Inhibitors of microsomal prostaglandin E synthase 1 (mPGES-1) are in the early phase of clinical development. Deletion of mPges-1 in mice confers analgesia, restrains atherogenesis, and fails to accelerate thrombogenesis, while suppressing prostaglandin E$_2$ (PGE$_2$), but increasing the biosynthesis of prostacyclin (PGI$_2$). In low-density lipoprotein receptor–deficient (Ldlr$^{-/-}$) mice, this last effect represents the dominant mechanism by which mPges-1 deletion restrains thrombogenesis, while suppression of PGE$_2$ accounts for its antiatherogenic effect. However, the effect of mPges-1 depletion on blood pressure (BP) in this setting remains unknown. Here, we show that mPges-1 depletion significantly increased the BP response to salt loading in male Ldlr$^{-/-}$ mice, whereas, despite the direct vasodilator properties of PGI$_2$, deletion of the I prostanoid receptor (Ipr) suppressed this response. Furthermore, combined deletion of the Ipr abrogated the exaggerated BP response in male mPges-1$^{-/-}$ mice. Interestingly, these unexpected BP phenotypes were not observed in female mice fed a high-salt diet (HSD). This is attributable to the protective effect of estrogen in Ldlr$^{-/-}$ mice and in Ipr$^{-/-}$ Ldlr$^{-/-}$ mice. Thus, estrogen compensates for a deficiency in PGI$_2$ to maintain BP homeostasis in response to high salt in hyperlipidemic female mice. In male mice, by contrast, the augmented formation of atrial natriuretic peptide (ANP) plays a similar compensatory role, restraining hypertension and oxidant stress in the setting of Ipr depletion. Hence, […]
Sex-dependent compensatory mechanisms preserve blood pressure homeostasis in prostacyclin receptor–deficient mice

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Inhibitors of microsomal prostaglandin E synthase 1 (mPGES-1) are in the early phase of clinical development. Deletion of mPges-1 in mice confers analgesia, restrains atherogenesis, and fails to accelerate thrombogenesis, while suppressing prostaglandin E2 (PGE2), but increasing the biosynthesis of prostacyclin (PGI2). In low-density lipoprotein receptor–deficient (Ldlr–/–) mice, this last effect represents the dominant mechanism by which mPges-1 deletion restrains thrombogenesis, while suppression of PGE2 accounts for its antiatherogenic effect. However, the effect of mPges-1 depletion on blood pressure (BP) in this setting remains unknown. Here, we show that mPges-1 depletion significantly increased the BP response to salt loading in male Ldlr–/– mice, whereas, despite the direct vasodilator properties of PGI2, deletion of the I prostanooid receptor (Ipr) suppressed this response. Furthermore, combined deletion of the Ipr abrogated the exaggerated BP response in male mPges-1–/– mice. Interestingly, these unexpected BP phenotypes were not observed in female mice fed a high-salt diet (HSD). This is attributable to the protective effect of estrogen in Ldlr–/– mice and in Ipr–/– Ldlr–/– mice. Thus, estrogen compensates for a deficiency in PGI2 to maintain BP homeostasis in response to high salt in hyperlipidemic female mice. In male mice, by contrast, the augmented formation of atrial natriuretic peptide (ANP) plays a similar compensatory role, restraining hypertension and oxidant stress in the setting of Ipr deletion. Hence, men with hyperlipidemia on a HSD might be at risk of a hypertensive response to mPGES-1 inhibitors.

Introduction

Both the adverse cardiovascular events associated with nonsteroidal antiinflammatory drugs (NSAIDs) and the opioid crisis have prompted interest in developing new analgesics (1–4). Several clinical trials have shown that the incidence and severity of hypertension from NSAID use are quite variable in humans (5–8). Inhibitors of microsomal prostaglandin synthase 1 (mPGES-1), an enzyme involved in the biosynthesis of prostaglandin E2 (PGE2), are in early clinical development as potential nonaddictive analgesics devoid of the cardiovascular hazards attributable to inhibition of COX-2 by NSAIDs.

Deletion of mPges-1 has a mild adverse cardiovascular profile in normolipidemic mice (3), and we have reported that redirevion of the mPGES-1 substrate prostaglandin H2 (PGH2) to prostacyclin (PGI2) synthase, augmenting PGI2, reduces thrombogenesis in hyperlipidemic mice (9). This is a point of distinction from COX-2 depletion or inhibition that suppresses the synthesis of this endogenous platelet inhibitor and predisposes mice to thrombogenic stimuli (3).

Sexual dimorphism in blood pressure (BP) homeostasis is at least partly explained by the endocrine system. For example, systolic blood pressure (SBP) is higher in boys from 13 years of age on compared with girls of the same age (10), and the hypertensive response to salt loading is more pronounced in apparently healthy men compared with premenopausal women at different ages (11). Similarly, in genetically and experimentally predisposed rodent models, hypertension develops more slowly in female mice than in male mice (12, 13). Deletion of the PGE2 receptor Epr1 reduced BP in male, but not female, mice (14). Besides, BP homeostasis is also closely linked to the immune system, inflammation, and the composition of gut microbiota (15–17). In both human and rodent studies, a high-salt diet (HSD) has been shown to increase BP while decreasing the α- and β-diversity of the microbiome (18). Among others, the Lactobacillus species has a negative association with BP responses (19).

Here, the BP response to a HSD was augmented in hyperlipidemic mice lacking the low-density lipoprotein receptor (Ldlr, referred to herein as Ldlr–/– mice). Both PGE2 and PGI2 may act as direct vasodilators, so we assumed that exaggeration of this response in mPges-1–deficient mice was attributable to the suppression of PGE2, despite augmented formation of PGI2 in these mice. To our surprise, deletion of the I pros-
showed a significant effect on DBP only by week 2 compared with baseline DPB for Ldlr–/– mutant mice and their littermate controls fed a HSD. A 4-way, repeated-measures ANOVA showed and abrogated the hypertensive phenotype in Ipr–/– mPges-1–/– Ldlr–/– mice. A 4-way, repeated-measures ANOVA showed a significant effect of the Ipr, mPges-1, phase, and a few of the 2- and 4-way interactions (Ipr:week, week:phase, Ipr:mPges-1:week:phase) on SBP. A post hoc pairwise test showed a significant effect on SBP in week 2 with respect to baseline SBP for Ldlr–/– mice. (C and D) Similar trends in DBP responses were observed in both the active and resting periods in all mutant mice and their littermate controls fed a HSD. A 4-way, repeated-measures ANOVA showed a significant effect of the Ipr, week, phase, and week:phase interaction on DBP. A pairwise test showed a significant effect on DBP only by week 2 compared with baseline DBP for Ldlr–/– mice. A pairwise t test was used to determine significant differences between Ldlr–/–, Ipr–/– mPges-1–/– Ldlr–/–, Ipr–/– Ldlr–/– (double-KO [DKO]), and mPges-1–/– Ldlr–/– mice. Genotypes and feeding periods with the same lowercase letter were significantly different (a–j, P < 0.05) at baseline, 1 week on a HSD, or 2 weeks on a HSD. For example, a – the baseline SBP (active phase) of mPges-1–/– Ldlr–/– (DKO) mice was significantly elevated compared with that of Ldlr–/– mice and b – Ipr–/– Ldlr–/– mice; f – the SBP (active phase) of mPges-1–/– Ldlr–/– mice was significantly elevated after 2 weeks on a HSD compared with baseline SBP. Data are expressed as the mean ± SEM, n = 13–16 mice per genotype.

Figure 1. Ipr deletion in mPges-1–deficient male hyperlipidemic mice abrogates salt-evoked hypertension. (A and B) SBP in male hyperlipidemic mice and mutants fed a HSD was measured via telemetry. A HSD led to a rise in SBP in Ldlr–/– (Ldlr-KO) mice in a time-dependent pattern, during both the active (night) and resting (day) periods. Deletion of mPges-1 in Ldlr–/– mice augmented salt-evoked hypertension. By contrast, deletion of the Ipr restrained salt-evoked hypertension and abrogated the hypertensive phenotype in Ipr–/– mPges-1–/– Ldlr–/– mutant mice. A 4-way, repeated-measures ANOVA showed a significant effect of the Ipr, mPges-1, phase, and a few of the 2- and 4-way interactions (Ipr:week, week:phase, Ipr:mPges-1:week:phase) on SBP. A post hoc pairwise t test showed a significant effect on SBP in week 2 with respect to baseline SBP for Ldlr–/– mice. (C and D) Similar trends in DBP responses were observed in both the active and resting periods in all mutant mice and their littermate controls fed a HSD. A 4-way, repeated-measures ANOVA showed a significant effect of the Ipr, week, phase, and week:phase interaction on DBP. A pairwise t test showed a significant effect on DBP only by week 2 compared with baseline DBP for Ldlr–/– mice. A pairwise t test was used to determine significant differences between Ldlr–/–, Ipr–/– mPges-1–/– Ldlr–/–, Ipr–/– Ldlr–/– (double-KO [DKO]), and mPges-1–/– Ldlr–/– mice. Genotypes and feeding periods with the same lowercase letter were significantly different (a–j, P < 0.05) at baseline, 1 week on a HSD, or 2 weeks on a HSD. For example, a – the baseline SBP (active phase) of mPges-1–/– Ldlr–/– (DKO) mice was significantly elevated compared with that of Ldlr–/– mice and b – Ipr–/– Ldlr–/– mice; f – the SBP (active phase) of mPges-1–/– Ldlr–/– mice was significantly elevated after 2 weeks on a HSD compared with baseline SBP. Data are expressed as the mean ± SEM, n = 13–16 mice per genotype.

The sexually dimorphic, exaggerated salt-evoked hypertension that we observed.

Results

Deletion of the Ipr in mPges-1–deficient hyperlipidemic mice abrogates salt-evoked hypertension. Hyperlipidemic mice (Ldlr–/–) were used in the current study to simulate more closely the atherosclerosis likely extant in elderly patients targeted for analgesia with mPGES-1 inhibitors. As shown in Supplemental Figure 1, A-D (supplemental material available online with this article; https://doi.org/10.1172/JCI136310DS1), despite being fed a chow diet, plasma cholesterol and/or triglyceride levels of Ldlr–/– and Ipr–/– and mPges-1–deficient Ldlr–/– mice were significantly elevated.

Male Ldlr–/– mice fed a HSD showed a time-dependent elevation of SBP in the active (night) period (Figure 1, A and B). The SBP was significantly elevated in week 2 compared with baseline during the active phase. Deletion of mPges-1 led to a further significant increase in the salt-evoked BP response. By contrast, deletion of the Ipr unexpectedly restrained the hypertensive response to the HSD in both Ldlr–/– mice and those also lacking mPges-1. At baseline, male mice lacking both mPges-1 and the Ldlr had elevated BPs compared with BPs of mice of the other genotypes (Figure 1). Thereafter, the attenuating effects of Ipr deletion became apparent: the SBPs of Ldlr–/–, mPges-1–/– Ldlr–/–, and Ipr–/– mPges-1–/– Ldlr–/– mice were significantly elevated compared with SBPs of Ipr–/– Ldlr–/– mice 1 week and/or 2 weeks after they were fed a HSD. We observed similar differences in diastolic blood pressure (DBP) responses in all mutants and their littermate controls fed a HSD during the active and resting periods (Figure 1, C and D). The DBPs in Ldlr–/– mice were significantly elevated in week 2 compared with baseline DBPs during the active phase. Compared with Ipr–/– Ldlr–/– mice, the DBPs of Ldlr–/–, mPges-1–/– Ldlr–/–, and Ipr–/– mPges-1–/– Ldlr–/– mice were significantly elevated at baseline as well as 1 week and/or 2 weeks after HSD feeding. However, we did not observe these HSD-evoked BP responses in female hyperlipidemic mice (Supplemental Figure 2, A–D). In addition, weight gain, the urinary output/fluid intake ratio, and urinary sodium levels did not appear to explain the sex differences in BP responses to the salt loading in our mice (Supplemental Figure 3, A–C). We were not able to accurately measure food intake in the current study, because the HSD was very hygroscopic.
Detailed statistical analyses of the interactions among genotypes, treatment (week), and phases for both sexes are described in the Supplemental Methods.

Impact of Ipr and mPges-1 deletion on prostaglandin biosynthesis in male hyperlipidemic mice on a HSD. Two weeks of HSD feeding suppressed PGE2 but increased PGI2 biosynthesis as reflected in their urinary (A) PGEM and (B) PGIM metabolites, respectively. Deletion of mPges-1 suppressed PGE2 but increased PGI2 biosynthesis in mPges-1–/– Ldlr–/– mice. Deletion of the Ipr did not alter PGEM or PGIM levels at baseline but increased PGIM levels on the HSD. (C) HSD feeding also increased urinary 2,3-dinor TxB2, (TxM) levels in DKO mutant mice. (D) After HSD feeding, urinary PGDM (11,15-dioxo-9α-hydroxy-2,3,4,5-tetranorprostan-1,20-dioic acid) levels were significantly elevated in Ipr–/– Ldlr–/– mice compared with levels in Ldlr–/– and mPges-1–/– Ldlr–/– mice. Deletion of the Ipr resulted in a reactive increase in the biosynthesis of PGI2, but also of TxB2 and PGD2, again apparent on a HSD.

Detailed statistical analyses of the interactions among urinary prostaglandin metabolites, mouse genotypes, and treatment (week) are described in the Supplemental Methods.

Pharmacological inhibition of the human mPGES-1 enzyme elevates SBP in hyperlipidemic male mice. To confirm the hypertensive phenotype of global mPges-1–/– Ldlr–/– mice, we administered the mPGES-1 inhibitor MF970 (10 mg/kg BW) concomitantly with a high-fat diet (HFD) for 39 weeks to humanized mPges-1–/– Ldlr–/– male mice. As shown in Supplemental Figure 4, inhibition of mPges-1 suppressed urinary PGEM (Supplemental Figure 4A) and increased the SBP response (Supplemental Figure 4B) as compared with control mice on a HFD alone.

A HSD activates ANP synthesis and release in Ipr-deficient mice. The unexpected suppression of the salt-evoked elevation of BP by Ipr deletion prompted us to compare gene expression profiles in the renal medullae of male Ldlr–/– and Ipr–/– Ldlr–/– mice by high-throughput RNA-Seq. We identified 2719 differentially expressed genes (DEGs), with a log fold change ranging from 2.64 to –3.83 between Ldlr–/– and Ipr–/– Ldlr–/– mice at a FDR cutoff of 0.12. One thousand ninety-seven of these 2719 DEGs were upregulated, and 1622 were downregulated in the renal medulla of Ipr–/– Ldlr–/– mice compared with Ldlr–/– mice. We used Ingenuity Pathway Analysis to assess changes in biological pathways associated with gene expression (Table 1), and the pathways most enriched with DEGs included eukaryotic initiation factor 2 (eIF2), eIF4/p70S6K signaling, mitochondrial dysfunction, and oxidative phosphorylation. Sixty-three of the 76 identified genes in the eIF2 pathway were downregulated in the Ipr–/– Ldlr–/– mice and were mostly members of the 60s and 40s ribosomal subunits involved in RNA binding (Figure 3A and Supplemental Table 1). Forty-five of 47 genes related to mitochondrial dysfunction and oxidative phosphorylation were downregulated in the Ipr–/– Ldlr–/– mice (Figure 3A and Supplemental Table 1). Most of these genes are components of mitochondrial complexes I–V, which are involved in electron transport and ATP synthesis. We validated 3 of the genes (downregulated: Cat, Atp5e, which are antioxidant enzymes) in the mitochondrial dysfunction and oxidative phosphorylation pathways by reverse transcription quantitative PCR (RT-qPCR) (Supplemental Figure 5, A and B). In addition, the RNA-Seq data were consistent with activation of the ANP pathway. We found that expression of nephrilysin (Mmem), which degrades natriuretic peptides, was elevated in Ipr–/– Ldlr–/– mice compared with expression levels in Ldlr–/– mice (Figure 3B). We confirmed by RT-qPCR that mRNA levels of corin (ANP-converting enzyme) and ANP, but not brain natriuretic peptide (BNP), were significantly increased in whole-heart lysates from Ipr–/– Ldlr–/– mice (Figure 3C–E). Moreover, renal medullary mRNA expression of Npr1, a receptor of ANP, was significantly increased (Figure 3F). Consistent with the gene expression data, urinary ANP levels were also elevated in Ipr–/– Ldlr–/– mice compared with levels
in Ldlr−/− mice after 2 weeks on the HSD (Figure 4, A and B). We did not observe a significant difference in creatinine levels in the urine samples between Ldlr−/− and Ipr−/− Ldlr−/− mutants (Supplemental Figure 3D). Thus, elevated urinary ANP levels were not likely to be confounded by differences in fluid intake. Consistent with the role of PG1 in restraining oxidative stress in atherosclerotic vasculature (23) and in salt-induced hypertension (24, 25) and the elevation of PG1 biosynthesis in mice on the HSD (Figure 2), excretion of a major urinary F2-isoprostane (F2iP), an index of lipid peroxidation, was not significantly elevated in Ldlr−/− mice after 2 weeks on a HSD (Figure 4, C and D). However, rather than increase with Ipr deletion, F2iP excretion, just like BP, unexpectedly fell, consistent with the changes in mitochondrial dysfunction and oxidative phosphorylation genes observed in the renal medulla of Ipr−/− Ldlr−/− mice (mostly downregulated in the Ipr−/− Ldlr−/− mice; Figure 4D). The reduction in urinary F2iP and elevated ANP levels consequent to Ipr deletion in the Ldlr−/− mice was abrogated by treatment with the ANP receptor antagonist A71915 (refs. 26–28 and Figure 4, E and F). This is consistent with evidence that ANP is both a vasodilator and a restraint on oxidative stress (27, 29).

The hypotensive phenotype of Ipr−/− Ldlr−/− mice was not associated with gross morphological changes in the kidneys (Supplemental Figure 6) or the vasculature (Supplemental Figure 7), as assessed by H&E staining. In male mice, deletion of the Ipr had no significant effect on urinary total nitrate plus nitrite (Supplemental Figure 8A) or on plasma renin levels (Supplemental Figure 8B) compared with Ldlr−/− mice.

In contrast to the males, expression levels of corin, ANP, and BNP mRNA in whole heart and of the 3 mitochondrial dysfunction and oxidative phosphorylation genes (Atp5e, Cat, and Sod2) in the renal medulla were not significantly altered between female Ldlr−/− and Ipr−/− Ldlr−/− mice fed a HSD for 2 weeks (Supplemental Figure 9, A–E). Urinary F2iP did not differ significantly in female Ipr−/− Ldlr−/− mice compared with Ldlr−/− mice at baseline (Supplemental Figure 10A) or after 2 weeks on a HSD (Supplemental Figure 10B). However, combined deletion of Ipr and ANP receptor blockade in female mice significantly increased urinary F2iP levels (Supplemental Figure 10C), whereas deletion of the Ipr significantly reduced baseline urinary ANP levels (Supplemental Figure 10D). This difference was abolished after 2 weeks on the HSD (Supplemental Figure 10E); blockade of the ANP receptor did not alter ANP levels between Ldlr−/− and Ipr−/− Ldlr−/− mice (Supplemental Figure 10F). These results were consistent with the failure of genotype to significantly influence the HSD-evoked BP response in female mice (Supplemental Figure 2).

Sex-dependent immunological responses induced by a HSD. Given our findings on sex differences in BP responses, we were interested in comparing the transcriptomic profiles of atria from female and male Ldlr−/− and Ipr−/− Ldlr−/− mice fed a HSD for 2 weeks. We identified 177 DEGs (136 are unique to females, 11 are unique to males, 30 are common between females and males), with a log fold change ranging from 5.00 to −3.84 at a FDR cutoff of 0.4 (Supplemental Figure 1A). In female mice, 110 of the 166 DEGs were downregulated and 56 were upregulated in Ipr−/− Ldlr−/− mice compared with Ldlr−/− mice. In male mice, 17 of the 41 DEGs were downregulated and 24 were upregulated in Ipr−/− Ldlr−/− mice. Ingenuity Pathway Analysis revealed the pathways most enriched with DEGs including the antigen presentation pathway, B cell development, and T cell receptor signaling (Supplemental Figure 1B and Table 2). In female mice, DEGs associated with the classical or nonclassical MHC class I molecules including C5ar2, Rfx5, H2-M3, H2-Q5, H2-Q6, C5ar1, H2-Aa, H2-Q7, H2-T22, H2-Dmb1, Nlr5, and H2-T10 were downregulated in Ipr−/− Ldlr−/− mice compared with Ldlr−/− mice, and only C5ar2 was downregulated in male Ipr−/− Ldlr−/− mice (Supplemental Data File 1, atrial DEGs between male and female Ldlr−/− and Ipr−/− Ldlr−/− mice on a HSD). We validated the H2-M3 DEG by RT-qPCR (Supplemental Figure 11C). Functional output analysis predicted inflammatory responses and chronic inflammatory disorders as downstream pathways likely to be affected by the DEGs. However, there was not a strong degree of consistency in the directions of the DEGs (Supplemental Figure 11D and Supplemental Table 2). Both Th17 cells and Tregs have been shown to modulate BP responses in hypertensive mouse models. Depletion of the Ipr significantly increased plasma levels of IL-17A (Supplemental Figure 11E) and cardiac mRNA levels of the IL-17 receptor A (IL-17ra) (Supplemental Figure 11F) and the transcription factor of Tregs (Foxp3) (Supplemental Figure 11G) in male Ldlr−/− mice.

An ANP antagonist rescues hypotension in Ipr-deficient hyperlipidemic mice on a HSD. Given the physiological constraint of implanting both radio telemetry probes and minipumps into mice to monitor BP and deliver the ANP antagonist during HSD feeding, we decided to use the tail-cuff system for the former, while delivering the antagonist by minipump. Despite its lower sensitivity, BP data collected using the tail-cuff system correlated with the data from radio telemetry (Figure 5, A and B).

Inhibition of the endogenous ANP signaling pathway with the antagonist A71915 (27) attenuated the hypotensive response to Ipr deletion in HSD-fed male Ipr−/− Ldlr−/− mice during both the night and day periods (Figure 5, A and B, and Supplemental Figure 12, sham-saline). Consistent with this, we observed no significant differences in mRNA levels of atrial or ventricular corin, ANP, or BNP between male Ldlr−/− and Ipr−/− Ldlr−/− mice treated with the antagonist (Supplemental Figure 13, A–F). Similarly, the differences in expression of the Npr1 receptor for ANP in renal medulla (Supplemental Figure 13G), and of the genes (Atp5e, Cat, and Sod2) in the mitochondrial dysfunction and oxidative phosphorylation pathways (Supplemental Figure 14, A–C) were abolished by antagonist administration. Administration of A71915 did not alter plasma creatinine levels.

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Table 1. Top canonical pathways predicted by Ingenuity Pathway Analysis for the 2719 DEGs in kidney medulla between male Ldlr−/− and Ipr−/− Ldlr−/− mice on a HSD

<table>
<thead>
<tr>
<th>Canonical pathway</th>
<th>P value</th>
<th>Overlap</th>
</tr>
</thead>
<tbody>
<tr>
<td>elf2 signaling</td>
<td>8.23 × 10−2</td>
<td>76/194</td>
</tr>
<tr>
<td>Oxidative phosphorylation</td>
<td>2.62 × 10−3</td>
<td>47/99</td>
</tr>
<tr>
<td>Mitochondrial dysfunction</td>
<td>8.26 × 10−8</td>
<td>60/159</td>
</tr>
<tr>
<td>Regulation of elf4 and p70S6K signaling</td>
<td>4.06 × 10−6</td>
<td>47/164</td>
</tr>
</tbody>
</table>

Seventy-six of the 194 genes associated with the elf2 signaling pathway were differentially expressed in Ipr−/− Ldlr−/− mice compared with Ldlr−/− mice.
Deletion of the Ipr augmented the SBP responses, and supplementation with estradiol (E2) significantly restrained these responses (Figure 5, C and D). Similar differences in DBP responses were observed in Ldlr–/– and Ipr-deficient Ldlr–/– mice (Figure 5, E and F). As expected, we detected no significant differences in BP responses among the sham-operated mice fed a HSD for 2 weeks (Supplemental Figure 16, A–D).

Detailed statistical analyses of the interactions among genotypes (Ldlr–/– and Ipr–/– Ldlr–/– mice) and E2 treatment and treatment week of OVX mice are described in the Supplemental Methods.

HSD alters gut microbiota composition in a sex-dependent manner
To study the impact of sex and Ipr depletion on the gut microbiota, we studied the impact of sex and Ipr depletion on the gut microbiota. As expected in female mice, we detected no differences in SBP or plasma creatinine between Ldlr–/– and Ipr–/– Ldlr–/– mice fed a HSD for 2 weeks in conjunction with ANP receptor blockade (Supplemental Figure 15, A and B).

Estrogen protects female hyperlipidemic mice from salt-evoked hypertension.
To address the female BP phenotypes, we performed the HSD experiment using OVX mice. The HSD significantly increased BP responses in OVX Ldlr–/– mice in week 2 compared with baseline during both the active and resting periods (Figure 5, C and D). Deletion of the Ipr augmented the SBP responses, and supplementation with estradiol (E2) significantly restrained these responses (Figure 5, C and D). Similar differences in DBP responses were observed in Ldlr–/– and Ipr-deficient Ldlr–/– mice (Figure 5, E and F). As expected, we detected no significant differences in BP responses among the sham-operated mice fed a HSD for 2 weeks (Supplemental Figure 16, A-D).

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Detailed statistical analyses of the interactions among genotypes (Ldlr–/– and Ipr–/– Ldlr–/– mice) and E2 treatment and treatment week of OVX mice are described in the Supplemental Methods.
female mice. Several taxa were changed over time in both sexes and genotypes: Bacteroidales S24-7 and Staphylococcus increased in relative abundance, whereas Mucispirillum and Helicobacter decreased. Corynebacterium was detected only in male mice and increased after HSD feeding (Supplemental Figure 18B).

When we examined the effect of sex on the gut microbiota after HSD feeding, we found that the α-diversity between female and male mice was not different (Supplemental Figure 1C), but we did detect differences in β-diversity (unweighted UniFrac) on day 0 in Ldlr−/− mice (P = 0.01) and on day 14 in Ipr+/− Ldlr−/− mice (P = 0.01, Supplemental Figure 1D). On day 14, the relative abundance of Lactobacillus was decreased (P = 0.03) in male Ipr+/− Ldlr−/− mice compared with females (Supplemental Figure 1E). To gain further insight into the types of Lactobacillus observed, we aligned representative sequences from our experiment to species-type strains and assigned species where our sequences matched within 2 bp. Thus, we observed a decrease in sequences consistent with Lactobacillus intestinatis (P = 1.6 × 10−4) in male Ldlr−/− mice relative to female mice (Supplemental Figure 1F). We found no differences in α-diversity or β-diversity between Ldlr−/− and Ipr+/− Ldlr−/− mice on day 0 or day 14 in female or male mice (Supplemental Figure 19, A and B).

A HSD alters microbiota-derived fecal indole metabolites and short-chain fatty acids. As a HSD significantly reduced the abundance of Lactobacillus in male mice compared with female mice, regardless of genotype, we were interested in measuring microbiota-derived fecal indole metabolites and short-chain fatty acids (SCFAs) by liquid chromatography tandem mass spectrometry (LC-MS/MS) and H-1-NMR, respectively. Since Ipr deletion did not alter both α- and β-diversity of the gut microbiota in female or male mice, we combined both genotypes in our analyses to determine the effect of a HSD on fecal metabolites. As shown in Figure 6D, a HSD significantly increased fecal indole-3-acetic acid (IAA), whereas indole-3-propionic acid (IPA) was decreased in both female and male mice. However, indole-3-lactic acid (ILA) was significantly decreased only in male mice. Consistent with the decreased abundance of Lactobacillus, we found that fecal lactic acid contents were significantly reduced in both female and male mice after HSD feeding (Supplemental Figure 20, A and B). We observed a similar pattern for fecal butyric acid, but only in male mice was a significant reduction attained. Fecal acetic and propionic acids were unaltered in both sexes.

A HSD differentially alters plasma metabolites in female and male mice. The effects of Ipr deletion and sex differences on metabolic activity were further analyzed using plasma samples and ultra-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) in a semitargeted approach. Orthogonal partial least-squares discriminant analysis (OPLS-DA) of genotype and sex revealed a distinct separation between female Ldlr−/− (red spheres) and male Ldlr−/− (green spheres) mice after 2 weeks on a HSD (Supplemental Figure 21A and Supplemental Data File 2, plasma

ome in our mouse model of salt-evoked hypertension, we subjected female and male Ldlr−/− and Ipr+/− Ldlr−/− mice to a HSD for 2 weeks. We analyzed fecal samples on day 0 and day 14 by 16S rRNA marker gene sequencing. The taxonomic identities of prominent amplicon sequence variants (ASVs) are presented in the heatmap in Supplemental Figure 17 (mean relative abundance of all parameters of >0.5%). A comparison of the microbiome on day 0 versus day 14 revealed that a HSD was associated with decreased α-diversity (Faith’s phylogenetic diversity [PD]) in female Ipr+/− Ldlr−/− mice (P = 0.034; Supplemental Figure 18A). The bacterial community, as analyzed by unweighted and weighted UniFrac, was different between day 0 and day 14 in both sexes and genotypes (Figure 6, A and B). At the genus level, the relative abundance of Lactobacillus decreased in male Ldlr−/− mice (P = 1.2 × 10−3; Figure 6C) and Ipr+/− Ldlr−/− mice (P = 2.5 × 10−4; Figure 6C), but not in

Figure 4. Combined deletion of Ipr and salt-evoked hypertension increases urinary ANP and reduces F,IPs. Urinary ANP levels at (A) baseline and (B) 2 weeks after a HSD were measured using an ELISA kit. Urinary ANP levels were elevated in Ipr+/− Ldlr−/− mice compared with levels in Ldlr−/− mice 2 weeks after HSD feeding. An abundant urinary F,IP (8,12-iso-IPF2a-VI) was analyzed by LC-MS/MS as described in Methods. (C and D) Urinary F,IP levels were not altered in Ipr+/− Ldlr−/− mice compared with Ldlr−/− mice at baseline, but urinary F,IP levels in Ipr+/− Ldlr−/− mice were significantly reduced after 2 weeks on a HSD. Treatment with the ANP receptor antagonist A71915 (50 μg/kg BW/day) abrogated the reduction of (E) urinary F,IPs and (F) urinary ANP in Ipr+/− Ldlr−/− mice. Data are expressed as the mean ± SEM. *P < 0.05, by 1-tailed parametric test with Welch’s correction. n = 9–15 mice per genotype. A 1-tailed parametric test was performed for urinary F,IP and ANP levels, because both mediators had already been shown to restrain oxidative stress in the vasculature.
metabolites between male and female \textit{Ldlr}–/– and \textit{Ipr}–/– \textit{Ldlr}–/– mice on a HSD). The OPLS-DA loadings plot showed that indoxyl sulfate, trimethylamine oxide, propylene glycol, and methyl adenosine metabolites were higher in female \textit{Ldlr}–/– mice (Supplemental Figure 21B). Metabolites such as orotate, deoxyuridine, cytidine, carnitine, and so on, on the right side of the plot (P1 >0) were significantly higher in the male \textit{Ldlr}–/– mice. MetaboAnalyst pathway analysis revealed several metabolic differences between female and male mice, including metabolic pathways for phenylalanine, tyrosine, tryptophan, and pyrimidine (Supplemental Figure 21C). Notably, “tryptophan metabolite” was one of the most affected metabolic pathways between female and male \textit{Ldlr}–/– mice after 2 weeks on a HSD. To corroborate with our microbiota-induced changes in indole metabolites, we focused on the tryptophan/indole pathway. Indeed, consistent with the reduction in abundance of \textit{Lactobacillus}, plasma \textit{Lactobacillus} levels of indoxyl sulfate/tryptophan (Figure 6E) were significantly decreased in male \textit{Ldlr}–/– mice compared with levels in female \textit{Ldlr}–/– mice, whereas tryptophan and kynurenine levels were not altered between female and male mice, regardless of their genotype (Supplemental Figure 21D).

**Discussion**

NSAIDs represent an alternative to opioid analgesics, but they confer a cardiovascular hazard attributable to suppression of COX-2–derived cardioprotective prostaglandins, especially \textit{PGI}1, \textit{PGI}2 limits thrombogenesis, while suppression of \textit{PGE}2 accounts for the restraint of atherogenesis (3, 33). This reflects redversion of the \textit{PGH}2 substrate of \textit{mPGES-1} to other prostaglandin synthases, most relevantly to \textit{PGI}1 biosynthesis (34). Depending on the genetic background, it may leave basal and evoked BP responses unchanged or modestly increased. On a hyperlipidemic background, increased \textit{PGI}1 biosynthesis, urinary \textit{F}2\textit{iP} was depressed (31, 32, 36). Here, we found that \textit{mPges-1} deletion predisposed hyperlipidemic male, but not female, mice to the pressor response to a HSD, consistent with the role of \textit{PGE}2, in fluid volume and BP homeostasis (37). Moreover, chronic exposure to a pharmacological inhibitor (MF970) specifically targeting human \textit{mPGES-1} resulted in elevated \textit{PGI}2 in hyperlipidemic mice on an HFD. These observations raise the possibility that, despite results in healthy volunteers (28), inhibition of \textit{mPGES-1} in male patients with hyperlipidemia may predispose them to an exaggerated BP response to a HSD.

To investigate whether the augmented \textit{PGI}1 biosynthesis resulting from \textit{mPges-1} deletion might be buffering the hypertensive phenotype, we used mice lacking the \textit{Ipr}. We were surprised to find that BP responses to salt loading in male, but not female, mice were attenuated (rather than exacerbated) in \textit{Ipr}–/– \textit{mPges-1}–/– mice. Deletion of the \textit{Ipr} resulted in a compensatory increase in the biosynthesis of \textit{PGI}1 consequent to salt loading. However, given the absence of its receptor, this would be unlikely to directly influence BP homeostasis. Rather, we found activation of another compensatory mechanism increased formation of the vasodilator ANP. Antagonism of its \textit{Npr1} receptor was sufficient to rescue the hypotensive response to a HSD in \textit{Ipr}-depleted mice. The ANP promoter contains \textit{CAMP} response element–binding sites. Normally, ligation of the \textit{Ipr} by \textit{PGI}1 results in activation of protein kinase A (PKA) and elevation of intracellular \textit{cAMP} (38). In the absence of its cognate receptor, augmented \textit{PGI}1 levels in male \textit{Ipr}–/– mice may activate other PKA-linked receptors, such as \textit{Epr2}, \textit{Epr4}, and \textit{Dpr1}. Indeed, the HSD also increased the biosynthesis of \textit{PGD}2 that, acting through the \textit{Dpr1}, may augment this effect. Thus, altered patterns of eicosanoid formation in \textit{Ipr}-deficient mice on a HSD may act via this mechanism to effect a compensatory elevation of ANP.

Hyperlipidemia in \textit{Ldlr}–/– mice is associated with oxidative stress, as reflected by increased generation of \textit{FIP}, a biomarker of lipid peroxidation (22). Both \textit{PGI}1 and ANP can act to restrain oxidative stress, which itself may contribute to an elevation of BP in response to a HSD (23, 39, 40). Here, we found that, despite an augmentation of \textit{PGI}1 biosynthesis, urinary \textit{FIP} was depressed in \textit{Ipr}–/– \textit{Ldlr}–/– mice compared with mice lacking the \textit{Ldlr} alone. To address the possibility that this reflected the compensatory augmentation of ANP, we treated the mice with an ANP receptor antagonist and found that, like the hypotensive phenotype, it rescued the suppression of \textit{FIP} in the \textit{Ipr}–/– \textit{Ldlr}–/– mice. Pathway enrichment analyses of RNA-Seq data also reflected a shift in the

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**Table 2. Top canonical pathways predicted by Ingenuity Pathway Analysis for the 177 DEGs in atria between female \textit{Ldlr}–/– and \textit{Ipr}–/– \textit{Ldlr}–/– mice on a HSD**

<table>
<thead>
<tr>
<th>Canonical pathway</th>
<th>P value</th>
<th>Overlap</th>
<th>Molecules represented</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antigen presentation</td>
<td>2.57 × 10^{-4}</td>
<td>6/27</td>
<td>H2-Q2, H2-DMb1, H2-Aa, H2-M3, NitrS, Psmb9</td>
</tr>
<tr>
<td>B cell development</td>
<td>2.45 × 10^{-5}</td>
<td>4/25</td>
<td>H2-Q2, H2-DMb1, H2-Aa, Ptprc</td>
</tr>
<tr>
<td>T cell receptor signaling</td>
<td>2.75 × 10^{-5}</td>
<td>9/148</td>
<td>Grap2, H2-T10, H2-T22, H2-Q2, H2-DMb1, H2-Aa, H2-M3, Ptkrc, Ptprc</td>
</tr>
</tbody>
</table>

Six of the 27 genes associated with antigen presentation signaling pathway were differentially expressed in \textit{Ipr}–/– \textit{Ldlr}–/– mice compared with \textit{Ldlr}–/– mice.
redox balance in the renal medulla of these Ipr–/– Ldlr–/– mice. Some 45 genes related to mitochondrial dysfunction and oxidative phosphorylation were downregulated, whereas genes encoding antioxidant enzymes, including mitochondrial SOD2 and catalase, were upregulated. Again, ANP antagonism rescued this signature, adding evidence consistent with an antioxidant effect of functional relevance. Although the ANP/Npr1 pathway plays an important role in regulating blood volume and pressure (41, 42), we failed to observe comparative diuresis or natriuresis in the Ipr–/– Ldlr–/– mice. Similarly, urinary total nitrate/nitrite and plasma renin levels were unaltered in the Ipr–/– Ldlr–/– mice compared with levels in Ldlr–/– controls.

These differences in the BP response to a HSD and the attendant changes in gene expression and activation of the ANP pathway were observed only in male mice. There is prior evidence for the influence of sex and genetic background on disruption of the prostaglandin pathways. For example, we have shown that deletion of the Ipr accelerates atherogenesis particularly in female mice because of the importance of PGI2 as a mediator of estrogen receptor–dependent cardioprotection (23).

Estrogen increases vasodilation partly by binding to its receptors in vascular endothelial and smooth muscle cells (SMCs) of the vasculature (43). Consistent with the findings that estrogen activates PGI2, biosynthesis in rat aortae (44), rat aortic SMCs (45), and human endothelial cells (46), ovariectomy augmented the hypertensive response to a HSD in Ldlr–/– and Ipr-deficient Ldlr–/– mice. Estradiol replacement restrained the elevation in BP in the OVX mice, consistent with our observation of sexual dimorphism in the response to Ipr deletion and the BP response to a HSD.

Increasing evidence suggests that T cells mediate inflammatory processes associated with hypertension in humans (16, 47, 48). Rodent models of hypertension have also been associated with the upregulation of pathogenic Th17 cells and the downregulation of protective Tregs (16, 49–51). Here,
Figure 6. A HSD alters gut microbiota composition in a sex-dependent manner. Fecal pellets were collected from singly housed mice at baseline (day 0) and 2 weeks (day 14) after HSD feeding. Bacterial DNA was extracted and analyzed by 16S RNA gene sequencing. Regardless of sex or genotype, Ldlr–/– (Ldlr-KO) versus Ipr–/– Ldlr–/– (Ipr Ldlr-DKO) mice, β-diversity of the gut microbiota was significantly different between day 0 and day 14 as assessed by (A) weighted UniFrac and (B) unweighted UniFrac. (C) The relative abundance of Lactobacillus was significantly reduced in male Ldlr–/– mice (P = 1.2 × 10–3) and Ipr–/– Ldlr–/– mice (P = 2.5 × 10–4) compared with female mice. (D) Fecal indole metabolites were analyzed by HPLC-MS/MS. HSD feeding significantly increased IAA in both female and male mice, whereas IPA decreased. ILA was significantly reduced in male mice only. As HSD reduces the abundance of Lactobacillus, we performed a 1-tailed test for fecal indole metabolites. Data are expressed as the mean ± SEM. *P < 0.05, **P < 0.01, ***P < 0.001, and ****P < 0.0001, by paired, 1-tailed parametric test. n = 18 female mice and n = 26 male mice per genotype. (E) Plasma levels of indoxyl sulfate/tryptophan were significantly reduced in male Ldlr–/– mice compared with levels in female Ldlr–/– mice after 2 weeks on a HSD. *P < 0.05. A 2-way ANOVA showed a significant effect of sex on plasma levels of indoxyl sulfate/tryptophan in Ldlr–/– mice. Sidak’s multiple comparison tests were used to test significant differences between the sexes. Data are expressed as the mean ± SEM. n = 9 female mice and n = 13 male mice per genotype. PCoA, principal coordinates analysis.
Additionally, DEGs associated with the classical or nonclassical MHC class I molecules were downregulated in female mice compared with male mice.

Although deletion of the Ipr failed to alter HSD-induced changes in the composition of gut microbiota in Ldlr−/− mice, HSD had a sexually dimorphic impact on the gut microbiome. Consistent with other studies (47, 53), we observed a substantial decrease in the abundance of Lactobacillus in male, but not female, mice fed a HSD for 2 weeks. Lactobacillus is able to metabolize tryptophan to indole metabolites, including IAA, IPA, and ILA, which act via binding to the aryl hydrocarbon receptor (AhR) to modulate innate and adaptive immune responses (54). For example, ILA-producing Lactobacillus murinus was reported to restrain HSD-induced inflammatory Th17 cells in the spleen, small intestine, and colon and rescue salt-sensitive hypertension in FVB/N male mice (47). Importantly, ILA induces the differentiation of CD4+ Th cells into double-positive intraepithelial lymphocytes via binding to the

Figure 7. Schema depicting the effect of a HSD on PGI2 receptor–deficient male and female mice. Deletion of mPges-1 suppressed PGE2 biosynthesis, while increasing PGI2, which contributed to the attenuation of thrombogenesis in hyperlipidemic mice. However, salt loading suppressed PGE2 biosynthesis and increased BP responses. Both PGI2 and ANP are vasodilators and restrain oxidative stress induced by a HSD. Deletion of the Ipr resulted in a compensatory increase in ANP/Npr1 signaling and reduced mitochondrial oxidative stress and BP responses. In female hyperlipidemic mice, estrogen protected Ldlr−/− and Ipr−/− Ldlr−/− mice from salt-evoked hypertension. A HSD suppressed the abundance of Lactobacillus in male mice and reduced the levels of indole metabolites such as ILA. ILA binds the AhR and activates signaling pathways that might restrain oxidative stress and hypertension. Large “X” symbols indicate inhibition or deletion of an enzyme, and horizontal lines with vertical end bars indicate restraint.
AhR. This effect was abrogated in AhR-deficient Th cells (55). Correspondingly, indoxyl sulfate/tryptophan was reduced in the plasma of male mice, consistent with a differential impact on tryptophan metabolism contributing to the sensitivity of BP to a HSD in male Ldlr–/– mice. Previous clinical studies have demonstrated that fecal microbiota-derived indole metabolites may activate the AhR to modulate different immune responses in health and disease (56, 57).

In summary, we report distinct sex-dependent compensatory mechanisms to preserve BP homeostasis in response to disruption of the receptor for the direct vasodilator PGI₂ (Figure 7). In male mice, deletion of the Ipr restrains salt-evoked hypertension via activation of the ANP/Npr1 pathway, thereby reducing the oxidative stress characteristic of hyperlipidemia. It remains to be seen whether this compensatory response wanes under conditions of chronic intake of a HSD, as might be most clinically relevant. In female mice, estrogen restrains the BP responses of both Ldlr–/– and Ipr–/– Ldlr–/– mice to salt-evoked hypertension. Irrespective of the impact of Ipr deletion, depletion of Lactobacillus in the gut results in perturbation of tryptophan metabolism that may exaggerate the hypertensive response of male mice to a HSD.

Finally, our findings with mPges-1 deletion or pharmacological inhibition of the enzyme in mice suggest that hyperlipidemic male patients consuming a HSD may be susceptible to hypertension when taking mPGES-1 inhibitors.

Methods

All reagents used were purchased from MilliporeSigma unless otherwise stated.

Detailed descriptions of the animal models and experimental methods are provided in the Supplemental Methods.

RNA-Seq data availability. RNA-Seq data were deposited in the NCBI’s Gene Expression Omnibus (GEO) database (GEO GSE115916).

Statistics. All animals were the same age and on the same Ldlr–/– background (C57BL/6). Where conclusions involved multiple factors, a 2-, 3-, or 4-way repeated-measures ANOVA was performed to assess changes in mean scores at multiple time points and differences in mean scores under multiple conditions. The residuals were normally distributed as required by ANOVA. The degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity. ANOVAs were repeated on multiple restricted models to assess the effects of combinations of factors. Post hoc analysis was performed using pairwise t tests with Bonferroni’s correction unless otherwise stated. A significance threshold of 0.05 was used for all tests. Significance of greater than 0.01 is indicated by double asterisks on the graphs and significance of greater than 0.001 is indicated by triple asterisks unless otherwise stated. Sample sizes were based on power analysis from estimates of the variability of the measurements, and the desire to detect a minimal 10% difference in the variables was assessed with α = 0.05 and the power (1-β) = 0.8.

Study approval. All animals in this study were housed according to University of Pennsylvania IACUC guidelines, and all experimental protocols were approved by the IACUC of the University of Pennsylvania (protocol 804754).

Author contributions

SYT, HM, STA, DS, SG, NFL, KNT, VT, and GRG acquired and analyzed the data. SYT, ER, EJH, KB, AW, and GAF contributed to data interpretation. SYT and GAF conceived the study and are responsible for the experimental design and manuscript preparation.

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