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

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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 ($PRR^{Adi/Y}$) with almost no detectable white adipose tissues. As a consequence, the livers of $PRR^{Adi/Y}$ mice were enlarged and demonstrated a marked accumulation of lipids. Adipocyte-specific deficiency of PRR increased systolic blood pressure (SBP) and the concentration of soluble PRR (sPRR) 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- and low-fat-fed $PRR^{Adi/Y}$ mice had elevated insulin levels. Interestingly, adipocyte-PRR deficiency improved glucose tolerance in high-fat-fed $PRR^{Adi/Y}$ mice. In response to feeding either low-fat or high-fat diets, SBP was greater in $PRR^{Adi/Y}$ mice compared with control mice. High-fat feeding elevated sPRR concentration in control and $PRR^{Adi/Y}$ mice. *In vitro* knock down of PRR by siRNA significantly decreased mRNA abundance of PPARγ, 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 SBP regulation.

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Disclosures

None.

Keywords

prorenin receptor; adipocytes; blood pressure; insulin; glucose; lipids

Hypertension is the major cause of cardiovascular diseases worldwide and, according to the 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 (AGT) 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 up-regulated 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 AGT 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.[7,11] In addition, PRR can be cleaved intracellularly by furin, resulting in the secretion of a soluble form of PRR (sPRR) in plasma[14] and urine,[15,16] which might bind renin and prorenin[15] 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[11] and 3T3-L1 preadipocytes[7,8] initiates an intracellular signaling cascade associated with the activation of the ERK1/2 pathway. In mesangial cells, the activation of the ERK1/2 pathway leads to the release of TGFβ1 and cytokines involved in inflammation. However, past attempts to generate complete PRR knock-out mice failed.[20] Specific cardiomyocyte or podocyte deletion of PRR led to animal lethality 3 weeks after birth due to heart or kidney failure.[21–24] Since 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] Additionally, 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 (1) to determine whether a specific adipocyte-PRR deficiency mouse model is viable, (2) to determine the physiological consequences of adipocyte-PRR deletion on blood pressure in normal physiology and during the development of obesity, and (3) to determine 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 ($PRR^{fl/fl}$) were bred to transgenic male mice ($PRR^{fl/Y}$) expressing Cre recombinase under the control of the adiponectin promoter (Strain Name: B6;FVB-Tg(Adipoq-cre)1Evdr/JB6;FVB-Tg(Adipoq-cre)1Evdr/J)[24] (Figure 1A). Diets, plasma measurements, histology, radiotelemetry and statistical analyses are described in the online-only Data Supplement.

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 $P<0.05$.

Results

Generation of mice with adipocyte-PRR deficiency

To confirm efficiency of PRR deletion in adipocytes, pre-adipocytes from the stromal vascular fraction of subcutaneous adipose tissue of control mice ($PRR^{fl/Y}$) 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 $PRR^{fl/Y}$ mice (Figure 1B; $P<0.05$) demonstrating the efficacy of the deletion.

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 $PRR^{fl/Y}$ mice (Figure 2B) and did not increase with age in $PRR^{Adi/Y}$ mice suggesting $PRR^{Adi/Y}$ mice do not accumulate adipose tissue (Figure S1). The mass of all white adipose tissues was significantly reduced in $PRR^{Adi/Y}$ mice compared with $PRR^{fl/Y}$ mice (Table S1, $P<0.05$). The analyses of epididymal adipose tissue morphology from $PRR^{fl/Y}$ 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 very 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). PPARγ, 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 PPARγ and Fabp4 were significantly decreased in differentiated siPRR cells compared with control cells.

Tissue weights of liver, spleen and pancreas were significantly higher in $PRR^{Adi/Y}$ mice compared with $PRR^{fl/Y}$ mice (Table S1). Leptin plasma concentrations were lower in $PRR^{Adi/Y}$ mice than in $PRR^{fl/Y}$ 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 $PRR^{Adi/Y}$ mice compared with $PRR^{fl/Y}$ mice (Figure S5). Neutral lipids were significantly increased in liver of $PRR^{Adi/Y}$ mice compared with $PRR^{fl/Y}$ 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 $PRR^{Adi/Y}$ mice compared with $PRR^{fl/Y}$ 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 AGT concentration (Figure 4A). Plasma renin activity (PRA) and plasma renin concentration (PRC) did not differ significantly between $PRR^{Adi/Y}$ mice and $PRR^{fl/Y}$ mice (Figure 4B), suggesting that adipocyte-specific deficiency of PRR did not influence AGT, PRA or PRC. Surprisingly, plasma sPRR levels increased by threefold in $PRR^{Adi/Y}$ mice compared with $PRR^{fl/Y}$ mice (Figure 4C).

Adipocyte-specific deficiency of PRR prevented the development of obesity and the accumulation of fat mass in high-fat-fed mice

$PRR^{Adi/Y}$ mice were resistant to HF-diet-induced obesity (Figure 5A). The fat mass of $PRR^{Adi/Y}$ mice was lower by about −70% compared with LF-fed $PRR^{fl/Y}$ mice and was lower by almost −80% compared with HF-fed $PRR^{fl/Y}$ mice (Figure 5B). When challenged with a HF diet, tissue weights of liver, heart, and kidney were significantly higher in $PRR^{Adi/Y}$ mice compared with $PRR^{fl/Y}$ mice (Table S3). Adipocyte-PRR deficiency did not significantly affect kidney structure (Figure S6). HF- and LF-fed $PRR^{Adi/Y}$ mice had increased lipid accumulation in liver compared with $PRR^{fl/Y}$ 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 $PRR^{Adi/Y}$ mice exhibited improved glucose tolerance compared with HF-fed $PRR^{fl/Y}$ mice. Fasting glucose levels were significantly lower in HF-or LF-fed $PRR^{Adi//Y}$ mice compared with $PRR^{fl/Y}$ 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 $PRR^{Adi/Y}$ 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 $PRR^{Adi/Y}$ mice regardless of diet (Table S4 and S5).

In obese mice, adipocyte-specific deficiency of PRR exaggerated the elevation of plasma sPRR levels

High-fat feeding induced a significant increase in plasma AGT concentrations in control $PRR^{fl/Y}$ mice (Figure 7A). However, plasma AGT concentrations did not differ between $PRR^{Adi/Y}$ and $PRR^{fl/Y}$ mice regardless of diet. PRA was not influenced by the diet or by adipocyte-specific PRR deficiency (Figure 7B). When challenged with HF diet, $PRR^{fl/Y}$ mice exhibited a lower PRC and a lower total prorenin/renin concentration than LF-fed $PRR^{fl/Y}$ mice (Figure 7B, Table S3). PRC and total prorenin/renin concentration in LF- and HF-fed $PRR^{Adi/Y}$ mice did not differ from those of LF-fed $PRR^{fl/Y}$ mice.

Plasma sPRR levels were significantly increased in LF-fed $PRR^{Adi/Y}$ mice compared with LF-fed $PRR^{fl/Y}$ mice. HF feeding induced a threefold increase in plasma sPRR levels in HF-fed $PRR^{fl/Y}$ mice compared with LF-fed $PRR^{fl/Y}$ mice (Figure 7C). Plasma sPRR levels were more than twofold higher in HF-fed $PRR^{Adi/Y}$ mice compared with HF-fed $PRR^{fl/Y}$ mice. Plasma sPRR concentration was positively correlated with SBP ($P<0.05$) in $PRR^{fl/Y}$ mice and $PRR^{Adi/}$ mice combined (Figure S10A) and in $PRR^{fl/Y}$ mice alone; the correlation was weaker ($P>0.05$) in $PRR^{Adi//Y}$ 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 appeared to be independent of systemic AGT 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. Additionally, since fatty acid binding proteins are important carriers for fatty acids uptake and fatty acids transport to sites of esterification into triglycerides,[27] 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 (fld) transgenic mouse models.[28,29] In contrast, $PRR^{Adi/Y}$ 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 hypertriglyceridemia are commonly observed. In addition, the plasma insulin levels in $PRR^{Adi/Y}$ mice increased modestly, and $PRR^{Adi/Y}$ mice did not present severe hyperinsulinemia. Our data are nevertheless in agreement with the phenotype of the $PPAR\gamma^{P465L/+}$ mouse model,[30] which had improved ability to respond to acute glucose overload compared with controls when challenged with HF feeding. As suggested by Tsai *et al.*,[30] the expansion of pancreatic islets likely could contribute to this increased responsiveness to glucose. Similar to our model, CGI-58β mouse[31] 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.

$PRR^{Adi/Y}$ mice exhibited elevated blood pressure similar to that reported in $PPAR\gamma^{P465L/+}$ mice and humans expressing FPLD2 and FPLD3 mutations.[28_30] In the latter instances, the cause of increased blood pressure is not well understood. Elevated leptin has been associated with elevated blood pressure[32] but could protect against nonalcoholic fatty liver disease[33]. Thus, while it is unlikely that low levels of circulating leptin in $PRR^{Adi/Y}$ 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 $PRR^{Adi/Y}$ mice.[28] 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.[18] Moreover, patients with heart failure have higher plasma sPRR levels than control subjects.[34] However, our demonstration of a positive correlation between sPRR and SBP when control mice and $PRR^{Adi/Y}$ mice are combined or in control mice only suggests that sPRR could play a role in blood pressure

control. Further investigation are needed regarding 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.[13–18,34]

Adipose tissue is one source of systemic angiotensin II, and both expansion or reduction of adipose tissue activates adipose RAS, thereby influencing blood pressure regulation.[3,4,30,35,36] Since Tsai *et al.*[30] demonstrated that expression of AGT and AT1R in adipose tissue is increased in $PPAR\gamma^{P465L/+}$ mice, the increase in blood pressure in $PRR^{Adi/Y}$ mice could be attributed to a local activation of adipose RAS. In $PRR^{Adi/Y}$ mice, the reduction of adipose tissue weight in conjunction with elevated plasma AGT 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 AGT deficiency induced profound reductions in blood pressure, systemic AGT, and angiotensin II, which influences adipose RAS content and secretion despite the continued presence of obesity.[4] Since the liver is an important source of renal angiotensin II,[37] it may also be possible that other local RAS are activated to compensate for the absence of adipose tissue. In contrast to liver AGT deficiency, $PRR^{Adi/Y}$ mice demonstrated a radical shift in lipid distribution resulting in hepatic steatosis, which might also have influenced adipose and other local RAS systems.[38] Indeed, despite lower body weights and adipose tissue weights in $PRR^{Adi/Y}$ mice, systemic AGT concentrations were not lower compared with those of obese control mice, suggesting substantial compensatory activation of systemic AGT from other tissues.

Perspectives

The remarkable phenotype of the adipocyte-PRR-deficient mouse model demonstrates the importance of adipocyte PRR in lipid and glucose and insulin homeostasis. Our results demonstrate the necessity of adipocyte PRR in the normal development of adipose tissue, beyond its potential role in local RAS activation. Further investigation is needed to determine the mechanism by which PRR regulates adipose cell formation and lipid homeostasis and blood pressure.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Novelty and Significance

What is new?

- Demonstration that adipocyte-derived 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 sPRR 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 (pro)renin receptor 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 sPRR levels.

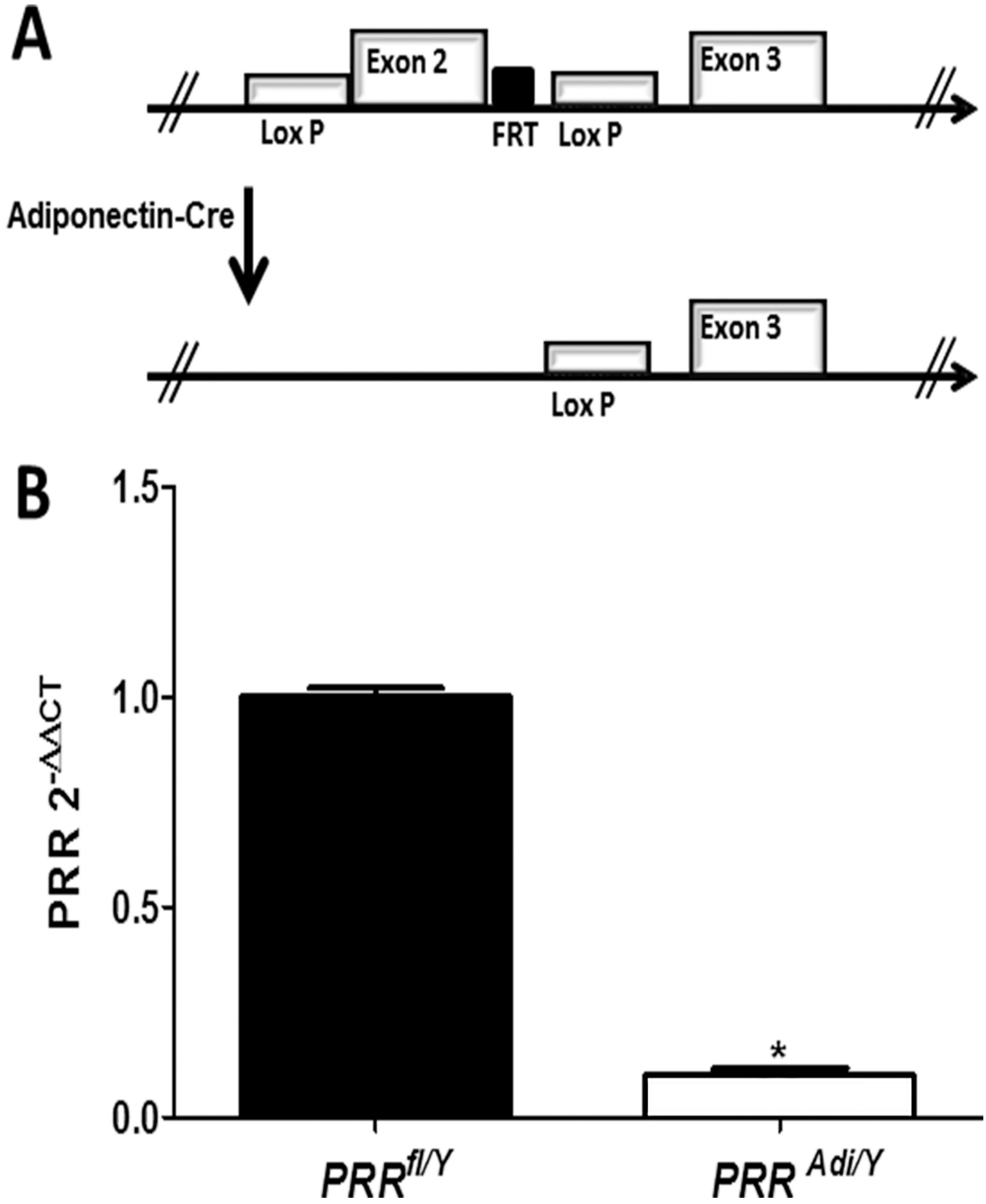

Figure 1.

(A) Schematic representation of the loxP-flanked 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 $PRR^{fl/Y}$ and $PRR^{Adi/Y}$ mice. Data are mean±SEM of 3 to 6 mice. * $P<0.05$ compared with $PRR^{fl/Y}$ mice.

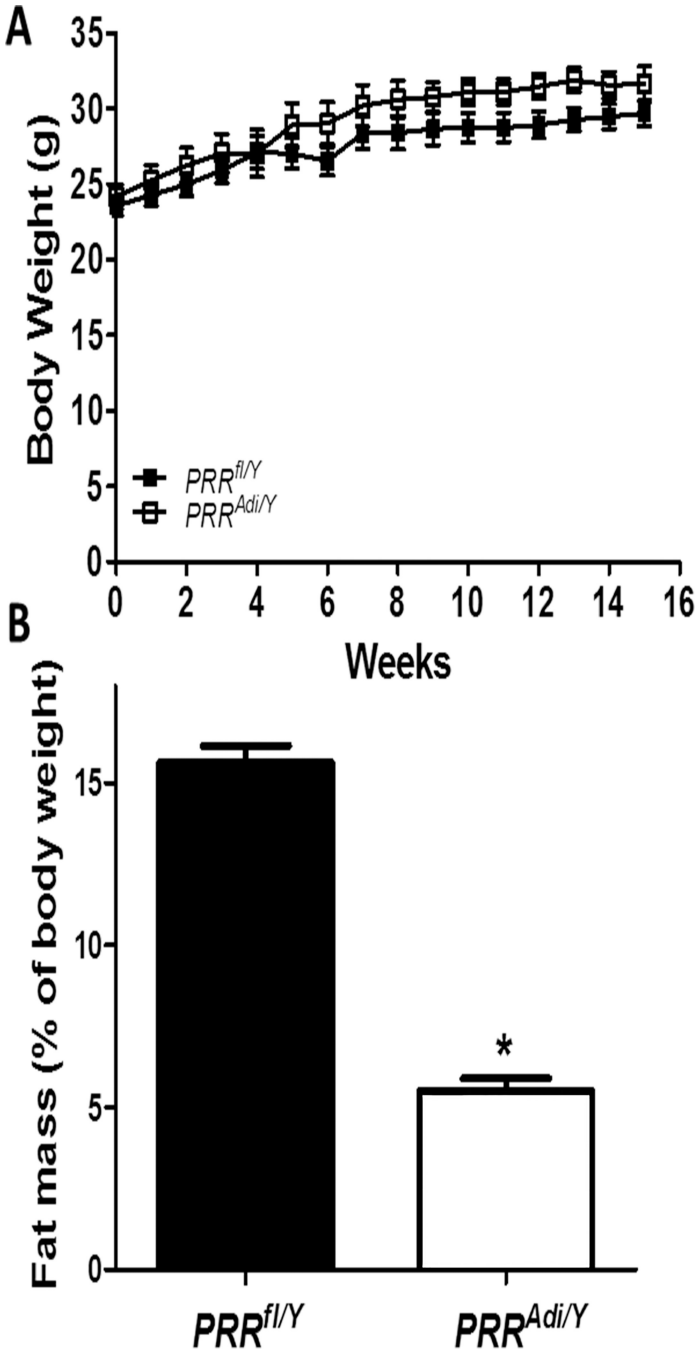

Figure 2.

Adipocyte PRR deficiency reduced fat mass in mice fed a standard diet. (A) Body weight curves of $PRR^{fl/Y}$ and $PRR^{Adi/Y}$ mice. Data are mean±SEM of 4 to 6 mice. (B) Fat mass (% of body weight) for mice in each group. Data are mean±SEM of 4 to 6 mice. * $P<0.05$ compared with $PRR^{fl/Y}$.

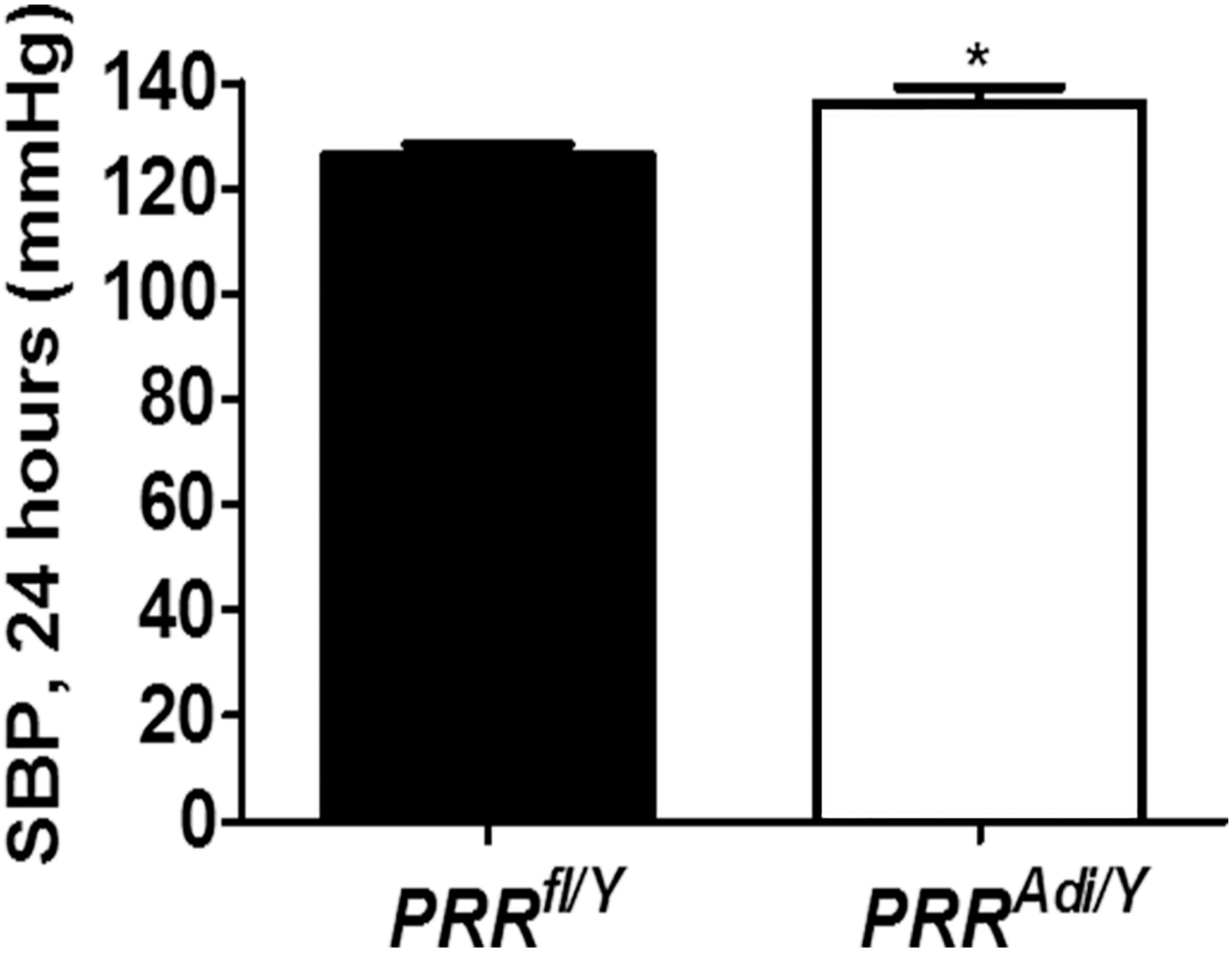

Figure 3.

Adipocyte PRR deficiency increased systolic blood pressure (SBP; 24 h) in mice fed a standard diet. (A) SBP (24 h) for male $PRR^{fl/Y}$ and $PRR^{Adi/Y}$ mice. Data are mean±SEM of 4 mice. *P<0.05 compared with $PRR^{fl/Y}$ mice.

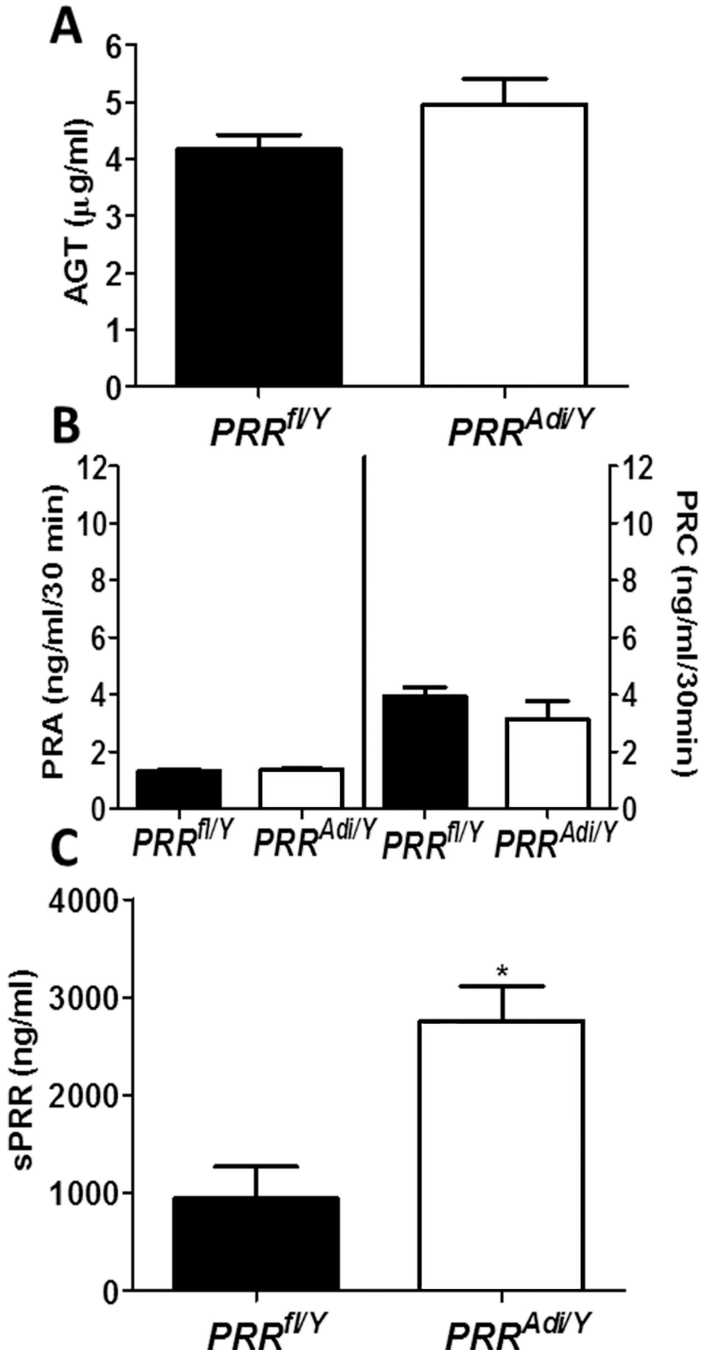

Figure 4.

Adipocyte PRR deficiency increased plasma sPRR. (A) Plasma AGT concentrations in male $PRR^{fl/Y}$ and $PRR^{Adi/Y}$ mice. (B) Plasma renin activity (PRA; left *y* axis) and concentrations (PRC; right *y* axis) in male $PRR^{fl/Y}$ and $PRR^{Adi/Y}$ mice. (C) Plasma sPRR concentrations in male $PRR^{fl/Y}$ and $PRR^{Adi/Y}$ mice. Data are mean±SEM of 4 to 6 mice. * $P<0.05$ compared with $PRR^{fl/Y}$ mice.

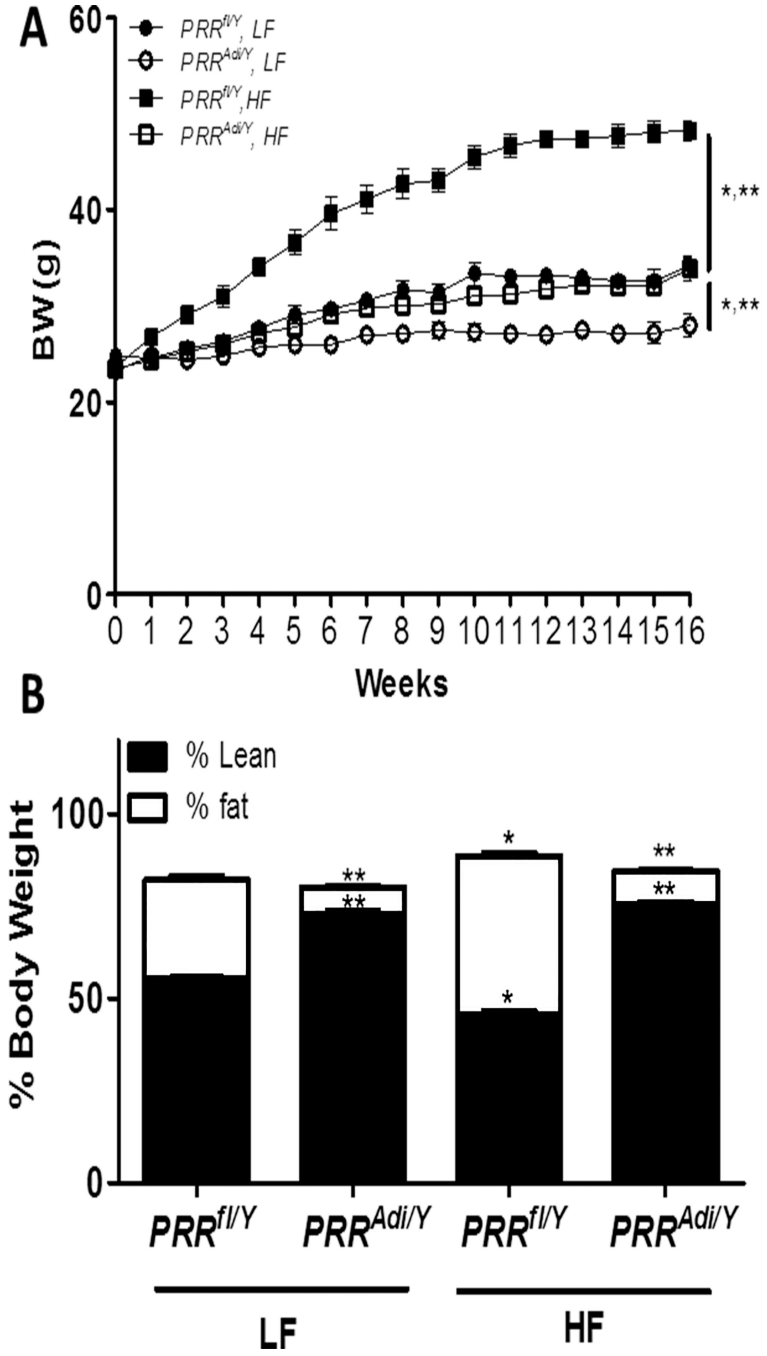

Figure 5.

Adipocyte PRR deficiency prevented the development of obesity and decreased fat mass. (A) Body weight curve of $PRR^{fl/Y}$ and $PRR^{Adi/Y}$ mice fed a low-fat (LF) or high-fat (HF) diet. Data are mean ± SEM from n = 5 to 8 mice/group. (B) Fat and lean mass (% of body weight) of $PRR^{fl/Y}$ and $PRR^{Adi/Y}$ mice fed a LF or HF diet. Data are mean±SEM of 3 to 8 mice.

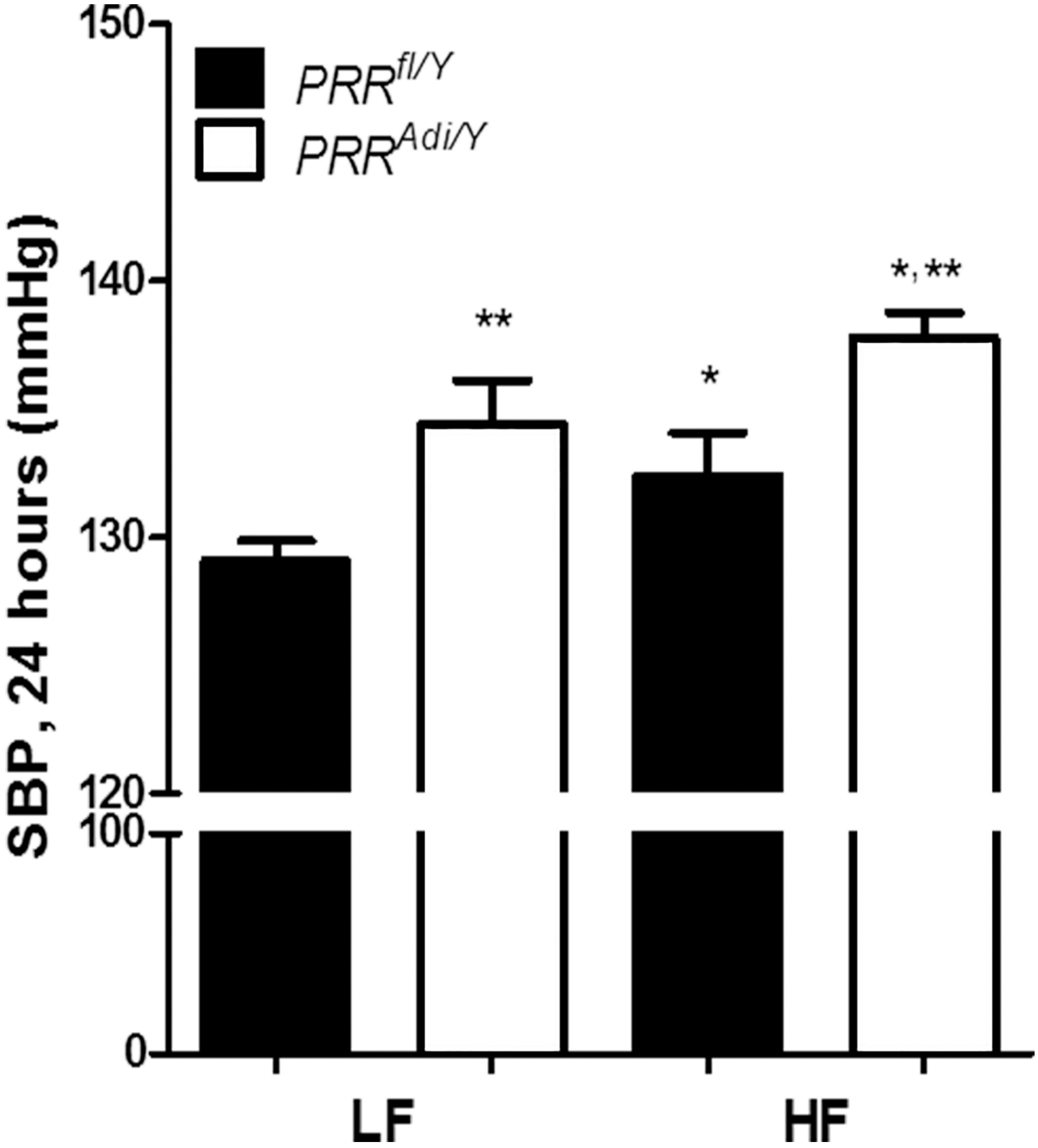

Figure 6.

Adipocyte PRR deficiency exaggerated diet-induced increase of blood pressure. SBP (24 h) of male $PRR^{fl/Y}$ and $PRR^{Adi/Y}$ mice fed a low-fat (LF) or high-fat (HF) diet. Data are mean ±SEM from n = 5 to 8 mice. * $P<0.05$ compared with LF diet. ** $P<0.05$ compared with $PRR^{fl/Y}$ mice.

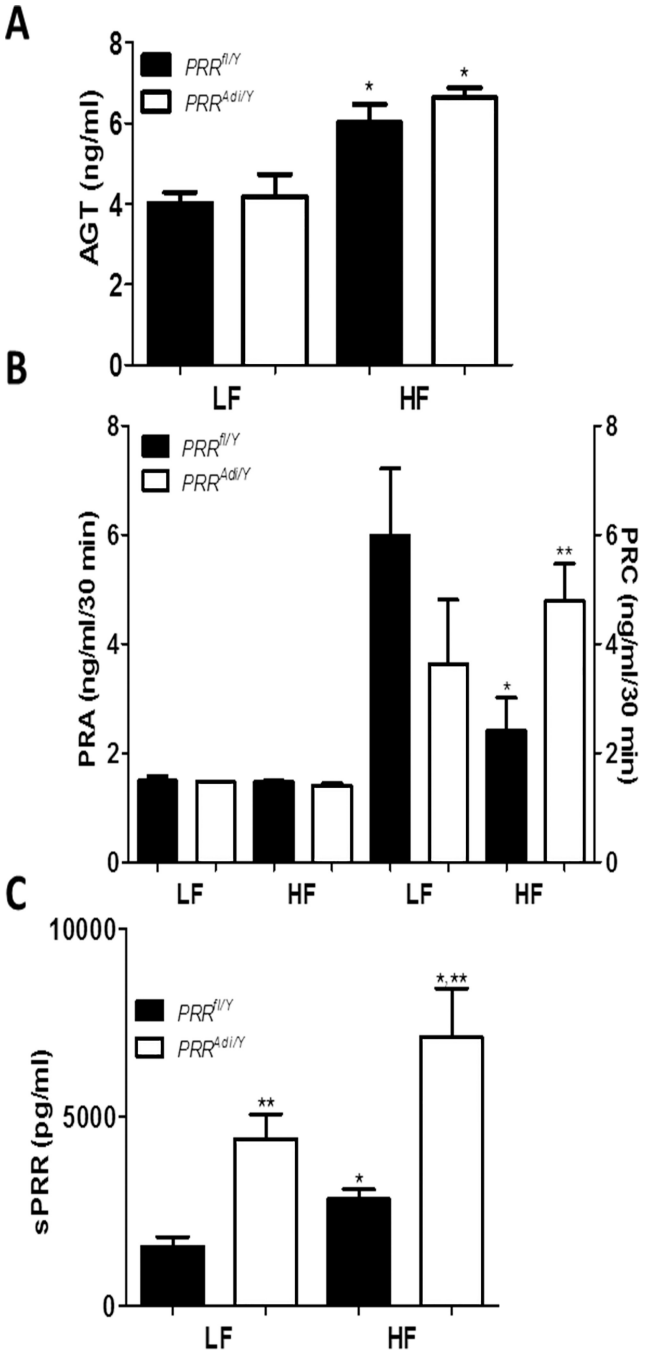

Figure 7.

(A) Plasma AGT concentrations in male $PRR^{fl/Y}$ and $PRR^{Adi/Y}$ mice fed a LF- or HF-diet. (B) Plasma renin activity (PRA; left *y* axis) and concentration (PRC; right *y* axis). (C) Plasma sPRR concentration. Data are mean±SEM of 5 to 8 mice. * $P<0.05$ compared with LF diet. ** $P<0.05$ compared with $PRR^{fl/Y}$ mice.