Maternal metabolism and vascular adaptation in pregnancy: the PPAR link

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Abstract

Current therapies for pregnancy-related hypertension and its complications remain inadequate although an increasing role of maternal susceptibility is becoming evident. Systemic vascular dysfunction in response to imbalances in angiogenic, inflammatory and constricting factors is implicated in the pathogenesis of gestational hypertension and growing evidence now links these factors with maternal metabolism. In particular, the crucial role of peroxisome proliferator-activated receptors (PPARs) in maternal vascular adaptation provides further insights into how obesity and gestational diabetes may be linked to pregnancy-induced hypertension and preeclampsia. This is especially important given the rapidly growing prevalence of obesity during pregnancy and highlights a new approach to treat pregnancy-related hypertension and its complications.

Keywords: pregnancy, preeclampsia, PPAR, metabolism, RGS5, blood vessel
Hypertensive disorders of pregnancy: an unresolved clinical problem

Pregnancy-associated hypertensive disease remains a common and serious clinical problem with far-reaching health implications for mother and child, even after birth. Hypertensive disorders of pregnancy comprise a group of clinical presentations which all share pathologically high blood pressure [1]. Amongst these, the pregnancy-specific syndrome preeclampsia is defined as hypertension, usually diagnosed after 20 weeks of gestation, in association with multiple other criteria including proteinuria or thrombocytopenia, liver and kidney insufficiencies and/or stroke [2]. Importantly, preeclampsia represents a spectrum of diseases with different etiologies and progressive clinical manifestations; 15-25% of women presenting with new onset or gestational hypertension, and 50% of women with pre-existing or chronic hypertension will eventually develop preeclampsia [3-4]. The heterogeneity of symptoms and disease progression has so far defied development of prognostic markers or specific treatment options beyond carefully timed delivery [2]. Considerable evidence supports a key role of ischemic placental injury (see Glossary) in the pathogenesis of preeclampsia [5]. Chronic imbalance of circulating inflammatory or angiogenic factors provides a plausible link between early placental stress and preeclampsia-mediated injury of the maternal vasculature (Box 1) [6-7]. However, as much as preeclampsia is multifaceted, disease manifestation involves diverse mechanisms that can act simultaneously or sequentially. Most likely, disease progression is shaped by reciprocal interactions between the placenta and the mother and thus pre-existing maternal genetic or metabolic conditions leading to endothelial dysfunction will influence pregnancy outcomes. For instance, maternal obesity predisposes to a significantly higher risk for gestational hypertension, gestational diabetes and preeclampsia [8]. Moreover, obesity combined with insulin resistance and gestational diabetes confers an even higher preeclampsia risk than either condition alone indicating additive effects of maternal metabolic stress and endothelial dysfunction on
disease development [9]. Thus, poor maternal vascular health is a substantial risk factor for pregnancy complications and warrants exploration of new and improved therapeutic interventions. PPARs are important regulators of lipid and glucose metabolism and PPAR agonists are used in the clinical management of type II diabetes [10]. PPAR ligands also control blood pressure and endothelial function and emerging evidence supports their application in gestational hypertension [11]; new mechanistic insights into how PPAR ligands could improve maternal cardiovascular health in complicated pregnancies are discussed below.

Maternal vascular health: lessons from epidemiology and genetics

Thus far, the strongest evidence for a crucial role of pre-existing maternal vascular dysfunction in complicated pregnancies comes from epidemiological and genetic studies. For instance, population-based studies show that pregnancy outcome is a predictor of long-term cardiovascular health in women. In particular, early onset and severe preeclampsia are associated with increased risk of maternal cardiovascular disease; this includes a fourfold higher risk for hypertension, a twofold increased risk for ischemic heart disease and stroke, and an overall higher mortality [19]. It is less clear, however, whether preeclampsia itself causes maternal vascular damage and metabolic changes that in turn lead to cardiovascular disease later in life. Alternatively or additionally, a genetic or metabolic predisposition in the mother may cause endothelial dysfunction first in response to pregnancy and later, in conjunction with other confounding factors such as age (Figure 1). A Norwegian population study measured cardiovascular risk factors before and after pregnancy in an attempt to differentiate between pregnancy-induced and pre-existing risk factors such as body mass index, high-density lipoprotein cholesterol and triglycerides, and blood pressure. These
results suggest that pre-existing cardiovascular risk factors are more important determinants of subsequent cardiovascular disease than the hypertensive pregnancy itself [20]. Moreover, familial predisposition for preeclampsia has motivated a plethora of genetic studies including linkage studies, candidate gene approaches and transcriptome analyses of maternal and fetal tissue [21-22]. Consistent with findings in other complex disorders, no single susceptibility gene for preeclampsia has so far been identified. Instead, there is accumulating evidence that genes associated with preeclampsia are also risk factors for cardiovascular disease in the general population and involve the renin-angiotensin system (RAS), body weight regulators or vasoactive and inflammatory factors; these findings further support the notion that pre-existing risk factors contribute to preeclampsia and long-term cardiovascular health in the mother [21, 23]. Interestingly, epigenetic gene modifications are also implicated in the development of preeclampsia. A comparative DNA methylation study in omental vessels, a vessel type which regulates maternal peripheral vascular resistance, from normal and preeclamptic pregnancies identified a suite of genes known to regulate vessel contraction, thrombosis, oxidative stress and inflammation [24]. For instance, one of the most hypomethylated genes is thromboxane synthase; reduced methylation of the thromboxane synthase gene promoter leads to increased gene expression in preeclamptic vessels and may increase synthesis of thromboxane A2, a potent vasoconstrictor and activator of platelets [25]. Furthermore, pathway analysis of preeclamptic omental vessels revealed methylation-induced retinoid X receptor (RXR) and PPAR gene silencing that links loss of RXR/PPAR protein dimers to pro-inflammatory effects in endothelial cells or vascular smooth muscle cells (vSMCs) and potential maternal vascular dysfunction [24]. Thus, DNA methylation patterns and therefore vascular function can potentially be modulated by pregnancy, or in response to maternal factors such as weight, age and other environmental stimuli [26]. Irrespective of whether pregnancy induces vascular damage or merely unmasks subclinical symptoms, the
maternal vasculature is critical for disease progression as well as clinical management before
and after delivery [6]. In fact, physiological adaptations to pregnancy which include increases
in cardiac output, heart rate and plasma volume represent a major cardiovascular challenge
for the mother and are a significant “stress test” to expose underlying cardiovascular
problems [27].

Cardiovascular adaptations and angiotensin signaling during pregnancy

The RAS is a crucial regulator of vascular constriction and increased activity causes
hypertension. Consistent with more relaxed blood vessels, pregnant women develop a marked
resistance to the vasoconstrictor peptide angiotensin II (AngII) as part of normal adaptive
processes [28]. In contrast, women with preeclampsia or gestational hypertension are highly
sensitive to the effects of AngII [6]. Furthermore, agonistic autoantibodies against AngII
receptor type 1 (ATR1) are present in the circulation of 70% of preeclamptic women and
antibody titers correlate with disease severity [29]. Indeed, these antibodies when injected
into pregnant mice elevate anti-angiogenic factors and induce preeclampsia-like symptoms
and intrauterine growth restriction (IUGR); these symptoms, including hypertension and
placental abnormalities, can be alleviated in mice with losartan, an ATR1 antagonist,
demonstrating the crucial role of the RAS in maintaining maternal vascular health and
uteroplacental perfusion [30-31]. Similarly, in a rodent model of RAS-induced preeclampsia,
features of human pregnancy disorders such as placental abnormalities, IUGR and aberrant
vascular reactivity are recapitulated [32]. Due to the crucial role AngII and RAS play in both
normal and pathological pregnancy, safeguard mechanisms are required to regulate the
delicate RAS balance. This includes potential downregulation of ATR1 in blood vessels and
subsequent desensitization of vSMCs to AngII stimulation; expression of Angiotensin-(1-7),
a peptide component of the RAS system generated by AngII converting enzyme 2 (ACE2),
which increases during pregnancy to counteract AngII signaling; or alternatively, balanced heterodimer formation of ATR1 with another G protein coupled receptor (GPCR) family member, \textbf{bradykinin receptor}. These heterodimers have been shown to increase AngII hypersensitivity in preeclamptic patients [33]. Recently, another mechanism has been identified which controls AngII signaling at a post-receptor level, and involves the family of regulator of G protein signaling (RGS) molecules ([11], see below). Whilst the physiology of maternal vascular adaptation to pregnancy is well delineated, it is less clear how maternal lifestyle and obesity is linked to RAS hyperactivity and vascular maladaptations.

\textbf{Pregnancy and metabolic syndrome}

Metabolic syndrome represents a spectrum of metabolic abnormalities that manifest as abdominal obesity, atherogenic dyslipidemia, insulin resistance, thrombosis and/or inflammation and confers increased risk for cardiovascular diseases such as hypertension, heart disease and type II diabetes. All factors associated with the metabolic cluster are linked to endothelial dysfunction, which is also a prominent feature of preeclampsia. The normal physiological response to pregnancy induces a transient state of metabolic adaptations; in complicated pregnancies, metabolic syndrome can precede gestation or develop during pregnancy [34]. For example, during normal pregnancy most plasma lipid components increase due to hormonal shifts, including cholesterol, triglycerides and various forms of low density (LDL) and high density lipoprotein (HDL) particles. Hypercholesterolemia and hypertriglyceridemia of pregnancy are physiologically important to meet increasing metabolic demands during gestation and milk production after delivery [35]. However, in preeclampsia triglyceride levels rise even further and are accompanied by an increased LDL/HDL ratio, thus reducing potentially cardioprotective HDL levels [36]. This ‘atherogenic’ lipid profile contributes to inflammation and oxidative stress [37]. Similarly,
insulin resistance develops as part of normal adaptive metabolic changes in pregnant women and correlates with elevated lipid concentrations, as gestation advances. Not surprisingly, insulin resistance increases in preeclampsia together with dyslipidemia, endothelial abnormalities and hypertension [34]. Upregulation of coagulation factors during pregnancy prevents excessive bleeding during delivery, but also increases thrombotic risk. Abnormal coagulation and resistance to natural anticoagulants such as protein C can cause thrombotic events in multiple organs during preeclampsia [38]. Unfortunately, the incidence of obesity and its consequent cardiovascular sequelae has now reached epidemic dimensions in the general population [39]. With approximately 60% of women of reproductive age being obese in the developed world, pregnancy complications associated with obesity or metabolic syndrome have become a major health concern [8].

**Role of PPARs in metabolic syndrome and pregnancy**

PPARs (PPARα, PPARβ/δ, PPARγ) are ligand-activated transcription factors that have emerged as crucial regulators of lipid and glucose homeostasis. They function as obligate heterodimers with RXR, bind to peroxisome proliferator response elements (PPRE) on the promoter of target genes, and control transcription of genes involved in metabolic function. Indeed, PPARγ modulation with synthetic ligands, members of the thiazolidinediones family (TZDs), e.g., rosiglitazone and troglitazone, has been clinically used as an insulin sensitizer in the treatment of type II diabetes [10]. Thus, the role of PPARs in metabolic disease is undisputed. Moreover, PPARs have been extensively studied in pregnancy. Most of these studies focus on early embryonic development and demonstrate a crucial role for a balanced PPARγ activity in trophoblasts (Box 2). However, beyond the fetal-placental interface, PPARs are crucial for maternal pregnancy adaptations by virtue of regulating inflammation,
angiogenesis and oxidative stress in reproductive and non-reproductive organs [26, 40].

During normal human pregnancy, potential PPARγ activators such as certain prostanoids or fatty acid derivatives are upregulated in maternal serum [41]. In preeclamptic pregnancies, circulating PPARγ ligands are suppressed even before the onset of maternal symptoms [42]. Consistently, administration of PPARγ antagonist from gestational day 11 to 15 in pregnant rats results in preeclampsia-like symptoms such as elevated blood pressure, proteinuria, endothelial dysfunction and increased platelet aggregation [43]. In contrast, PPARγ agonist treatment improved pregnancy outcome in the reduced uterine perfusion pressure (RUPP) model of rat preeclampsia by ameliorating oxidative stress in a heme oxygenase (HO)-1-dependent pathway [44]. This is consistent with HO-1 being a PPARγ target gene in human vSMC and endothelial cells [45]. Interestingly, women with rare dominant-negative PPARγ mutations, not only display symptoms such as dyslipidaemia, early onset insulin resistance, gestational diabetes, hypertension and polycystic ovarian syndrome, but also pre-eclamptic pregnancies [46] (Table 1). So far, maternal PPARγ function during pregnancy, its role in gestational hypertension, and potential target genes is less explored.

PPARs regulate endothelial function and blood pressure homeostasis

That PPARγ agonists lower blood pressure in diabetic patients and, conversely, early onset hypertension develops in humans with rare genetic PPARγ defects, implies a regulatory role of PPARs beyond lipid metabolism. PPARγ is expressed in endothelial cells and vSMCs of the vasculature that further supports a possible direct effect in blood pressure homeostasis [53]. Indeed, TZD treatment of spontaneously hypertensive or AngII-induced hypertensive rodents improves vascular function and lowers blood pressure. A series of murine studies using vessel-specific PPARγ knockout or transdominant negative mutants demonstrate that
PPARγ in endothelial cells protects from oxidative stress and increases NO production [54]. Loss of PPARγ specifically in vSMCs in adult mice results in exacerbated AngII-induced remodeling of mesenteric arteries, oxidative stress and impaired endothelial relaxation [55]. Similarly, vSMC-specific expression of dominant negative PPARγ mutants increases blood pressure, reduces NO-mediated aortic relaxation, and increases myogenic tone and AngII-induced constriction of small resistance arteries [56-57]. Whilst these findings provide a rationale for the protective cardiovascular effects of TZDs, the identity of downstream PPARγ effectors in blood vessels remains largely elusive. Equally, little is known about how PPARγ may affect maternal vascular adaptations during pregnancy. Interestingly, PPARγ mRNA expression in rodent pregnant or non-pregnant uterine arteries is increased over mesenteric vessels, and PPARγ inhibition in the second half of pregnancy specifically impairs uterine vessel dilation in vitro. These findings, together with decreased fetal birth weight as a result of PPARγ inhibition, indicate a role for PPARγ in uteroplacental blood flow [58].

RGS5 and pregnancy-associated hypertension

The ATR1 receptor is a prototypic GPCR that associates with heterotrimeric G proteins to transduce signals from the cell surface into the cytoplasm. Duration and signal intensity of GPCRs can be modified by RGS molecules [59]. RGS5 is a family member that is highly expressed in vSMCs and plays a pivotal role in vascular pathologies (Box 3). RGS5 is a negative modulator of AngII/ATR1 signaling in vitro and in humans RGS5 gene polymorphism has been associated with hypertension in the general population [60-62] (Table 1). Moreover, RGS5-deficient mice are hypertensive due to vascular hyper-responsiveness to AngII. Elevated blood pressure in knockout mice can be normalized with anti-ATR1 treatment, demonstrating that RGS5 regulates blood pressure homeostasis.
downstream of ATR1 and RGS5 levels determine vascular AngII sensitivity [63]. Increased vascular AngII reactivity predicts for gestational hypertension and preeclampsia [64]. Interestingly, RGS5 expression is dynamically regulated during murine pregnancy, and also highly suppressed in myometrial vessels from hypertensive or preeclamptic women; these findings suggest that RGS5-mediated inhibition of ATR1 signaling may be an integral part of vascular adaptations during pregnancy. Indeed, hypertensive RGS5-deficient mice develop severe gestational hypertension when pregnant [11]. Mechanistically, hypertension is associated with increased vascular constriction and blunted relaxation indicative of systemic peripheral vascular dysfunction. Vascular reactivity can be restored with antioxidant treatment in vivo, which is consistent with reduced NO bioavailability and impaired NO/cGMP signaling secondary to oxidative stress in RGS5 knockout mice. Embryo weight or litter size are not affected in these females even though circulating levels of sFLT are increased. Interestingly, RGS5 heterozygote knockout mice are normotensive but develop hypertension during pregnancy. If homo- or heterozygote RGS5 mice are further challenged with AngII infusion to mimic circulating agonistic ATR1 autoantibodies [29], mice develop preeclampsia-like syndrome with proteinuria and IUGR. Importantly, preeclampsia associated features directly correlate with RGS5 levels in the mother indicating that maternal vascular health is a strong determinant of pregnancy outcome [11]. It is not yet known whether RGS5 polymorphisms are associated with preeclampsia. However, mutations in another RGS family member, RGS2, are associated with enhanced risk to develop preeclampsia and post-pregnancy hypertension [65] (Table 1). Whilst RGS2 plays a crucial role in controlling vessel constriction and relaxation [66], mechanistic insights into potential pregnancy-related disorders are so far lacking.

**Linking PPAR signaling with RGS molecules**
A functional link between PPARs and RGS molecules, in particular RGS4 and 5, has been suggested in rodent models of atherosclerosis. PPARβ agonists ameliorate disease in mouse models of experimentally enhanced atherosclerosis by suppressing inflammation. This beneficial effect correlates with upregulation of RGS transcripts [74-75]. Similarly, lipid lowering and anti-inflammatory drugs of the statin family such as the 3-hydroxy-3methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor pravastatin, upregulate RGS5 in aortas of ApoE-deficient mice subjected to a high fat diet. Indeed, a direct role of RGS5 in protecting from atherosclerotic plaque formation has been demonstrated in RGS5 gene deficient mice [73]. Similar to PPARs, RGS5 appears to protect from sequelae of the metabolic syndrome such as diet-induced obesity, hepatic steatosis, inflammation, and insulin resistance [76]. Intriguingly, PPARβ agonists lower blood pressure in spontaneously hypertensive rats in correlation with increased RGS5 vascular expression, thus providing the first evidence for a potential PPAR-RGS link in controlling blood pressure [77]. In contrast, enhanced vasoconstriction in dominant negative PPARγ mutants reduces RGS5 expression. Importantly, identification of a PPRE element in the murine RGS5 gene suggests a potential direct transcriptional control of RGS5 by PPARs [57].

**The AngII-PPAR-RGS5 signaling axis**

If RGS5 regulates vascular sensitivity to AngII in response to PPARs, PPAR activation should result in higher RGS5 levels and thus suppression of AngII signaling. Indeed, hypertension seen in pregnant heterozygote but not homozygote RGS5-deficient mice is abolished by treatment with the PPAR-γ agonist, troglitazone, which also improves endothelial function and vascular responsiveness in maternal arteries [11]. This treatment increases vascular RGS5 expression in heterozygote mice to wild type levels. Of clinical
interest, PPARγ agonist treatment is effective in heterozygote RGS5 knockout mice in a therapeutic setting (from mid-gestation to term) when hypertension has already developed, but treatment itself does not cause placental abnormalities or reduced birth weight [11]. These findings are consistent with earlier observations that PPARγ agonist treatment improves pregnancy outcome in preeclamptic rats [44], and provide the first genetic evidence for a direct control of blood pressure homeostasis by PPAR-mediated transcriptional regulation of RGS5. Furthermore, PPARβ agonist treatment also ameliorates AngII-induced experimental hypertension in mice via upregulation of RGS5 [78].

Chronic and gestational hypertension in RGS5-deficient mice are abolished when AngII signaling is blocked for instance by using ATR1 or ACE inhibitors. However, direct targeting of the RAS in the management of pregnancy-related hypertension remains difficult because ACE inhibitors and ATR blockers are toxic to the embryo. Thus, controlling AngII signaling indirectly by modulating RGS5 levels becomes an appealing therapeutic approach for hypertensive disorders of pregnancy. Identification of a direct link between PPARs and RGS5-regulated vascular hypersensitivity to AngII strongly suggests that PPAR agonists or potentially statins might be a useful therapy to treat gestational hypertension and preeclampsia (Key figure 2).

Concluding Remarks and Future Perspectives

Control of hypertension during pregnancy is currently restricted to short-acting antihypertensive agents such as general vSMC relaxants, alpha/beta adrenergic antagonists and calcium channel blockers (e.g., hydralazine, labetalol, nifedipine, respectively). These treatments are recommended for use during pregnancy when benefits outweigh risks [79]. Experimental therapeutic interventions for preeclamptic women or those at risk of developing preeclampsia include supplementation with anti-oxidants such as vitamin C and E, and
improvement of vasodilator activities, e.g., by providing NO precursor L-arginine, **CO and hydrogen sulphide (H₂S)** donors [80]; these interventions are so far of variable benefits. Cholesterol-lowering statins (HMG-CoA reductase inhibitors), that are already widely used to prevent primary and secondary cardiovascular disease in the general population are also considered in preeclampsia [81]. Besides their lipid-lowering effects, statins target inflammation, oxidative stress and coagulation which are all features of the metabolic syndrome [82]. Furthermore, statins induce HO-1 which in turn lowers sFLT-1 and sEng in the circulation [83]. More recently, statins have also been shown to lower blood pressure [84]. Thus, the distinctive anti-inflammatory and antioxidant effects of statins, together with lipid-lowering activity, could have substantial vascular benefits in pregnancies complicated by obesity, type II diabetes and hypertension (see Outstanding Questions). Although a clinical trial (Statins to Ameliorate Early Onset Preeclampsia, StAmP, EudraCT 2009-012968-13) into safety during pregnancy has not concluded, preliminary evidence suggests that there are no increased risks for fetal development with early exposure [85-86].

Strikingly similar to statins, PPARs have key regulatory capabilities in metabolic processes, inflammation, oxidative stress and blood pressure. Intriguingly, recent evidence demonstrates that statins may exert their lipid-lowering and cardioprotective effects via PPARs [87]. In obese, hypertensive LDL receptor/leptin double knockout mice, treatment with statins normalized blood pressure in correlation with upregulation of PPARγ [88]. Mechanistically, statins induce cyclooxygenase (COX)-2, an enzyme involved in the synthesis of prostanoids, which increases the native PPARγ ligand prostaglandin J2 for instance in vSMCs [89]. Consistent with RGS5 being a direct PPARγ target, RGS5 is amongst the most upregulated genes in aorta following statin treatment in mice [90], protects from inflammation and metabolic dysfunction [76], and regulates blood pressure homeostasis [11]. Overall, this provides a compelling reason to further explore PPARγ agonist treatment in pregnancy
complications, in particular for managing maternal symptoms. PPARγ has been proposed as a potential therapeutic target to ameliorate placental deficiencies [47, 91]. In mice, there is some controversy over potential adverse effects of the PPARγ agonist rosiglitazone on the placenta when applied during early embryonic development at high dose (100 mg/kg/day) rather than moderate doses (5-10 mg/kg/day) [40, 44, 92]. In humans, early inadvertent rosiglitazone exposure in pregnant women had no adverse effect on fetal development [85, 93-94]. Whilst encouraging, drug safety needs to be assessed in appropriate clinical trials. Treatment with PPARγ agonist troglitazone as late as mid-gestation (10 mg/kg/day) is highly effective in normalizing blood pressure in mice [11]. Thus, targeting PPARs at the time when hypertension arises may improve maternal symptoms without affecting early placental development. Currently, second generation PPAR modulating agents with improved safety profiles are in clinical trials and combined statin/PPAR agonist treatment shows synergistic therapeutic efficacy in dyslipidemic patients [95]; if safe, these effects may be extended to preeclamptic patients.

With deeper understanding of various molecular pathways associated with hypertensive pregnancy complications it becomes evident that no single treatment will cure preeclampsia. There is also compelling evidence for a crucial role of maternal factors in the clinical manifestation of a disease that shares striking features with the metabolic syndrome. Exploring new treatment options that simultaneously target multiple maternal symptoms may prevent disease progression, reduce preterm deliveries and minimize long-term health risks for mother and child.
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Glossary:

Atherosclerosis: narrowing of arteries through fat deposition (plaque build-up) that reduces blood flow and can lead to plaque rupture, a major cause of myocardial infarction/stroke.

Bradykinin receptor (BDKRB2): G protein coupled receptor (GPCR) that binds the vasodepressor hormone bradykinin. ATR1/BDKRB2 heterodimerization may confer AngII hypersensitivity.

Cholesterol, triglycerides and lipoproteins: two types of lipoproteins that carry cholesterol to and from cells are low-density lipoprotein (LDL), and high-density lipoprotein (HDL). Triglycerides are fatty acid esters of glycerol and represent the main lipid component of dietary fat. High levels of either LDL cholesterol or triglycerides increase the risk for cardiovascular disease.

Carbon monoxide (CO) and hydrogen sulphide (H2S): gaseous vasodilators that maintain the vascular tone. Unlike NO and CO, which mediate vasorelaxation largely by activating the cGMP pathway in endothelial cells, the vasorelaxant effect of H2S acts on vascular smooth muscle cells (vSMCs) independent of the cGMP pathway.

Epigenetic gene modifications: changes in gene expression without alterations of the genetic code. Involves covalent DNA modification such as methylation (addition of methyl (CH3) group to DNA) and can be transitory or heritable.
**Insulin resistance:** when muscle, fat, and liver cells do not respond properly to insulin secreted by the pancreas and fail to absorb glucose from the blood. Chronic insulin resistance can lead to type II diabetes.

**Ischemic placental injury:** placental malperfusion due to higher pressure/fluctuating oxygen supply that causes ischemia-reperfusion type injury. The resulting oxidative stress may induce placental secretion of inflammatory and antiangiogenic factors.

**Leptin:** hormone that regulates energy balance and food intake.

**Myogenic tone:** intrinsic property of vSMCs, which enables vSMCs to contract in response to an increase in blood pressure without innervation or hormonal stimulation.

**Myometrial vessels:** blood vessels of the myometrium, the middle layer of the uterine wall that induce uterine contractions. Contractility of radial arteries of the uterus is highly significant for pregnancy and delivery.

**Nitric oxide (NO)/cGMP singaling:** NO activates soluble guanylate cyclase (sGC), an enzyme that converts guanosine triphosphate (GTP) to cyclic guanosine monophosphate (cGMP); cGMP is an intracellular messenger that in vSMCs stimulates relaxation.

**Oxidative stress:** imbalance between the production of reactive oxygen species (for instance free radicals, superoxide anions, hydrogen peroxide) and antioxidant defences. Oxidative stress in the placenta can arise due to organ malperfusion.

**Prostanoids:** relate predominantly to the products of the cyclooxygenase pathway, prostaglandins, prostacyclins, and thromboxanes. Certain prostaglandin related compounds are natural PPARγ agonists, the most notable PPAR ligand being 15-deoxy-Delta12-14-Prostaglandin J2 (15d-PGJ2).

**Renin angiotensin system (RAS):** hormone system that regulates blood pressure and fluid balance. Renin converts angiotensinogen into angiotensin I. Angiotensin converting enzyme (ACE) metabolizes angiotensin I into the active vasoconstrictor hormone angiotensin II
(AngII) which binds to two subtypes of cell surface receptors, type I and type II (ATRI and ATR2).

**Trophoblasts**: are peripheral cells of the blastocyst. They adhere to the maternal uterus and are precursors of the placenta. The inner cellular layer is the cytotrophoblast and the outer layer is the syncytiotrophoblast. Trophoblasts form the major part of the placenta and provide nutrients to the embryo.

**Box1: Circulating factors and maternal vascular dysfunction**

Impaired or shallow trophoblast invasion in the early stages of gestation causes insufficient utero-placental blood flow and placental hypoxia/ischemia. In response to this oxidative stress, the placenta releases inflammatory factors and microparticles which are implicated in systemic maternal vascular dysfunction and clinical symptoms such as hypertension and end organ damage [2]. While low-grade systemic inflammation during pregnancy is normal, increased inflammatory factors such as Interleukin (IL)-1β, IL-6, C-reactive protein and tumor necrosis factor (TNF)α are associated with preeclampsia [12]. Placental factors such as small membrane-coated vesicles and micro RNA have also been implicated in chronic inflammation and vascular dysfunction [13]. In preeclampsia, excessive inflammation correlates with an imbalance of angiogenic factors. For instance, vascular endothelial growth factor (VEGF) and placental growth factor (PIGF) levels are reduced. In contrast, soluble VEGF receptor 1 (or fms-related tyrosine kinase-1, s-Flt-1) increases and competes with VEGF and PIGF for receptor binding. This in turn induces an anti-angiogenic state which is associated with systemic vascular dysfunction [7]. sFLT-1 can be secreted by the placenta following oxidative injury, but is also induced by platelet-monocyte aggregates [14]. Tissue factor (TF), an essential regulator of hemostasis, mediates release of sFLT-1 by monocytes following inflammation, thus linking inflammation, thrombosis and defective angiogenesis.
Moreover, utero-placental oxidative stress increases local and systemic levels of reactive oxygen species (ROS) which includes molecules such as hydrogen peroxide, superoxide and peroxynitrite, and the free radical nitric oxide (NO). Superoxide and peroxynitrite formation uncouple endothelial NO synthase (eNOS) activity and restrict bioavailability of NO with profound effects on maternal vascular relaxation [16]. Vessel dilatation can be further decreased by soluble endoglin (sEng), an anti-angiogenic factor released by the stressed placenta; sEng inhibits vascular signaling through transforming growth factor (TGF)-β receptor and reduces NO production. Heme oxygenase (HO)-1 is a key enzyme that regulates blood pressure homeostasis through the production of carbon monoxide (CO); reduced HO-1 levels in preeclamptic placentas further promotes release of anti-angiogenic factors such as sFLT-1 and sEng, and peripheral pathogenesis [17]. Interestingly, HO-1 is also a potent anti-inflammatory and anti-oxidative molecule which improves insulin signaling and dyslipidemia in a model of essential hypertension and thus links the placenta with systemic immunological and metabolic dysfunction [18].

Box 2: Role of PPARγ in placental maturation

Besides controlling glucose homeostasis, PPARγ is crucial for early placentation events, in particular trophoblast invasion and differentiation [47]. PPARγ is highly expressed in cytotrophoblasts and syncytiotrophoblasts in human placentas and in the trophoblast labyrinthine zone in the mouse. Murine studies have confirmed the importance of PPAR signaling in vivo. Deletion of PPARγ or RXR genes in mice results in premature fetal loss at midgestation (embryonic day 9.5 to 10.5) due to substantial placental abnormalities related to trophoblast differentiation and vascularization. These defects are phenocopied in both PPARγ and RXR knockout strains, thus demonstrating the functional relevance of heterodimeric signaling in early placental development [48]. Among potential PPARγ target genes that
execute trophoblast differentiation and function are genes encoding mucin-1 (MUC-1) and glial cell missing-1 (Gcm-1). The MUC1 protein is localized to the apical surface of the labyrinthine trophoblast around maternal blood sinuses and an active PPAR binding site has been identified in the proximal Muc1 promoter [49]. Gcm-1 deficient mice die at midgestation due to the absence of syncytiotrophoblasts and a placental labyrinth, and there is emerging evidence for regulation of Gcm-1 by synthetic PPARγ ligands [50]. PPARβ/δ and PPARα are also expressed in placenta, but gene knockout studies show less severe phenotypes. For instance, PPARβ/δ knockout mice can give birth to viable offspring (>10%) and PPARα-deficient mothers have a higher spontaneous abortion rate but surviving embryos develop to term [51-52].

**Box 3: RGS5 and its role in the cardiovascular system**

GPCRs are prominently involved in physiological and pathological signaling. Due to their regulatory role in a wide spectrum of diseases, including cardiovascular disease, they are prime pharmacological targets. Ligand binding of GPCRs induces the Gα subunit of associated heterotrimeric G proteins to exchange guanidine diphosphate (GDP) for guanidine triphosphate (GTP) and to dissociate from the Gβγ unit; this process enables both subunits to signal autonomously. The family of RGS molecules acts predominantly as GTPases (GAPs) for Gα proteins (Gαi/o, Gαq/11, Gαs) and accelerates the hydrolysis of GαGTP to GαGDP [59]. Thus, RGS molecules promote signal termination and therefore are considered negative regulators of GPCR signaling. RGS5 is prominently expressed in vascular tissue, in particular arteries [67] and has been shown to regulate Gαq, Gαi and Gα12/13 signaling [63, 68]. Human and mouse RGS5 mRNA are 90% identical indicative of conserved regulatory functions across species [59]. Murine RGS5 is encoded by 5 exons, and intron 1 contains important regulatory elements including a functional PPRE [57]. Moreover, there is evidence
that transcriptional regulation of the RGS5 gene may also be influenced by promoter methylation and epigenetic mechanisms [69]. RGS5 is a small protein which consists of a 120 aa conserved RGS domain which confers GTPase activity and a 33 aa N-terminus with a cysteine (C2) residue which targets it for rapid degradation. As a substrate for the N-end rule degradation pathway, RGS5 protein degradation/stabilization provides responsiveness to incoming environmental cues such as changing oxygen levels [70]. Indeed, RGS5 expression levels are dynamically regulated in cardiovascular pathologies indicative of a role in adaptive processes. Besides control of blood pressure homeostasis, this includes a crucial role in atherosclerosis, tumor angiogenesis, arteriogenesis and cardiac adaptation following pressure overload [63, 68, 71-73].
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Figure legends:

Figure 1: Pregnancy- and age-related hypertension.
Genetic or metabolic predisposition in a normotensive mother causes endothelial dysfunction which in turn may trigger hypertension during pregnancy, followed by post-menopausal hypertension induced by age-related changes in hormone and metabolic status. This hypothesis is consistent with a high risk of long-term cardiovascular disease in women with hypertensive/preeclamptic pregnancies. Graph has been modified from [6].

Key figure 2: RGS5 is a key mediator of vascular PPARγ effects.
Endogenous PPARγ ligands or synthetic agonists (TZD, thiazolidinediones) exert pleiotrophic non-vascular and vascular effects, similar to statins. PPARγ activation of vSMCs increases translocation into the nucleus and transcription of target genes such as RGS5. RGS5 is a negative regulator of GPCRs including the AngII receptor (ATR1). Reduction in MAP kinase signalling (ERK1/2), nuclear factor (NFκB) and NADPH oxidase activities improves vascular function and vessel relaxation. RGS5 mediates vascular AngII resistance. Thus, increasing RGS5 expression via TZD treatment decreases AngII sensitivity and blood pressure during pregnancy [11]. Statins by virtue of increasing PPARγ vascular expression via COX-2 dependent synthesis of the prostaglandin 15d-PGJ2 may regulate similar signalling pathways [87].
Table 1. Association of human PPARγ, RGS2 and RGS5 gene polymorphisms with hypertensive and metabolic disorders.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Genetic variation</th>
<th>Population</th>
<th>Phenotype</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPARγ1</td>
<td>Pro467Leu Val290Met</td>
<td>3 individuals (2 females/1 male)</td>
<td>Early onset hypertension, type II diabetes, insulin resistance</td>
<td>[46]</td>
</tr>
<tr>
<td>PPARγ2</td>
<td>Pro12Pro homozygotes</td>
<td>Caucasian</td>
<td>Postmenopausal hypertension</td>
<td>[96]</td>
</tr>
<tr>
<td></td>
<td>Pro12Ala</td>
<td>Chinese</td>
<td>Hypertension, metabolic lipid disorder</td>
<td>[97]</td>
</tr>
<tr>
<td>PPARγ3</td>
<td>C681G (rs10865710)</td>
<td>Chinese</td>
<td>Hypertension</td>
<td>[98]</td>
</tr>
<tr>
<td>RGS2</td>
<td>C1114G (rs4606)</td>
<td>Caucasian (Norwegian HUNT2 study)</td>
<td>Preeclampsia, Hypertension after pregnancy</td>
<td>[65], [99]</td>
</tr>
<tr>
<td>RGS5</td>
<td>rs2815272</td>
<td>African Americans</td>
<td>Hypertension</td>
<td>[60]</td>
</tr>
<tr>
<td></td>
<td>rs12041294C/T</td>
<td>Chinese</td>
<td>Hypertension, Lipid metabolism</td>
<td>[61]</td>
</tr>
<tr>
<td></td>
<td>rs10917690A/G</td>
<td>Chinese</td>
<td>Hypertension</td>
<td>[62]</td>
</tr>
<tr>
<td></td>
<td>rs10917695T/C</td>
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<td>[62]</td>
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<td></td>
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<td></td>
<td>rs16849802</td>
<td>Chinese</td>
<td>Hypertension</td>
<td>[62]</td>
</tr>
</tbody>
</table>
Blood pressure

- Subclinical
- Hypertensive
- Normal

Genetic/metabolic predisposition
Normal

Pre-pregnancy
Pregnancy
Post-menopausal

Age
Inflammation
Oxidative stress
Vascular tone

Non vascular effects

Insulin sensitization
Glucose homeostasis
Lipid metabolism

Endogenous PPARγ ligands, TZDs

Statins, e.g., pravastatin

Vascular effects

COX-2/15d-PGJ2

Vascular effects

PPARγ
RXR

RGS5

ATR1

ERK 1/2 NFκB
NADPH oxidase

Inflammation
Oxidative stress
Vascular tone

Endogenous PPARγ ligands, TZDs

Statins, e.g., pravastatin

vSMC membrane
cytoplasm
nucleus

vSMC

Insulin sensitization
Glucose homeostasis
Lipid metabolism

Non vascular effects