insulin resistance are associated with glucocorticoid excretion in the elderly8. Patients with incidentally discovered adrenal adenomas, now common with the ubiquitous use of imaging techniques, may have subclinical Cushing syndrome and increased cardiovascular risk9. Local production of active glucocorticoids in critical tissues promotes insulin resistance10. Understanding the mechanisms of insulin resistance induced by glucocorticoid treatment may provide insight into the current epidemic of obesity-related diabetes and hypertension.

Abnormal fatty acid metabolism contributes to the pathogenesis of insulin resistance11. Glucocorticoids perturb fatty acid metabolism12. Lowering fatty acids reverses insulin resistance in humans treated with glucocorticoids13, suggesting that lipid signaling is involved in steroid-induced side effects. PPARs, transcription factors activated by fatty acids, transduce metabolic and nutritional signals into transcriptional responses14. Of the known members of this subset of the nuclear receptor superfamily, PPAR-α is the most likely potential mediator of the effects of glucocorticoids on insulin sensitivity. It is expressed in liver, muscle, kidney and the vasculature, which are key tissues for glucose metabolism and regulation of blood pressure. Glucocorticoids induce PPAR-α expression in cultured hepatocytes and intact liver from rodents15,16. Hepatic PPAR-α expression is increased by physiological stress, a response inhibited by the antiglucocorticoid RU486 (ref. 16). PPAR-α deficiency prevents insulin resistance caused by high-fat feeding in two different mouse backgrounds17,18.

To determine whether PPAR-α mediates glucocorticoid-induced insulin resistance, we crossed PPAR-α-deficient (Ppara−/−) mice with LDLR–null (Ldlr−/−) mice, hyperlipidemic animals that are susceptible to insulin resistance induced by a high-fat diet19,20. We also used Ppara−/− mice in a wild-type genetic background (C57BL/6). Our results suggest that hepatic expression of PPAR-α is responsible for steroid-induced diabetes and, surprisingly, for the elevated blood pressure caused by glucocorticoid therapy.

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RESULTS
Dexamethasone and insulin sensitivity

*Para*+/+ *Ldlr*+/– (n = 43) and *Para*+/+ *Ldlr*−/− (n = 29) littermate mice were treated with the synthetic glucocorticoid dexamethasone (DEX; 1 mg/kg) by intraperitoneal injection every other day for 5 months. PPAR-α genotype had no effect on plasma dexamethasone levels assayed by a commercial laboratory. Separate cohorts of *Para*+/+ *Ldlr*−/− (n = 8) and *Para*+/+ *Ldlr*−/− (n = 8) littermates were injected with normal saline every other day for 5 months.

Weight gain was the same in DEX- (Fig. 1a) and saline-injected (Fig. 1b) mice. There was no effect of genotype or drug treatment on food intake (g per mouse per d): 3.18 ± 0.35 for DEX-treated *Para*+/+ *Ldlr*−/−, 3.20 ± 0.15 for DEX-treated *Para*−/− *Ldlr*−/−, 3.97 ± 0.30 for saline-treated *Para*+/+ *Ldlr*−/− and 3.20 ± 0.50 for saline-treated *Para*−/− *Ldlr*−/− mice (not significant by ANOVA).

Body composition and serum chemistries in the same animals at baseline and after DEX treatment are shown in Supplementary Table 1 online. Fasting glucose and insulin increased in DEX-treated mice treated with DEX, and body weight of eight *Para*+/+ *Ldlr*−/− and eight *Para*−/− *Ldlr*−/− mice treated with DEX. Data are presented as mean ± s.e.m. of equal numbers of age-matched males and females (there was no gender-specific effect on the response to injections). C. Glucose tolerance testing of 52 mice (18 for each genotype treated with DEX and 8 for each genotype treated with saline). Data are presented as mean ± s.e.m. of equal numbers of age-matched males and females (there was no gender-specific effect on the response to injections). C. Glucose tolerance testing of mice treated with DEX and saline. Data are presented as mean ± s.e.m. of equal numbers of age-matched males and females (there was no gender-specific effect on the response to injections).

**Figure 1** Impaired glucose tolerance and insulin responsiveness in DEX-treated *Para*+/+ but not *Para*−/– mice. (a–d) LDLR-null mice were injected with DEX or saline every other day beginning at the age of 2 months (day 0). (a) Body weight of 29 *Para*+/+ *Ldlr*−/− (□) and 43 *Para*−/− *Ldlr*−/− (●) mice treated with DEX. b. Body weight of eight *Para*+/+ *Ldlr*−/− (○) and eight *Para*−/− *Ldlr*−/− (●) mice treated with saline. Data are presented as mean ± s.e.m. of equal numbers of age-matched males and females (there was no gender-specific effect on the response to injections). c. Glucose tolerance testing of 52 mice (18 for each genotype treated with DEX and 8 for each genotype treated with saline). Data are presented as mean ± s.e.m. of equal numbers of age-matched males and females (there was no gender-specific effect on the response to injections). d. Insulin tolerance testing of mice treated with DEX and saline. Data are presented as mean ± s.e.m. of equal numbers of age-matched males and females (there was no gender-specific effect on the response to injections).

**Figure 2** Adenoviral treatment caused overexpression in *Para*+/+ mice but not *Para*−/– mice. (a) Body weight of 29 *Para*+/+ *Ldlr*−/− (□) and 43 *Para*−/− *Ldlr*−/− (●) mice treated with DEX and saline. (b) Glucose tolerance testing of 52 mice (18 for each genotype treated with DEX and 8 for each genotype treated with saline). Data are presented as mean ± s.e.m. of equal numbers of age-matched males and females (there was no gender-specific effect on the response to injections). c. Glucose tolerance testing of 52 mice (18 for each genotype treated with DEX and 8 for each genotype treated with saline). Data are presented as mean ± s.e.m. of equal numbers of age-matched males and females (there was no gender-specific effect on the response to injections). d. Insulin tolerance testing of mice treated with DEX and saline. Data are presented as mean ± s.e.m. of equal numbers of age-matched males and females (there was no gender-specific effect on the response to injections)

**Figure 3** Wild-type C57BL/6 mice were injected with DEX every other day beginning at the age of 2 months. e. Glucose tolerance testing of mice treated with DEX and saline. Data are presented as mean ± s.e.m. of equal numbers of age-matched males and females (there was no gender-specific effect on the response to injections). f. Insulin tolerance testing of mice treated with DEX and saline. Data are presented as mean ± s.e.m. of equal numbers of age-matched males and females (there was no gender-specific effect on the response to injections). g. Insulin tolerance testing of mice treated with DEX and saline. Data are presented as mean ± s.e.m. of equal numbers of age-matched males and females (there was no gender-specific effect on the response to injections). h. Insulin tolerance testing of mice treated with DEX and saline. Data are presented as mean ± s.e.m. of equal numbers of age-matched males and females (there was no gender-specific effect on the response to injections). i. Insulin tolerance testing of mice treated with DEX and saline. Data are presented as mean ± s.e.m. of equal numbers of age-matched males and females (there was no gender-specific effect on the response to injections). j. Insulin tolerance testing of mice treated with DEX and saline. Data are presented as mean ± s.e.m. of equal numbers of age-matched males and females (there was no gender-specific effect on the response to injections). k. Insulin tolerance testing of mice treated with DEX and saline. Data are presented as mean ± s.e.m. of equal numbers of age-matched males and females (there was no gender-specific effect on the response to injections). l. Insulin tolerance testing of mice treated with DEX and saline. Data are presented as mean ± s.e.m. of equal numbers of age-matched males and females (there was no gender-specific effect on the response to injections). m. Insulin tolerance testing of mice treated with DEX and saline. Data are presented as mean ± s.e.m. of equal numbers of age-matched males and females (there was no gender-specific effect on the response to injections). n. Insulin tolerance testing of mice treated with DEX and saline. Data are presented as mean ± s.e.m. of equal numbers of age-matched males and females (there was no gender-specific effect on the response to injections). o. Insulin tolerance testing of mice treated with DEX and saline. Data are presented as mean ± s.e.m. of equal numbers of age-matched males and females (there was no gender-specific effect on the response to injections). p. Insulin tolerance testing of mice treated with DEX and saline. Data are presented as mean ± s.e.m. of equal numbers of age-matched males and females (there was no gender-specific effect on the response to injections). q. Insulin tolerance testing of mice treated with DEX and saline. Data are presented as mean ± s.e.m. of equal numbers of age-matched males and females (there was no gender-specific effect on the response to injections). r. Insulin tolerance testing of mice treated with DEX and saline. Data are presented as mean ± s.e.m. of equal numbers of age-matched males and females (there was no gender-specific effect on the response to injections). s. Insulin tolerance testing of mice treated with DEX and saline. Data are presented as mean ± s.e.m. of equal numbers of age-matched males and females (there was no gender-specific effect on the response to injections). t. Insulin tolerance testing of mice treated with DEX and saline. Data are presented as mean ± s.e.m. of equal numbers of age-matched males and females (there was no gender-specific effect on the response to injections). u. Insulin tolerance testing of mice treated with DEX and saline. Data are presented as mean ± s.e.m. of equal numbers of age-matched males and females (there was no gender-specific effect on the response to injections). v. Insulin tolerance testing of mice treated with DEX and saline. Data are presented as mean ± s.e.m. of equal numbers of age-matched males and females (there was no gender-specific effect on the response to injections). w. Insulin tolerance testing of mice treated with DEX and saline. Data are presented as mean ± s.e.m. of equal numbers of age-matched males and females (there was no gender-specific effect on the response to injections). x. Insulin tolerance testing of mice treated with DEX and saline. Data are presented as mean ± s.e.m. of equal numbers of age-matched males and females (there was no gender-specific effect on the response to injections). y. Insulin tolerance testing of mice treated with DEX and saline. Data are presented as mean ± s.e.m. of equal numbers of age-matched males and females (there was no gender-specific effect on the response to injections). z. Insulin tolerance testing of mice treated with DEX and saline. Data are presented as mean ± s.e.m. of equal numbers of age-matched males and females (there was no gender-specific effect on the response to injections).
with AdPGC-1α developed fasting hyperglycemia (160 ± 13 versus 123 ± 5 mg/dl; P = 0.022).

Glucose and insulin increased in Ppara<sup>+/-</sup>Ldlr<sup>-/-</sup> but not Ppara<sup>-/-</sup>Ldlr<sup>-/-</sup> mice after 5 months of DEX treatment (Fig. 3b,c). Normoglycemic DEX-treated Ppara<sup>+/-</sup>Ldlr<sup>-/-</sup> mice with normal insulin levels became hyperglycemic and hyperinsulinemic with AdPPAR-α, but not AdNull (Fig. 3b,c). In glucose tolerance tests, glucose excursions were higher in mice treated with DEX and AdPPAR-α than in those treated with DEX and AdNull (P < 0.05; data not shown). However, reconstitution of PPAR-α in the livers of Ppara<sup>-/-</sup>Ldlr<sup>-/-</sup> mice in the absence of DEX treatment did not affect fasting glucose or glucose tolerance (Fig. 3d). Despite hepatic overexpression of PGC-1α (Fig. 2d) similar to that seen in Ppara<sup>+/-</sup> mice treated chronically with DEX (see below), AdPGC-1α did not affect glucose metabolism (Fig. 3b,c).

**PPAR-α and hepatic gluconeogenesis**

AdPPAR-α-mediated induction of insulin resistance in PPAR-α-null mice was not caused by nonphysiological expression of PPAR-α. Hepatic Ppara mRNA was fivefold greater in DEX-treated than in saline-treated Ppara<sup>−/-</sup> mice (Fig. 4a; P < 0.002), and the same level was seen in the livers of DEX-treated Ppara<sup>+/+</sup> mice after receiving AdPPAR-α (Fig. 4b). Expression of Ppargc1 was tenfold higher in DEX-treated Ppara<sup>+/+</sup>Ldlr<sup>-/-</sup> than in DEX-treated Ppara<sup>-/-</sup>Ldlr<sup>-/-</sup> mice (Fig. 4c). Expression of the key gluconeogenic enzymes phosphoenolpyruvate carboxykinase (Pck; Fig. 4e) and glucose 6-phosphatase (G6pc; Fig. 4g) was also induced by DEX in Ppara<sup>+/+</sup>Ldlr<sup>-/-</sup> mice. However, reconstitution of PPAR-α in DEX-treated Ppara<sup>−/-</sup>Ldlr<sup>-/-</sup> mice induced Pck and G6pc expression (Fig. 4f) without affecting expression of Ppargc1 (Fig. 4d).

**PPAR-γ (Pparg) expression in DEX-treated mice**

Hepatic Pparg2, but not Pparg1, was elevated in DEX-treated mice with insulin resistance. Pparg2 expression was 5.9 ± 2.0 for Ppara<sup>+/+</sup>Ldlr<sup>-/-</sup> versus 0.6 ± 0.2 for Ppara<sup>-/-</sup>Ldlr<sup>-/-</sup> mice; P = 0.04. Pparg1 expression was 7.4 ± 1.1 for Ppara<sup>+/+</sup>Ldlr<sup>-/-</sup> versus 7.8 ± 1.0 for Ppara<sup>-/-</sup>Ldlr<sup>-/-</sup> mice (n = 4 for each; arbitrary units normalized to Gapd). In DEX-treated adipose tissue, Pparg1 expression was 1.2 ± 0.7 for Ppara<sup>+/+</sup>Ldlr<sup>-/-</sup> versus 1.5 ± 0.7 for Ppara<sup>-/-</sup>Ldlr<sup>-/-</sup> mice; Pparg2 expression was 4.7 ± 0.7 for Ppara<sup>+/+</sup>Ldlr<sup>-/-</sup> versus 5.1 ± 1.5 for Ppara<sup>-/-</sup>Ldlr<sup>-/-</sup> mice.

**PPAR-α-dependent elevation of blood pressure**

Changes in blood pressure (Fig. 3a) paralleled those for glucose and insulin in mice after DEX and adenoviral treatment. Blood pressure increased in Ppara<sup>+/+</sup>Ldlr<sup>-/-</sup>, but not Ppara<sup>-/-</sup>Ldlr<sup>-/-</sup>, mice treated with DEX (Fig. 3a). Systolic pressure increased by 17 mmHg and diastolic pressure by 15 mmHg in DEX-treated Ppara<sup>+/+</sup>Ldlr<sup>-/-</sup> mice compared with baseline (P < 0.0001 for each). Saline had no effect on blood pressure.

Normotensive DEX-treated Ppara<sup>+/+</sup>Ldlr<sup>-/-</sup> mice (Fig. 3a) were infused with AdPPAR-α or control virus and DEX treatment was continued. Beginning 4 d later, blood pressures were measured in 23 AdPPAR-α- and 18 AdNull-treated mice by an observer blinded to treatment status. Systolic and diastolic blood pressure increased by ~12 mmHg in DEX-treated Ppara<sup>+/+</sup>Ldlr<sup>-/-</sup> mice after AdPPAR-α (Fig. 3a) but did not change in AdNull-infected mice. Infusion of AdPGC-1α in a separate group of DEX-treated Ppara<sup>+/+</sup>Ldlr<sup>-/-</sup> mice did not affect blood pressure (Fig. 3a).

These results, obtained by noninvasive techniques in conscious mice, were confirmed by invasive techniques in anesthetized mice. In DEX-treated Ppara<sup>+/+</sup>Ldlr<sup>-/-</sup> mice, systolic blood pressure was 92 ± 1.5 mmHg with AdPPAR-α versus 82 ± 1.6 mmHg with AdNull (P = 0.002), and diastolic pressure was 66 ± 1.3 mmHg with AdPPAR-α versus 59 ± 1.4 mmHg with AdNull (P = 0.01). Vascular compliance in these mice, measured using graded infusions of angiotension II...
and phenylephrine, was identical in the two groups, arguing against a direct effect of PPAR-α on the vascular wall.

Effects of PPAR-α on renal sodium balance

Plasma renin activity, the major determinant of renal salt balance, more than doubled in dexamethasone-treated PPAR-α-null mice infused with AdPPAR-α, as compared with AdNull-infused mice (Table 1). Urinary excretion of norepinephrine, a marker of sympathetic nervous system activity, was also higher in Ad-PPAR-α-treated mice (Table 1). Renin activity and sympathetic tone independently promote renal sodium retention, volume expansion, and hypertension. Urinary sodium excretion decreased by nearly half in AdPPAR-α-treated mice (Table 1), providing a mechanism underlying the elevated blood pressure detected by both noninvasive and invasive techniques after reconstitution of hepatic PPAR-α expression.

Effects of PPAR-α activation on HDL cholesterol

We measured high-density lipoprotein (HDL) cholesterol to provide an independent marker of PPAR-α activation. Cholesterol was higher in DEX-treated Ppara−/−Ldlr−/− mice (Supplementary Table 1 online), mostly because of HDL cholesterol (207 ± 6 mg/dl for DEX-treated Ppara−/−Ldlr−/− versus 45 ± 4 mg/dl for DEX-treated Ppara+/+Ldlr−/− mice). HDL cholesterol was unchanged when these mice received AdNull (184 ± 9 mg/dl; not significant compared with the same mice before infection) but decreased by 47% after AdPPAR-α (110 ± 6 mg/dl; P < 0.0001 compared with the same mice before infection), which was expected knowing that PPAR-α activation decreases HDL cholesterol in mice.

Effects in human hepatocytes

To determine whether glucocorticoids interact with PPAR-α and induce gluconeogenesis in humans, cultured human hepatocytes were incubated for 6 h with dexamethasone (10−8 M) and the PPAR-α agonist Wy14,643 (100 µM). The combination tripled expression of the gluconeogenic genes PCK (Fig. 5a) and PPARA (Fig. 5b).

DISCUSSION

Patients treated with glucocorticoids frequently trade one disorder, such as symptomatic asthma, for another, such as insulin resistance. Insulin resistance was induced by glucocorticoid treatment in Ppara+/+, but not Ppara−/−, mice. Hepatic reconstitution of PPAR-α in steroid-treated, but not saline-treated, Ppara+/+ mice produced the insulin-resistant phenotype. These data show that hepatic expression of the nuclear receptor PPAR-α participates in the induction of several distinct features of insulin resistance by glucocorticoids in mice.

Insulin resistance can lead to hyperglycemia resulting from impaired insulin-stimulated glucose disposal in peripheral tissues, the failure of insulin to suppress endogenous glucose production by the liver, or both. Glucocorticoids increase hepatic glucose production and decrease peripheral uptake of glucose25,26. DEX-treated Ppara+/+ mice were resistant to suppression of endogenous glucose production but were not resistant to glucose disposal in clamp experiments using a high rate of insulin infusion. Maximal glucose transport induced by high insulin levels may have obscured PPAR-α-dependent differences in glucose disposal existing under more physiologic conditions. However, DEX-treated Ppara+/+ mice had more adiposity than Ppara−/− mice (Supplementary Table 1 online). Because increased adiposity can decrease peripheral glucose disposal27, greater fat in Ppara−/− animals could negate the potentially beneficial effect of PPAR-α deficiency on glucose disposal. Regardless, the inability to suppress glucose production (even with high rates of insulin infusion), as well as the restoration of the insulin-resistant phenotype by hepatic expression of PPAR-α, strongly suggests an important role for the liver.
Glucocorticoids increase hepatic glucose production by stimulat-
ing gluconeogenesis and G6pc (catalyzing the final step in de novo glucose production) mRNA levels were elevated in the livers of DEX-treated Ppara+/+, but not DEX-treated Ppara−/−, mice. Pck and G6pc were increased when hepatic PPAR-α expression was reconstituted in DEX-treated Ppara+/+Ldlr−/− mice. Reconstitution of PPAR-α in the livers of DEX-treated, but not saline-treated, Ppara+/+Ldlr−/− mice caused hyperglycemia and hyperinsulinemia. Collectively, these results suggest that the mechanism of insulin resistance in our mouse model involves induction of gluconeogenesis through the combination of glucocorticoid and PPAR-α signaling.

There are clear differences in PPAR-α biology between mice and humans. PPAR-α expression is lower in human compared with rodent liver, and humans seem to be resistant to the potentially adverse effects of peroxisomal proliferators in the liver. However, the interaction between DEX and PPAR-α identified in mice may also be operative in humans. The combination of DEX and the potent PPAR-α ligand Wy14,643 increased expression of PPARα and PCK in human hepatocytes (Fig. 5).

The transcriptional coactivator PGC-1α is an important regulator of gluconeogenesis. In the current study, overexpression of PGC-1α in liver caused hyperglycemia in Ppara+/+Ldlr−/−, but not Ppara−/−Ldlr−/−, mice (Fig. 3). Hepatic expression of PPAR-α in Ppara+/+Ldlr−/− mice activated gluconeogenesis without induction of PGC-1α (Fig. 4). These data provide evidence that glucocorticoid-induced gluconeogenesis is PPAR-α dependent, but do not exclude the contribution of other factors, including PPAR-γ. Hepatic Pparg2 was tenfold higher in DEX-treated mice with insulin resistance. Feeding obese mice with a high-fat diet induces hepatic Pparg2 expression and deficiency of hepatic PPAR-γ improves liver insulin sensitivity, consistent with a possible role for this factor in the stimulation of phosphoenolpyruvate carboxykinase and glucose 6-phosphatase.

There is evidence for negative feedback between PPAR-α and the glucocorticoid receptor. PPAR-α activation suppresses hepatic 11β-hydroxysteroid dehydrogenase type 1 (HSD-1), which converts glucocorticoids from inactive to active forms. HSD-1 overexpression promotes fatty acids and HSD-1 deficiency prevents insulin resistance. DEX, which does not require activation, bypasses this feedback loop, allowing cross talk between PPAR-α and the glucocorticoid receptor to enhance gluconeogenesis.

Ppara+/+Ldlr−/− mice were also protected from glucocorticoid-induced hypertension; reconstitution of hepatic PPAR-α elevated blood pressure (Fig. 3). Renin-angiotensin and sympathetic nervous system activation caused sodium retention (Table 1), a mechanism contributing to hypertension in insulin resistance. Fatty acids bind to PPAR-α, promoting β-oxidation and ketogenesis. Hepatic fatty acid oxidation and ketogenesis modulate the activity of vagal afferent nerve fibers that signal the central nervous system, a potential pathway for the regulation of sympathetic tone by the liver.

Table 1  PPAR-α and renal sodium balance

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<th>AdPPAR-α</th>
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<td>Urine norepinephrine</td>
<td>219 ± 30</td>
<td>116 ± 32</td>
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<td>Urine sodium (μmol/g/d)</td>
<td>1.8 ± 0.4</td>
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DEX-treated Ppara+/+Ldlr−/− mice were infused with AdPPAR-α or AdNull. Urine results for a single animal were recorded as the mean of three assays. Data are expressed as mean ± s.e.m. P values represent comparisons by unpaired two-tailed t test.

Figure 4  Expression of Ppara and hepatic gluconeogenesis genes. Hepatic expression of Ppara (a,b), Pparag1 (c,d), Pck (e,f) and G6pc (g,h) was assayed in mice after DEX treatment (a,c,e,g) and after infusion of DEX-treated Ppara+/+ mice with AdPPAR-α (b,d,f,h). Levels of mRNA were assayed by quantitative RT-PCR and corrected to Gapd. a, DEX-treated Ppara+/+Ldlr−/− samples were compared with normal saline (NS)-treated Ppara+/+Ldlr−/− samples because PPAR-α signal was undetectable in Ppara+/+Ldlr−/− mice. b, AdPPAR-α-treated samples were also compared with NS-treated Ppara+/+ samples because PPAR-α signal was undetectable in Ppara+/+Ldlr−/− mice treated with AdNull. c,e,g, mRNA levels were compared in DEX-treated Ppara+/+Ldlr−/− (△) and Ppara−/−Ldlr−/− (□) mice. d,f,h, mRNA levels were compared in DEX-treated Ppara+/+Ldlr−/− mice after administration of AdPPAR-α (●) or AdNull (○). The y-axis indicates relative mRNA expression in arbitrary units, normalized to Gapd. Data represent mean ± s.e.m. *, presence of Ppara expression; □, absence of Ppara expression (a-h). * P < 0.01 compared with the bar in the same panel (a-g) or P < 0.05 (h).
Figure 5 Gene expression in human hepatocytes, as determined by quantitative RT-PCR. (a) Expression of PCK. *, P < 0.001 by ANOVA. (b) Expression of PPARα. *, P < 0.02 by ANOVA. Data were combined from hepatocytes from two separate human donors. Results were also significant when cells from each donor were analyzed separately. HCM, hepatocyte culture medium.

Gastrointestinal vagal afferent activity can increase renin activity37. Portal infusion of long-chain fatty acids induces insulin resistance, sympathetic activation and hypertension38,39. PPARα-null mice on a high-fat diet are protected from hypertension17,40. Taken together, these data suggest that hepatic PPAR-α is an unexpected modulator of blood pressure.

Proven agents such as gemfibrozil and fenofibrate are weak PPAR-α agonists. When more potent agonists become available, our data suggest that their use might affect glucose metabolism and blood pressure in patients receiving glucocorticoids. Our results also raise the possibility that glucose and blood pressure responses to the administration of a dexamethasone/fibrate combination could identify individuals at risk for insulin resistance and its sequelae.

METHODS

Animals. PPAR-α-null mice of mixed genetic background (C57BL/6 and 129Sv) were crossed with LDLR-null mice in the C57BL/6 background to generate Ppara−/−Ldlr−/− animals and Ppara+/−Ldlr−/− littermates. Mice of both sexes were treated with either DEX (1 mg/kg) or normal saline every other day for 45 min in PBS with 1% BSA, 0.3% Triton-X 100 and 5% horse serum. Slides were incubated for 60 min with a 1:200 dilution of goat polyclonal antibody to PPAR-α (SC-1982; Santa Cruz Biotechnology) followed by a 1:200 dilution of biotinylated antibody to goat immunoglobulin (BA-9500; Vector Laboratories), then treated with streptavidin peroxidase followed by aminoethyl carbazole solution (Zymed).

Human hepatocytes. Cryopreserved human hepatocytes from two independent subjects were obtained from Cambrex Bio Science. Aliquots of cells (n = 3–4 per condition in each experiment) were replenished in hepatocyte culture medium from the manufacturer, then treated with vehicle, 10−8 M DEX (after dose-response experiments identified an optimal dose), 100 µM Wy14,643, or both compounds. Six hours later, cells were washed, RNA was prepared and human PCK2 and PPARα expression was determined by quantitative RT-PCR.

Quantitative RT-PCR–based gene expression. Analyses were done in a GeneAmp 5700 or 7700 Sequence Detector (Applied Biosystems). RNA not subjected to reverse transcription was included in each assay as a negative control. We used the following mouse oligonucleotides: Ppara forward, 5′-GGGCAAAGGAAATCCAGGAGA-3′; Ppara reverse, 5′-GGTTGTGTCTGGTCTCTCCC-3′; Pparg1 forward, 5′-CAGGAAATCATATCACCACAG-3′; Pparg1 reverse, 5′-TGAGGACGGCCTGAGG-3′; Gapdh forward, 5′-TGACAGTCGGTCAAGACGTG-3′; Gapdh reverse, 5′-GAGGACGCGGAAGAAGAGA-3′. The amplified bands were sequenced to verify their identity.

Human plasma. Noninvasive determinations of systolic and diastolic blood pressures were made over several days in conscious mice using a tail-cuff system (Kent Scientific) as described41. For mice treated with adenoviruses, measurements began 4 d after the injection. Noninvasive results were confirmed using invasive monitoring. To determine vascular compliance, angiotensin II (0.1–2.0 nmol/kg), phenylephrine (1–100 nmol/kg) or saline was infused as described42. Adenoviruses. AdPPAR-α (containing mouse Ppara cDNA), AdPGC-1α (containing mouse Ppargc1 cDNA) and control adenoviruses were generated, purified and expanded as described43,44. Viruses were administered by slow intravenous infusion at a dose of 6 × 109 PFU for AdPPAR-α and 8 × 109 PFU for AdPGC-1α, each in a total volume of 200 µl. Ppargc1 mRNA was detected by northern blotting. RT-PCR for Ppara and Gapdh was done using the following primers: Ppara forward, 5′-TCGGCCTGGCTTCTAACA-3′; Ppara reverse, 5′-GTTCACACATCTTCAGAAGCT-3′; Gapdh forward, 5′-CCCCATCGCATTTCCAGAAGG-3′; Gapdh reverse, 5′-GTCAGGATCCCTTG-GCCAGGG-3′. Amplified bands were sequenced to verify their identity.

Immunohistology. Formalin-fixed, paraffin-embedded sections were treated with xylene followed by ethanol. Endogenous peroxidase activity was quenched with 0.6% hydrogen peroxide in methanol. Slides were blocked for 45 min in PBS with 1% BSA, 0.3% Triton-X 100 and 5% horse serum. Slides were incubated for 60 min with a 1:200 dilution of goat polyclonal antibody to PPAR-α (SC-1982; Santa Cruz Biotechnology) followed by a 1:200 dilution of biotinylated antibody to goat immunoglobulin (BA-9500; Vector Laboratories), then treated with streptavidin peroxidase followed by aminoethyl carbazole solution (Zymed).
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