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

1 results suggest that pre-existing cardiovascular risk factors are more important determinants
2 of subsequent cardiovascular disease than the hypertensive pregnancy itself [20]. Moreover,
3 familial predisposition for preeclampsia has motivated a plethora of genetic studies including
4 linkage studies, candidate gene approaches and transcriptome analyses of maternal and fetal
5 tissue [21-22]. Consistent with findings in other complex disorders, no single susceptibility
6 gene for preeclampsia has so far been identified. Instead, there is accumulating evidence that
7 genes associated with preeclampsia are also risk factors for cardiovascular disease in the
8 general population and involve the **renin-angiotensin system** (RAS), body weight regulators
9 or vasoactive and inflammatory factors; these findings further support the notion that pre-
10 existing risk factors contribute to preeclampsia and long-term cardiovascular health in the
11 mother [21, 23]. Interestingly, **epigenetic gene modifications** are also implicated in the
12 development of preeclampsia. A comparative DNA methylation study in omental vessels, a
13 vessel type which regulates maternal peripheral vascular resistance, from normal and
14 preeclamptic pregnancies identified a suite of genes known to regulate vessel contraction,
15 thrombosis, oxidative stress and inflammation [24]. For instance, one of the most
16 hypomethylated genes is thromboxane synthase; reduced methylation of the thromboxane
17 synthase gene promoter leads to increased gene expression in preeclamptic vessels and may
18 increase synthesis of thromboxane A₂, a potent vasoconstrictor and activator of platelets [25].
19 Furthermore, pathway analysis of preeclamptic omental vessels revealed methylation-induced
20 retinoid X receptor (RXR) and PPAR gene silencing that links loss of RXR/PPAR protein
21 dimers to pro-inflammatory effects in endothelial cells or vascular smooth muscle cells
22 (vSMCs) and potential maternal vascular dysfunction [24]. Thus, DNA methylation patterns
23 and therefore vascular function can potentially be modulated by pregnancy, or in response to
24 maternal factors such as weight, age and other environmental stimuli [26]. Irrespective of
25 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, **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.

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 (PPAR α , PPAR β/δ , PPAR γ) 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, PPAR γ 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 PPAR γ 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 PPAR γ activators such as certain **prostanoids** or fatty acid derivatives are upregulated in maternal serum [41]. In preeclamptic pregnancies, circulating PPAR γ ligands are suppressed even before the onset of maternal symptoms [42]. Consistently, administration of PPAR γ 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, PPAR γ 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 PPAR γ target gene in human vSMC and endothelial cells [45]. Interestingly, women with rare dominant-negative PPAR γ 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 PPAR γ function during pregnancy, its role in gestational hypertension, and potential target genes is less explored.

PPARs regulate endothelial function and blood pressure homeostasis

That PPAR γ agonists lower blood pressure in diabetic patients and, conversely, early onset hypertension develops in humans with rare genetic PPAR γ defects, implies a regulatory role of PPARs beyond lipid metabolism. PPAR γ 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 PPAR γ knockout or transdominant negative mutants demonstrate that

PPAR γ in endothelial cells protects from oxidative stress and increases NO production [54]. Loss of PPAR γ 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 PPAR γ 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 PPAR γ effectors in blood vessels remains largely elusive. Equally, little is known about how PPAR γ may affect maternal vascular adaptations during pregnancy. Interestingly, PPAR γ mRNA expression in rodent pregnant or non-pregnant uterine arteries is increased over mesenteric vessels, and PPAR γ 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 PPAR γ inhibition, indicate a role for PPAR γ 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. PPAR β 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, PPAR β 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 PPAR γ 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

1 interest, PPAR γ agonist treatment is effective in heterozygote RGS5 knockout mice in a
2 therapeutic setting (from mid-gestation to term) when hypertension has already developed,
3 but treatment itself does not cause placental abnormalities or reduced birth weight [11]. These
4 findings are consistent with earlier observations that PPAR γ agonist treatment improves
5 pregnancy outcome in preeclamptic rats [44], and provide the first genetic evidence for a
6 direct control of blood pressure homeostasis by PPAR-mediated transcriptional regulation of
7 RGS5. Furthermore, PPAR β agonist treatment also ameliorates AngII-induced experimental
8 hypertension in mice via upregulation of RGS5 [78].

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

19 **Concluding Remarks and Future Perspectives**

20 Control of hypertension during pregnancy is currently restricted to short-acting
21 antihypertensive agents such as general vSMC relaxants, alpha/beta adrenergic antagonists
22 and calcium channel blockers (e.g., hydralazine, labetalol, nifedipine, respectively). These
23 treatments are recommended for use during pregnancy when benefits outweigh risks [79].
24 Experimental therapeutic interventions for preeclamptic women or those at risk of developing
25 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 PPAR γ [88]. Mechanistically, statins induce cyclooxygenase (COX)-2, an enzyme involved in the synthesis of prostanoids, which increases the native PPAR γ ligand prostaglandin J2 for instance in vSMCs [89]. Consistent with RGS5 being a direct PPAR γ 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 PPAR γ agonist treatment in pregnancy

1 complications, in particular for managing maternal symptoms. PPAR γ has been proposed as a
2 potential therapeutic target to ameliorate placental deficiencies [47, 91]. In mice, there is
3 some controversy over potential adverse effects of the PPAR γ agonist rosiglitazone on the
4 placenta when applied during early embryonic development at high dose (100 mg/kg/day)
5 rather than moderate doses (5-10 mg/kg/day) [40, 44, 92]. In humans, early inadvertent
6 rosiglitazone exposure in pregnant women had no adverse effect on fetal development [85,
7 93-94]. Whilst encouraging, drug safety needs to be assessed in appropriate clinical trials.
8 Treatment with PPAR γ agonist troglitazone as late as mid-gestation (10 mg/kg/day) is highly
9 effective in normalizing blood pressure in mice [11]. Thus, targeting PPARs at the time when
10 hypertension arises may improve maternal symptoms without affecting early placental
11 development. Currently, second generation PPAR modulating agents with improved safety
12 profiles are in clinical trials and combined statin/PPAR agonist treatment shows synergistic
13 therapeutic efficacy in dyslipidemic patients [95]; if safe, these effects may be extended to
14 preeclamptic patients.

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

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3 Hospital Medical Research Foundation, the Western Australian Cancer Council, the
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5 the Harry Perkins Institute of Medical Research.

6

7 **Glossary:**

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

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

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

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

22 **Epigenetic gene modifications:** changes in gene expression without alterations of the
23 genetic code. Involves covalent DNA modification such as methylation (addition of methyl
24 (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 signaling: 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 PPAR γ 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)-1 β , 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 (PlGF) levels are reduced. In contrast, soluble VEGF receptor 1 (or fms-related tyrosine kinase-1, s-Flt-1) increases and competes with VEGF and PlGF 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

[15]. 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 PPAR γ in placental maturation

Besides controlling glucose homeostasis, PPAR γ is crucial for early placentation events, in particular trophoblast invasion and differentiation [47]. PPAR γ 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 PPAR γ 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 PPAR γ and RXR knockout strains, thus demonstrating the functional relevance of heterodimeric signaling in early placental development [48]. Among potential PPAR γ 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 PPAR γ ligands [50]. PPAR β/δ and PPAR α are also expressed in placenta, but gene knockout studies show less severe phenotypes. For instance, PPAR β/δ knockout mice can give birth to viable offspring (>10%) and PPAR α -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 $G\alpha$ subunit of associated heterotrimeric G proteins to exchange guanine diphosphate (GDP) for guanine triphosphate (GTP) and to dissociate from the $G\beta\gamma$ unit; this process enables both subunits to signal autonomously. The family of RGS molecules acts predominantly as GTPases (GAPs) for $G\alpha$ proteins (*Gai/o*, *Gaq/11*, *Gas*) and accelerates the hydrolysis of $G\alpha_{GTP}$ to $G\alpha_{GDP}$ [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 *Gaq*, *Gai* and *Ga12/13* 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

1 that transcriptional regulation of the RGS5 gene may also be influenced by promoter
2 methylation and epigenetic mechanisms [69]. RGS5 is a small protein which consists of a
3 120 aa conserved RGS domain which confers GTPase activity and a 33 aa N-terminus with a
4 cysteine (C2) residue which targets it for rapid degradation. As a substrate for the N-end rule
5 degradation pathway, RGS5 protein degradation/stabilization provides responsiveness to
6 incoming environmental cues such as changing oxygen levels [70]. Indeed, RGS5 expression
7 levels are dynamically regulated in cardiovascular pathologies indicative of a role in adaptive
8 processes. Besides control of blood pressure homeostasis, this includes a crucial role in
9 atherosclerosis, tumor angiogenesis, arteriogenesis and cardiac adaptation following pressure
10 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 PPAR γ effects.

Endogenous PPAR γ ligands or synthetic agonists (TZD, thiazolidinediones) exert pleiotrophic non-vascular and vascular effects, similar to statins. PPAR γ 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 (NF κ B) 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 PPAR γ vascular expression via COX-2 dependent synthesis of the prostaglandin 15d-PGJ2 may regulate similar signalling pathways [87].

1 Table 1. Association of human PPAR γ , RGS2 and RGS5 gene polymorphisms with
2 hypertensive and metabolic disorders.

Gene	Genetic variation	Population	Phenotype	Ref
PPAR γ 1	Pro467Leu Val290Met	3 individuals (2 females/1 male)	Early onset hypertension, type II diabetes, insulin resistance	[46]
PPAR γ 2	Pro12Pro homozygotes	Caucasian	Postmenoposal hypertension	[96]
	Pro12Ala	Chinese	Hypertension, metabolic lipid disorder	[97]
PPAR γ 3	C681G (rs10865710)	Chinese	Hypertension	[98]
RGS2	C1114G (rs4606)	Caucasian (Norwegian HUNT2 study)	Preeclampsia, Hypertension after pregnancy	[65], [99]
RGS5	rs2815272	African Americans	Hypertension	[60]
	rs12041294C/T rs10917690A/G, rs10917695T/C, rs10917696T/C, rs2662774G/A in combination	Chinese	Hypertension, Lipid metabolism	[61]
	rs16849802	Chinese	Hypertension	[62]

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