Regulator of G protein signaling 5 is determinant of gestational hypertension and preeclampsia

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One Sentence Summary: Vascular RGS5 regulates AngII sensitivity during pregnancy and, when reduced, causes hypertension and preeclampsia.

Abstract:

Preeclampsia is a systemic vascular disorder of pregnancy and is associated with increased sensitivity to angiotensin II (AngII) and hypertension. The cause of preeclampsia remains unknown. Here, we identified the role of Regulator of G protein Signaling 5 (RGS5) in blood pressure regulation during pregnancy and preeclampsia. *RGS5* expression in human myometrial vessels is dramatically suppressed in gestational hypertension and/or preeclampsia. In pregnant RGS5-deficient mice, reduced vascular RGS5 expression causes gestational hypertension by enhancing vascular sensitivity to AngII. Further challenge by increasing AngII results in preeclampsia-like symptoms, namely more severe hypertension, proteinuria, placental pathology and reduced birth weight. In pregnant heterozygote null mice, treatment with peroxisome proliferator activated receptor (PPAR) agonists normalizes vascular function and blood pressure through effects on RGS5. These findings highlight a key role of RGS5 at the interface between AngII and PPAR signaling. Because preeclampsia is refractory to current standard therapies, our study opens an unrecognized and urgently needed opportunity for treatment of gestational hypertension and preeclampsia.

Introduction

Preeclampsia and associated hypertension is a serious disease which affects 5-10 % of pregnancies (1). Clinical intervention is limited to pre-term delivery and causes long-term cardiovascular problems in the mother and child. Healthy pregnancy requires extensive adaptation of maternal cardiovascular physiology. Despite increased cardiac output and plasma volume, blood pressure decreases due to reduced peripheral resistance (2-3). The renin-angiotensin system is crucial for these adaptations (4). Paradoxically, pregnant women develop a marked resistance to pressor effects of infused AngII, even though components of the renin-angiotensin system increase in the circulation. This is in stark contrast to women with gestational hypertension or preeclampsia, where hyper-reactivity to AngII and failure of compensatory reduction in peripheral vascular resistance occur (5-6). Pharmacological inhibition of AngII receptor type 1 (ATR1) or angiotensin-converting enzyme (ACE) is contraindicated during pregnancy due to fetopathy (7-8), highlighting the need for alternative treatment options.

The mechanism for enhanced AngII sensitivity is poorly understood. Pregnancy-related hypertension and preeclampsia can arise from autoimmune-mediated ATR1 hyper-stimulation effected by agonistic anti-ATR1 antibodies (9-10). Furthermore, G protein-coupled receptor (GPCR) heterodimer formation, for instance between ATR1 and bradykinin receptor, have been shown to contribute to AngII hypersensitivity (11). Alternatively, we hypothesized that AngII signaling could be regulated at a post-receptor level. RGS molecules tightly control GPCR activation, including ATR1, by acting as intracellular GTPase-activating (GAP) proteins for heterotrimeric G proteins (12-14). We recently identified RGS5 as a key regulator of angiogenic vessel remodeling in cancer and critical modulator of cardiovascular function (14-15). We therefore speculated that RGS5 might be involved in vascular changes induced by pregnancy.

PPARs are ligand-activated nuclear transcription factors important in lipid metabolism and glucose homeostasis, and have recently been implicated in blood pressure control (16). PPARs are also important for early placental development (17). There is circumstantial evidence that vascular RGS5 expression correlates with PPAR activation (18-19). This establishes a potential link between vascular RGS5 signaling, metabolism, and pregnancy.

Here, we demonstrate a post-receptor regulatory role for RGS5 in pregnancy-induced hemodynamic adaptations. Reduction in RGS5 causes systemic vascular dysfunction leading to high arterial pressure, severe maternal hypertension, and increased preeclampsia risk. PPAR agonists emerge as effective regulators of RGS5 and blood pressure during pregnancy.

Results

RGS5 deficiency causes severe gestational hypertension

To test the hypothesis that RGS5 is involved in pregnancy-related vascular adaptation, we analyzed myometrial arteries from women with normal or hypertensive/preeclamptic pregnancies. *RGS5* expression was significantly ($P = 0.0015$) reduced in hypertensive/preeclamptic pregnancies, suggesting that low *RGS5* levels contribute to the pathophysiology (Fig. 1A, table S1). To gain further mechanistic insights, we employed *RGS5* knockout mice and recorded blood pressure daily throughout pregnancy using telemetry. Non-pregnant *RGS5* knockout mice were hypertensive when compared to wild type controls (14) (Fig. 1B). With pregnancy, *RGS5* deficiency caused more severe gestational hypertension, with daytime mean arterial blood pressure (MAP) of 145 ± 3.7 mmHg around day 12-14 of gestation (Fig. 1B). *RGS5* heterozygote knockout mice were normotensive but developed hypertension during pregnancy (116.9 ± 3.7 mmHg, d12) in a gene-dose-dependent manner (Fig. 1B). During

the nocturnal activity phase MAP values reached 182.6 ± 6.1 mmHg in RGS5 knockout females (Fig. 1, C-E). Activity and heart rate remained unchanged between groups (Fig. 1 C and D, and fig. S1, A and B), and hypertension was not dependent on genetic background (Fig. 1E and fig. S1C). From day 14, MAP decreases but only reaches pre-pregnancy levels 7 days after delivery (Fig. 1B). Consistent with a regulatory role during pregnancy, RGS5 levels increase in uterine arteries and peak around d12 (Fig. 1, F and G), whereas mRNA expression of vasoconstrictor and vasodilator receptors remains unchanged between wild type and RGS5 knockout mice (fig. S2, A-E).

RGS5 regulates vascular adaptation during pregnancy

During normal pregnancy, vasodilation reduces systemic vascular resistance and decreases blood pressure. Consistent with gestational hypertension in RGS5-deficient mice, the pregnancy-associated increase in blood flow in femoral and uterine vascular beds is halved in RGS5 knockout females (Fig. 2A). Moreover, reactivity to vasoactive factors is altered in uterine, mesenteric and femoral arteries (Fig. 2, B-E uterine arteries, and fig S3, A-D mesenteric and femoral arteries). Specifically, in pregnant RGS5-deficient mice the AngII-evoked reduction in uterine vascular conductance *in vivo* and constriction of isolated arteries is markedly enhanced (Fig. 2, B and D). Likewise, endothelium-mediated vasodilation of uterine arteries is diminished (Fig. 2, C and E). Thus, enhanced vasoconstriction and reduced endothelium-mediated vasodilation in RGS5-deficient vessels contribute to reduced blood flow and elevated blood pressure. The passive stress-strain relationship of uterine arteries is specifically altered in pregnant knockout mice, and is shifted to the right, indicating reduced stiffness, consistent with

remodeling as observed in small arterioles during hypertension (20) (Fig. 2F uterine arteries, and fig. S3, E and F mesenteric and femoral arteries).

Excessive AngII signaling causes oxidative stress in RGS5-deficient mice

Chronic treatment of RGS5 knockout pregnant females with ATR1 antagonists normalizes blood pressure demonstrating that ATR1 hyper-activation in the absence of RGS5 is responsible for gestational hypertension (Fig. 3A). AngII activates NADPH oxidases to generate reactive oxygen species (ROS). Excessive oxidative stress secondary to enhanced AngII signaling in RGS5 knockout vessels could explain endothelial vasodilator dysfunction (21). Indeed, arteries from d18 pregnant RGS5 knockout females produce more ROS (Fig. 3B uterine arteries, and fig. S4A mesenteric arteries). Treatment of RGS5 knockout mice with the anti-oxidant apocynin restores vascular responsiveness to ACh but not AngII *in vivo* (Fig. 3, C and D uterine arteries and fig. S4, B and C femoral arteries). The nitric oxide (NO) donor sodium nitroprusside (SNP) evokes larger increases in blood flow and vasodilation in arteries from d18 pregnant RGS knockout mice than wild type controls (Fig. 3, E and F uterine arteries, and fig. S4, D and E femoral and mesenteric arteries). Hypersensitivity to SNP can be an indicator of chronic endogenous NO deficiency (22), and indeed activation of eNOS in RGS5-deficient arteries is impaired (Fig. 3G). Moreover, vasodilator-stimulated phosphoprotein (VASP) serine 239 phosphorylation, a marker for the activity state of the NO-cGMP pathway (23), is enhanced under SNP stimulation (Fig. 3H). Collectively, these findings demonstrate a critical role for RGS5 in regulating AngII signaling and systemic and uteroplacental vascular tone in pregnancy.

RGS5 controls vascular adaptation in response to volume loading

The pathological rise in blood pressure in pregnant RGS5-deficient females mimics the kinetics of plasma volume expansion during murine and human pregnancy (24). Plasma volume expansion during pregnancy in wild type females is comparable to RGS5 knockout mice. Similarly, weight gain does not differ between genotypes, thus excluding high fluid retention as cause for MAP elevation in pregnant RGS5 knockout females (fig. S5, A and B). Instead, we hypothesized that failure to decrease peripheral resistance when plasma volume increases may cause hypertension on a RGS5-deficient background. To test this, we assessed blood pressure responses to chronic [deoxycorticosterone acetate (DOCA)/salt intake] and acute (saline infusion) hypervolemia. During periods of increased salt intake elevations of plasma volume, cardiac output and arterial pressure are expected (25). However, the rise in MAP after DOCA/salt intake, which is exacerbated in RGS5 knockout mice compared to controls, can be abrogated by ATR1 blockade (Fig. 4A). Similarly, RGS5 knockout mice demonstrate greater volume-induced (saline infusion) hypertension, which is abolished by ATR1 inhibition, whereas wild type mice show only mild volume-induced hypertension and non-significant reduction with ATR1 blockade (Fig. 4B). The loss of one RGS5 gene copy is sufficient to increase the MAP response to DOCA/salt intake and acute volume expansion (fig. S6, A and B). These results demonstrate that RGS5 regulates cardiovascular adaptation in response to hypervolemia, which is also an integral part of pregnancy.

RGS5 deficiency predisposes to preeclampsia

Despite hypertension, RGS5 homo- and heterozygote knockout females give birth to normal offspring (Table 1). However, significantly ($P = 0.001$) elevated soluble fms-like tyrosine

kinase-1 (sFLT), a factor which can be released by the ischemic placenta in hypertensive and/or preeclamptic pregnancies (26), in serum from RGS5 knockout mothers is indicative of underlying placental/vascular abnormalities (27) (Fig. 5A). To mimic high-risk pregnancies with strongly activated AngII signaling, such as in the presence of ATR1 autoantibodies (9) on a RGS5-deficient background, mice were infused with AngII from d10 to d18 of pregnancy (28); this increased d18 blood pressure more in hetero- or homozygote knockout compared with wild type mice (Fig. 5B). Proteinuria was induced with AngII infusion in RGS5-deficient females only (loss of one or two gene copies, Fig. 5C). Offspring from AngII-treated RGS5 hetero- or homozygote knockout females were more likely to experience intra-uterine growth restriction (IUGR) as compared to offspring born to wild type females (Fig. 5D, Table 1). Similarly, RGS5 deficiency caused a higher incidence of embryonic death with AngII hyper-stimulation (Table 1). Placental weight remained unchanged between groups (fig. S7), however, IUGR correlated with placental abnormalities including reduction in the embryonic labyrinth zone and expansion of the spongiotrophoblast layer (Fig. 5 E and F). Fetal capillaries in the labyrinthine layers were significantly ($P = 0.0001$) smaller in RGS5-deficient placentas and further constricted with AngII infusion (Fig. 5G and H) indicating that fetal and hence placental loss of RGS5 is a confounding factor in inducing IUGR, most likely by contributing to reduced fetal perfusion. However, fetal complications only arose in AngII-infused RGS5 hetero- or homozygote females (for example, maternal -/- x paternal +/+ pregnancies produced 24% dead embryos and IUGR, as compared to asymptomatic pregnancies seen in +/+ maternal x -/- paternal matings, Table 1), suggesting that alterations of the renin-angiotensin system in conjunction with maternal RGS5 reduction are an important risk factor. This is consistent with human pregnancies where onset of hypertension remote from term increases the risk of preeclampsia (29).

PPAR agonists act upstream of Rgs5 to abolish gestational hypertension and vessel dysfunction

RGS5 heterozygote knockout mice developed hypertension during pregnancy (Fig. 1B). Given the proposed role of PPARs in increasing vascular *Rgs5* expression (18-19), we assessed whether PPAR γ agonist treatment can rescue heterozygote mice from gestational hypertension. Chronic treatment of mice with a PPAR γ agonist (troglitazone) throughout pregnancy abolished hypertension in heterozygote but not homozygote RGS5 knockout mice (Fig. 6A). Troglitazone treatment did not change heart rate (fig. S8A). To further evaluate the therapeutic potential, RGS5 heterozygote mice were treated i.p. or by oral gavage with troglitazone or highly selective PPAR γ (GW1929) or PPAR β/δ (GW0742) agonists or a PPAR γ antagonist (GW9662), from day 10 of pregnancy when hypertension is evident. During the 8 days of treatment in pregnant heterozygote mice MAP was normalized to wild type levels (Fig. 6B i.p. treatment and fig. S8B oral gavage). Moreover, 8 days of troglitazone treatment abolished exacerbated hypertension in AngII-infused heterozygote d18 pregnant mice and normalized birth weight (fig. S8, C and D). PPAR γ agonists increased uterine *Rgs5* mRNA expression in heterozygote pregnant mice to wild type levels (Fig. 6C). Consistent with a protective role of PPAR γ in AngII-induced vascular damage (30), troglitazone improved endothelial function and normalized vascular responsiveness in uterine arteries *in vivo* (Fig. 6, D and E) and *ex vivo* (fig. S8, E and F). The vasculature of RGS5 knockout mice was not protected by PPAR γ agonists (Fig. 6, D and E, *in vivo* and fig. S8, G and H, *ex vivo*), providing evidence that PPARs act upstream of *Rgs5*. This establishes an important role of RGS5 in linking AngII and PPAR signaling to cardiovascular adaptation during pregnancy (Fig. 6F).

Systemic troglitazone treatment of heterozygote pregnant females also increases *Rgs5* mRNA expression in d18 placentas (fig. S9A). However, prolonged treatment over 25 days was

required to normalize the fetal blood supply (fig. S9B). Anti-hypertensive PPAR γ agonist treatment from mid- to end-term did not induce placental abnormalities or reduce birth weight in RGS5 heterozygote mice (fig. S9, C-F) and may thus be a potential therapy for severe cases of gestational hypertension or preeclampsia.

Discussion

The mechanisms contributing to peripheral vascular events of pregnancy remain poorly understood. Here, we establish RGS5 as a regulator of peripheral resistance to AngII and identify a mechanism of ATR1 signaling desensitization during pregnancy. In healthy pregnancy, vascular RGS5 concentration increases with plasma volume expansion and antagonizes AngII signaling. In contrast, reduced RGS5 expression causes severe endothelial dysfunction secondary to pathologically enhanced AngII signaling in a sequence of events involving superoxide production, eNOS uncoupling and impaired NO/cGMP signaling. Systemic vascular dysfunction then causes gestational hypertension and most likely reduces maternal-placental perfusion. This correlates with decreased fetal capillary sizes in RGS5 homozygote or heterozygote embryos, which may contribute to placental inefficiency. Despite vascular abnormalities, RGS5 homozygote and heterozygote knockout females produce normal litter sizes. This is consistent with lack of maternal proteinuria or organ damage. The degree of maternal and fetal vascular hyperconstriction and thus utero-placental perfusion in RGS5-deficient mice is strictly gene dose-dependent and only reaches pathological levels when superimposed with enhanced AngII stimulation. This could occur in pregnancies with elevated ATR1-activating autoantibodies, for instance (9). Further restriction of blood supply to embryos with enhanced AngII stimulation then results in preeclampsia-like symptoms such as proteinuria, intrauterine growth restriction

and loss of embryos. Although fetal RGS5 deficiency is a confounding factor, intrauterine growth restriction with enhanced AngII stimulation was only observed in mothers with reduced expression of RGS5.

In humans, increased sensitivity to AngII signaling is a hallmark of preeclampsia and also persists *post partum* (31), raising the possibility that it may precede pregnancy and pose an increased risk for pregnancy complications. Indeed, reduced RGS5 expression, as occurs in RGS5 heterozygote mice, triggers gestational hypertension in otherwise asymptomatic mice and increases the risk of preeclampsia. Therefore, our study demonstrates that pre-existing, sub-clinical maternal vascular dysfunction can result in a spectrum of pregnancy-related cardiovascular complications. This may be independent of early placentation events, therefore challenging the dogma that maternal endothelial dysfunction and preeclampsia arise exclusively due to poor placentation (1). Our data thus support the idea that cardiovascular complications arising well after pregnancy may be constitutional rather than consequent upon the hypertensive pregnancy (32-33).

So far, RGS molecules have not been linked to pregnancy-induced vascular adaptation or preeclampsia. However, *RGS5* expression in human myometrial vessels is reduced in gestational hypertension and/or preeclampsia (Fig. 1A) which provides clinical support for our murine findings. Another RGS family member, RGS2, blunts AngII signaling in patients with Bartter's/Gitelman's syndrome who present with normotension or hypotension and reduced peripheral resistance despite high levels of circulating AngII and aldosterone (34); this provides evidence for a delicate role of RGS molecules in regulating peripheral vascular resistance in response to increased renin-angiotensin activity.

Our data also show that anti-hypertensive effects of PPAR agonists during pregnancy are exclusively mediated by RGS5. PPARs are important in normal pregnancy because of their specific role in trophoblast invasion and placental development (17, 35). However, the role of PPARs in the maternal and/or uteroplacental vasculature in later pregnancy is less explored. PPAR γ inhibition during pregnancy impairs uteroplacental blood flow and induces preeclampsia-like symptoms in rats (36-37). In contrast, PPAR γ agonist treatment in a rat model of reduced uterine perfusion improves vascular function and hypertension (38). There is also emerging evidence that PPAR expression is associated with maternal metabolism and adverse pregnancy outcomes related to obesity and gestational diabetes mellitus (GDM) (39-40). Thus, the PPAR-RGS5-AngII signaling network presented here provides insights into how obesity and GDM may be linked to gestational hypertension and preeclampsia; this is important given the rapidly growing prevalence of obesity during pregnancy (41).

How RGS5 expression is regulated during normal pregnancy is currently unknown. There is evidence for transcriptional regulation of the *Rgs5* gene as part of dynamic cardiovascular adaptations to diverse stimuli, such as hypoxia, growth factor expression, or chronic AngII exposure (14, 42-43). However, there is no evidence for transcriptional regulation of *Rgs5* by GPCR agonists such as AngII (43-44), thus making it unlikely that RGS5 is directly regulated by the renin angiotensin system during pregnancy. Given the direct link between PPAR agonists and *Rgs5* vascular transcripts, it is possible that *RGS5* expression rises with increasing PPAR activation. PPAR γ activators such as fatty acids and lipid metabolites are upregulated in human serum during normal gestation and reduced with preeclampsia (39, 45). Circulating PPAR activator concentrations could be linked to the dynamic RGS5 expression we report here.

Furthermore, gene polymorphisms for both *PPAR* γ and *RGS5* have been implicated in human essential hypertension (46-49) and may also increase the risk for pregnancy-related disorders.

Our study suggests that optimal PPAR agonist treatment for RGS5-related hemodynamic maladaptations may be during the second half of pregnancy at the peak of volume expansion. PPAR γ agonist treatment from mid- to end-term did not induce placental abnormalities or reduce birth weight in our mouse model. Despite earlier clinical setbacks, the use of PPAR agonists remains attractive, and next generation drugs replacing thiazolidinediones are already in clinical use to treat type II diabetes and female infertility (50-51).

Current strategies for managing preeclampsia are inadequate and reflect a fundamental lack of understanding of the causes and pathogenesis of the disease (52). Consistent with other murine gene knockout studies (53), the RGS5 knockout model described here mimics part of the clinical spectrum and thus addresses only some aspects of the complex mechanisms underlying the disease. Nevertheless, our study identifies mechanisms that potentially contribute to preeclampsia and provides mechanism-guided treatment options for pregnancy-related hypertensive disorders, which warrant clinical investigation.

Direct targeting of the renin-angiotensin system in the management of pregnancy-related hypertension remains an important but so far unmet clinical need. Our study demonstrates that modulating RGS5 expression with PPAR agonists provides an opportunity to improve hypertension and pregnancy outcomes.

Materials and Methods

Study design

The purpose of the study was to demonstrate a specific vascular role of RGS5 in pregnancy. The hypothesis was based on our previous findings that RGS5 regulates angiogenic vessel remodeling and AngII signaling in mice (14-15); both features are integral parts of pregnancy. Human myometrial vessels from healthy pregnancies and women with pregnancy complications (inclusion criteria: gestational hypertension, MAP >105 mmHg, and preeclampsia, hypertension, MAP >105 mmHg, and at least 0.5 g protein/24 h urine collection after week 20 of gestation; exclusion criteria: diabetes, intrauterine growth restriction without hypertension/proteinuria) were analyzed to test the clinical relevance of RGS5. Human myometrial arteries from pregnant women (table S1) were obtained at delivery after informed written consent. The South Eastern Sydney Local Health District review board approved the human study. Quantitative *RGS5* analysis in human samples was done blindly. Mechanistic studies were conducted on RGS5 knockout and wild type mice of identical genetic background (C3HeBFe) with the exception of one control experiment where RGS5 knockout and wild type mice bred on a C57BL/6 genetic background were analyzed (fig. S1C). All experimental protocols involving mice were approved by the Animal Ethics Committee of the University of Western Australia. No randomization was used in animal experiments. No statistical method was used to predetermine sample size. No data (outliers) were excluded. At least three independent experiments were conducted. All data are presented directly, or as a representative figure, with quantification of experiments. Murine placental histopathology was conducted blindly. Drug studies are based on initial reports demonstrating a correlation between *Rgs5* expression and PPAR γ or PPAR δ agonist treatment (18-19). For clinical translation, drug experiments were conducted with different compounds

targeting the same pathway, different application routes (i.p. and oral gavage), and at different intervention times (prophylactic versus therapeutic).

Mice

Homo- or heterozygote RGS5 knockout females (54) on a C3HeBFe background (> 30 generations backcrossed) were studied between weeks 8 and 16 of age. MAP in pregnant RGS5 knockout females on a C57BL/6 background was analysed in comparison (fig. S1C). Mice were kept under specific pathogen-free conditions at the University of Western Australia. Timed matings were performed using standard operating procedures and mating plugs identified on day 1. On d18, embryo genotyping with the following RGS5 primers (RGS51: CTCCCTTAATGGACTCACTGAAGAA, RGS52: ACCATGGATTATCTCCTTTTGAATCT) was performed before placental analyses.

Chemicals and antibodies

The following drugs and doses were used: angiotensin II (Merck Millipore), 10 µg/kg for acute experiments, 1 mg/kg/day in minipumps, 10^{-10} to 10^{-5} M for myography; ATR1 antagonist (candesartan, Astra Zeneca), 10 mg/ml in drinking water; ACh (Sigma) 10 µg/kg *in vivo*, 10^{-10} to 10^{-5} M for myography, 5 µM for western blot analysis; SNP (Sigma) 200 µg/kg *in vivo*, 10^{-10} to 10^{-5} M for myography, 5 µM for western blot analysis; apocynin (Cayman Chemical) 1.5 mmol/L, added daily to the drinking water; troglitazone (Cayman Chemical) 10 µg/kg/day i.p. or 50 µg/kg/day oral gavage; GW9662 (Cayman Chemicals) 10 µg/kg/day i.p.; GW1229 (Adipogen) 10 µg/kg/day i.p. or 50 µg/kg/day oral gavage; GW0742 (Tocris) 5 µg/kg/day i.p. or 20 µg/kg/day oral gavage; lucigenin (Cayman Chemical) 5 µM. The following antibodies were purchased from Cell Signaling Technology: eNOS (rabbit polyclonal, 9572), phospho-eNOS

(Ser1177, rabbit polyclonal, 9571), VASP (9A2, rabbit monoclonal antibody); other antibodies were phosphoVASP-biotinylated (Ser239, 16C2, mouse monoclonal, Enzo Life Sciences), RGS5 (chicken polyclonal, XW-7534, ProSci Inc.), α SMA-FITC (1A4, mouse monoclonal, Sigma), CD31 (rabbit polyclonal, ab124432, Abcam).

Western blot analysis

Uterine arteries: vessels were snap-frozen and pulverized with mortar and pestle under liquid nitrogen, dissolved in 0.1% Triton lysis buffer (10 mM HEPES, pH 7.4, 50 mM sodium pyrophosphate, 50 mM NaF, 5 mM EDTA, 5 mM EGTA, 100 μ M Na₃VO₄, 50 mM NaCl, 0.1% Triton X-100, 500 μ M phenylmethylsulfonyl fluoride, and 10 μ g/ml leupeptin), sonicated and cleared by centrifugation. 100 μ g protein/lane was separated and transferred onto a nitrocellulose membrane and the bands were visualized with a chemiluminescence detection system (ECL Western Blotting Detection Reagents; Amersham Bioscience) and quantified. Mesenteric arteries: *ex vivo* phosphorylation studies and western blot analysis were performed as previously described (14).

Gene expression analysis

RNA from uterine or mesenteric arteries, murine placenta (dissected labyrinth layer), or human myometrial arteries was prepared using Trizol reagent (Invitrogen). Gene expression was quantified by qPCR using the Rotor Gene Real-Time PCR detection system (Qiagen). All reactions were normalized to hypoxanthine-guanine phosphoribosyltransferase (*Hprt/HPRT*).

Primer sequences: *Atr1a/b*: FP: TGTAAGATCGCTTCGGCCA, RP: AGGTAGCGATCGATGCTGAGA; *Atr2*: FP: GCATATGTCGGAAATGAATTCTGA, RP:

TCGAAAGCCCACGATTCACT; *BdkrB1*: FP: CGAAGCTTGGCACTTTGTAAGG , RP: AGCCAATGGGAGGAGGAAA; *BdkrB2*: FP: CAAGTCCTCGGGTCTGCTCTT, RP: CGGTGTCTGGGCAGTTGTC;

EtrA: FP: AACTAACCCTCCGCAGAAACAC, RP: GCGTAATGGTTGTTTCCATCCT; *EtrB*: FP: GTGGCTTCTTGGGGGTATGG, RP: TCTTAGTGGGTGGCGTCATTA; human *HPRT*: FP: CGTCTTGCTCGAGATGTGATG, RP: GCACACAGAGGGCTACAATGTG; mouse *Hprt*: FP: ACACCTGCTAATTTTACTGGCAACA, RP: TGGAAAAGCCAAATACAAAGCCTA; human *RGS5*: CAGAAAACCTCGCTGGACGA, RP: TGAAACTGGCAAGTCCATAGTTGT; mouse *Rgs5*: FP: GCTTTGACTTGGCCCAGAAA, RP: CCTGACCAGATGACTACTTGATTAGCT.

ELISA

ELISA for mouse VEGFR-1/FLT-1 (R&D Systems) was performed according to the manufacturer's instructions. Blood samples were diluted 1:25. Absorbance was determined at 450 nm and at 540 nm for wavelength correction (Biotek PowerWave XS2). All assays were performed in duplicates, and the protein concentrations were calculated using a standard curve derived from known concentrations of recombinant protein standards.

Histology and immunohistochemistry

Placentas were isolated from d18 pregnant females, fixed in 10% neutral buffered formalin, and embedded in paraffin. Five μ m sections were stained with hematoxylin & eosin (Sigma). For α SMA staining, placentas were deparaffinized, stained with primary antibody, and signals amplified using the mouse on mouse (M.O.M.) kit (Vector). For CD31 staining, deparaffinized

sections were stained with antibody followed by the ABC kit (Vector) and counterstained with methyl green. Stained sections were photographed with a Nikon microscope (Eclipse Ni) and 5 random fields/placenta were quantified using Nis Elements software (Nikon, version 4).

Hemodynamic measurements

Radiotelemetry devices (PA-C10, Data Sciences International, DSI) were implanted into females as previously described (14). Ten days later, females were timed mated, and their blood pressure, heart rate and locomotor activity (counts per min, as recorded by telemetry device and counted by the data acquisition system) were measured during pregnancy and 7 days *post partum*. Data were collected using the DataQuest ART system, version 4.1 (DSI). Blood pressure was recorded for 30 min/day (starting at 11 am). In addition, continuous (15 min sampling/hour) day and night recordings (12 h light to dark cycle) were collected over 24 h. Seven day release Alzet osmotic pumps (1007D) were implanted s.c. as described by the manufacturer at d10 of pregnancy. Intra-arterial blood pressure under general anesthesia (urethane, 1.1 g/kg) was recorded using a transducer-tipped catheter. Briefly, the right common carotid artery was catheterized with a Millar 1.4 French blood pressure probe and transducer (ADInstruments). The catheter was connected to a Millar transducer amplifier and PowerLab data acquisition system (PowerLab 16/35 and Lab Chart Pro Data Acquisition Software, ADInstruments). Vasoactive drugs were applied via intravenous bolus injections through a catheter in the femoral vein. Blood flow in femoral and uterine arteries was measured under general anesthesia (urethane, 1.1 g/kg) using the transit-time ultrasonic system (Transonic Systems, probe: 0.5 mm PS series). Flow was recorded with a T206 flowmeter (Transonic Systems). Conductance was calculated by dividing

the maximal blood flow (ml/s) by the mean arterial pressure (mmHg) recorded with a Millar 1.4-F probe and transducer inserted in the carotid artery.

Chronic and acute volume expansion

Chronic (high salt intake): mice were implanted with deoxycorticosterone acetate (DOCA) pellets (Innovative Research of America, 50 mg, 21 day release) following manufacturer instructions and provided with 1% (w/v) NaCl in the drinking water. Intra-arterial blood pressure was measured after 3 weeks in anesthetized mice. Acute (saline infusion): the volume expansion protocol was modified from (55). Mice were anesthetized (urethane, 1.1 g/kg), and the right common carotid artery was catheterized with a Millar 1.4 French blood pressure probe and transducer to record blood pressure throughout the procedure. A release catheter was introduced into the bladder. Mice were infused with lactated Ringer's solution with 4.5% BSA at a rate of 4.3 μ l/g/h through the femoral artery using an infusion pump (AL-2000, World Precision Instrument Inc.) for 15 min. This was followed by a higher infusion rate of 114 μ l/g/h for 30 min, which results in a volume increase corresponding to approximately 3% of body weight (55). The infusion rate was then lowered again to 4.3 μ l/g/h for 15 min. ATR1 inhibitor was provided via bolus injection through the femoral vein.

NADPH oxidase activity

Superoxide production in mesenteric and uterine arteries was measured using lucigenin-enhanced chemiluminescence. Arteries of 3 mm length and equal diameter were dissected using a graticule microscopic eyepiece. Vessels were pre-incubated in carbonated Krebs buffer containing NADPH (100 μ M) alone or in the presence of AngII (100 μ M) or apocynin (100 μ M) for 30 min

at 37°C in a 96-well plate (white, flat bottom, Nunc). Solutions were replaced with lucigenin (5 µM) containing buffers, and bioluminescence was detected in a microplate reader every second over 30 min (PHERAstar, BMG Labtech). Solutions without vessels served as control.

Determination of plasma volume

Plasma volume was determined by Evans Blue dye solution as published (56). After measurement of baseline absorbance at 620 nm, 30 µl of a 5 mg/ml Evans Blue (Sigma) saline solution was injected into the tail vein of pregnant mice at the indicated days. After 10 and 30 minutes, blood samples were collected by tail-vein puncture for determination of circulating Evans Blue concentration. Plasma was separated by centrifugation, and Evans Blue absorbance was read at 620 nm at a 1:5 dilution with a Nanodrop ND-1000 Spectrophotometer. Plasma Evans Blue concentration was calculated by extrapolation from an Evans Blue standard curve prepared in mouse plasma and is expressed in µl/g body weight.

Vascular reactivity and stress-strain relationship

Artery reactivity: first order mesenteric or uterine arteries 1-2 mm in length were mounted on a 4 channel wire myograph (Danish MyoTechnology) as described (14). Contractions are expressed as a percentage of the contraction evoked by high potassium physiological saline solution (KPSS, isotonic replacement of Na⁺ with K⁺). ACh-induced relaxation was assessed in arteries submaximally (70% of maximum) constricted with phenylephrine (PE) and relaxation was expressed as the percent decrease from pre-constriction levels. Curves were constructed using Prism (v.5.0; GraphPad Software), and sigmoidal curves were fitted to the data using the least squares method. Passive mechanical wall properties were assessed in femoral, mesenteric and

uterine arteries (3-4 mm long) mounted on a pressure myograph (Living Systems Instrumentation) as described previously (14). Briefly, arteries were continuously superfused at 15 mL/min with zero-Ca²⁺ physiological saline solution containing EGTA (2mM) bubbled with 95% O₂, 5% CO₂ at 36°C. Each artery was pressurised from 0 to 200 mmHg in 10 mmHg increments. Arterial dimensions (length, outside diameter and wall thickness (WT)) were measured at each 10 mmHg increment. Wall stress and strain were derived: wall stress (kPa) = (intraluminal pressure x internal diameter)/(2 x WT); wall strain = (internal diameter – internal diameter extrapolated to 5 mmHg pressure)/internal diameter extrapolated to 5 mmHg pressure.

Statistics

All data are presented as mean ± SEM or mean ± SD as indicated in the figure legends, and numbers of mice or tissue samples/group (n) are provided in figure legends. Comparisons between groups were performed by 1-way or 2-way ANOVA followed by the Tukey *post hoc* test for significance, unless state otherwise in figure legends. A *P* value ≤0.05 was considered significant.

Supplementary Materials

Fig. S1. Diurnal heart rate and genetic background in pregnant RGS5 knockout mice.

Fig. S2. *Gpcr* gene expression during pregnancy.

Fig. S3. Peripheral vascular dysfunction in pregnant RGS5 knockout mice.

Fig. S4. Oxidative stress and peripheral vascular function in RGS5-deficient mice.

Fig. S5. Volume expansion during pregnancy.

Fig. S6. RGS5 gene-dose-dependent blood pressure regulation in response to volume expansion.

Fig. S7. Placental weight in pregnancies with AngII infusion.

Fig. S8. PPAR agonist-mediated improvement of vascular function in HET mice.

Fig. S9. Troglitazone treatment and placental phenotype.

Table S1. Patient characteristics.

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Acknowledgments: We thank E. Bolitho and N. Johansen for technical assistance, G. Bergers and H. Ee for critical reading of the manuscript. **Funding:** This work was supported by the Royal Perth Hospital Medical Research Foundation (project grants to R.G., equipment grant to R.G. and D.J.M.), the Harry Perkins Institute of Medical Research, the National Health and Medical Research Council (NHMRC) of Australia (project grants to R.G. and L.F.A. APP1042446, fellowship to R.G., 634304), the Australian Government and the University of Western Australia (postgraduate scholarship to Y.S.C.). **Author contributions:** Conception and design: R.G., L.F.A.; development of methodology: V.H., Y.S.C., M.T., J.B., B.H.; analysis and interpretation of data: V.H., Y.S.C., J.B., M. P., M.T., B.H., A.K.C., D. J. M.; writing, review and revision of the manuscript: R.G., L.F.A., M.T., A.K.C.; administrative, technical, or material support: L.L., T.V.M, S.L.S. **Competing interests:** the authors declare that they have no competing interests.

Figure Legends:

Fig. 1. Loss of RGS5 correlates with preeclampsia in humans and causes gestational hypertension in mice. (A) Relative *RGS5* expression (normalized to *HPRT* mRNA) in human myometrial arteries from normal (n = 13) or hypertensive/preeclamptic pregnancies (n = 10), mean $\pm$ SEM. *P* value determined by Student's *t*-test. (B) MAP recordings during pregnancy and *post partum* in wild type (WT), *RGS5* homozygote (KO) and heterozygote (HET) knockout mice. A summary of 3 independent radiotelemetry experiments is shown (n = 14 for WT, n = 8 for HET, n = 15 for KO mice). Error bars represent mean $\pm$ SEM of 30 min recordings/day during daytime. (C) and (D) Day/night blood pressure recordings and activity as measured by telemetry in conscious (C) day 12-13 pregnant, and (D) day 18-19 pregnant WT and KO mice, n = 5 for all groups. (E) MAP differences (Δ MAP) between day and night in 12-13 day and 18-19 day pregnant females (night peak MAP on pregnancy day 12-13: WT 109.1 ± 4.2 , KO 182.6 ± 6.1 mmHg; pregnancy day 18-19, WT 103.2 ± 5.2 , KO 169.8 ± 3.8 mmHg), mean $\pm$ SEM, n = 5 mice for all groups. (F) Relative *Rgs5* mRNA expression (normalized to *Hprt* mRNA) in uterine arteries from non-pregnant (d0, n = 6 pools of 3 vessels each), pregnant day 6 (n = 4 pools), day 12 (n = 6 pools) and day 18 WT mice (n = 7 pools), d12 compared to d0; mean $\pm$ SEM; *P* value determined by Student's *t*-test. (G) *RGS5* protein expression in uterine arteries of WT mice during pregnancy as assessed by immunoblotting. *RGS5* protein expression was normalized to tubulin. Three independent experiments were quantified, mean $\pm$ SEM. A representative Western blot is shown below the graph.

Fig. 2. RGS5 regulates vascular adaptations during pregnancy. (A) Basal blood flow in femoral and uterine vascular beds as measured in anesthetized WT and KO mice, non-pregnant

(d0) or at d18 of pregnancy, $n = 3$ mice per group, mean $\pm$ SEM. **(B)** and **(C)** MAP and blood flow in uterine arteries of d18 pregnant WT and KO mice were measured to calculate conductance after bolus injection of **(B)** AngII (10 μ g/kg, indicated by arrow), or **(C)** acetylcholine (ACh, 10 μ g/kg, indicated by error) $n = 4$ mice per group, mean $\pm$ SEM (for ACh, error bars are within symbols). **(D)** AngII-mediated contraction in uterine arteries from d18 pregnant WT or KO mice, mean $\pm$ SEM, $n = 6$ vessels per group. **(E)** Endothelium-dependent relaxation induced by ACh in uterine arteries from d18 pregnant WT and KO mice submaximally constricted with phenylephrine (PE), mean $\pm$ SEM, $n = 6$ vessels per group. **(F)** Stress-strain relationship for uterine arteries from d18 pregnant mice, mean $\pm$ SEM, $n = 6$ vessels for WT, $n = 5$ vessels for KO.

Fig. 3. Excessive AngII signaling causes endothelial dysfunction on a RGS5-deficient background. **(A)** Telemetric recordings of MAP in KO treated with ATR1 inhibitor (candesartan) in the drinking water, $n = 6$ mice, mean $\pm$ SEM from 30 min recordings/day during daytime. Untreated groups are provided as reference. **(B)** NADPH oxidase activity in uterine arteries from WT and KO mice, untreated (control) or *ex vivo* treated with AngII or apocynin, expressed as % WT control. A summary of three independent experiments is shown, mean $\pm$ SD, $n = 8$ arteries per group. **(C)** and **(D)** WT and KO females were treated with apocynin in the drinking water from d10 to d18 of pregnancy. Conductance in uterine arteries after bolus injection of **(C)** ACh (10 μ g/kg), or **(D)** AngII (10 μ g/kg), mean $\pm$ SEM (for ACh, error bars are within symbols), $n = 4$ mice per group. **(E)** Conductance in uterine arteries from d18 pregnant WT and KO mice after stimulation with SNP (200 μ g/kg), mean $\pm$ SEM, $n = 3$ mice per group. **(F)** SNP evoked concentration-dependent relaxation in isolated uterine arteries from d18

pregnant WT and KO mice, mean $\pm$ SEM, n = 6 vessels per group. Negative logarithm of half maximal effective SNP concentration (pEC50): WT 7.1 ± 0.2 , KO 7.5 ± 0.2 , $P = 0.05$. **(G)** Activation of eNOS as determined by the ratio of phosphorylated (Ser 1177)/total eNOS in mesenteric arteries from d18 pregnant females, untreated (-) or after 15 min incubation with ACh (+, 5 μ M). **(H)** Ratio of phosphorylated (Ser 239)/total VASP in mesenteric arteries from d18 pregnant females, untreated (-) or after 15 min incubation with SNP (+, 5 μ M). **(G)** and **(H)**, densitometric phosphorylation data are presented as mean $\pm$ SD of 3 independent experiments. Representative Western blots are shown below the graphs.

Fig. 4. RGS5 deficiency exacerbates hypertension in response to volume loading. **(A)** MAP in anesthetized mice, untreated or 3 weeks after DOCA/salt-induction (difference in MAP WT: 40.1 ± 3.0 , KO: 63.6 ± 5.8 mmHg) or after DOCA/salt treatment combined with ATR1 inhibitor (candesartan) in the drinking water, mean $\pm$ SEM, n = 6 mice per group. **(B)** Changes from baseline MAP in WT and KO mice during saline infusion at 114 μ l/min for 30 min (Δ MAP WT: 6.5 ± 2.8 , KO: 20.4 ± 4.5 mmHg), or saline infusion in combination with bolus injection of ATR1 inhibitor, mean $\pm$ SEM, n = 3 mice per group.

Fig. 5. RGS5-deficiency increases the risk of developing preeclampsia. **(A)** Circulating sFLT concentrations in serum from non-pregnant (d0) and d18 pregnant females, mean $\pm$ SEM, n = 8-10 mice per group. **(B)** Mice were implanted with AngII minipumps (1 mg/kg/day) from d10 to d18 of pregnancy. MAP was measured in anesthetized mice at d18 (Δ MAP untreated versus AngII infusion: WT: 29.9 ± 2.0 ; HET: 43.0 ± 2.8 ; KO: 53.2 ± 3.3 mmHg), mean $\pm$ SEM, n = 5 mice per group, average litter size all pregnancies: n = 7. **(C)** Urinary albumin/creatinine ratio

($\mu\text{g}/\text{mg}$) for all genotypes after AngII infusion, $n=12-13$ mice. **(D)** Embryo weights at d18 of pregnancy with or without AngII infusion, mean $\pm$ SD, $n = 5$ mice, 27 embryos per group. **(E)** Ratio of labyrinth/total placental area, mean $\pm$ SD, $n = 5$ mice, 27 placentas per group. **(F)** Representative images from H&E stained placentas from AngII-treated WT, HET, and KO mice, with labyrinth zone delineated in black (left), or αSMA -stained sections (right), sp: spongiotrophoblast layer, la: labyrinthine layer. Scale bar, 1000 μm . **(G)** Quantification of the total area of fetal capillary lumens in placental labyrinthine layers from untreated or AngII-infused mice of different genotypes, $n = 8$ mice (3 placentas/mouse), mean $\pm$ SEM. **(H)** Representative staining of CD31^+ lined fetal vessels in the labyrinthine layer from three independent experiments. One vessel per microscopic field is delineated (dotted line) to illustrate vessel size. Scale bar, 10 μm . KO refers to $-/-$ females and $-/-$ embryos, HET to $+/-$ females and selective analysis of $+/-$ embryos.

Fig. 6. PPAR agonist treatment abolishes hypertension and vascular dysfunction in RGS5 HET but not KO mice. **(A)** Telemetric MAP recordings in heterozygote (HET, $n = 6$) and homozygote (KO, $n = 4$) RGS5-deficient mice, treated with 10 $\mu\text{g}/\text{kg}/\text{day}$ troglitazone (trog) throughout pregnancy, mean $\pm$ SEM from 30 min recordings/day during daytime. **(B)** HET mice were left untreated or treated from d10 to d18 of pregnancy with the following drugs delivered i.p.: GW1969 (PPAR γ agonist, 10 $\mu\text{g}/\text{kg}/\text{day}$), GW9662 (PPAR γ antagonist, 10 $\mu\text{g}/\text{kg}/\text{day}$), GW0742 (PPAR β/δ agonist, 5 $\mu\text{g}/\text{kg}/\text{day}$), troglitazone (PPAR γ agonist, 10 $\mu\text{g}/\text{kg}/\text{day}$). MAP was recorded in anesthetized mice on d18, mean $\pm$ SEM, $n = 3$ mice per group. **(C)** Relative *Rgs5* mRNA expression (normalized to *Hprt* mRNA) in uterine arteries from d18 pregnant WT, untreated HET, or HET mice after troglitazone injection (i.p., 10 $\mu\text{g}/\text{kg}$), $n = 6$ mice for all groups, mean $\pm$ SEM. **(D)** and **(E)** Conductance in uterine arteries of untreated and troglitazone-

treated HET or KO mice after bolus injection of **(D)** AngII (10 µg/kg), or **(E)** ACh (10 µg/kg), mean ± SEM (for ACh, error bars are within symbols), n = 4 mice per group. **(F)** Schematic summary of the PPAR-RGS5-AngII signaling network in pregnancy: (i) circulating PPAR ligands (35) and components of the renin-angiotensin system such as AngII (6) are upregulated during normal pregnancy, (ii) activated PPARs increase *Rgs5* gene transcription, (iii) increased RGS5 decreases AngII sensitivity.

Table 1. Genotype and survival of offspring from RGS5 mutant mice.

Parental genotype: maternal x paternal	Total number of offspring d18	Number of offspring grouped by genotype:			Number of resorbed embryos	IUGR
		+/+	+/-	-/-		
<i>untreated</i>						
+/+ x +/+	86	86	NA	NA	2	no
+/- x +/-	66	17	34 (1)*	14	0	no
-/- x +/+	50	NA	50	NA	1	no
+/+ x -/-	42	NA	42	NA	0	no
+/- x -/-	87	NA	43	41 (3)	0	no
-/- x -/-	110	NA	NA	110	5	no
<i>AngII infusion</i>						
+/+ x +/+	49	49	NA	NA	4	no
+/- x +/-	61	16	28 (2)	11 (4) [†]	8	yes
-/- x +/+	45	NA	34 (11) ^{‡ §}	NA	8	yes
+/+ x -/-	40	NA	40	NA	2	no

+/- x -/-	108	NA	51	45 (12) [¶]	5	yes
-/- x -/-	75	NA	NA	48 (27) [¶]	12	yes

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* number of dead embryos is shown in parentheses. [†] $P = 0.037$ compared to +/- x +/- mating without AngII infusion. [‡] $P = 0.001$ compared to +/+ x -/- AngII matings and [§] $P < 0.001$ compared to -/- x +/+ untreated matings. [¶] $P = 0.046$ compared to +/- x -/- untreated matings. ^{||} $P < 0.001$ compared to -/- x -/- untreated matings. Embryonic death is independent of parental genotype in untreated groups ($P = 0.091$), but significantly dependent on parental genotype in AngII-treated groups ($P < 0.001$), Pearson's χ^2 test. IUGR: intrauterine growth restriction; NA: not applicable.

Regulator of G protein signaling 5 is a determinant of gestational hypertension and preeclampsia

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Supplementary Materials

Fig. S1

Fig. S1. Diurnal heart rate and genetic background in pregnant RGS5 knockout mice. (A and B) Day/night heart rate recordings as measured by telemetry in conscious (A) day12-13 or (B) day 18-19 pregnant wild type (WT) and RGS5 knockout (KO) mice, $n = 5$ for all groups. (C) MAP recordings in anaesthetized d18 pregnant WT and KO females bred on a C57BL/6 background, mean $\pm$ SEM, $n = 6$ mice for all groups.

Fig. S2

Fig. S2. *Gpcr* gene expression during pregnancy. (A-E) Relative mRNA expression (normalized to *Hprt* mRNA) in uterine arteries from wild type (WT) and RGS5 knockout (KO) mice, non-pregnant (d0), pregnant day 6 (d6), day 12 (d12), or day 18 (d18) of (A) *Atr1*; (B) endothelin receptor type A (*EtrA*); (C) endothelin receptor type B (*EtrB*); (D) bradykinin receptor B1 (*BdkrB1*), *P* values determined by Student's *t*-test; (E) bradykinin receptor B2 (*BdkrB2*), mean $\pm$ SEM, $n = 9$ pools of 3 vessels each for all groups. *Atr2* expression was below detection level in all groups.

Fig. S3

Fig. S3. Peripheral vascular dysfunction in pregnant RGS5 knockout mice. (A and B)

Conductance in femoral arteries of d18 pregnant WT and KO mice after bolus injection of (A) AngII (10 µg/kg), or (B) ACh (10 µg/kg), $n = 4$ mice per group, mean $\pm$ SEM (for ACh, error bars are within symbols). (C) AngII-mediated contraction in mesenteric arteries from d18 pregnant WT or KO mice. Contractions are expressed as a percentage of the contraction evoked by high-potassium physiological salt solution, mean $\pm$ SEM, $n = 5$ per group, $**P = 0.01$. (D) Endothelium-dependent relaxation induced by ACh in mesenteric arteries submaximally

constricted with PE from d18 pregnant WT and KO mice, mean $\pm$ SEM, n = 6 vessels per group.

(**E** and **F**) Stress-strain relationships for (**E**) mesenteric and (**F**) femoral arteries from d18 pregnant mice, mean $\pm$ SEM, n = 5 vessels for KO, n = 6 vessels for WT.

Fig. S4

Fig. S4. Oxidative stress and peripheral vascular function in RGS5-deficient mice. (A) NADPH oxidase activity in mesenteric vessels from wild type (WT) and RGS5 knockout (KO) mice, untreated (control) or *ex vivo* treated with AngII or apocynin, expressed as % WT control. A summary of three independent experiments is shown, mean $\pm$ SD, $n = 6$ per group. (B and C) Pregnant WT and KO females were treated with apocynin from d10 to d18 of pregnancy. Conductance in femoral arteries after bolus injection of (B) ACh (10 μ g/kg), or (C) AngII (10

$\mu\text{g/kg}$) was determined, mean $\pm$ SEM (for ACh, error bars are within symbols), $n = 4$ mice per group. **(D)** Conductance in femoral arteries from d18 pregnant females after SNP bolus injection ($200 \mu\text{g/kg}$), mean $\pm$ SEM, $n = 3$ mice per group. **(E)** SNP-evoked concentration-dependent relaxation in mesenteric arteries from d18 pregnant WT and KO mice, mean $\pm$ SEM, $n = 5$ vessels per group, ($p\text{EC}_{50}$ WT: 7.0 ± 0.1 , KO: 7.6 ± 0.1 , $P = 0.05$).

Fig. S5

Fig. S5. Volume expansion during pregnancy. **(A)** Plasma volume in wild type (WT) and RGS5 knockout (KO) mice was determined during the course of pregnancy and *post partum* using the Evans Blue dye test, mean $\pm$ SD, $n = 5$ mice per group. **(B)** Percent weight gain from d1 to d18 of pregnancy in females treated with or without AngII infusion from d10 to d18, mean $\pm$ SEM, $n = 6$ mice per group; ns, not statistically significant between groups.

Fig. S6

Fig. S6. *Rgs5* gene-dose-dependent blood pressure regulation in response to volume expansion. (A) MAP changes relative to untreated baseline values (Δ MAP) recorded in anesthetized wild type (WT), RGS5 knockout (KO), and heterozygote (HET) mice 3 weeks after DOCA/salt-induction, mean $\pm$ SEM, $n = 6$ mice per group. (B) Changes in MAP from baseline (Δ MAP) in WT, KO, and HET mice recorded during acute volume expansion with saline infusion at a low rate of 4.3 μ l/g/h for 15 min, followed by a high rate of 114 μ l/g/h for 30 min and again 4.3 μ l/g/h for 15 min, mean $\pm$ SEM, $n = 3$ mice per group.

Fig. S7

Fig. S7. Placental weight in pregnancies with AngII infusion. Wild type (WT), RGS5 knockout (KO) and heterozygote (HET) mice were infused with AngII from d10 to d18 of pregnancy and placental weight was determined at d18 of pregnancy.

Fig. S8

Fig. S8. PPAR agonist-mediated improvement of vascular function in HET mice. (A) Heart rate in RGS5 homozygote (KO) or heterozygote (HET) mice with or without troglitazone treatment. Depicted are average heart rates measured during daytime in non-pregnant (d0) and pregnant mice at day 12 or day 18 of pregnancy, mean $\pm$ SEM, $n = 6$ mice for HETS, $n = 4$ mice for KO. (B) HET mice were left untreated or treated from d10 to d18 of pregnancy with the

following drugs given by oral gavage: GW1969 (PPAR γ agonist, 50 μ g/kg), GW0742 (PPAR β/δ agonist, 20 μ g/kg), troglitazone (PPAR γ agonist, 50 μ g/kg). MAP was recorded in anesthetized mice. HET untreated are shown as reference, mean $\pm$ SEM, n = 3 mice per group. (C) MAP in d18 pregnant untreated WT mice, HET treated with AngII (reference only, data shown in Fig. 5B), or d18 pregnant HET infused with AngII (from d10 to d18) with daily i.p. troglitazone (10 μ g/kg) injections, n = 3 mice. (D) Weights of untreated WT embryos, heterozygote embryos at d18 of pregnancy with AngII infusion (reference only, data shown in Fig. 5D), or AngII infusion combined with troglitazone i.p. injections, mean $\pm$ SD, n = 3 mice, 21 embryos for HET AngII + trog. (E) and (F) Uterine arteries from untreated d18 pregnant HET mice, or HET mice treated with troglitazone from d10 to d18 of pregnancy (HET + troglitazone). (E) AngII-mediated contraction. (F) Endothelium-dependent relaxation induced by ACh in arteries submaximally constricted with PE, mean $\pm$ SEM, n = 5 vessels per group, *** P = 0.05. (G) and (H) Uterine arteries derived from KO mice on d18 of pregnancy, or KO mice treated with troglitazone from d10 to d18 (KO + trog). (G) AngII-mediated contraction. (H) Endothelium-dependent relaxation induced by ACh in arteries submaximally constricted with PE, mean $\pm$ SEM., n = 6 vessels per group.

Fig. S9

Fig. S9. Troglitazone treatment and placental phenotype. (A) Relative *Rgs5* mRNA expression (normalized to *Hprt* mRNA) in mouse placenta (labyrinth) after troglitazone (trog) i.p. injection. HET, n= 6 mice (12 placentas), HET trog, n=3 (6 placentas), *P* value determined

by Student's *t*-test. **(B)** Quantification of the total area of fetal capillary lumens in placental labyrinthine layers from untreated d18 pregnant WT and HET (reference only, data as shown in Fig. 5G) or HET treated for 8 days (d10 to d18 of pregnancy) with troglitazone +/- AngII infusion, or HET treated with troglitazone for 25 days throughout pregnancy, *n* = 8 mice (3 placentas/mouse), mean $\pm$ SEM. **(C)** Embryo weight on day 18 of pregnancy in heterozygote RGS5 knockout mice (HET) without treatment or treated for 8 days (d10 to d18) with 50 μ g/kg/d troglitazone by oral gavage, mean $\pm$ SEM, *n* = 4 mice, 26 embryos for untreated, and *n* = 7 mice, 43 embryos for treated groups. **(D)** Corresponding placental weights, mean $\pm$ SEM, *n* = 4 mice. **(E)** Representative H&E stained placental sections with labyrinth zone delineated in black (left), or α SMA-stained sections (right) for untreated or troglitazone-treated HET mice; sp: spongiotrophoblast layer, la: labyrinthine layer. Scale bar, 1000 μ m. **(F)** Ratio of labyrinth/total placental area measured on midline sections of H&E stained placentas from HET mice (*n* = 3 mice, 16 placentas), troglitazone-treated HET from d10 to d18 of pregnancy (HET trog 8 days, 50 μ g/kg/d, oral gavage, *n* = 4 mice, 23 placentas), and HET treated with troglitazone throughout pregnancy (HET trog 25 days, 10 μ g/kg/d, i.p. , *n* = 4 mice, 23 placentas), mean $\pm$ SD.

Table S1: Patient characteristics

Pregnancy status	Age	Body mass index [kg/m²]	MAP [mmHg]	Gestation [days]	<i>RGS5</i> [relative mRNA]
normal	37	23.1	83	268	46.5
normal	39	18.5	76	271	14.7
normal	32	23.8	83	267	15.5
normal	30	22.5	85	272	6.3
normal	42	17.5	83	270	17.0
normal	40	25.9	78	278	17.0
normal	29	27.9	85	272	9.6
normal	36	18.0	70	272	7.2
normal	31	25.3	78	266	28.3
normal	39	25.6	83	270	9.7
normal	33	31.3	100	270	7.3
normal	42	21.3	87	273	14.7
normal	35	22.6	83	270	11.4
hypertension	40	27.6	121	264	3.8
hypertension	31	26.1	107	268	2.1
hypertension	42	26.8	122	280	5.2
hypertension	37	29.0	128	278	3.2
hypertension	31	42.0	110	267	1.9

hypertension	31	28.0	108	259	2.6
hypertension/preeclampsia*	34	22.9	133	259	0.7
hypertension/preeclampsia*	30	28.4	130	214	4.5
hypertension/preeclampsia*	40	24.3	120	244	4.1
hypertension/preeclampsia*	34	24.7	123	250	2.2

*At least 0.5 g protein/24 h urine collection after week 20.

All pregnancies: no pre-existing hypertension; singleton fetuses; delivery via Caesarean section. Sample collection: myometrial vessels were collected in oxygenated physiological salt solution and snap frozen. Normal pregnant: age, 35.8 ± 4.5 ; body mass index: $23.3 \pm 4.0 \text{ kg/m}^2$; MAP $82.6 \pm 6.9 \text{ mmHg}$; gestation: $270.7 \pm 3.0 \text{ days}$, $n = 13$. Hypertensive/preeclamptic pregnant: age, 35.0 ± 4.4 ; body mass index: $28 \pm 5.3 \text{ kg/m}^2$, $P = 0.032$ compared to normal pregnancies, Mann-Whitney U test; MAP $120.2 \pm 9.1 \text{ mmHg}$, $P < 0.001$ compared to normal pregnancies, Mann-Whitney U test; gestation: $258.3 \pm 19 \text{ days}$, $n = 10$, mean $\pm$ SD.