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# Soluble Prorenin Receptor Increases Blood Pressure in High-Fat Fed Male Mice

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## Abstract

Obesity-related hypertension is a major public health concern. We recently demonstrated that plasma levels of the soluble form of the prorenin receptor (sPRR) were elevated in obesity-associated hypertension. Therefore, in the present study, we investigated the contribution of sPRR to blood pressure elevation in the context of obesity. High-fat fed C57BL/6 male mice were infused with vehicle or sPRR (30 μg/kg/day) via subcutaneously implanted osmotic minipump for 4 weeks. Blood pressure parameters were recorded using radiotelemetry devices. Male mice infused with sPRR exhibited higher systolic blood pressure and mean arterial pressure and lower spontaneous baroreflex sensitivity than mice infused with vehicle. To define mechanisms involved in systolic blood pressure elevation, mice were injected with an angiotensin-II type 1 receptor antagonist (losartan), a muscarinic receptor antagonist (atropine), a β-adrenergic antagonist (propranolol) and a ganglionic blocker (chlorisondamine). Losartan did not blunt sPRR-induced elevation in systolic blood pressure. Chlorisondamine treatment exacerbated the decrease in mean arterial pressure in male mice infused with sPRR. These results demonstrated that sPRR induced autonomic nervous dysfunction. Interestingly, plasma leptin levels were increased in high-fat fed C57BL/6 male mice infused with sPRR. Overall, our results indicated that sPRR increased systolic blood pressure through an impairment of the baroreflex sensitivity and an increase in the sympathetic tone potentially mediated by leptin in high-fat fed C57BL/6 male mice.

## Graphical Abstract

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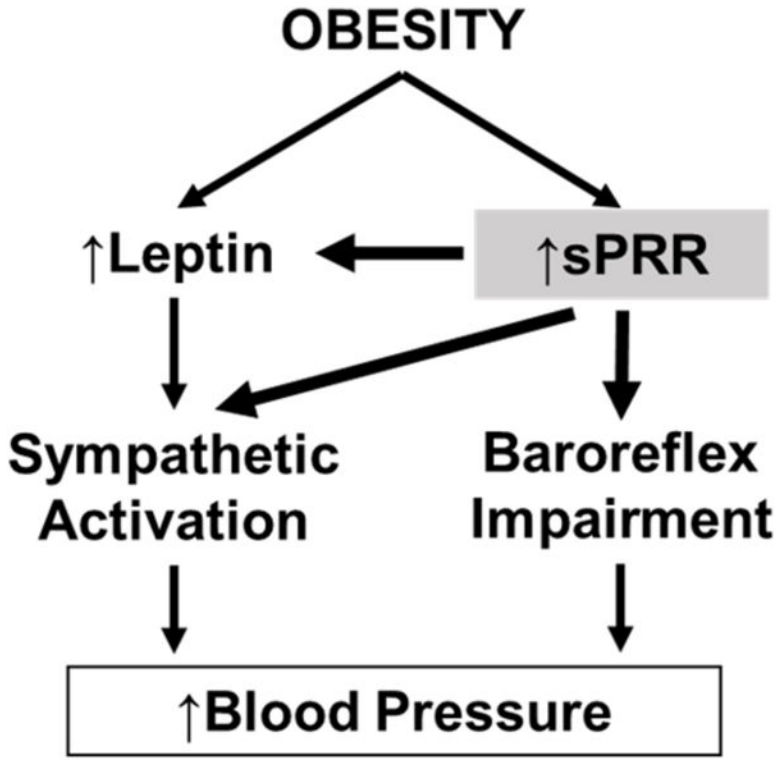
Disclosures

None.




## Summary

The soluble form of the prorenin receptor elevates blood pressure during the development of obesity, demonstrating an important role for sPRR in the control of blood pressure in obese male mice. In obese male mice, sPRR may elevate blood pressure primarily through a dysregulation of the autonomic nervous system via an impairment of the baroreflex sensitivity and an increase in the sympathetic tone.

### Keywords

soluble prorenin receptor; blood pressure; obesity

## Introduction

Obesity is one of the major causes of the rise in prevalence of hypertension[1]. According to the National Health and Nutrition Examination Survey (2011–14), 36.5% of adults are obese and 29.1 % are hypertensive in the US [2,3].Treating hypertension associated with obesity has become the most challenging healthcare epidemic for the medical field. Despite treatments (Angiotensin-converting enzyme inhibitors, angiotensin-II type 1 receptor blockers, adrenergic receptor antagonists and calcium channel blockers), 20–30% of the patients have resistant hypertension [4,5]. Therefore, to advance medical care, a clear need exists for better understanding the mechanisms responsible for the pathogenesis of hypertension associated with obesity.

The renin angiotensin system (RAS) plays a pivotal role in blood pressure control and fluid homeostasis. We and others recently found that (pro)renin receptor (PRR), a component of the RAS, is up-regulated during the development of obesity [6–9]. The PRR, a 350 amino protein with a single transmembrane domain, is the receptor for (pro)renin, renin in its active form and prorenin in its inactive form [10,11]. PRR can be cleaved to generate a soluble form of PRR (sPRR). The sPRR can be retained inside cells [12] and secreted into plasma [13], urine and into the extra-cellular space [14,15]. An increasing body of evidence suggested that sPRR has a biological function. Previous studies have found that sPRR increased renin activity of prorenin in vitro [16]. sPRR up-regulated renal aquaporin 2 gene through LRP6/FZD8 and participated in urine concentration [17]. Moreover, we demonstrated that infusion of sPRR activated the RAS by increasing plasma renin levels and by up-regulating renal and hepatic angiotensinogen (AGT) genes in C57BL/6 female mice fed a standard diet [18]. In human and rodents, sPRR has also been shown to be a potential biomarker of cardiovascular pathologies. For instance, plasma sPRR levels were elevated in patients with heart failure [19]. In patients with essential hypertension, circulating sPRR levels correlated positively with urinary AGT excretion and negatively with glomerular filtration rate (GFR) [20]. High circulating levels of plasma sPRR in early pregnancy predicted elevated systolic blood pressure (SBP) and high sPRR levels at delivery were associated with preeclampsia [21]. Interestingly, we recently demonstrated that sPRR levels are elevated during the development of obesity-hypertension and in mouse models of lipodystrophy [9,18]. However, whether sPRR is directly involved in blood pressure control during the development of obesity remained to be investigated.

Therefore, the objectives of our study were to determine whether sPRR infusion affects the blood pressure regulation during the development of obesity in male mice and to investigate whether elevated blood pressure was mediated by an AngII-dependent mechanism in male mice.

## Methods and Animals

The data, analytic methods, and study materials that support the findings of this study are available from the corresponding author upon reasonable request.

### Experimental protocol.

All animal protocols described below were in compliance 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 (IACUC protocol number 2013–1109).

Six weeks old C57BL/6 male mice (The Jackson Laboratory, DIO C57BL/6J, Jax # 380050) were fed a high-fat diet (HF, 60% fat; catalog #D12492, Research Diets Inc, New Brunswick, NJ). Mice were provided ad libitum access to food and drinking water. Body weight was recorded once a week. After 15 weeks of HF feeding, a total of 12 out of 14 male mice were implanted with a catheter connected to a telemetry transmitter (PA-C10 model, DSI, St Paul, MN) in the left carotid artery for blood pressure measurement. Surgery was performed under isoflurane anesthesia and followed by carprofen injection for 3 days

(10mg/kg daily, i.p). After radiotelemetry device implantation, mice were housed individually. Blood pressure (BP) was recorded prior vehicle or sPRR infusion to design 2 groups of mice with similar BP. On week 17 of HF feeding, all mice (n=14) were implanted with an osmotic pump (Alzet Mini osmotic pump, Model 2004, Durect Corporation, Cupertino, CA) under isoflurane anesthesia and infused for 4 weeks with vehicle (saline) (Veh, n=7) or sPRR (mouse recombinant sPRR-HisTag, 30 μg/kg/day) (sPRR, n=7), as previously described [18]. On day 27 of infusion, mice were placed in individual metabolic cages for 24h with free access to food and water (Tecniplast Solo Mouse Metabolic Cages, Tecniplast USA, Exton, PA) for urine collection.

### Quantification of plasma and urine parameters.

Plasma AGT levels were determined using mouse total AGT assay kit (IBL Co, Minneapolis, MN). Plasma renin activity was evaluated using Renin Assay Kit (Sigma, St Louis, MO) according to the manufacturer's instructions. Results were expressed in renin equivalent calculated from the change in relative fluorescence units for each sample compared with that of recombinant renin provided in the kit. Plasma leptin levels were measured using Mouse/Rat Leptin ELISA (Alpco, Salem, NH). Plasma total prorenin/renin levels were assessed using Mouse Prorenin and Renin Total Antigen ELISA Kit (Molecular Innovation, Novi, MI). Urinary vasopressin levels were determined with Arg8-Vasopressin ELISA kit (Enzo Life Sciences, Farmingdale, NY).Urinary sodium excretion was quantified using a dual channel flame photometer (Model 2655–10, Cole-Parmer Instrument Company, Vernon Hills, IL).

### LC-MS/MS based quantification of equilibrium plasma angiotensin levels.

Angiotensins levels were measured by Attoquant Diagnostics as previously described [18]. Equilibrium angiotensin concentrations were analyzed by mass spectrometry following 30 min of equilibration of conditioned heparin plasma at 37°C and subsequent stabilization of equilibrium peptide levels. Stabilized samples were spiked with 200 pg of stable isotope-labeled internal standard for each individual angiotensin metabolite (AngI, AngII, Ang1–7, Ang1–5, Ang2–8, Ang3–8, Ang2–10, Ang2–7, Ang1–9, and Ang3–7). Following C18-based solid-phase-extraction, samples were subjected to LC-MS/MS analysis using a reversed-phase analytical column (Acquity UPLC® C18, Waters) operating in line with a XEVO TQ-S triple quadrupole mass spectrometer (Waters) in MRM mode.

### Immunostaining.

Retroperitoneal fat pads were fixed in paraformaldehyde and embedded in paraffin blocks. Sections were stained with hematoxylin and eosin and examined with a Nikon Eclipse 80i light microscope. Cells size and number were determined at 10x magnification using NIS Elements BR.3.10 software.

### RNA extraction and quantitative RT-PCR.

RNA was extracted from kidney using the SV Total RNA Isolation System (Promega, Madison, WI) and quantified with a NanoDrop 2000 spectrophotometer (Wilmington, DE). PerfeCTa SYBR Green FastMix (Quanta BioSciences, Gaithersburg, MD) was used to

perform real-time quantitative PCR after cDNA synthesis using qscript cDNA SuperMix (Quanta Biosciences, Gaithersburg, MD).

### Western blotting.

Protein from frozen brain and kidney were extracted in ice-cold Tris buffer with a Geno/Grinder® 2010 (SPEX SamplePrep, Metuchen, NJ) and were submitted to SDS-Page on precast polyacrylamide gel (Mini-PROTEAN® TGX™, 4–20%, Bio-Rad Laboratories, Hercules, CA). After transfer to polyvinylidene difluoride membrane and blocking in 5% non-fat dried milk in Tris-buffered saline with 0.1 % Tween 20 (TBST), membranes were incubated with anti-PRR antibody (Sigma, St Louis, MO), anti-AQP2 antibody (Cell Signaling Technology, Inc., Danvers, MA) or anti-GAPDH (Cell Signaling Technology, Inc., Danvers, MA) in TBST 5% non-fat dried milk. Following incubation with HRP-conjugated anti-rabbit secondary antibody (Jackson ImmunoResearch Laboratories, West Grove, PA), proteins were imaged using a Syngene PXi imager (Syngene, Frederick, MD). The levels of proteins were quantified using ImageJ software (National Institutes of Health, Bethesda, MD, USA) and normalized to GAPDH levels.

### Assessment of cardiovascular parameters using radiotelemetry.

Systolic blood pressure (SBP), mean arterial pressure (MAP), diastolic blood pressure (DBP) and heart rate (HR) were examined for 5 consecutive days after 2 weeks of infusion with vehicle or sPRR. The spontaneous baroreflex sensitivity (SBRS) was analyzed with HemoLab Software Ver. 20.7, using the sequence method described by Bertinieri et al[22] after 2 weeks of infusion with vehicle or sPRR. After 3 weeks of infusion, the contribution of the RAS to BP control was evaluated using losartan (20 mg/kg body weight, i.p). The autonomic function was assessed using atropine sulfate (5 mg/kg body weight, i.p), propranolol (5 mg/kg body weight, i.p) and chlorisondamine diiodide (1 mg/kg body weight, i.p).

### Statistical Analysis.

Data are represented as means ± the standard error of the mean (SEM). Statistical differences between groups were assessed by one-way ANOVA or two-way ANOVA followed by Holm-Sidak post-hoc analysis for multiple comparisons. The normal distribution of the data was tested using Shapiro-Wilk test. When required, data were transformed to ensure normal distribution. Statistical outliers were identified using Grubbs test (GraphPad QuickCalcs). Based on Grubbs test outcomes, one male mouse infused with vehicle was excluded from blood pressure measurement. Values of $P<0.05$ were considered statistically significant.

## Results

### sPRR infusion tends to increase fat mass in high-fat fed male mice

To determine whether sPRR influenced body weight and adipose deposition during the development of obesity, male mice were fed a high fat diet for 17 weeks and infused with sPRR or with vehicle. After 4 weeks of infusion, there was no significant difference in body weight of HF-fed C57BL/6 male mice infused with sPRR compared with HF-fed male mice

infused with vehicle (Figure 1A). White fat pad weights (retro-peritoneal and subcutaneous fat) tended to increase in HF-fed C57BL/6 male mice infused with sPRR compared with HF-fed male mice infused with vehicle (Figure 1B), likely due to an increased number of enlarged adipocytes (Supplemental figure S1, please see http://hyper.ahajournals.org). sPRR infusion did not change liver, kidney and heart weights but slightly affected the weights of the pancreas and the spleen (Supplemental Table S1).

### sPRR infusion induced an increase in blood pressure during the development of obesity

After 2 weeks of infusion, SBP and MAP were significantly increased during light and dark cycles in HF-fed male mice infused with sPRR compared to male mice infused with vehicle (Figure 2A and Supplemental Table S2), suggesting that sPRR infusion potentiated the elevation of blood pressure during the development of obesity. We next examined the participation of the autonomic nervous system to SBP elevation by assessing the baroreflex sensitivity. HF-fed mice infused with sPRR displayed impaired baroreflex function compared to vehicle suggesting that sPRR might affect baroreflex sensitivity during the development of obesity in male mice (Figure 2B).

### Blood pressure response to losartan was not affected by sPRR infusion in HF-fed mice

We next investigated the mechanism by which sPRR elevated SBP. To examine whether this elevation was mediated by an Angiotensin II (AngII)-dependent mechanism, male mice were treated with an angiotensin-II type 1 receptor (AT1R) antagonist (Losartan). The decrease in SBP induced by losartan was similar in HF-fed male mice infused with sPRR compared to male mice infused with vehicle suggesting that sPRR-induced increase in SBP is not primarily mediated by AT1R (Figure 2C). Plasma total prorenin/renin and AGT levels were not influenced by sPRR infusion in HF-fed male mice (Figure 3A and Figure 3C), while plasma renin activity tended to increase in HF-fed male mice infused with sPRR compared to male mice infused with vehicle (Figure 3B). The quantification of plasma angiotensin peptides in HF-fed male mice infused with sPRR did not reveal significant changes in angiotensin I, angiotensin II, angiotensin III, angiotensin 1–5 and angiotensin IV levels compared to mice infused with vehicle (Figure 3D). Interestingly, plasma Ang2–10 level was significantly decreased in HF-fed male mice infused with sPRR compared to mice infused with vehicle (Figure 3D). The infusion of sPRR in HF-fed male mice did not change urinary vasopressin (Supplemental figure S2A), urine volume (Supplemental figure S2B), nor urine sodium excretion (Supplemental figure S2C). sPRR infusion had no effect on PRR or aquaporin 2 mRNA abundance or protein levels in kidney (Supplemental figures S2D, S2E and S2F). In addition, sPRR infusion did not change PRR and sPRR protein contents in the brain (Supplemental figure S3).

### The elevation of blood pressure is likely mediated by the autonomic nervous system

We next determined the contribution of the autonomic nervous system to SBP elevation by assessing changes in MAP (ΔMAP) and in HR (ΔHR) using a non-selective muscarinic receptor antagonist (atropine-sulfate), a β-adrenergic receptor antagonist (propranolol) and a nicotinic receptor antagonist (chlorisondamine) as previously published [23]. The diminution in MAP, observed after the injection of chlorisondamine, was amplified by sPRR infusion indicating that sPRR might compromise the autonomic nervous function likely by increasing

vascular sympathetic activity (Figure 4A). The bradycardic response after propranolol injection (Figure 4B) and the tachycardic response after atropine injection (Figure 4C) were similar in HF-fed male mice infused with sPRR compared to male mice infused with vehicle. Interestingly, sPRR infusion increased plasma leptin levels in obese male mice (Figure 5) suggesting that sPRR effect on sympathetic activity could be partly mediated by leptin.

## Discussion

To our knowledge, no prior studies examined the role of sPRR in blood pressure regulation during diet-induced obesity in male mice. In this present study, we found that sPRR infusion elevated blood pressure in HF-fed male mice but that losartan treatment failed to restore SBP in male mice infused with sPRR. Interestingly, sPRR infusion impaired baroreflex sensitivity and autonomic nervous function potentially through leptin.

Our present study is in agreement with previous studies showing an association between sPRR levels and blood pressure. Indeed, we previously demonstrated that the deletion of adipocyte PRR led to an increase in plasma levels of sPRR, which were positively correlated with the increase in blood pressure in male mice [9]. In addition, reduced PRR gene dosage in nephron progenitors cells resulted in an increase in urinary sPRR levels which were thought to contribute to developmental programming of hypertension in mice [24]. In human, Narita et al., reported that plasma sPRR levels were higher in preeclamptic women than in normotensive pregnant women [25].

The role of the sympathetic nervous system in obesity-related hypertension is well recognized and has been extensively described in several studies [26–29]. The underlying mechanisms involved endothelial dysfunction [30], increased leptin levels [31], decreased adiponectin and ghrelin levels [32,33] and baroreflex dysfunction [34]. In the present study, the impairment of the baroreflex sensitivity observed in male mice infused with sPRR could partly explain the autonomic nervous system dysfunction. In addition, our data demonstrated that, chlorisondamine treatment induced a larger decrease in MAP in mice infused with sPRR than in mice infused with vehicle, suggesting that sPRR could increase vascular sympathetic tone. Since leptin has been reported to cause sympathetic activation leading to an increase in blood pressure [35–37], and since our data showed that sPRR infusion elevated plasma leptin, one could speculate that plasma leptin mediates sPRR effect on sympathetic tone.

Previous studies showed that the activation of PRR in the hypothalamic paraventricular nucleus induced sympathoexcitation in Sprague-Dawley rats [38]. Additionally, PRR knockdown in the brain of human renin-AGT double transgenic hypertensive mice or intracerebroventricular infusion of PRO20 in DOCA salt treated mice improved baroreflex sensitivity and lowered cardiac and vasomotor sympathetic tone [39,40]. However, in HF-fed male mice infused with sPRR, brain full length and sPRR protein contents were not increased indicating that the activation of the sympathetic activity is likely independent of cerebral PRR.

Losartan reduced SBP to the same extent in male mice infused with sPRR compared with male mice infused with vehicle. Moreover, plasma total prorenin/renin, AGT and Ang II levels at equilibrium were not increased in mice infused with sPRR compared with mice infused with vehicle. Together our data suggested that, in HF-fed male mice, sPRR-induced SBP increase is not likely mediated by an AT1R-dependent mechanism. Interestingly, the equilibrium peptide level analysis revealed that sPRR reduced Ang2–10 concentration, an angiotensin peptide known to induce opposite effect to AngII through AT1R and thus promote cardioprotection [41–43]. Therefore, Ang2–10 reduction could also participate to SBP elevation.

A body of evidence suggested sex-specific mechanisms in the development of obesity-associated hypertension [44,45]. Notably, it is recognized that obese males have a higher propensity to exhibit elevated sympathetic nerve activity than obese females [46,47]. In the present study, we demonstrated that sPRR elevated sympathetic activity in HF-fed male mice. In addition, losartan did not blunt the elevation of blood pressure in C57BL/6 male mice infused with sPRR. Similar results from our group were observed in HF-fed adipose PRR KO male mice exhibiting elevated sPRR levels (unpublished data: ΔSBP after losartan injection, WT mice= −7.9 ± 1.5 mmHg; adipose PRR KO mice= −6.4 ± 3.1 mmHg). In contrast, we recently showed that the elevation of blood pressure in HF-fed adipose PRR KO female mice was mediated by an AngII/AT1R-dependent mechanism but not through the autonomic nervous system [18]. Therefore, our data indicated that sex differences in sPRR-induced elevation in blood pressure could exist during the development of obesity. The underlying sex-specific mechanism needs further investigation.

The infusion of sPRR did not induce significant changes in body weight or organ weights. However, the presence of enlarged adipocytes, in line with a trend for an expanded adipose tissue suggested that sPRR plays a role in adipose tissue morphology and remodeling. In agreement with these results, we previously showed that adipose PRR KO significantly decreased adipose tissues weights [9,18]. It would be interesting in future studies to determine whether higher dose of infused sPRR exacerbated those effects.

### Perspectives

We demonstrated that, in obese male mice, sPRR participates in blood pressure regulation primarily through impairment of baroreflex sensitivity and elevation of the sympathetic tone likely mediated by leptin. Because of sexual dimorphism in obesity-hypertension, further investigation is needed to decipher sex-specific mechanism. Future studies should also investigate whether the inhibition of sPRR could prevent obesity-hypertension.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgments

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## Novelty and Significance

**What is new?**

- Demonstration that sPRR is involved in obesity-hypertension in male mice
- Demonstration that sPRR impairs the baroreflex sensitivity and increases the sympathetic tone

**What is relevant?**

- This study demonstrates that sPRR is a contributor to the control of blood pressure in obese male mice
- This study demonstrates that sPRR could represent a new therapeutic target for the treatment of hypertension related to obesity

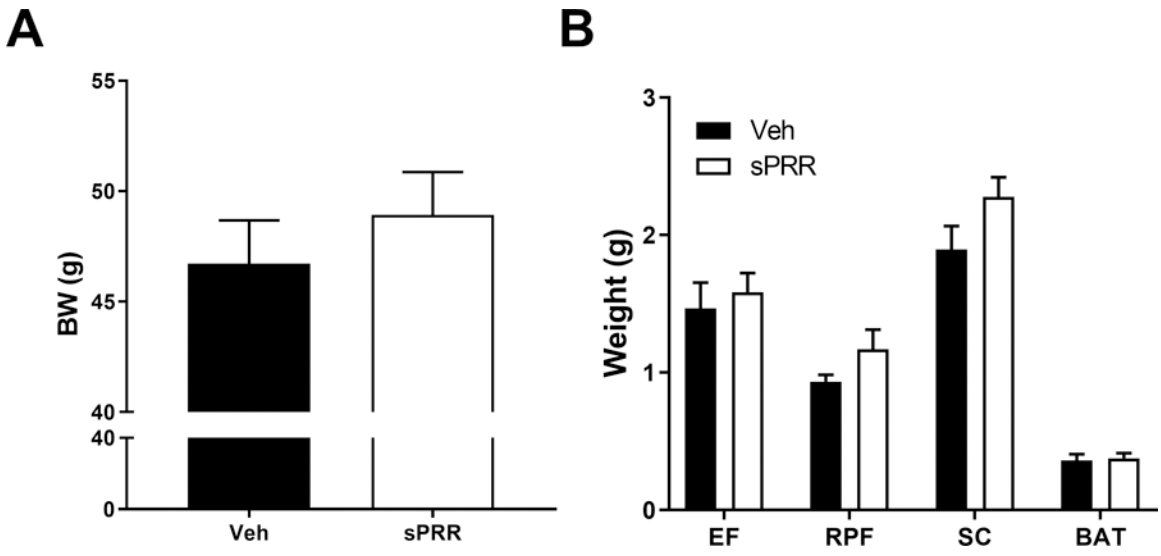


**Figure 1.**

The infusion of sPRR tended to increase adipose depot weight in obese male mice. (A) Body weight of HF-fed C57BL/6 male mice after 4 weeks of infusion with vehicle (Veh, n=7) or sPRR (sPRR, n=7). (B) Weight of epididymal fat (EF), retroperitoneal fat (RPF), subcutaneous fat (SC) and brown adipose tissue (BAT) of mice after 4 weeks of infusion. Values are mean±SEM. One–way ANOVA with post hoc Holm-Sidak multiple comparison was performed to detect differences between groups.

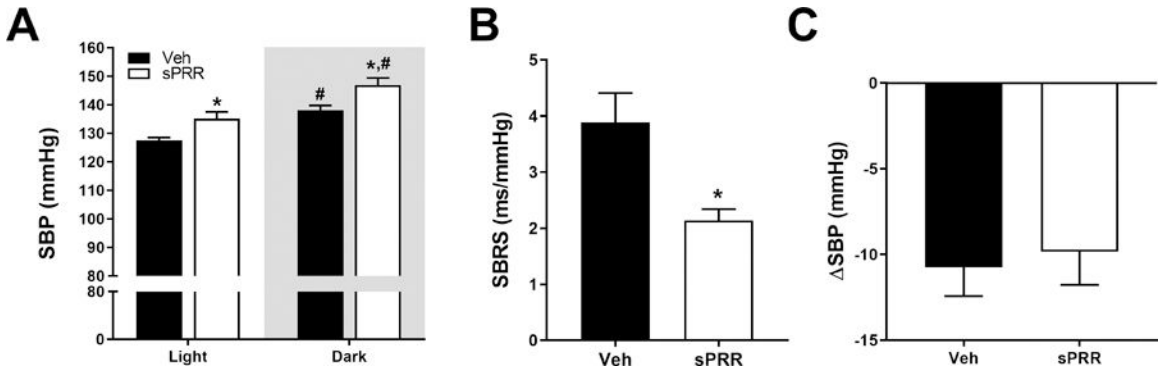


**Figure 2.**

The infusion of sPRR increased systolic blood pressure and impaired baroreflex sensitivity in obese male mice. (A) Systolic blood pressure (SBP) of HF-fed C57BL/6 male mice during light and dark cycles infused with vehicle (Veh, n=5) or sPRR (sPRR, n=6). Differences were revealed using Two–way ANOVA followed by post hoc Holm-Sidak multiple comparison. *P<0.05 compared with Veh, #P<0.05 compared with light SBP. (B) Spontaneous baroreflex sensitivity (SBRS) of HF-fed C57BL/6 male mice infused with vehicle or sPRR. (C) SBP response (ΔSBP) represents the difference in SBP before and after 2 days of losartan injection (20 mg/kg body weight, i.p) in mice infused with vehicle or sPRR. Values are mean±SEM. Differences between groups were revealed using One–way ANOVA followed by post hoc Holm-Sidak multiple comparison. *P<0.05 compared with Veh.

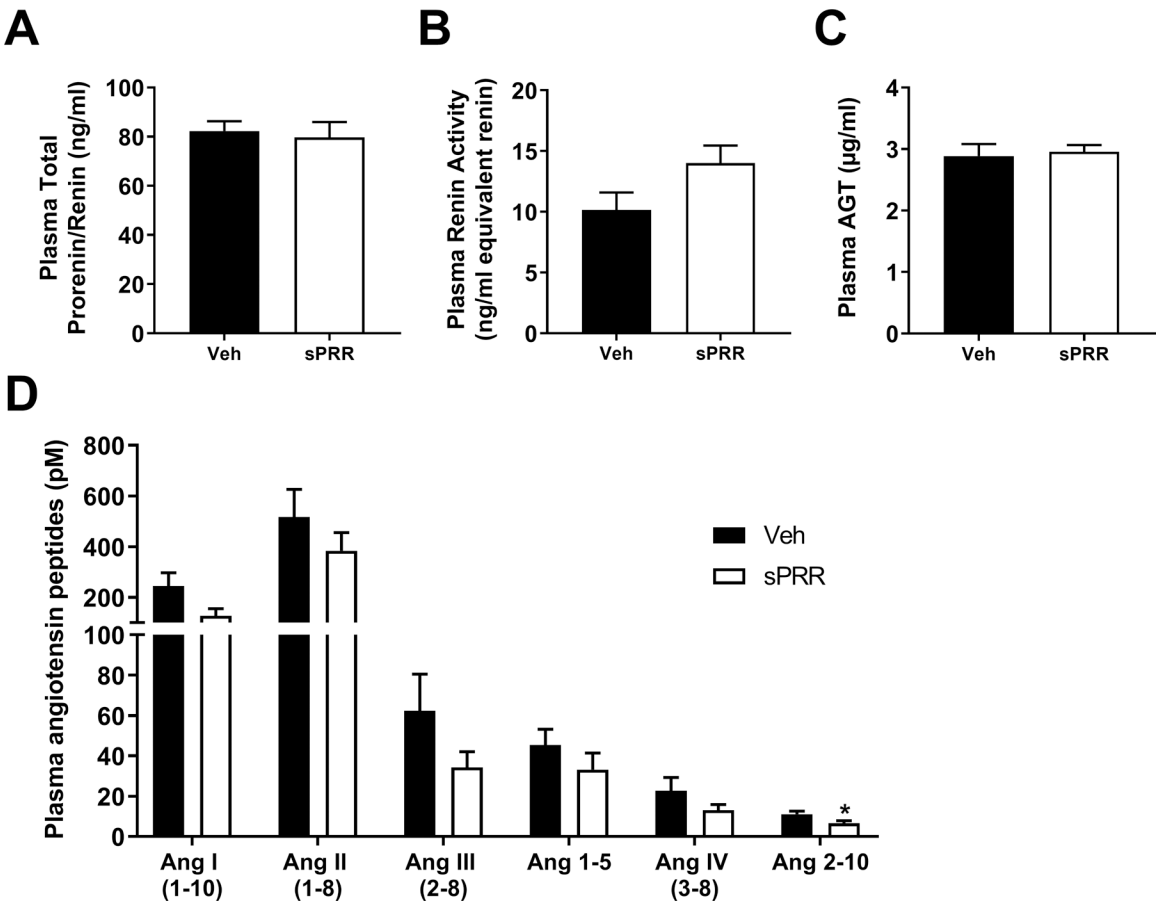


**Figure 3.**

sPRR infusion in obese male mice did not change systemic RAS. (A) Plasma total prorenin/renin in HF-fed C57BL/6 male mice infused for 4 weeks with vehicle (Veh, n=7) or sPRR (sPRR, n=7). (B) Plasma renin activity. (C) Plasma angiotensinogen (AGT). (D) Angiotensin peptides levels at equilibrium. Values are mean±SEM. Differences between groups were revealed using One–way ANOVA followed by post hoc Holm-Sidak multiple comparison. *P<0.05 compared with Veh.

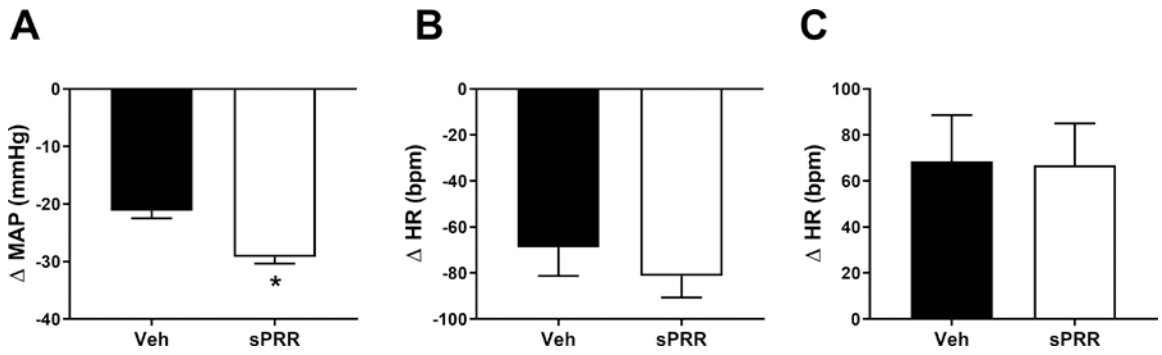


**Figure 4.**

Infusion of sPRR impaired autonomous nervous system in obese male mice. (A) Mean arterial pressure response (ΔMAP) to chlorisondamine injection (1mg/kg body weight, i.p) represents the difference in MAP one hour before and one hour after drug injection in HF-fed C57BL/6 male mice infused with vehicle (Veh, n=5) or sPRR (sPRR, n=6). (B) Heart rate response (ΔHR) to propranolol injection (5mg/kg body weight, i.p) represents the difference in HR one hour before and one hour after drug injection. (C) HR response (ΔHR) to atropine injection (5mg/kg body weight, i.p) represents the difference in HR one hour before and one hour after drug injection. Values are mean±SEM. Differences between groups were revealed using One–way ANOVA followed by post hoc Holm-Sidak multiple comparison. *P<0.05 compared with Veh.

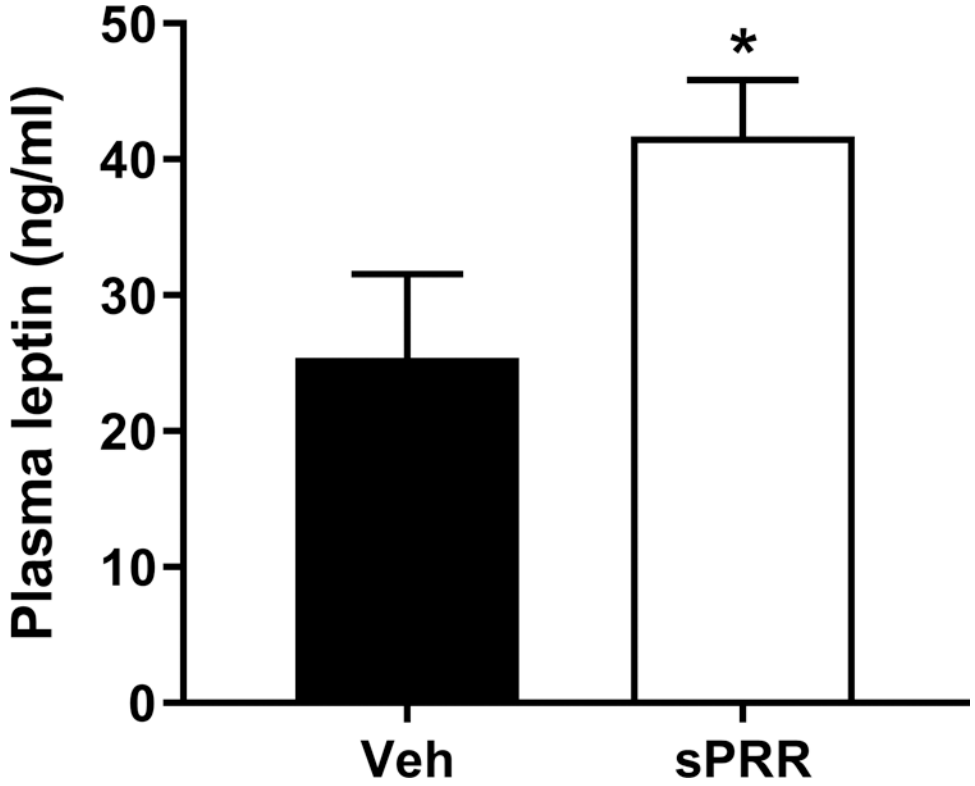


**Figure 5.**

Infusion of sPRR increased plasma leptin levels in obese male mice. Plasma leptin levels in HF-fed C57BL/6 male mice infused for 4 weeks with vehicle (Veh, n=7) or sPRR (sPRR, n=7). Values are mean±SEM. Differences between groups were revealed using One–way ANOVA followed by post hoc Holm-Sidak multiple comparison. *P<0.05 compared with Veh.