Adipocyte (Pro)Renin-Receptor Deficiency Induces Lipodystrophy, Liver Steatosis and Increases Blood Pressure in Male Mice

Chia-Hua Wu, Shayan Mohammadmoradi, Joel Thompson, Wen Su, Ming Gong, Genevieve Nguyen, Frédérique Yiannikouris

Abstract—Adipose tissue dysfunction related to obesity is overwhelmingly associated with increased risk of developing cardiovascular diseases. In the setting of obesity, (pro)renin receptor (PRR) is increased in adipose tissue of mice. We sought to determine the physiological consequences of adipocyte-PRR deficiency using adiponectin-Cre mice. We report a unique model of adipocyte-PRR–deficient mice (PRR<sup>Adi/Y</sup>) with almost no detectable white adipose tissues. As a consequence, the livers of PRR<sup>Adi/Y</sup> mice were enlarged and demonstrated a marked accumulation of lipids. Adipocyte-specific deficiency of PRR increased systolic blood pressure and the concentration of soluble PRR in plasma. To determine whether adipocyte-PRR was involved in the development of obesity-induced hypertension, mice were fed a low-fat or a high-fat diet for 16 weeks. Adipocyte-PRR–deficient mice were resistant to diet-induced obesity. Both high-fat– and low-fat–fed PRR<sup>Adi/Y</sup> mice had elevated insulin levels. Interestingly, adipocyte-PRR deficiency improved glucose tolerance in high-fat–fed PRR<sup>Adi/Y</sup> mice. In response to feeding either low-fat or high-fat diets, systolic blood pressure was greater in PRR<sup>Adi/Y</sup> mice than in control mice. High-fat feeding elevated soluble PRR concentration in control and PRR<sup>Adi/Y</sup> mice. In vitro knockdown of PRR by siRNA significantly decreased mRNA abundance of PPAR<sub>γ</sub> (peroxisome proliferator-activated receptor gamma), suggesting an important role for PRR in adipogenesis. Our data indicate that adipocyte-PRR is involved in lipid homeostasis and glucose and insulin homeostasis, and that soluble PRR may be a predictor of metabolic disturbances and play a role in systolic blood pressure regulation. (Hypertension. 2016;68:213-219. DOI: 10.1161/HYPER.115.06954.) • Online Data Supplement

Key Words: adipocytes ■ blood pressure ■ glucose ■ insulin ■ lipids ■ prorenin receptor

Hypertension is the major cause of cardiovascular diseases worldwide and, according to the National Health and Nutrition Examination Survey III (NHANES III), the prevalence of hypertension continues to increase.1,2 Obesity is an important risk factor for hypertension.1 The renin angiotensin system (RAS) is recognized for playing a critical role in the regulation of blood pressure and sodium and water homeostasis. The deletion of components of the RAS, for instance angiotensinogen in liver or adipose tissue, prevents obesity-related hypertension.3,4

Among the components of the RAS expressed in adipose tissue,5,6 (pro)renin receptor (PRR) is abundant and is upregulated during the development of obesity.3,7-9 PRR is a 350-amino acid protein with a single transmembrane domain and has been first identified as the receptor for renin in its active form and for prorenin in its inactive form.10,11 Treatment of preadipocytes, extracted from adipose tissue, with angiotensinogen and renin results in a dose-dependent increase in angiotensin I (AngI) generation. Specific deletion of PRR in the brain attenuates angiotensin II–dependent hypertension, whereas human PRR transgenic rats exhibit elevated systolic blood pressure.12,13 Taken together, these results suggest that adipose PRR may potentially play a role in blood pressure control.1,14 In addition, PRR can be cleaved intracellularly by furin, resulting in the secretion of a soluble form of PRR (sPRR) in plasma14 and urine,15,16 which might bind renin and prorenin15 and participate in AngI formation.14-17 Previous studies have shown that increased plasma sPRR levels in early pregnancy are associated with the development of preeclampsia,18 a hypertension-related complication. Conversely, lower plasma sPRR levels were observed in patients treated with angiotensin II receptor blockers.19 The physiological consequences of changes in plasma sPRR levels during the development of obesity and hypertension remain unclear.

The function of PRR is not restricted to AngI generation and hypertension. The binding of renin or prorenin to PRR...
in mesangial cells \(^1\) and 3T3-L1 preadipocytes \(^7,8\) initiates an intracellular signaling cascade associated with the activation of the extracellular signal-regulated kinase (ERK) 1/2 pathway. In mesangial cells, the activation of the ERK1/2 pathway leads to the release of transforming growth factor-β1 and cytokines involved in inflammation. However, past attempts to generate complete PRR knockout mice failed.\(^{20}\) Specific cardiomyocyte or podocyte deletion of PRR led to animal lethality 3 weeks after birth caused by heart or kidney failure.\(^{21–24}\) Because PRR interacts with V-ATPase, the deletion of PRR may trigger destabilization of V-ATPase activity, leading to a decrease in vacuolar acidification and to the lethality of cells.\(^{21–24}\) In addition, recent studies in xenopus have shown that PRR may link V-ATPase to the Wnt receptor protein, LRP6, and induce phosphorylation of LRP6.\(^{25,26}\) The objectives of our study were to determine (1) whether a specific adipocyte-PRR deficiency mouse model is viable, (2) the physiological consequences of adipocyte-PRR deletion on blood pressure in normal physiology and during the development of obesity, and (3) the relationship between adipocyte-PRR, plasma sPRR concentrations, the RAS, and blood pressure.

**Methods and Animals**

All procedures involving animals were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee at the University of Kentucky (University of Kentucky IACUC protocol number: 2013-1109). Female mice with loxP sites flanking exon 2 of the PRR gene (PRRfl/Y) were bred to transgenic male mice (PRRAdi/Y expressing Cre recombinase under the control of the adiponectin promoter (Strain Name: B6;FVB-Tg(Adipoq-cre)1Evdr/J)\(^{24}\) (Figure 1A). PRR mRNA abundance was markedly reduced in adipocytes differentiated from PRRfl/Y mice compared with control PRR\(^{0/0}\) mice (Figure 1B; \(P<0.05\)) demonstrating the efficacy of the deletion.

**Statistical Analysis**

Results are expressed as mean±SEM. All data were analyzed using Sigma Plot and Graph Prism. ANOVAs (and ANOVA repeated measures when appropriate) were used to compare diet and genotype effects, followed by post hoc tests using Holm–Sidak or Bonferroni corrections for multiple comparisons. When the assumptions underlying the ANOVAs were not otherwise met, data were nonlinearly transformed; however, for ease of illustration, figures show untransformed data. GraphPad QuickCalcs (Grubbs test) was used to determine statistical outliers, and a \(t\) test was used to compare mean insulin levels between high-fat (HF)– and low-fat (LF)–fed mice. Statistical significance was defined as \(P<0.05\).

**Results**

**Generation of Mice With Adipocyte-PRR Deficiency**

To confirm efficiency of PRR deletion in adipocytes, preadipocytes from the stromal vascular fraction of subcutaneous adipose tissue of control mice (PRR\(^{0/0}\)) and adipocyte-PRR-deficient mice (PRR\(^{Adi/Y}\)) were differentiated into adipocytes (Figure 1A). PRR mRNA abundance was markedly reduced in adipocytes differentiated from PRR\(^{Adi/Y}\) mice compared with control PRR\(^{0/0}\) mice (Figure 1B; \(P<0.05\)).

**Adipocyte Deficiency of PRR Drastically Decreased Adipose Tissue Mass in Male Mice Fed a Standard Diet**

Body weight increased with age (Figure 2A) with no significant difference between genotypes. The fat mass was significantly reduced in PRR\(^{Adi/Y}\) compared with control PRR\(^{0/0}\) mice (Figure 2B) and did not increase with age in PRR\(^{Adi/Y}\) mice, suggesting that PRR\(^{Adi/Y}\) mice do not accumulate adipose tissue (Figure S1 in the online-only Data Supplement). The mass of all white adipose tissues was significantly reduced in PRR\(^{Adi/Y}\) mice compared with PRR\(^{0/0}\) mice (Table S1; \(P<0.05\)). The analyses of epididymal adipose tissue morphology from PRR\(^{0/0}\) mice revealed the presence of differentiated adipocytes throughout the section, whereas histological analysis of residual adipose tissue from around the epididymis of PRR\(^{Adi/Y}\) mice showed an unexpectedly small number of differentiated adipocytes (Figure S2). To determine whether adipocyte-PRR was involved in adipocyte differentiation, PRR was silenced in vitro in 3T3-L1-cells (Figure S3). Peroxisome proliferator-activated receptor gamma (PPAR\(\gamma\)), an important gene involved in adipocyte differentiation, and fatty acid–binding protein 4 (Fabp4 or aP2), a marker for differentiated adipocytes and a carrier protein for fatty acids, were evaluated. The abundance of mRNA PPAR\(\gamma\) and Fabp4 was significantly decreased in differentiated siPRR cells compared with control cells.

![Figure 1. A. Schematic representation of the loxp-flanked (pro) renin receptor (PRR) allele before (a) and after recombination with adiponectin-driven Cre expression (b). B. mRNA PRR abundance of differentiated adipocyte from subcutaneous adipose tissue of PRR\(^{0/0}\) and PRR\(^{Adi/Y}\) mice. Data are mean±SEM of 3 to 6 mice. *\(P<0.05\) compared with PRR\(^{0/0}\) mice. FRT indicates flippase recognition target.](http://hyper.ahajournals.org/content/early/2017/07/12/HYPERTENSIONAHA.117.110947.full.html)
Tissue weights of liver, spleen, and pancreas were significantly higher in PRR\textsuperscript{Adi/Y} mice than in PRR\textsuperscript{fl/Y} mice (Table S1). Leptin plasma concentrations were lower in PRR\textsuperscript{Adi/Y} mice than in PRR\textsuperscript{fl/Y} mice (Table S1). Glucose tolerance did not differ between genotypes at week 5, 9, or 13 of the experiment (Figure S4).

**Adipocyte-Specific Deficiency of PRR Triggered Lipid Accumulation in Livers of Male Mice Fed a Standard Diet**

Microscopic examination of liver sections revealed an increase in hepatic fat accumulation comprising small and large fat vacuoles in PRR\textsuperscript{Adi/Y} mice compared with PRR\textsuperscript{fl/Y} mice (Figure S5). Neutral lipids were significantly increased in liver of PRR\textsuperscript{Adi/Y} mice compared with that of PRR\textsuperscript{fl/Y} mice. Plasma triglycerides did not differ significantly between groups (Table S1).

**Adipocyte-Specific Deficiency of PRR Increased Systolic Blood Pressure of Male Mice Fed a Standard Diet**

The SBP and the pulse pressure were significantly higher in PRR\textsuperscript{Adi/Y} mice than in PRR\textsuperscript{fl/Y} mice (Figure 3). Mean arterial blood pressure, diastolic blood pressure, and heart rate did not differ significantly between groups (Table S2).

**Adipocyte-Specific Deficiency of PRR Increased Plasma sPRR Concentrations**

Adipocyte-specific deficiency of PRR did not change plasma angiotensinogen concentration (Figure 4A). Plasma renin activity and plasma renin concentration did not differ significantly between PRR\textsuperscript{Adi/Y} mice and PRR\textsuperscript{fl/Y} mice (Figure 4B), suggesting that adipocyte-specific deficiency of PRR did not influence angiotensinogen, plasma renin activity, or plasma renin concentration. Surprisingly, plasma sPRR levels increased by 3-fold in PRR\textsuperscript{Adi/Y} mice compared with PRR\textsuperscript{fl/Y} mice (Figure 4C).

**Adipocyte-Specific Deficiency of PRR Prevented the Development of Obesity and the Accumulation of Fat Mass in HF-Fed Mice**

PRR\textsuperscript{Adi/Y} mice were resistant to HF-diet–induced obesity (Figure 5A). The fat mass of PRR\textsuperscript{Adi/Y} mice was lower by about −70% compared with LF-fed PRR\textsuperscript{fl/Y} mice and was lower by almost −80% compared with HF-fed PRR\textsuperscript{fl/Y} mice (Figure 5B). When challenged with a HF diet, tissue weights of liver, heart, and kidney were significantly higher in PRR\textsuperscript{Adi/Y} mice than in PRR\textsuperscript{fl/Y} mice (Table S3). Adipocyte-PRR deficiency did not
significantly affect kidney structure (Figure S6). HF- and LF-fed PRR<sup>Adi/Y</sup> mice had increased lipid accumulation in liver compared with PRR<sup>fl/Y</sup> mice (Figure S7). Adipocyte-PRR deficiency did not change PRR mRNA levels in kidney and liver (Figure S8A and S8B). The HF-diet induced a significant increase in plasma cholesterol, which did not differ between genotypes.

**When Challenged With HF Diet, Adipocyte-Specific Deficiency of PRR Improved Glucose Homeostasis**

Glucose tolerance did not differ between genotypes after 16 weeks of LF diet (Figure S9A and S9B). However, HF-fed PRR<sup>Adi/Y</sup> mice exhibited improved glucose tolerance compared with HF-fed PRR<sup>fl/Y</sup> mice. Fasting glucose levels were significantly lower in HF- or LF-fed PRR<sup>Adi/Y</sup> mice than in PRR<sup>fl/Y</sup> mice (Figure S9C). Adipocyte-PRR deficiency induced a significant increase in plasma insulin levels regardless of diet (Table S3).

**Despite the Resistance to HF-Diet–Induced Obesity, Adipocyte-Specific Deficiency of PRR Further Increased SBP**

Adipocyte-specific deficiency of PRR induced a significant increase in SBP in LF-fed mice (Figure 6). The increase in SBP, resulting from PRR deficiency, was further exacerbated when PRR<sup>Adi/Y</sup> mice were fed a HF diet. These data suggest that adipocyte-specific deficiency of PRR aggravated HF-diet–induced elevation of SBP. Mean arterial pressure and heart rate were higher in PRR<sup>Adi/Y</sup> mice regardless of diet (Table S4 and S5).

**In Obese Mice, Adipocyte-Specific Deficiency of PRR Exaggerated the Elevation of Plasma sPRR Levels**

HF feeding induced a significant increase in plasma angiotensinogen concentrations in control PRR<sup>fl/Y</sup> mice (Figure 7A). However, plasma angiotensinogen concentrations did not differ between PRR<sup>Adi/Y</sup> and PRR<sup>fl/Y</sup> mice regardless of diet. Plasma renin activity was not influenced by the diet or by adipocyte-specific PRR deficiency (Figure 7B). When challenged with HF diet, PRR<sup>fl/Y</sup> mice exhibited a lower plasma renin concentration and a lower total prorenin/renin concentration than LF-fed PRR<sup>fl/Y</sup> mice (Figure 7B; Table S3). Plasma renin concentration and total prorenin/renin concentration in LF- and HF-fed PRR<sup>Adi/Y</sup> mice did not differ from those of LF-fed PRR<sup>fl/Y</sup> mice.

Plasma sPRR levels were significantly increased in LF-fed PRR<sup>Adi/Y</sup> mice compared with LF-fed PRR<sup>fl/Y</sup> mice. HF feeding induced a 3-fold increase in plasma sPRR levels in HF-fed PRR<sup>fl/Y</sup> mice compared with LF-fed PRR<sup>fl/Y</sup> mice (Figure 7C). Plasma sPRR levels were >2-fold higher in HF-fed PRR<sup>Adi/Y</sup> mice than in HF-fed PRR<sup>fl/Y</sup> mice. Plasma sPRR concentration was positively correlated with SBP (P<0.05) in PRR<sup>fl/Y</sup> mice and PRR<sup>Adi/Y</sup> mice combined (Figure S10A) and in PRR<sup>fl/Y</sup> mice alone; the correlation was weaker (P>0.05) in PRR<sup>Adi/Y</sup> mice alone. However, plasma insulin levels were not correlated with SBP (Figure S10B).

**Discussion**

This study examined the role of adipocyte-derived PRR in blood pressure control and the physiological consequences of the deletion of PRR in adipocytes of male mice during the development of obesity. The deletion of adipocyte-PRR induced a marked reduction in all white adipose tissues with no abnormal distribution of adipose tissue pads. In vitro
studies demonstrated that PRR regulated PPARγ and Fabp4. The lipodystrophy was accompanied by hepatic steatosis. When challenged with HF feeding, adipose PRR-deficient mice were resistant to the development of obesity and had improved glucose tolerance. Despite the absence of white adipose tissue and the resistance to diet-induced obesity, mice with adipocyte-PRR deficiency had elevated blood pressure. This blood pressure elevation in adipocyte-PRR-deficient mice seemed to be independent of systemic angiotensinogen and renin concentrations. Surprisingly, plasma sPRR concentrations were increased with HF diet and markedly elevated in adipocyte-PRR-deficient mice.

Deletion of adipocyte-PRR led to a reduction of adipose tissue mass and an increase in lipid deposition in liver, suggesting lipodystrophy accompanied by liver steatosis. In vitro PRR silencing revealed a significantly decrease of PPARγ and Fabp4, suggesting that PRR is a master regulator of adipocytes differentiation. In addition, because fatty acid–binding proteins are important carriers for fatty acids uptake and fatty acids transport to sites of esterification into triglycerides, our data suggest an important role of PRR in fatty acid trafficking and storage in adipocytes.

Our phenotype has been observed in other models of lipodystrophy such as the A-ZIP/F, aP2/DTA, SREBP-1c, or fatty liver dystrophy transgenic mouse models. In contrast, PRRAdi/Y mice fed a HF diet demonstrated much greater glucose sensitivity than HF-fed control mice. Our results differ from those of other mouse models of lipodystrophy, in which hyperglycemia and hypertriglycerideremia are commonly observed. In addition, the plasma insulin levels in PRRAdi/Y mice increased modestly, and PRRAdi/Y mice did not present severe hyperinsulinemia. Our data are nevertheless in agreement with the phenotype of the PPARγP465L/+ mouse model, which had improved ability to respond to acute glucose overload compared with controls when challenged with HF feeding. As suggested by Tsai et al., the expansion of pancreatic islets likely could contribute to this increased responsiveness to glucose. Similar to our model, CGI-58β mouse model developed hepatic steatosis but were protected against obesity and glucose intolerance. A reduction of body weight may also have contributed to better glucose sensitivity.

PRRAdi/Y mice exhibited elevated blood pressure similar to that reported in PPARγP465L/+ mice and humans expressing FPLD2 and FPLD3 mutations. In the latter instances, the cause of increased blood pressure is not well understood. Elevated leptin has been associated with elevated blood pressure but could protect against nonalcoholic fatty liver disease. Thus, although it is unlikely that low levels of circulating leptin in PRRAdi/Y mice could have contributed to elevated blood pressure, low levels of circulating leptin may have contributed to the development of liver steatosis.

Insulin resistance can cause increased blood pressure, thus elevated insulin levels could have participate to the elevation of SBP in PRRAdi/Y mice. However, our results demonstrated that insulin levels were not correlated with SBP suggesting that elevated insulin might not be the origin of elevated blood pressure. In contrast, we have demonstrated that plasma sPRR levels increased with the development of obesity-induced hypertension. Surprisingly, the elevation of plasma sPRR concentrations was exacerbated by adipocyte-PRR deficiency. The elevation of plasma sPRR concentration during early pregnancy has been reported to predict both hypertension and preeclampsia risk in pregnant woman. Moreover, patients with heart failure have higher plasma sPRR levels than control subjects. However, our demonstration of a positive correlation between sPRR and SBP when control mice and PRRAdi/Y mice are combined or in control mice only suggests that sPRR could play a role in blood pressure control. Further investigation are needed about a direct effect of sPRR on SBP. The heart, brain, liver, kidney, and smooth muscle express PRR gene and could potentially participate to the release of sPRR or be potential target tissues.

Adipose tissue is one source of systemic angiotensin II, and both expansion and reduction of adipose tissue activate adipose RAS, thereby influencing blood pressure regulation. Because Tsai et al. demonstrated that the expression of angiotensinogen and AT1R in adipose tissue is increased in

**Figure 7.** A. Plasma angiotensinogen (AGT) concentrations in male PRRY/+ and PRRAdi/Y mice fed a low-fat (LF) or high-fat (HF) diet. B. Plasma renin activity (PRA; left y axis) and concentration (PRC; right y axis). C. Plasma soluble form of (pro)renin receptor (sPRR) concentration. Data are mean±SEM of 5 to 8 mice. *P<0.05 compared with LF diet. **P<0.05 compared with PRRY/+ mice.
PPARγ<sup>−/−</sup> mice, the increase in blood pressure in PRR<sup>−/−</sup> mice could be attributed to a local activation of adipose RAS. In PRR<sup>−/−</sup> mice, the reduction of adipose tissue weight in conjunction with elevated plasma angiotensinogen may have activated local adipose RAS. Unfortunately, due to the severe reduction in adipose tissue, we lacked sufficient adipose tissue weight to confirm this hypothesis. Hepatocyte angiotensinogen deficiency induced profound reductions in blood pressure, systemic angiotensinogen, and angiotensin II, which influences adipose RAS content and secretion despite the continued presence of obesity. Because the liver is an important source of renal angiotensin II, it may also be possible that other local RAS are activated to compensate for the absence of adipose tissue. In contrast to liver angiotensinogen deficiency, PRR<sup>−/−</sup> mice demonstrated a radical shift in lipid distribution resulting in impaired lipid and glucose and insulin homeostasis. Our results demonstrate the necessity of adipocyte-PRR in the normal development and function of adipose tissue and lipid homeostasis and blood pressure. We thank Vickie English from the laboratory of Dr Cassis for her assistance in the measurement of renin.

**Sources of Funding**

These studies were supported by grants from the American Heart Association (13SDG17230008), the National Institute of General Medical Sciences (P20-GM103527), and the University of Kentucky, Center for Clinical and Translational Sciences (UL1TR000117).

**Acknowledgments**

We thank Vickie English from the laboratory of Dr Cassis for her assistance in the measurement of renin.

**Disclosures**

None.

**References**


Novelty and Significance

What Is New?

- Demonstration that adipocyte-derived (pro)renin receptor (PRR) deficiency regulates fat mass growth.
- Demonstration that adipocyte-derived PRR influences lipid homeostasis and glucose and insulin homeostasis.
- Demonstration that adipocyte-derived PRR deficiency increases plasma soluble PRR and blood pressure.

What Is Relevant?

- This study demonstrates that adipocyte-PRR is essential for the development of adipose tissue.
- This study demonstrates that adipocyte-PRR contributes to the control of blood pressure.

Summary

The effect of PRR to reduce fat mass is profound in mice with diet-induced obesity, demonstrating the important role of PRR in fat mass growth. Adipose PRR-deficient mice show elevated plasma insulin. In obese mice, adipocyte-PRR deficiency improves glucose tolerance. adipocyte-PRR deficiency elevates SBP and increases plasma soluble PRR levels.
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Hypertension. 2016;68:213-219; originally published online May 16, 2016; doi: 10.1161/HYPERTENSIONAHA.115.06954
Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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ADIPOCYTE (PRO)REIN-RECEPTOR DEFICIENCY INDUCES LIPODYSTROPHY, LIVER STEATOSIS AND INCREASES BLOOD PRESSURE IN MALE MICE

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Short title: Adipocyte PRR Deficiency and Obesity
Word count of manuscript: 5995
Word count of abstract: 232
Total number of figures: 7
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Methods

Animals
All procedures involving animals were conducted with the approval of the University of Kentucky Institutional Animal Care and Use Committee (University of Kentucky IACUC protocol number: 2013-1109) and in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Male mice (Strain Name: B6;FVB-Tg(Adipoq-cre)1Evd/J;B6;FVB-Tg(Adipoq-cre)1Evd/J) were generated at our facility for experimental use by breeding female mice with loxP sites flanking exon 2 of the PRR gene (PRRfl/fl) (Figure 1A) (kindly provided by Dr. Nguyen) to transgenic male mice (PRRfl/Y) expressing Cre recombinase under the control of the Adiponectin promoter (Figure 1A).

Experimental protocol
Characterization of the model: Male PRRAdi/Y mice (n= 4) and PRRfl/Y mice (n= 6) 2 months of age were fed a standard laboratory diet (18% protein, Global Diet; Teklad Harlan Madison, WI) for 16 weeks. Mice were provided water and diet ad libitum. Fat, lean and water mass were measured monthly on conscious mice using NMR spectroscopy (Echo MRI, Houston, TX).
Diet-induced obesity study: Male PRRAdi/Y mice (n= 13) and PRRfl/Y control mice (n= 13) (2 months of age) were randomly assigned to low-fat (LF) (10% of total calories as fat; catalog #D12450J) or high-fat (HF) (60% total calories as fat; catalog #D12492) diets from Research Diets Inc (New Brunswick, NJ) for 16 weeks. Mice were provided water and diet ad libitum. At study endpoint, mice were anesthetized with a mixture of ketamine/xylazine (100/10 mg/kg, i.p) and exsanguinated. Blood was collected in tubes (4°C) containing EDTA (0.2 mol/L), centrifuged at 5,000 rpm for 10 min, and plasma was stored at -80°C. Tissue was harvested, snap frozen in liquid nitrogen, and stored at -80°C.

Quantification of glucose tolerance
Intraperitoneal glucose tolerance tests were performed monthly as previously described. Blood glucose concentrations were quantified with a hand-held glucometer before (0 min) and after (15, 30, 60 and 120 min) glucose administration (1 mg/kg body weight, i.p).
Quantification of plasma components
Determination of plasma renin activity (PRA). Plasma (8 µL) was incubated at 37°C (30 min) in phosphate buffer containing EDTA (0.05 mol/L) and enalapril (10 µmol/L). The angiotensin I generated was then quantified by radioimmunoassay as described previously.
Determination of plasma renin concentrations (PRC). Plasma (8 µL) was incubated at 37°C (30 min) with exogenous AGT (25 nmol/L; generated from nephrectomized rats) followed by quantification of angiotensin I by radioimmunoassay.
Quantification of plasma AGT. Plasma (10 µL) concentrations of AGT were quantified using a mouse total AGT assay kit (Immuno-Biological Laboratories Co, Minneapolis, MN).
Quantification of plasma sPRR. Plasma (10 µL) concentrations of sPRR were quantified using a soluble (Pro)renin Receptor ELISA kit (Immuno-Biological Laboratories Co, Minneapolis, MN).
Quantification of plasma cholesterol and triglycerides were assessed by using a Wako kit (Wako Chemicals, Richmond, VA), plasma insulin using ultra-sensitive mouse insulin ELISA kit (Crystal Chem Inc, Downers Grove, IL), plasma mouse prorenin-renin total antigen ELISA kit.
(Molecular innovation, Novi, MI), leptin plasma using a Mouse Leptin ELISA kit (Millipore, Billerica, MA), and plasma of C-Reactive protein using a mouse C-Reactive protein antigen ELISA kit (Molecular innovation, Novi, MI).

**Quantification of blood pressure by radiotelemetry**

After 15 weeks of diets, mice were anesthetized with isoflurane and implanted with a carotid artery catheter connected to a telemetry device (model PA-C10). After recovery (range, 7-10 days), mean arterial, systolic and diastolic blood pressure, pulse pressure and locomotor activity were recorded continuously for 5 days. Blood pressure was quantified as described previously.\(^1\)

**Kidney, adipose tissue and liver immunostaining**

Kidney were fixed with formalin and embedded in paraffin and processed through paraffin. Sections (4 µm) were stained for Masson’s trichrome (Sigma, St. Louis, MO) and visualized at 40x magnification using a Nikon Eclipse 80i light microscope and Nikon NIS-elements visualization software. 40x fields (9 to 32 fields per animal) were captured and collagen content was quantified using NIH imageJ software.

For adipose tissue, gonadal adipose tissues were fixed with formalin and embedded in paraffin and processed through paraffin. Sections were stained with hematoxylin and eosin. Sections of adipose tissue from each mouse were photographed under 10X magnification.

Livers were collected, fixed in paraformaldehyde and embedded in paraffin blocks. Sections were stained with hematoxylin and eosin and examined by light microscopy. For neutral lipids analysis, liver sections were stained with Oil Red O and quantified using NIS Elements BR.3.10 software.

**Tissue RNA extraction and quantitative RT-PCR**

RNA was extracted from cells using the SV Total RNA Isolation System (Promega, Madison, WI). Total RNA was quantified with a NanoDrop 2000 spectrophotometer (Wilmington, DE) and cDNA was synthesized using qscript cDNA SuperMix (Quanta Bioscience, Gaithersburg, MD). Real-time quantitative PCR was performed with PerfeCTa SYBR Green FastMix, Low Rox (Quanta BioSciences, Gaithersburg, MD). PRR primers sequences are: forward: 5’-TGGTGGCGGGTGCTTTAGGA-3’ and reverse: 5’-AGCCCGTGGCGGTGAATA-3’. PPARγ primers sequences are: forward: 5’-GATGGGAA GACCACTCGCATT-3’ and reverse: 5’-AACCATTGGGTACCTCGTG-3’. Fatty acid binding protein 4 primers sequences are: forward 5’-GGAACCTGGAAAGCTTGTCTC-3’ and reverse 5’-TGATGCTCTTACCTCTTGG-3’. Mineralocorticoid receptor primers sequences are: forward 5’-GTGGACAG TCCTTTCACTACCG-3’ and reverse 5’-TGACACCCAGAAGCCTCATCTC-3’. 18s primers sequences are: forward 5’-GTAACCCGTTAGACCGTATT-3’ and reverse 5’-CATTTTCCGTTAGCGTATT-3’.

**In vitro PRR silencing in 3T3-L1 cells**

3T3-L1 pre-adipocytes were cultured in the presence of Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% (v/v) fetal bovine serum and 1% (v/v) of a mixture of penicillin and streptomycin. PRR was silenced before differentiation by mouse Stealth RNA interference designed for Atp6ap2 gene (PRR) using lipofectamineTM 2000 (Life technology) according to the procedure recommended by the manufacturer.

**Statistical analysis**

Results are expressed as mean±SEM. All data were analyzed using Sigma Plot and Graph Prism. ANOVAs (and ANOVA repeated measures when appropriate)
were used to compare diet and genotype effects, followed by post-hoc tests using Holm-Sidak or Bonferroni corrections for multiple comparisons. When the assumptions underlying the ANOVAs were not otherwise met, data were nonlinearly transformed; however, for ease of illustration, figures show untransformed data. GraphPad QuickCalcs (Grubbs' test) was used to determine statistical outliers and a $t$-test was used to compare mean insulin levels between HF- and LF-fed mice. Statistical significance was defined as $P<0.05$. 
References
Table S1. Characteristics of \(\text{PRR}^{fl/Y}\) and \(\text{PRR}^{Adi/Y}\) male mice.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>(\text{PRR}^{fl/Y})</th>
<th>(\text{PRR}^{Adi/Y})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organ weight, g</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epididymal fat</td>
<td>0.504±0.066</td>
<td>0.015±0.002*</td>
</tr>
<tr>
<td>Retro-peritoneal fat</td>
<td>0.178±0.027</td>
<td>0.020±0.004*</td>
</tr>
<tr>
<td>Subcutaneous fat</td>
<td>0.265±0.036</td>
<td>0.035±0.005*</td>
</tr>
<tr>
<td>Brown adipose tissue</td>
<td>0.129±0.011</td>
<td>0.120±0.021</td>
</tr>
<tr>
<td>Liver</td>
<td>1.67±0.05</td>
<td>2.82±0.16*</td>
</tr>
<tr>
<td>Pancreas</td>
<td>0.270±0.018</td>
<td>0.434±0.044*</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.080±0.003</td>
<td>0.153±0.014*</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.431±0.018</td>
<td>0.444±0.043</td>
</tr>
<tr>
<td>Heart</td>
<td>0.164±0.006</td>
<td>0.178±0.006</td>
</tr>
<tr>
<td>Soleus</td>
<td>0.016±0.001</td>
<td>0.019±0.002</td>
</tr>
<tr>
<td>Plasma</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>44±12</td>
<td>28±3</td>
</tr>
<tr>
<td>Leptin, ng/mL</td>
<td>3.52±0.6</td>
<td>0.26±0.10*</td>
</tr>
<tr>
<td>Fasting glucose mg/dL</td>
<td>142±7</td>
<td>109±14*</td>
</tr>
</tbody>
</table>

Data are mean±SEM for 4 to 6 mice per group. *, \(P<0.05\) compared with control \(\text{PRR}^{fl/Y}\) mice.
Table S2. Blood pressure parameters (24 h) of PRR\textsuperscript{fl/Y} and PRR\textsuperscript{Adi/Y} male mice.

<table>
<thead>
<tr>
<th>Telemetry Parameters</th>
<th>PRR\textsuperscript{fl/Y}</th>
<th>PRR\textsuperscript{Adi/Y}</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP, mmHg</td>
<td>112±1</td>
<td>117±3</td>
</tr>
<tr>
<td>DBP, mmHg</td>
<td>97±2</td>
<td>99±3</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>564±9</td>
<td>552±17</td>
</tr>
<tr>
<td>PP, mmHg</td>
<td>29±2</td>
<td>37±1*</td>
</tr>
</tbody>
</table>

Data are mean±SEM of 4 to 6 mice per group. * $P<0.05$ compared with control PRR\textsuperscript{fl/Y} mice.
Table S3. Characteristics of PRR\textsuperscript{fl/Y} and PRR\textsuperscript{Adi/Y} male mice fed a low-fat (LF) or high-fat (HF) diet.

<table>
<thead>
<tr>
<th>Organ weights (g)</th>
<th>LF Diet</th>
<th>HF Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PRR\textsuperscript{fl/Y}</td>
<td>PRR\textsuperscript{Adi/Y}</td>
</tr>
<tr>
<td>Body Weight</td>
<td>33.9±1.6</td>
<td>29.7±0.6\textdagger</td>
</tr>
<tr>
<td>EF</td>
<td>1.36±0.240</td>
<td>0.02±0.002\textdagger</td>
</tr>
<tr>
<td>RPF</td>
<td>0.503±0.090</td>
<td>0.012±0.003\textdagger</td>
</tr>
<tr>
<td>Subc</td>
<td>0.513±0.051</td>
<td>0.042±0.004\textdagger</td>
</tr>
<tr>
<td>BAT</td>
<td>0.220±0.012</td>
<td>0.243±0.018</td>
</tr>
<tr>
<td>Liver</td>
<td>1.50±0.08</td>
<td>3.02±0.37\textdagger</td>
</tr>
<tr>
<td>Pancreas</td>
<td>0.282±0.008</td>
<td>0.331±0.021</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.083±0.007</td>
<td>0.145±0.013\textdagger</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.356±0.015</td>
<td>0.370±0.008</td>
</tr>
<tr>
<td>Heart</td>
<td>0.148±0.004</td>
<td>0.154±0.004\textdagger</td>
</tr>
<tr>
<td>Soleus</td>
<td>0.017±0.001</td>
<td>0.016±0.001</td>
</tr>
</tbody>
</table>

Plasma (mg/ml)

<table>
<thead>
<tr>
<th></th>
<th>LF Diet</th>
<th>HF Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triglycerides</td>
<td>48±6</td>
<td>42±7</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>108±10</td>
<td>133±19</td>
</tr>
<tr>
<td>CRP (μg/ml)</td>
<td>5.4±0.3</td>
<td>6.1±0.5</td>
</tr>
<tr>
<td>Insulin (ng/ml)</td>
<td>0.40±0.05</td>
<td>2.11±0.98\textdagger</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>13.4±1.5</td>
<td>0.8±0.1\textdagger</td>
</tr>
<tr>
<td>Prorenin/renin</td>
<td>9.1±0.6</td>
<td>8.4±1.2\textdagger</td>
</tr>
</tbody>
</table>

Data are mean±SEM. N=5-8/group. * P<0.05 compared to LF within genotype. † P<0.05 compared to PRR\textsuperscript{fl/Y} within diet. (BW) Body weight, (EF) epididymal fat, (RPF) retroperitoneal fat, (Subc) subcutaneous fat, (BAT) brown adipose tissue, C-R Reactive protein (CRP).
<table>
<thead>
<tr>
<th>Blood pressure parameters</th>
<th>LF Diet</th>
<th>HF Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$PRR^{fl/Y}$</td>
<td>$PRR^{Adi/Y}$</td>
</tr>
<tr>
<td>Light SBP, mmHg</td>
<td>122±1</td>
<td>128±2†</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>105±1</td>
<td>110±1†</td>
</tr>
<tr>
<td>DBP, mmHg</td>
<td>88±0</td>
<td>91±1</td>
</tr>
<tr>
<td>PP, mmHg</td>
<td>34±1</td>
<td>37±1†</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>528±8</td>
<td>555±11</td>
</tr>
<tr>
<td>Dark SBP, mmHg</td>
<td>136±1</td>
<td>141±2†</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>118±0</td>
<td>121±1</td>
</tr>
<tr>
<td>DBP, mmHg</td>
<td>99±0</td>
<td>102±1</td>
</tr>
<tr>
<td>PP, mmHg</td>
<td>37±1</td>
<td>39±1</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>568±8</td>
<td>599±14†</td>
</tr>
</tbody>
</table>

Data are mean±SEM of 5 to 8 mice per group. *, $P<0.05$ compared with week 0. †, $P<0.05$ compared with $PRR^{fl/Y}$. 

Table S4. Blood pressure parameters during light and dark cycle in low-fat (LF)- and high-fat (HF)-fed mice.
Table S5. Blood pressure parameters (24 h) of \( PRR^{fl/Y} \) and \( PRR^{Adi/Y} \) male mice fed a low-fat (LF) or high-fat (HF) diet.

<table>
<thead>
<tr>
<th>Telemetry Parameters</th>
<th>LF Diet</th>
<th>HF Diet</th>
<th>LF Diet</th>
<th>HF Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( PRR^{fl/Y} )</td>
<td>( PRR^{Adi/Y} )</td>
<td>( PRR^{fl/Y} )</td>
<td>( PRR^{Adi/Y} )</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>112±0</td>
<td>116±1†</td>
<td>117±1*</td>
<td>120±1*:†</td>
</tr>
<tr>
<td>DBP, mmHg</td>
<td>94±0</td>
<td>96±1</td>
<td>101±2*</td>
<td>102±2*</td>
</tr>
<tr>
<td>PP, mmHg</td>
<td>35±1</td>
<td>38±1</td>
<td>32±2</td>
<td>36±2</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>543±7</td>
<td>577±12†</td>
<td>608±12*</td>
<td>616±6*:†</td>
</tr>
</tbody>
</table>

Data are mean±SEM of 5 to 8 mice per group. * \( P<0.05 \) compared with low-fat (LF) within genotype. †, \( P<0.05 \) compared with \( PRR^{fl/Y} \) within diet.
Figure S1. Fat and lean mass evolution of $PRR^{fl/Y}$ and $PRR^{Adi/Y}$ male mice fed a standard diet. Data are mean±SEM of 4 to 6 mice per group. *, $P<0.05$ compared with week 0. **, $P<0.05$ compared with $PRR^{fl/Y}$. 
Figure S2. Representative histological section of adipocyte morphology in epididymal adipose tissue of $PRR^{fl/Y}$ and $PRR^{Adi/Y}$ male mice fed a standard diet. Magnification (10x). Scale bar, 200 µm.
Figure S3. mRNA abundance of PRR, PPARγ, Fabp4 and MR in 3T3-L1 cells transfected with either vehicle or siPRR (2 independent experiment, n=3/experiment), * P<0.05 compared to vehicle.
Figure S4. Intraperitoneal glucose tolerance tests in $PRR^{fl/Y}$ and $PRR^{Adi/Y}$ male mice fed a standard diet. Blood glucose levels after administration of glucose (2 mg/kg body weight) after 5 (A), 9 (C), 13 (E) weeks of standard diet. Area under the curve (AUC) after 5 (B), 9 (D), 13 (F) weeks of standard diet. Data are mean±SEM of 4 to 6 mice.
Figure S5. (A) Representative histological sections of liver from \textit{PRR}^{fl/Y} and \textit{PRR}^{Adi/Y} male mice fed a standard diet stained by H&E (magnification 10x) and Oil Red O staining (magnification 20x). Scale bar, 200 μm. (B) Quantification of Oil Red O staining. Data are mean±SEM of 4 to 6 mice. *, \textit{P<0.05} compared with \textit{PRR}^{fl/Y}. 
Figure S6. Renal histology of low-fat (LF)- and high-fat (HF)-fed of PRR^ii/Y and PRR^Adi/Y male mice. (A) Masson trichrome staining (40x). (B) Quantification of collagen deposition. Scale bar, 50 μm. Data are mean±SEM of 3 to 6 tissue sections.
Figure S7. (A) Representative histological sections of liver from low-fat (LF)- and high-fat (HF)-fed $PRR^{fl/Y}$ and $PRR^{Adi/Y}$ male mice (magnification 10x). Scale bar, 200 μm.
Figure S8. mRNA abundance of PRR in liver (A), and kidney (B) from low-fat (LF)- and high-fat (HF)-fed PRR\textsuperscript{fl/Y} and PRR\textsuperscript{Adi/Y} male mice. Data are mean±SEM, N=5-8.
Figure S9. Adipocyte PRR deficiency improved glucose homeostasis in HF-fed groups. (A) Intraperitoneal glucose tolerance test and (B) Area under the curve. (C) Fasting blood glucose. Data are mean±SEM from N = 5-8 mice/group. *, P<0.05 compared to LF-diet. **, P<0.05 compared to PRR<sup>N/Y</sup> mice.
Figure S10. Linear regression between sPRR and SBP (A) and between insulin and SBP (24 h) (B) of male $PRR^{l/l}$ and $PRR^{Aa/Ad}$ mice from all studies together (N=19).