Human Soluble Prorenin Receptor Expressed in Adipose Tissue Improves Insulin Sensitivity and Endothelial Function in Obese Female Mice

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ABSTRACT

Increased circulating levels of the soluble prorenin receptor (sPRR), a component of the renin angiotensin system (RAS), plays a role in obesity, glucose, and insulin homeostasis. However, elevated plasma sPRR in diabetic patients has been shown correlated with hyperglycemia in women but not men. Hence, the current study sought to understand the contribution of human sPRR (HsPRR) produced in the adipose tissue (Adi) on adipogenesis, and glucose and insulin balance in obesity settings. Adi-HsPRR mice were generated by breeding human sPRR-Myc-tag transgenic mice with mice expressing Adiponectin/Cre. The mouse model was validated by detecting 28kDa myc-tagged HsPRR by western blotting. Adipose HsPRR expression did not change circulating sPRR in female mice fed a standard chow diet or high fat diet (HFD) but increased plasma sPRR in male Adi-HsPRR mice fed a HFD compared to HFD-fed controls. Yet, Adi-HsPRR improved insulin sensitivity, vascular relaxation and the vasodilator agent Ang 1-7 in obese female mice but not in the male counterparts. Moreover, Adi-HsPRR expression reduced the expression of the adipogenic genes SREBP1C and CD36 only in gonadal white adipose from obese female mice, signifying that adipose tissue-derived HsPRR exerts a sex-specific effect on insulin sensitivity and endothelial function which seems independent of circulating sPRR.

KEYWORDS: Soluble prorenin receptor, insulin sensitivity, renin angiotensin system, vascular function, sex differences

Introduction

The prevalence of obesity is increasing worldwide. According to the World Health Organization (WHO), about 39% of the world's populace is overweight, of which more than 13% are obese (1). In the United States, 2 in 5 adults are obese, accounting for 41.9% of the population (2). Obesity leads to insulin resistance and hyperinsulinemia, leptin resistance, and hyperleptinemia. Obesity also activates the sympathetic nervous system (SNS) the renin angiotensin system (RAS) through the upregulation of angiotensinogen and renin, which are necessary precursors (3-5). As such, RAS activation contributes to the development of obesity-induced diabetes, hypertension and cardiovascular diseases (6)

Prorenin receptor is a component of the RAS that is shown to be involved in adipogenesis and obesity (7). PRR exists in the full-length transmembrane protein, the soluble PRR (sPRR), and the truncated form (8). The full-length PRR can bind to renin and prorenin and participate in the generation of angiotensin (Ang)-I by increasing the catalytic activity of renin and promoting prorenin nonproteolytic activation (9, 10). PRR can also initiate intracellular signals, especially the activation of mitogen activated protein (MAP) kinases through an Ang II-independent pathway (11, 12). Furthermore, PRR can interact with the membrane receptor complex of the Wnt/β-catenin pathway and with the vacuolar-type H-ATPase (13, 14).

PRR is necessary for adipogenesis; hence the knockdown of PRR in the adipose tissue leads to lipodystrophy in mice (15, 16). In humans, obesity is known to increase adipose tissue PRR levels (16, 17). The functional role of PRR in adipogenesis is due to its role in Ang II generation, extracellular signal-regulated kinase (ERK1/2) activation and

regulation of the peroxisome proliferator-activated receptor gamma ($PPAR_{\gamma}$), known as the master regulator of adipogenesis (15, 18). The loss of PRR in adipose tissue reduces peroxisome proliferator activated receptor gamma ($PPAR_{\gamma}$) expression (15) and $PPAR_{\gamma}$ knockdown decreases adipose PRR expression (19). Studies have demonstrated that PRR regulates metabolic homeostasis. In the pancreas, the knockdown of PRR in β-cells resulted in diabetes by reducing insulin secretion and impairing glucose sensitivity (20). In the adipose tissue, PRR knockdown improved fasting glucose and glucose sensitivity in male and female mice fed a high fat diet (16). Together, these studies show that optimal PRR expression is needed for a normal adipogenesis.

However, the role of sPRR in obesity, glucose and insulin homeostasis is still obscure. It has been shown that exacerbated sPRR expression secondary to obesity induce pathophysiological signaling in metabolic tissues leading to glucose intolerance and insulin resistance. For instance, in a mouse model of whole-body loss of sPRR, body weight was reduced in male mice (21) and infusion of sPRR increased body weight in male mice fed a HFD (22). Furthermore, HFD-induced obesity in male mice, elevated plasma sPRR leading to hyperglycemia, hyperinsulinemia and glucose intolerance, characteristic of type 2 diabetes (T2D) (23). Yet, in another model of HFD-induced obesity, sPRR infusion in male mice reduced body weight and improved glucose and insulin sensitivity by increasing adipose glucose transporter type 4 (Glut 4) and the phosphorylation of proteins kinase B (Akt) (19). sPRR has also been demonstrated to mediate blood pressure regulation and endothelial function. Infusion of sPRR in high fat diet-fed male mice increased blood pressure (BP) (22, 24) and induced endothelial dysfunction in male mice via the activation of angiotensin II type 1 (AT1) receptor (24).

In humans, obesity is known to elevate circulating sPRR (25) whereas weight loss after bariatric surgery reduced circulating sPRR (25). Additionally, plasma sPRR is elevated in women with type 2 diabetes (26) and in hypertensive patients on RAS blockers suggesting that elevated circulating sPRR in humans is associated with obesity and BP regulation, but little is known about its role in sex-specific protective or detrimental effects on cardiometabolic function during obesity.

To address these questions, we developed a humanized mouse model that specifically expresses human sPRR (HsPRR) in the adipose tissue (Adi-HsPRR). Male and female Adi-HsPRR mice were kept on low and high fat diets to assess the contribution of Adi-HsPRR on adipogenesis, glucose and insulin balance. In addition, we assessed Adi-HsPRR's effect on blood pressure and endothelial function.

Methods

The original data that support the findings of this study are available from the corresponding author upon reasonable request.

Transgenic mouse model

All animal studies described below complied with the University of Kentucky Institutional Animal Care and Use Committee (Protocol No. 2022-4059) and followed the "Guide for the Care and Use of Laboratory Animals" issued by the National Research Council.

Mice were on a 14:10 hours, light: dark cycle, starting at 7:00 am for the light cycle and at 9:00 pm for the dark cycle. Mice had *ad libitum* access to water and diet.

Human sPRR-Myc-tag transgenic mice were developed by cloning myc epitope-tagged sPRR into the CAG-GFP vector (27). The fragment was sequenced to the confirm

accuracy of the construct and transfected into HEK293 cells stably expressing Cre recombinase and screened with a myc monoclonal antibody (clone Vli01, MaineHealth Institute for Research, Scarborough, ME) to verify the expression of the fragment in the media. Upon validation, the construct was injected into oocytes of C57BL/6J mice to generate male and female Human sPRR-Myc-tagged transgenic mice.

Adi-HsPRR male and female mice were generated by breeding heterozygote female human sPRR-Myc-tag transgenic mice with male mice expressing Cre recombinase under the control of the Adiponectin promoter (B6; FVB-Tg (AdipoCre)1Evdr/J, Jax No. 010803, The Jackson Laboratory, Figure 1A).

Experimental Design

Adi-HsPRR and control littermates (CTL) male and female mice (Males= 8-16/group; Females=8-14/group) were weaned and kept on regular chow diet for 8 weeks (18% protein, Teklad Global Diet, ENVIGO Harlan, Madison, WI). Then, a subset of mice was switched to either a low-fat diet (10% kcal from fat; D12450J, Research Diets, Inc. USA) or a high-fat diet (60% kcal from fat; D12492, Research Diets, Inc. USA) for 20 weeks. Body weight was recorded weekly and body composition (EchoMRI) was assessed monthly. The intraperitoneal glucose tolerance test (ipGTT) was performed at 12 weeks and insulin tolerance test (ITT) was performed at 14 weeks.

At 16 weeks, mice were housed individually and implanted in the left carotid artery with a catheter connected to a telemetry transmitter (PA-C10 model; DSI; Saint Paul, MN). Surgery was performed under isoflurane anesthesia and mice were treated with meloxicam for 2 days (4mg/kg daily, subcutaneous injection). One week after recovery, systolic blood pressure (SBP), mean arterial pressure (MAP), diastolic blood pressure

(DBP) and heart rate (HR) were measured for 5 consecutive days using the DSI Ponemah software. At the end of the study, tissues and plasma were collected for biochemical analysis and vascular studies.

Intraperitoneal Glucose and Insulin Tolerance Test

Intraperitoneal glucose tolerance test (ipGTT) was performed on mice after a 6-h fast and intraperitoneal insulin tolerance test (ITT) was performed after a 3-h fast. Blood samples were drawn at different times following glucose (1mg/kg, i.p) and insulin injection (i.p). Insulin dose is as follows, LFD males: 0.75 U/kg; LFD females: 0.4 U/kg; HFD males: 1 U/kg; HFD females: 0.9 U/kg. Blood glucose concentrations were measured using a glucometer (Accu-Chek Aviva Plus, Roche Diagnostics).

Vascular Reactivity

Mouse thoracic aorta was isolated, cleaned from perivascular adipose tissue. Rings were cut into 4 concentric rings (~3 mm each), mounted on pins for wire myography (Danish Myo Technology A/S, Aarhus, Denmark) and tested for viability; preconstricted with serotonin (SE, 1×10^{-4} M) and relaxed with acetylcholine (ACh, 1×10^{-3} M). Cumulative concentration-response (CCR) curves to potassium chloride (KCl, 4.7 mM to 100 mM) were utilized to assess constriction. Endothelial-dependent relaxation was assessed, aortic segments were pre-constricted with SE ($1x10^{-4}$ M, 5 ul), and CCR curves to ACh (1×10^{-9} to 1×10^{-5} M) were performed. Following recovery, vasorelaxation to the endothelium-independent vasodilator sodium nitroprusside (SNP; 1×10^{-10} M to 1×10^{-5} M) was assessed in the same aortic segments. Vasorelaxation data are presented as percent relaxation from SE-induced constriction. For all CCR curves, maximum response (Emax) was calculated using Prism Software (GraphPad, San Diego, CA).

Immunostaining

Gonadal white adipose tissue (gWAT) was fixed in paraformaldehyde and embedded in paraffin blocks. Sections were stained with hematoxylin and eosin and examined with a Nikon Eclipse 80i light microscope. All sections were photographed and quantified using NIS Elements BR.3.10 software to determine cell size and count.

Quantification of Plasma Parameters

Plasma sPRR was determined by soluble (Pro)renin Receptor ELISA (IBL, Minneapolis, MN). Plasma renin was determined by Mouse Renin ELISA (IBL, Minneapolis, MN). Plasma RAS levels were assessed using LC-MS/MS at the University of Kentucky RAAS Analytical Laboratory as previously described (28).

Immunoblotting

Protein from frozen retroperitoneal adipose tissue was extracted and 40µg of protein was run on precast polyacrylamide gel as previously described (29) and then transferred to a polyvinylidene difluoride membrane via a semi-wet transfer equipment (Trans-Blot Turbo Transfer System; Bio-Rad Laboratories, Hercules, CA) as recommended by the manufacturer. Membranes were blocked in 5% nonfat dried milk in Tris-buffered saline with 0.1% Tween 20, membranes were incubated with C-myc antibody (MaineHealth Institute for Research, clone Vli01), pAKT (Cell Signaling, #9271), AKT (Cell Signaling, #9272) and HSP90 (Cell signaling, #4874) in 5% nonfat dried milk in Tris-buffered saline with 0.1% Tween 20. After incubation with handle region decoy peptide-conjugated antirabbit secondary antibody (Jackson ImmunoResearch Laboratories, AB2313567), proteins were imaged using ChemiDoc Imaging System (Bio-Rad Laboratories, Hercules, CA). The levels of proteins were quantified using Image Lab software (Bio-Rad Laboratories, Hercules, CA) and normalized to GAPDH.

Tissue RNA Extraction and Quantitative RT-PCR

RNA from gonadal white adipose tissue was extracted with the SV Total RNA Isolation System (Promega, Madison, WI). RNA concentration was measured by a NanoDrop (Thermo-Fisher Scientific, Madison, WI), and cDNA was prepared from 500ng of RNA using Qscript cDNA SuperMix (Quanta Biosciences, Gaithersburg, MD). mRNA expression of genes (Supplemental Table 1) was evaluated by quantitative RT-PCR using PerfeCTa SYBR Green FastMix (Quanta Biosciences) and normalized to 18S.

Statistical Analysis

Data are represented as means±SEM. Statistical analysis was performed using Graph Prism. Statistical differences between groups were assessed by a two-way ANOVA followed by Tukey post hoc analysis for multiple comparisons. Grubbs test (GraphPad QuickCalcs) was used to determine statistical outliers. Values of $P<0.05$ were considered statistically significant.

Results

Characterization of Adi-HsPRR mouse model

Figure 1A shows the breeding strategy to generate Adi-HsPRR mice. To confirm the expression of human myc-tagged sPRR in the adipose tissue of mice, western blot analysis of c-myc was performed. The protein expression of myc-tagged HsPRR was detected at 28 KDa in perirenal, gonadal and subcutaneous adipose tissue of male and female Adi-HsPRR mice (Figure 1B) and was absent in control male and female littermate mice. Expression of sPRR in the adipose tissue (perirenal) was increased in male and female Adi-HsPRR mice (Figure 2A-B). However, body weight, fat and lean mass were similar between CTL and Adi-HsPRR mice on a low or high-fat diet (Supp Table 1 and 2).

In addition, no differences were observed in fat weights nor adipose tissue morphology (Figure 2C-F). Further, plasma leptin was also similar between groups (Supp Figure 1A-B).

Adi-HsPRR modulates circulating RAS in mice fed an HFD.

In males, Adi-HsPRR increased further plasma sPRR in response to a HFD (Figure 3A); however, the increase in plasma sPRR in female littermates was similar compared to control mice (Figure 3B).

Plasma renin concentration (PRC) was reduced similarly between genotype and sex in response to HFD (Supp Figure 2A-B). Similarly, RAS components in mice fed a HFD were similar between groups, including plasma renin activity, plasma aldosterone and angiotensin peptides: Ang II (1-8), Ang I (1-10), Ang III (2-8), Ang 1-5, Ang IV (3-8), Ang 1-9, Ang 2-10 were similar between HFD groups (Figure 3C-D). However, expression of HsPRR in adipose tissue reduced Ang 1-7 in Adi-HsPRR males compared to CTL whereas levels were increased in Adi-HsPRR female mice.

Adi-HsPRR improves insulin sensitivity in female mice fed a HFD.

Fasting blood glucose (3 and 6 h) was similar between CTL and Adi-HsPRR mice on an LFD or HFD (Supp Figure 3A-D). Furthermore, when challenged with glucose, time-dependent glucose clearance remained similar between groups on LFD or HFD (Figure 5A-B, Supp Figure 4A-B). Glucose utilization upon insulin challenge was similar in male CTL and HsPRR mice fed a HFD (Figure 4C, Supp Figure 4C). However, female Adi-HsPRR mice fed an HFD displayed improved blood glucose utilization compared to HFD CTL (Figure 4D, Supp Figure 4D), which suggest better insulin sensitivity in HFD Adi-HsPRR female mice.

Adi-HsPRR improves endothelial function in female Adi-HsPRR mice fed an HFD.

Endothelium-dependent relaxation with acetylcholine (ACh) of the thoracic aorta was similarly impaired in male CTL and Adi-HsPRR fed a HFD (Figure 5A). However, female Adi-HsPRR counterparts fed a HFD had better endothelium-dependent vascular relaxation compared to CTLs (Figure 5B). Endothelium-independent relaxation with sodium nitroprusside (SNP) was similar between genotypes in male and female mice (Figure 5A-B). In addition, maximal potassium chloride (KCl) was similar between groups in males and females (Supplementary Figure 5A-B).

Obesity-induced hypertension was present in both male and female mice regardless of the genotype (Figure 5B-C).

Adi-HsPRR in HFD females decreases transcription factors important for lipid synthesis and shuttling.

Lastly, we assessed mRNA expression of transcription factors and peptides that are important for glucose and lipid metabolism, adipogenesis, thermogenesis, beigeing and inflammation in gonadal WAT in mice fed a HFD. mRNA expression of $PPAR_{\gamma}$, phosphoglycerate kinase 1 (PGK1), protein kinase A (PKA), uncoupling protein (UCP) 1 and 2, PR domain containing 16 (PRDM16), CCAAT enhancer binding protein beta (CEBP/β), and peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1α) were similar between groups in male and female mice (Figure 6A-B). Also, genes involved in lipid metabolism, scavenger receptor class B member 3 (CD36), sterol regulatory element-binding transcription factor 1C (SREBP1C), fatty acid binding protein 4 (FABP4), fatty acid synthase (FAS), hormone-sensitive lipase (HSL), adipose

triglyceride lipase (AGTL) and perilipin were similar between groups in males (Figure 6A). However, Adi-HsPRR female mice had a significant decrease in CD36 and SREBP1c compared to CTL (Figure 6B).

Discussion

In the present study, we show for the first time the biological effects of human sPRR expressed in adipose tissue on circulating sPRR, adipogenesis and obesity, glucose and insulin homeostasis, blood pressure and endothelial function in mice fed a low or high-fat diet. Adipose HsPRR expression increased tissue sPRR expression but did not change body weight, adipose tissue morphology or plasma leptin in mice on both low and high fat-diets. Circulating sPRR was similar between groups on a low or high-fat diet but male Adi-HsPRR mice showed an increase in circulating sPRR on a HFD. Yet, Adi-HsPRR improved insulin sensitivity and vascular relaxation in HFD Adi-HsPRR female mice but not males. Further analysis showed that Adi-HsPRR increased vasodilator agent, Ang 1-7 in HFD females, which was decreased in males. Moreover, gene expression of CD36 and SREBP1C were reduced in gonadal white adipose of HFD females, which was unchanged in males. Taken together, these findings suggest that HsPRR in adipose tissue exerts a sex-specific effect on insulin balance and endothelial function which are independent of circulating sPRR.

The adipose tissue and liver have been previously demonstrated to be important sources of circulating sPRR (29, 30). In clinical studies, plasma sPRR is elevated in obese subjects (25, 31), and studies in mice show elevated plasma sPRR in obese male but not female mice (23). Likewise, in our study, obese Adi-HsPRR male but not female mice

displayed increased plasma sPRR signifying that sPRR in adipose tissue may affect circulating sPRR levels in males but not females.

Plasma sPRR has been associated with changes in visceral fat area after weight loss in bariatric surgery subjects (31). In obese male mice, infusion of sPRR was shown to reduce gonadal white adipose tissue (19). However, expression of human sPRR in the adipose tissue did not change body weight or body composition between CTL and Adi-HsPRR mice on a low or high-fat diet, nor did it affect adipose tissue morphology even though plasma sPRR was elevated in HFD Adi-HsPRR male mice. Reasons for this discrepancy could be the length of HFD feeding (20 weeks in our study vs 32 weeks used in other studies), method of elevated plasma sPRR generation (increased tissue derived vs infusion) or species differences (mouse vs human sPRR). Nevertheless, our findings indicate that other factors such as PRR and $PPAR_{\gamma}$ may play a bigger role in adipogenesis and obesity than sPRR. Indeed, in a lipodystrophy model, induced by knockdown of PRR in adipose tissue, plasma sPRR was elevated (15). Moreover, infusion of sPRR in $PPAR_{\gamma}$ KO mice, another model of lipodystrophy, did not affect body weight (19).

Clinical studies have shown that plasma sPRR is elevated and associated with hyperglycemia in women but not men with type 2 diabetes (26). Moreover, increased circulating sPRR is associated with gestational diabetes (32). However, none of these studies showed a direct mechanistic link between elevated plasma sPRR and glucose and insulin balance, drawing the question of whether elevated plasma sPRR is solely a biomarker in these conditions. Interestingly, we found that HFD Adi-HsPRR females, which show increased tissue but not plasma sPRR levels compared to CTL have improved responses to insulin. Meanwhile, HFD Adi-HsPRR males which have increased

plasma sPRR levels did not show any change in glucose or insulin metabolism. This data suggests that the influence of circulating sPRR on glucose and insulin metabolism could be sex and location (local vs circulating) dependent.

Clinical studies do not show an association between plasma sPRR and glucose metabolism in men (26). Still studies in male mice show that plasma sPRR could be implicated in glucose and insulin homeostasis. In obese male mice with hyperglycemia and hyperinsulinemia, plasma sPRR was elevated (23). However, exogenous addition of sPRR has been demonstrated to promote adipose tissue beigeing in male mice (33) and improve glucose and insulin balance in morbidly obese male mice (19). In the present study, we found that human sPRR expression in adipose tissue did not affect glucose and insulin metabolism irrespective of circulating sPRR levels, suggesting that plasma human sPRR in males may not play a role in glucose and insulin homeostasis, similar to reported findings in human studies (26). The discrepancies in findings between human and mouse sPRR may be due to specie differences. Although the sPRR gene has more than 90% identity between humans and mice, slight differences could lead to variations in signaling and response.

Adi-HsPRR also reduced SREBP1c and CD36 gene expression and increased vasodilator agent Ang 1-7 in female mice. Ang 1-7, produced from the cleavage of Ang II by ACE2 and the cleavage of Ang I by neprilysin (NEP) upon binding to Mas receptor induces vasodilatation, antihypertrophic, antiinflammation, anti-remodeling and antiapoptotic effects (34). Recent evidence shows that Ang 1-7 improves metabolic function and its levels could be a marker of metabolic syndrome (35). Increased Ang 1-7 levels have been shown to improve skeletal muscle insulin sensitivity (36) and protect

against streptozotocin-induced diabetes mellitus in rats through improvements in pancreatic β cell survival, insulin secretion and utilization (37). Moreover, as a potent vasodilator antihypertrophic, antiinflammation and anti-remodeling agent, Ang 1-7 has been shown to improve endothelial function. Ang 1-7 decreases Ang II-induced matrix metalloproteinase 8 (MMP-8) expression which drives atherosclerosis (38). In humans, an imbalance between Ang II and Ang 1-7 has been associated with endothelial dysfunction and inflammation in diabetics (39). Hence, the increase in circulating Ang 1-7 in female Adi-HsPRR mice could be a primary pathway or mechanism for improved insulin response and endothelial function in Adi-HsPRR female mice. Notably, male Adi-HsPRR mice, which show decreased Ang 1-7 levels, do not have an improvement in insulin response and endothelial function. Future studies will investigate the mechanism of HsPRR-Ang 1-7 mediated insulin signaling and vascular functioning.

The role of SREBP1c in lipid metabolism and insulin signaling has been well reported (40, 41). Increased expression of SREBP1c in the adipose tissue of mice has been shown to lead to insulin resistance and diabetes (42). In humans, hyperinsulinemia increased SREBP1c gene expression (43). Similarly, our findings show that insulin sensitivity induced by HsPRR expression in the adipose tissue decreased SREBP1c expression. SREBP1c is downstream of insulin signaling (44), and hence, its presence is stimulated by either the insulin receptor-Akt pathway or the insulin receptor-PKCλ pathway. The exact mechanism of SREBP1c reduction in Adi-HsPRR female mice would be the focus of future studies.

CD36 is an important factor in fatty acid uptake. Increased expression of CD36 in the adipose tissue has been linked to insulin resistance in *in vitro (45)* and humans (46, 47).

Likewise, Adi-HsPRR in females decreased CD36 expression in gonadal WAT which is linked to the improved insulin response observed.

Our findings provide insights into the potential interaction between adipose-derived human sPRR and RAS signaling that affects insulin signaling and vascular function in a sex-specific manner. Thus, our model could be a useful tool to investigating the potential antidiabetic effects of adipose sPRR in obese women.

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Disclaimers and Disclosures

None

Supplemental Materials

Tables 1-2

S1-S5

Figures and Figure Legends

Figure 1 Generation and validation of adipose-specific human sPRR (Adi-HsPRR) mouse model. Adipose tissue-derived human sPRR male and female mice were generated by breeding heterozygote male mice that expressed Cre recombinase under the control of the Adiponectin promoter with female heterozygote mice that express the human soluble prorenin receptor (HsPRR/+) (Figure 1A). Expression of Human sPRR was validated by the detection of myc-tagged HsPRR in adipose tissue (peri-renal, gonadal, and subcutaneous) (Figure 1B).

Figure 2 Effect of Adi-HsPRR expression of Adipose Tissue

Tissue sPRR expression assessed via western blot in (A) Male and (B) Female mice. Data are mean ± SEM of 7-1 mice/group. A 2-way ANOVA was performed to detect differences. Tissue morphology of gonadal white adipose tissue was analyzed and quantified in (C) Male HFD and (D) Female HFD mice using Nikon NIS Elements BR.3.10 software at 10X (25µm). Data are mean ± SEM of 5-15 mice/group. A t-test was

performed to detect differences. Fat pads at takedown in (E) Male HFD and (F) Female HFD mice. Data are mean ± SEM of 8-16 mice/group. A t-test was performed to detect differences.

Figure 3 Adi-HsPRRR expression modulates circulating RAS

Plasma sPRR in (A) Male and (B) Female mice. Data are mean ± SEM of 7-17 mice/group. A 2-way ANOVA was performed to detect differences. Plasma RAS in (C) Male HFD and (D) Female HFD mice. Data are mean ± SEM of 4 mice/group. A t-test was performed to detect differences.

Figure 4 Adi-HsPRR improves insulin sensitivity in HFD females.

AUC for time-dependent glycemia after glucose injection in (A) Male and (B) Female mice. Data are mean ± SEM of 9-14 mice/group. AUC for time dependent glycemia after insulin injection in (C) Male and (D) Female mice. Data are mean ± SEM of 6-13 mice/group. A 2-way ANOVA was performed to detect differences.

Figure 5 Adi-HsPRR improves endothelium-dependent vascular relaxation in HFD females.

Vascular relaxation in (A) Male and (B) Female mice. Data are mean ± SEM of 5 mice/group. A 2-way repeat ANOVA was performed to detect differences. Day and night systolic blood pressure in (A) Male and (B) Female mice. Data are mean ± SEM of 4-9 mice/group. A 3-way ANOVA was performed to detect differences.

Figure 6 Adi-HsPRR decreases transcription factors for lipid synthesis and trafficking in HFD females.

Gene expression analysis was performed in gonadal WAT of (A) Male HFD and (B) Female HFD mice. Data are mean ± SEM of 8-15 mice/group. A t-test was performed to detect differences.

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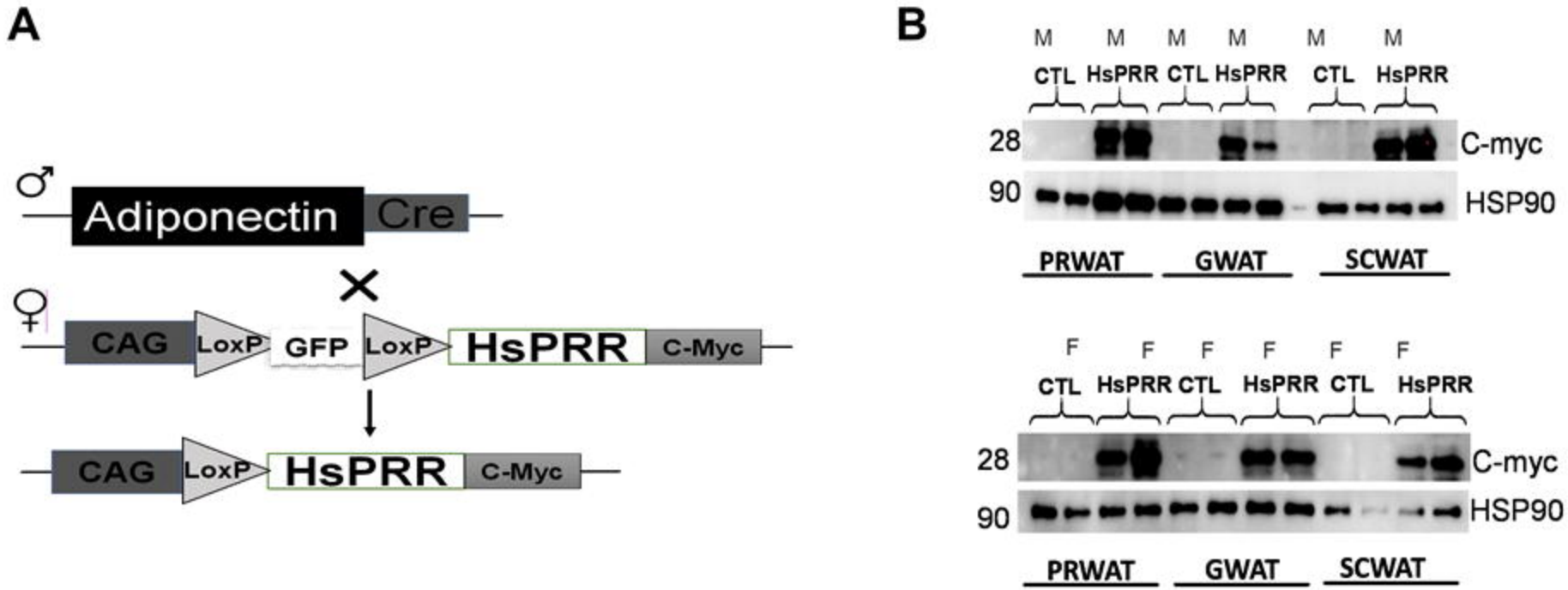
Figure 1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♂
Adiponectin
Cre
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♀
CAG
LoxP
GFP
LoxP
HsPRR
C-Myc
CAG
LoxP
HsPRR
C-Myc
B
M M M M M M
CTL HsPRR CTL HsPRR CTL HsPRR
28
90
C-myc
HSP90
PRWAT
GWAT
SCWAT
F F F F F F
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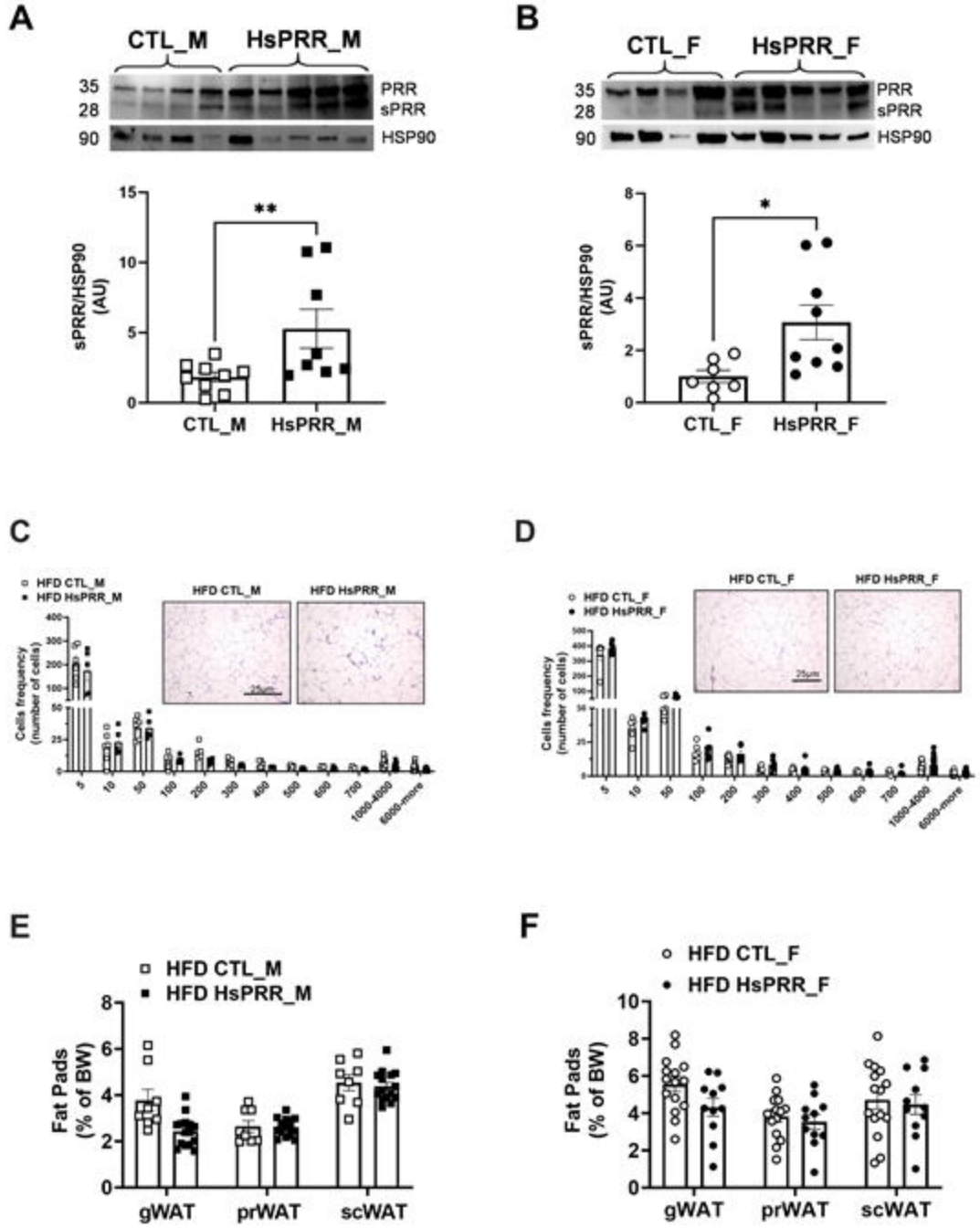
28
90
C-myc
HSP90
PRWAT
GWAT
SCWAT

Figure 2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CTL_M
HsPRR_M
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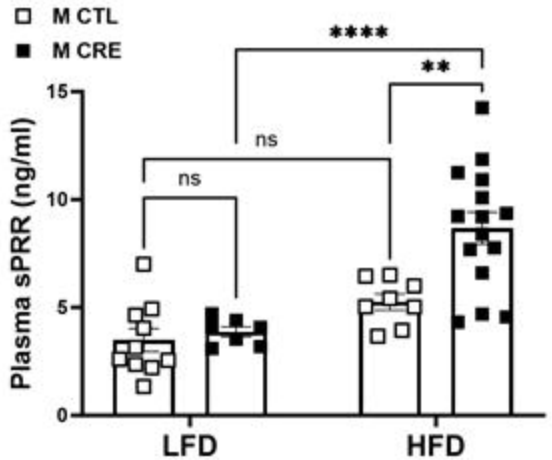
90
PRR
sPRR
HSP90
sPRR/HSP90 (AU)
**
CTL_M
HsPRR_M
B
CTL_F
HsPRR_F
35
28
90
PRR
sPRR
HSP90
sPRR/HSP90 (AU)
*
CTL_F
HsPRR_F
C
HFD CTL_M
HFD HsPRR_M
Cells frequency (number of cells)
D
HFD CTL_F
HFD HsPRR_F
Cells frequency (number of cells)
E
HFD CTL_M
HFD HsPRR_M
Fat Pads (% of BW)
gWAT
prWAT
scWAT
F
HFD CTL_F
HFD HsPRR_F
Fat Pads (% of BW)
gWAT
prWAT
scWAT

Figure 3

A



Source of Variation	% of total variation	P value
Interaction	5.968	0.0299
Row Factor	27.98	<0.0001
Column Factor	9.385	0.0075

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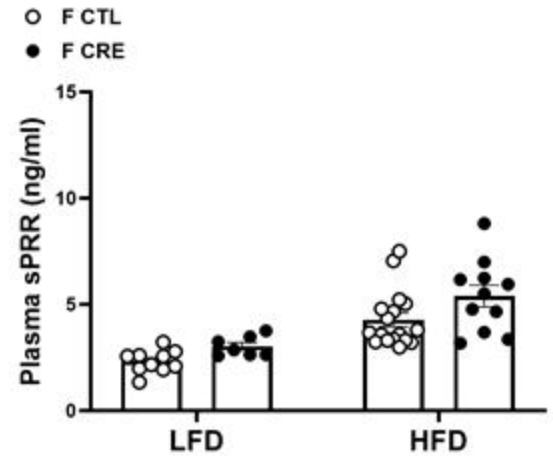

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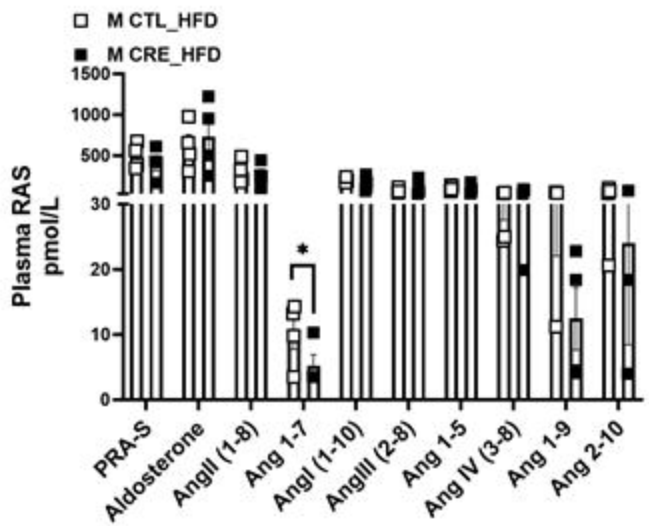
Interaction	0.3720	0.5897
Row Factor	40.23	<0.0001
Column Factor	7.176	0.0217

C



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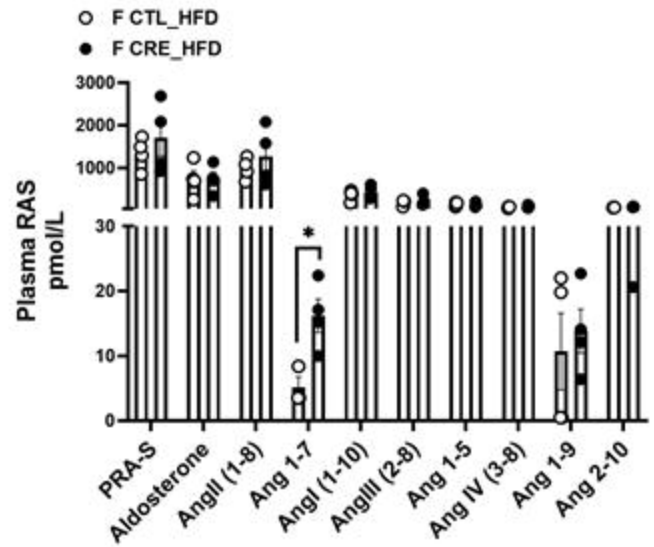
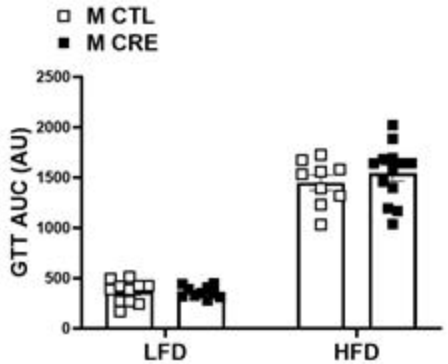


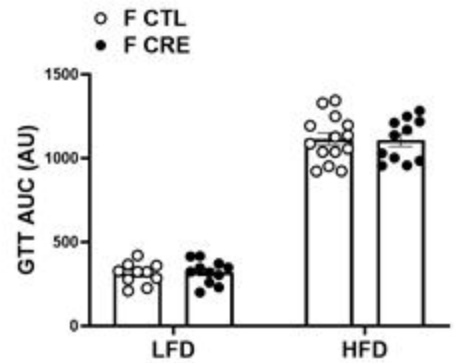
Figure 4

A



Source of Variation	% of total variation	P value
Interaction	0.1526	0.4400
Row Factor	87.05	<0.0001
Column Factor	0.1414	0.4571

B



Source of Variation	% of total variation	P value
Interaction	0.007445	0.8253
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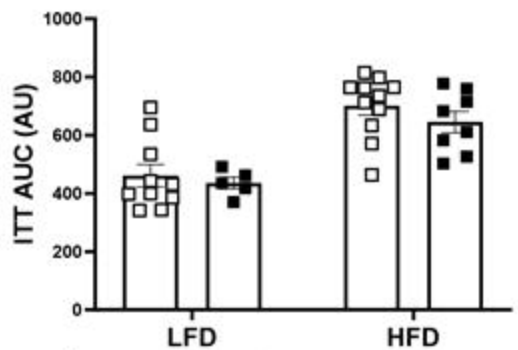
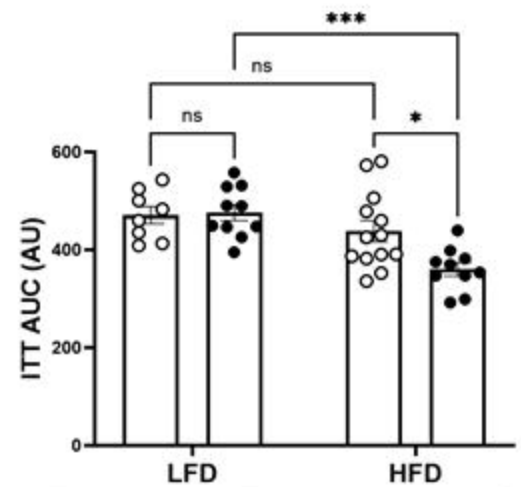
Column Factor	0.0005743	0.9511

C



Source of Variation	% of total variation	P value
Interaction	0.2398	0.6854
Row Factor	50.63	<0.0001
Column Factor	1.659	0.2906

D



Source of Variation	% of total variation	P value
Interaction	7.904	0.0357
Row Factor	25.50	0.0004
Column Factor	6.020	0.0649

Figure 5

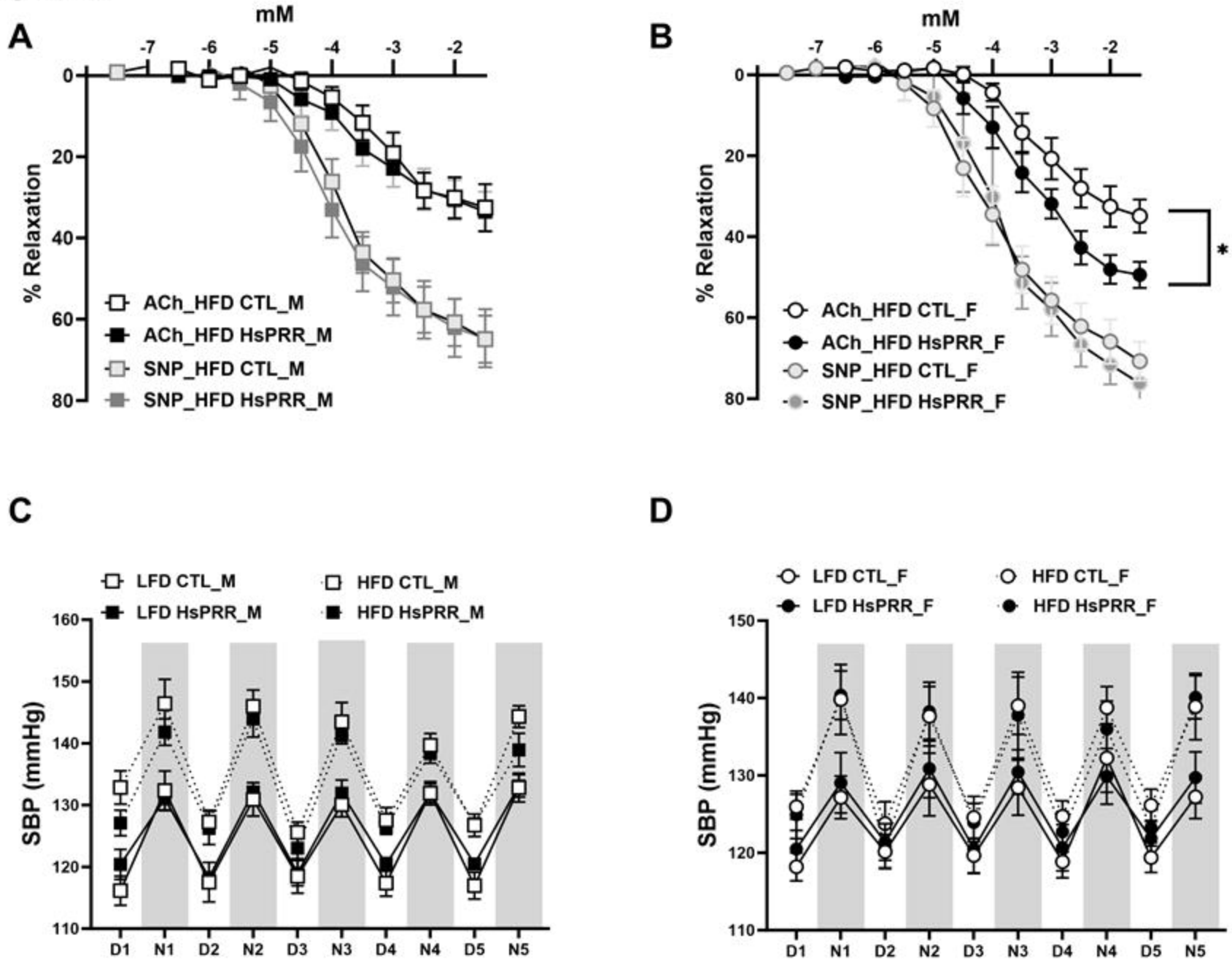
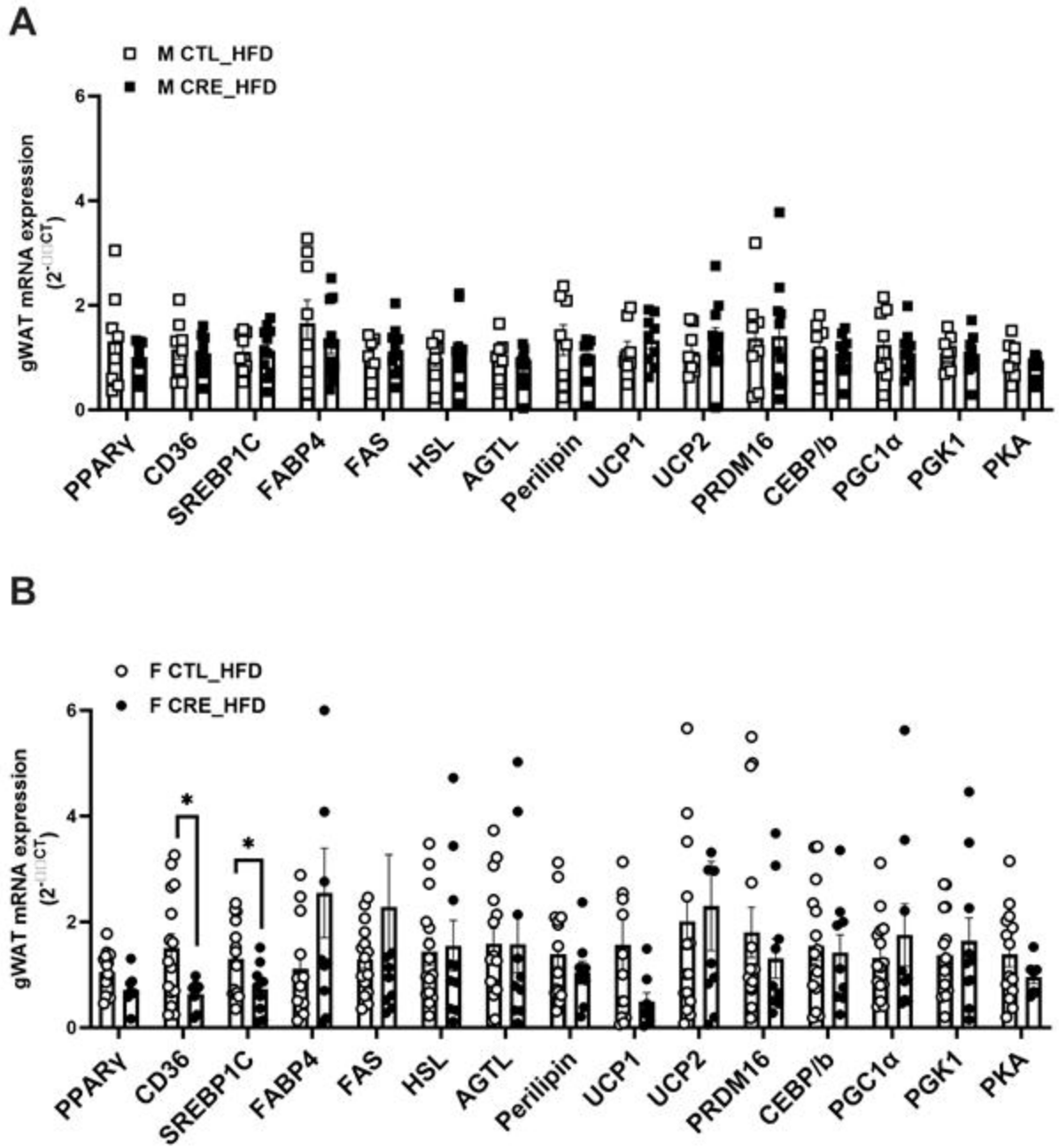

A
mM
-7
-6
-5
-4
-3
-2
0
20
40
60
80
% Relaxation
ACh_HFD CTL_M
ACh_HFD HsPRR_M
SNP_HFD CTL_M
SNP_HFD HsPRR_M
B
mM
-7
-6
-5
-4
-3
-2
0
20
40
60
80
% Relaxation
ACh_HFD CTL_F
ACh_HFD HsPRR_F
SNP_HFD CTL_F
SNP_HFD HsPRR_F
*
C
LFD CTL_M
HFD CTL_M
LFD HsPRR_M
HFD HsPRR_M
160
150
140
130
120
110
SBP (mmHg)
D1
N1
D2
N2
D3
N3
D4
N4
D5
N5
D
LFD CTL_F
HFD CTL_F
LFD HsPRR_F
HFD HsPRR_F
150
140
130
120
110
SBP (mmHg)
D1
N1
D2
N2
D3
N3
D4
N4
D5
N5

Figure 6


A
M CTL_HFD
M CRE_HFD
gWAT mRNA expression
0
2
4
6
PPARγ
CD36
SREBP1C
FABP4
FAS
HSL
AGTL
Perilipin
UCP1
UCP2
PRDM16
CEBP/b
PGC1α
PGK1
PKA
B
F CTL_HFD
F CRE_HFD
gWAT mRNA expression
0
2
4
6
*
*
PPARγ
CD36
SREBP1C
FABP4
FAS
HSL
AGTL
Perilipin
UCP1
UCP2
PRDM16
CEBP/b
PGC1α
PGK1
PKA