Role of microbial metabolites in the pathogenesis of hypertensive disorders of pregnancy: From short-chain fatty acids to tryptophan metabolites (Review)

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Abstract. Hypertensive disorders of pregnancy (HDP) are a leading cause of maternal and perinatal morbidity worldwide, and their pathogenesis involves complex interactions among vascular dysfunction, placental ischemia and immune dysregulation. The gut microbiota has been highlighted as a key upstream modulator, with microbial metabolites serving as key functional mediators rather than microbial composition alone. The present review focused on the roles of short-chain fatty acids (SCFAs) and tryptophan metabolites in HDP pathogenesis. SCFAs and tryptophan-derived metabolites modulate vascular function, immune tolerance and placental development via G protein-coupled receptors, histone deacetylase inhibition and aryl hydrocarbon receptor (AhR) signaling. Their crosstalk underscores integrated regulatory networks at the maternal-fetal interface. For example, butyrate promotes regulatory T cell differentiation via histone deacetylase inhibition, while indole-3-lactic acid activates the AhR; these pathways may synergistically enhance immune tolerance, yet competition for AhR binding between different tryptophan metabolites could produce antagonistic effects. Despite key progress, notable challenges remain regarding causal inference, methodological standardization and translational barriers. The present review aimed to synthesize current mechanistic insights and evaluate the clinical translation potential of targeting microbial metabolites for HDP prevention and management.

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Abbreviations: AhR, aryl hydrocarbon receptor; BPH/5, blood pressure high/5; GPR41, G protein-coupled receptor 41; GPR43, G protein-coupled receptor 43; HDAC, histone deacetylase; HDP, hypertensive disorders of pregnancy; IDO, indoleamine 2,3-dioxygenase; KYNA, kynurenic acid; LC-MS, liquid chromatography-mass spectrometry; SCFAs, short-chain fatty acids; sFlt-1, soluble fms-like tyrosine kinase-1; TMAO, trimethylamine N-oxide; Treg, regulatory T cell; VCAN, versican

Key words: gut microbiota, microbial metabolites, SCFAs, tryptophan, HDP, preeclampsia, maternal-fetal interface, metabolic crosstalk

Contents

1. Introduction

Hypertensive disorders of pregnancy (HDP), including gestational hypertension, preeclampsia and eclampsia, are a leading cause of maternal and perinatal morbidity and mortality worldwide, affecting 5-10% of all pregnancies (1). Beyond the immediate adverse outcomes (2), including preterm birth, fetal growth restriction and perinatal mortality, affected women face markedly elevated long-term risks of cardiovascular disease (including ischemic heart disease, heart failure, stroke and cardiomyopathy) (3), metabolic syndrome (characterized by hypertension, obesity, dyslipidemia and insulin resistance) (4) and chronic kidney disease, whereas offspring exhibit increased susceptibility to neurodevelopmental disorders such as autism spectrum disorder, attention-deficit/hyperactivity disorder, cerebral palsy and cognitive impairments (1,5), as

well as cardiometabolic disorders, including elevated blood pressure and obesity in childhood and adolescence across the life course (4). Despite decades of research, the precise pathogenic mechanisms underlying HDPs remain incompletely understood and current clinical management largely focuses on blood pressure control and timely delivery rather than on disease-modifying interventions. The recognition that HDP pathogenesis involves complex interactions among maternal vascular dysfunction, placental ischemia, systemic inflammation and immune dysregulation has prompted growing interest in the role of the gut microbiota as an upstream modulator of these processes (2).

The gut microbiota undergoes notable physiological remodeling during pregnancy, with longitudinal studies demonstrating gestational stage-specific shifts in microbial composition, diversity and functional capacity that support maternal metabolic adaptation and immune modulation (3,5). Using bibliometric analysis of 1,268 publications, Chen *et al* (3) mapped global research trends and identified that the gut microbiota during pregnancy shifts toward increased Proteobacteria and reduced *Faecalibacterium* in the third trimester, supporting its role in metabolic adaptation. These pregnancy-induced microbial changes are characterized by increased inter-subject variability and altered abundances of taxa involved in short-chain fatty acids (SCFAs) production (5). Notably, disruptions in this normal remodeling process have been documented in women who develop HDP, with reduced α-diversity and distinct taxonomic signatures reported in affected pregnancies (6,7). A systematic review and meta-analysis by Colonetti *et al* (7) pooled data from 11 case-control studies (total n=1,020) and reported that preeclamptic women had notably lower gut microbial α-diversity (standardized mean difference, -0.67; 95% CI, -1.02-0.32) and a higher Firmicutes/Bacteroidetes ratio, confirming a consistent association between dysbiosis and preeclampsia. Similarly, Jordan *et al* (8) conducted a scoping review concluding that microbiota dysbiosis is reproducibly associated with increased preeclampsia risk, although the directionality of this relationship remains a topic of debate, as multiple studies have explicitly questioned whether microbial alterations represent a causative factor or merely an epiphenomenon secondary to disease pathophysiology (2,9). The importance of understanding site-specific microbial changes during pregnancy has been emphasized in the study by Flores Ventura *et al* (10), which highlighted opportunities for targeted interventions based on distinct microbial signatures. Specifically, distinct site-specific signatures in the gut, vaginal and oral microbiotas are differentially associated with pregnancy outcomes (for example, preterm birth, pre-eclampsia and gestational diabetes), providing a rational basis for the development of site-specific probiotic interventions tailored to each biological niche.

Notably, the functional consequences of microbiota-host interactions are primarily mediated through microbial-derived metabolites rather than microbial composition *per se*. Among these, SCFAs and tryptophan metabolites have emerged as particularly notable signaling molecules at the maternal-fetal interface (2,11,12). SCFAs, generated through bacterial fermentation of dietary fiber, exert pleiotropic effects on host physiology through activation of G protein-coupled receptors and histone deacetylase (HDAC) inhibition, thereby modulating vascular tone, inflammatory responses and immune cell function (13,14). A scoping review by Zhao *et al* (14) synthesized evidence from 11 case-control studies (total of 2,314 participants) and concluded that reduced SCFAs concentrations, particularly butyrate (median reduction, 37%; range, 22-54%), are consistently associated with preeclampsia. The tryptophan pathway represents another major route of host-microbiota metabolic interaction, with gut bacteria metabolizing dietary tryptophan to generate indole derivatives, while host enzymes process the majority through the kynurenine pathway (9). A systematic review by van Zundert *et al* (9) examined 42 studies (18 human and 24 animal studies) and reported that alterations in the kynurenine pathway [increased kynurenine/tryptophan ratio and reduced kynurenic acid (KYNA)] are consistently observed in HDP, although causal relationships remain to be established. The placental enzyme indoleamine 2,3-dioxygenase (IDO), which catalyzes the rate-limiting step in tryptophan degradation, serves a key role in maintaining maternal-fetal immune tolerance and its dysregulation has been implicated in preeclampsia pathogenesis (15). A previous review by Kudo and Sugimoto (15) summarized data from 23 human placental studies and reported that IDO activity is reduced by 40-60% in preeclamptic placentas compared with normotensive controls, associating IDO deficiency with impaired immune tolerance.

Emerging evidence has begun to elucidate the mechanistic convergence of SCFAs and tryptophan metabolite signaling pathways in HDP. Both metabolite classes influence shared downstream effectors including immune cell polarization, endothelial function and placental development, suggesting that integrated analysis of these pathways may yield insights beyond investigating each pathway in isolation (2,12). The aryl hydrocarbon receptor (AhR) has been identified as a central hub integrating signals from tryptophan-derived microbial metabolites, with recent studies demonstrating that indole-3-lactic acid alleviates preeclampsia-like phenotypes through AhR activation (16-18). Concurrently, SCFAs modulate macrophage polarization and trophoblast function via G protein-coupled receptor 43 (GPR43)-mediated signaling, highlighting the existence of parallel yet interconnected regulatory networks at the maternal-fetal interface (11,13). The concept of a gut-placenta axis has been proposed, wherein microbial metabolites serve as key mediators associating maternal gut ecology with placental function and fetal outcomes (12,19). A comprehensive review by Ma *et al* (12) integrated data from 86 studies and proposed that the gut-placenta axis operates through SCFAs, tryptophan metabolites and trimethylamine N-oxide (TMAO), each influencing placental autophagy, inflammation and angiogenesis. Mechanistic insights from animal models have further supported this framework, with previous studies revealing that microbial metabolites regulate placental autophagy and ferroptosis, processes notably involved in trophoblast survival and placental development (13,20,21).

Despite key progress, notable knowledge gaps and methodological challenges persist in the field. Majority of clinical studies are cross-sectional, to the best of our knowledge, precluding the determination of whether microbial alterations precede or result from disease onset (2,8,22). Mendelian

randomization (MR) studies, including those by Li *et al* (23), have provided genetic evidence supporting causal relationships between the gut microbiota and preeclampsia; however, these approaches cannot fully exclude residual confounding or reverse causation. In a two-sample MR study using summary statistics from 18,340 individuals for gut microbiota and 5,976 preeclampsia cases, Li *et al* (23) identified that the genus *Ruminococcaceae* (OR=1.28; 95% CI, 1.10-1.49) and order Clostridiales (OR=0.81; 95% CI, 0.68-0.96) demonstrated causal associations with preeclampsia, but horizontal pleiotropy could not be fully excluded. Technical challenges associated with distinguishing microbial-derived from host-derived metabolites, the need for absolute quantification methods and the absence of standardized analytical protocols across studies further complicate interpretation and cross-study comparability (14,24). Furthermore, the extent to which findings from preclinical animal models translate to human pregnancy physiology remains to be elucidated due to notable species differences in placental structure, gestational timing and metabolic exchange (25). Unresolved questions regarding the mechanisms of maternal-fetal metabolite transfer and the potential in intergenerational programming of disease risk represent key frontiers for future investigation (26). The present review aimed to synthesize current evidence on the roles of SCFAs and tryptophan microbial metabolites in HDP pathogenesis, with a particular focus on the functional mechanisms underlying these associations, the emerging crosstalk between these metabolite classes and the clinical translation potential for biomarker development and therapeutic intervention. The three novel contributions are as follows: i) A comprehensive mechanistic synthesis; ii) a hypothetical framework for SCFA-tryptophan crosstalk (presented in the following section, with detailed mechanistic evidence for synergistic amplification and competitive antagonism provided in the corresponding subsections); and iii) a key appraisal of clinical translation barriers, including biomarker specificity and safety.

2. Overview of HDP and gut microbial metabolites in pregnancy

HDP represent a spectrum of conditions characterized by new-onset hypertension after 20 weeks of gestation, encompassing gestational hypertension, preeclampsia and eclampsia. The pathophysiological hallmarks include systemic vascular dysfunction, placental ischemia, maternal systemic inflammation and disruption of immune tolerance mechanisms. In parallel with advances in understanding HDP pathogenesis, there has been increasing recognition that the maternal gut microbiota undergoes notable physiological remodeling during pregnancy (27-29), with emerging evidence implicating alterations in this ecosystem in HDP development. Of note, the functional consequences of microbiota-host interactions are largely mediated through microbial-derived metabolites, particularly SCFAs and tryptophan metabolites, which serve as key signaling molecules at the maternal-fetal interface. These metabolites act on the vasculature, immune system and placenta through GPR41/43, HDAC inhibition and AhR signaling, collectively influencing HDP pathogenesis (Fig. 1).

Clinical classification and pathophysiological hallmarks of HDP. HDP encompass a heterogeneous group of conditions with overlapping clinical features but distinct diagnostic criteria and pathophysiological underpinnings. According to the American College of Obstetricians and Gynecologists guidelines, these include gestational hypertension (new-onset hypertension after 20 weeks without proteinuria) and preeclampsia (hypertension with proteinuria or end-organ dysfunction) (30). Preeclampsia, the most extensively studied disorder within this spectrum, is characterized by *de novo* hypertension after 20 weeks of gestation accompanied by proteinuria or maternal end-organ dysfunction. The core pathophysiological hallmarks include impaired spiral artery remodeling, placental ischemia-reperfusion injury, excessive oxidative stress and a generalized maternal systemic inflammatory response with endothelial dysfunction as a unifying feature (31). In a nested case-control study (124 preeclamptic women and 124 normotensive controls), Lin *et al* (31) measured gut microbial composition and reported that the genus *Bifidobacterium* was significantly reduced in preeclampsia (fold change, 0.52; P=0.008) and that this reduction correlated positively with systolic blood pressure (r=-0.41; P=0.003), supporting the association between gut dysbiosis and HDP severity. Increasing evidence from MR studies have provided genetic support for causal relationships between gut microbiota composition and HDP susceptibility, suggesting that microbial factors may contribute directly to disease pathogenesis rather than merely reflecting epiphenomena. Wu *et al* (32) performed an MR analysis using genome-wide association studies (GWAS) data for 211 microbial taxa and 7,025 preeclampsia cases, identifying 12 taxa with notable causal associations (for example, Pasteurellales, OR=1.19; 95% CI, 1.06-1.34), thereby reinforcing the biological plausibility of microbiota-host interactions in HDP development. Of note, HDP encompasses heterogeneous subtypes including gestational hypertension, early-onset preeclampsia (delivery <34 weeks) and late-onset preeclampsia (delivery ≥34 weeks), which have distinct pathophysiological features (for example, placental ischemia vs. maternal metabolic syndrome) and may exhibit different microbial metabolite signatures; however, to the best of our knowledge, majority of current studies do not stratify by these subtypes (33,34). In a prospective cohort study, Lv *et al* (33) analyzed fecal samples from 60 women with early-onset preeclampsia and 40 healthy pregnant women and reported that α-diversity (Shannon index) was reduced by 28% (P<0.001) in the preeclampsia group, and these alterations persisted into the postpartum period. This finding suggests that HDP-associated dysbiosis is not merely a consequence of acute disease. Notably, the majority of existing research, including all studies cited in sections 3 and 4 on SCFAs and tryptophan metabolites, has focused exclusively on preeclampsia, to the best of our knowledge. Evidence specific to gestational hypertension or eclampsia is virtually absent. Therefore, in the present review, the term 'HDP' was used generically to refer to the broader disorder category; however, the underlying data derive almost entirely from preeclampsia cohorts unless stated otherwise.

Physiological gut microbiota remodeling in pregnancy and dysregulation in HDP. Normal pregnancy is associated with

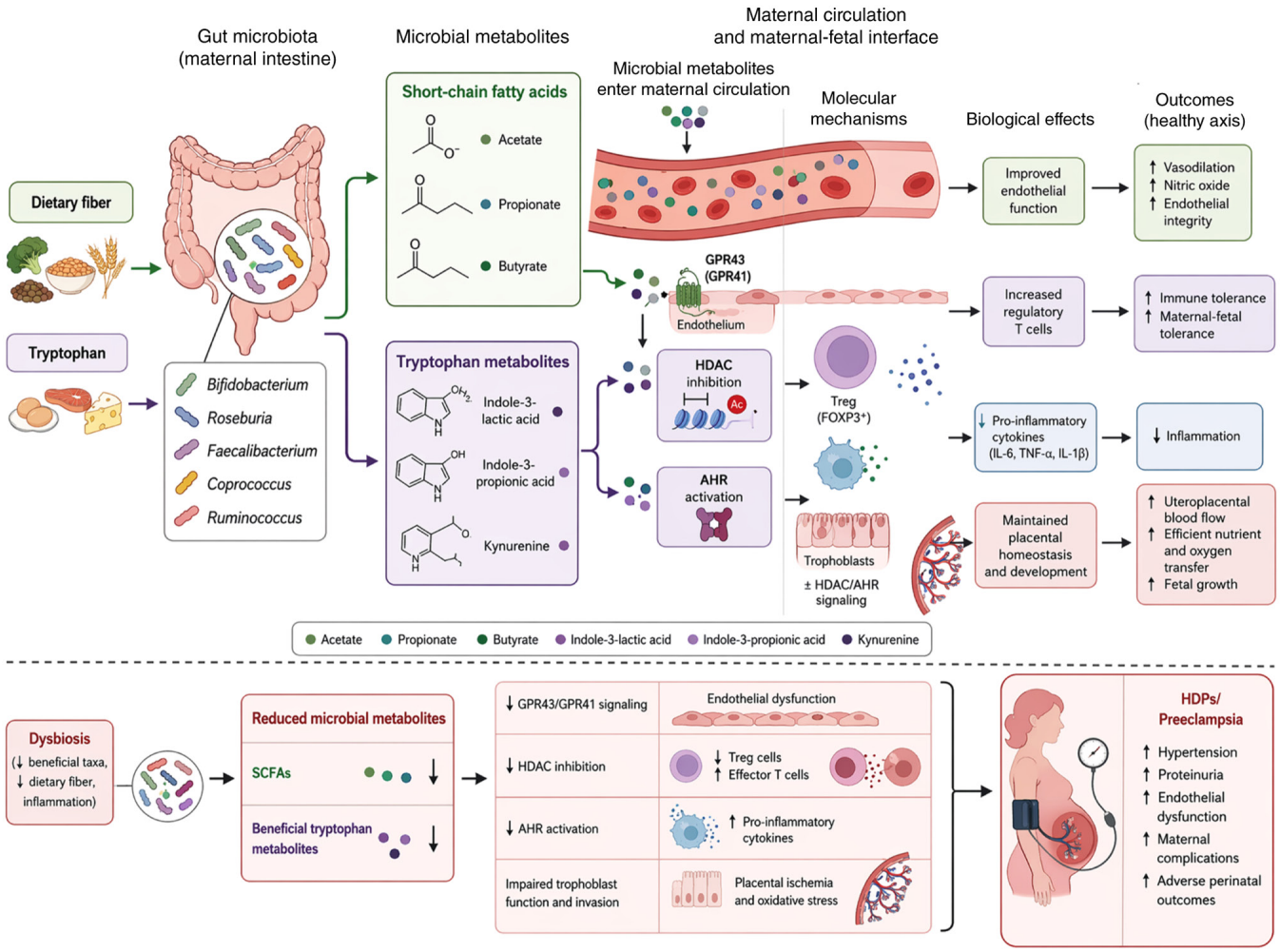

Figure 1. Schematic overview of the maternal gut microbiota-metabolite-HDP axis during pregnancy. The gut microbiota undergoes gestational stage-specific remodeling, producing short-chain fatty acids via dietary fiber fermentation and tryptophan metabolites via host (kynurenine pathway) and microbial (indole pathway) routes. These metabolites act on the vasculature, immune system and placenta through GPR41/43, HDAC inhibition and AhR signaling, collectively influencing HDP pathogenesis. AhR, aryl hydrocarbon receptor; GPR41/43, G protein-coupled receptors 41/43; HDAC, histone deacetylase; HDP, hypertensive disorders of pregnancy.

notable and dynamic alterations in maternal gut microbiota composition, structure and functional capacity across gestation. Using a longitudinal design with samples collected at gestational weeks 12, 24 and 36, Crusell *et al* (35) sequenced the gut microbiota of 50 pregnant women and reported that the relative abundance of *Faecalibacterium* significantly increased by 2.4-fold from the first to third trimester (P=0.003), and that these changes were more marked in women who later developed gestational diabetes compared with those who remained normoglycaemic during pregnancy, suggesting that pregnancy complications amplify physiological microbial adaptations. The metabolic potential of the maternal gut microbiota also undergoes gestational stage-specific reprogramming, for example, Gosalbes *et al* (36) performed metagenomic sequencing on 40 pregnant women at three time points and demonstrated that genes involved in SCFA production (for example, butyrate kinase) significantly increased by 34% in the third trimester compared with the first trimester (P=0.002), extending beyond taxonomic shifts to encompass functional metabolic adaptation.

In the context of HDP, emerging evidence indicates that the normal physiological remodeling of the maternal gut microbiota is disrupted, with distinct compositional and functional signatures observed in affected women. Lv *et al* (33) reported that in antepartum women with early-onset preeclampsia, the abundance of the butyrate-producing genus *Roseburia* was significantly reduced by 76% compared with normotensive controls (P<0.001) and this reduction persisted at 6-weeks postpartum, indicating long-lasting dysbiosis. Similarly, Liu *et al* (37) conducted a cross-sectional study of 52 patients with severe preeclampsia and 52 matched controls, using 16S ribosomal RNA (rRNA) sequencing and targeted metabolomics. The study reported that fecal butyrate levels correlated negatively with systolic blood pressure (r=-0.58; P<0.001) and identified 18 microbial species that discriminated severe preeclampsia with an area under the curve (AUC) of 0.92 (95% CI, 0.87-0.97). These findings collectively support the concept that HDP-associated gut dysbiosis extends beyond simple compositional changes to encompass functional metabolic alterations that may contribute to disease pathogenesis.

Key gut microbial metabolites in maternal-fetal health. Among the diverse array of bioactive molecules produced by the gut microbiota, SCFAs and tryptophan metabolites have emerged as particularly key mediators of maternal-fetal health due to their pleiotropic effects on host physiology and their capacity to reach biologically relevant concentrations in the maternal circulation and at the maternal-fetal interface (11,38). SCFAs, primarily acetate, propionate and butyrate, are generated through bacterial fermentation of dietary fiber and resistant starch, with their production contingent upon substrate availability and the metabolic capacity of specific microbial communities (39). In a cross-sectional study of 120 pregnant women (60 women in the third trimester and 60 women in the first trimester), Ziętek *et al* (39) measured serum SCFAs concentrations and reported that acetate, propionate and butyrate were significantly higher in the third trimester compared with those in the first trimester (mean increases, 42, 38 and 55%, respectively; all $P<0.01$), and that butyrate levels correlated negatively with total cholesterol ($r=-0.34$; $P=0.02$), supporting the relevance of SCFAs to maternal metabolic regulation. The tryptophan pathway represents another major route of host-microbiota metabolic interaction, with gut bacteria metabolizing dietary tryptophan through multiple pathways to generate bioactive metabolites including indole derivatives and kynurenine pathway intermediates (40). In a prospective cohort study of 1,057 women with first-trimester blood sampling, van Zundert *et al* (41) measured 12 tryptophan metabolites and reported that lower KYNA levels (≤25th percentile) were associated with a 2.3-fold increased risk of subsequent HDP (95% CI, 1.4-3.8), highlighting the potential predictive value of these metabolites in early gestation.

General biological mechanisms of microbial metabolite action. Microbial metabolites act on maternal vascular homeostasis, immune tolerance and placental function via multiple convergent mechanisms that influence HDP pathogenesis (42). SCFAs signal through G protein-coupled receptors, particularly G protein-coupled receptor 41 (GPR41) and GPR43, which are expressed on vascular endothelial cells, immune cells and placental tissues, activating intracellular signaling cascades that modulate inflammatory responses, vascular tone and tissue perfusion. In addition to receptor-mediated effects, SCFAs function as histone deacetylase inhibitors, thereby influencing epigenetic regulation of gene expression in host cells and modifying transcriptional programs relevant to inflammation, oxidative stress and metabolic function (43). Tryptophan-derived microbial metabolites, particularly those activating the AhR, represent a parallel signaling axis with notable effects on maternal immune tolerance and vascular function (40). Wei *et al* (17) treated pregnant mice with soluble fms-like tyrosine kinase-1 (sFlt-1; to induce a preeclampsia-like phenotype) and co-administered indole-3-lactic acid (50 mg/kg/day, oral) or vehicle. The treated group demonstrated normalization of blood pressure (from 158±9 to 131±6 mmHg; $P<0.001$), reduced urinary protein (from 345±42 to 187±31 mg/dl; $P<0.001$) and increased placental AhR target gene expression [cytochrome P450, family 1, subfamily A, polypeptide 1 (CYP1A1) increased 3.2-fold], providing mechanistic evidence for the protective effects of this AhR pathway. The interplay between SCFAs and tryptophan metabolite signaling pathways at the maternal-fetal interface represents an emerging area of investigation with potential implications in understanding the integrated effects of microbial metabolites on pregnancy outcomes.

Methodological advances in microbial metabolite research. Investigation of microbial metabolites in obstetric disease has been advanced by methodological developments enabling more precise detection, quantification and functional validation of these molecules in relevant biological matrices (44). Mass spectrometry-based metabolomic platforms have facilitated comprehensive profiling of microbial metabolites in maternal serum, plasma, urine and fecal samples, while emerging evidence suggests that microbial metabolites may also be detectable in amniotic fluid and even in fetal tissues, indicating transplacental transfer and potential direct effects on fetal development (45). Wang *et al* (45) analyzed 20 human fetal intestinal samples (gestational age, 10-20 weeks) using mass spectrometry and detected 83 bacterial-derived metabolites, including SCFAs, indole derivatives and secondary bile acids, in the fetal gut lumen, providing direct evidence for transplacental transfer and fetal exposure to maternal microbial metabolites. However, the field faces notable technical challenges, including the distinction between microbial- and host-derived metabolites, the need for absolute quantification rather than relative abundance measures and the complexities of establishing causal relationships in observational human studies (46). Bihl *et al* (46) performed a key re-analysis of putative fetal microbiome data and demonstrated that up to 80% of bacterial sequences in certain studies could be attributed to contamination, underscoring the need for strict negative controls in microbiome research. The integration of multi-omics approaches, including metagenomics, metabolomics and transcriptomics, together with advanced bioinformatic and statistical methods, offers opportunities to address these challenges and advance understanding of the role of microbial metabolites in HDP pathogenesis (47). Huang *et al* (47) performed integrated multi-omics analysis (16S rRNA sequencing, targeted metabolomics and cytokine profiling) on 150 pregnant women and identified 49 metabolites and 12 cytokines that are associated with gut microbial shifts, with butyrate and tryptophan metabolites accounting for 38% of the variance in inflammatory markers, demonstrating the power of integrative approaches.

To further interpret the strength of the evidence, the following framework is used throughout: i) Prospective cohort studies provide the strongest human evidence for temporality and risk prediction; ii) case-control and cross-sectional studies can identify associations but cannot establish causality due to risk of reverse causation; and iii) preclinical studies (animal models and cell lines) offer mechanistic insights but their translation to human pregnancy warrants caution. As appropriate, the study design of human studies cited in the present review is explicitly noted.

3. Role of SCFAs in the pathogenesis of HDP

The involvement of SCFAs in HDP pathogenesis is supported by converging evidence from clinical observational studies, mechanistic investigations and preclinical intervention trials.

SCFAs (acetate, propionate and butyrate) are produced by gut bacterial fermentation of dietary fiber. In normal pregnancy, SCFAs levels change dynamically across gestation; in HDP, butyrate is consistently reduced. SCFAs signal via G protein-coupled receptors (such as GPR43) and inhibit HDACs, leading to enhanced regulatory T cell development, reduced inflammation and improved vascular function (Fig. 2). The figure provides a schematic overview of SCFAs biosynthesis, their dynamic changes across gestation and the multi-level mechanisms through which these metabolites influence maternal vascular function, immune homeostasis and placental development.

Biosynthesis, microbial sources and gestational dynamics of SCFAs. SCFAs, predominantly acetate, propionate and butyrate, are generated through bacterial fermentation of dietary fiber and resistant starch in the distal gut, with their production contingent upon substrate availability and the metabolic capacity of specific microbial communities (48). A comprehensive review by Mukhopadhya and Louis (48) summarized that 500-600 mmol of SCFAs are produced daily in the human colon, with a typical acetate/propionate/butyrate ratio of 60:20:20 and that butyrate is the primary energy source for colonocytes. During normal pregnancy, the maternal gut microbiota undergoes physiological remodeling that includes functional metabolic adaptation, with altered capacity for SCFAs production across gestation (49). In a cross-sectional study of 90 pregnant women (30 women each in the first, second and third trimesters), Ziętek *et al* (49) reported that serum butyrate concentrations significantly increased from 4.1±1.2 μM in the first trimester to 7.3±2.1 μM in the third trimester (P<0.001) and that these levels correlated positively with gestational age (r=0.52; P<0.001), supporting a notable increase in SCFA bioavailability across gestation. The biosynthesis of SCFAs is not uniform but rather reflects the compositional dynamics of SCFAs-producing bacterial populations, including genera such as *Coprococcus*, *Roseburia* and *Faecalibacterium*, which exhibit gestational stage-specific abundance patterns that influence the availability of these metabolites at the maternal-fetal interface.

Clinical evidence of SCFA alterations in HDP. Multiple observational studies have consistently demonstrated notable alterations in circulating, fecal and placental SCFA profiles in women with preeclampsia compared with normotensive pregnancies. In a case-control study of 35 preeclamptic and 35 normotensive pregnant women, Chang *et al* (50) used gas chromatography-mass spectrometry to quantify fecal SCFAs and reported that butyrate was significantly reduced by 54% (36.2±8.4 vs. 78.9±12.5 μmol/g; P<0.001) and propionate by 41% (23.1±5.2 vs. 39.3±7.8 μmol/g; P<0.001) in the preeclampsia group, and these reductions significantly correlated with increased systolic blood pressure (r=-0.61 for butyrate; P<0.001), providing the first comprehensive evidence associating reduced fecal SCFAs with preeclampsia. These findings were corroborated in a previous study by Altemani *et al* (51), which performed 16S rRNA sequencing on fecal samples from 40 women who developed preeclampsia and 50 normotensive controls, and reported that the abundance of the butyrate-producing genus *Coprococcus* was 67% lower in the preeclampsia group compared with that in normotensive pregnant women (relative abundance, 0.8 vs. 2.4%; P=0.002), directly associating a taxonomic shift with a functional metabolic consequence. Building on this clinical evidence, Li *et al* (52) conducted a diagnostic study of 95 preeclamptic and 98 healthy pregnant women, measuring plasma SCFAs using liquid chromatography-mass spectrometry (LC-MS), and reported that a panel of acetate and butyrate levels distinguished preeclampsia from healthy pregnancy with an AUC of 0.94 (95% CI, 0.89-0.98) and at a cut-off value of butyrate ≤4.2 μM, the sensitivity was 86% and specificity 91%. Chen *et al* (42) further extended these observations by systematically examining SCFAs expression across multiple pregnancy complications, confirming that altered SCFA profiles represent a common feature of adverse pregnancy outcomes with particular relevance to hypertensive disorders. In a cross-sectional study of 200 pregnant women (50 women each with preeclampsia, gestational diabetes mellitus, intrauterine growth restriction and 50 healthy controls), Chen *et al* (42) reported that butyrate was significantly lower only in the preeclampsia group (P<0.001 vs. each of the other groups), suggesting that SCFA reduction is relatively specific to hypertensive disorders rather than a general marker of pregnancy complications. A recent scoping review by Zhao *et al* (14) synthesized evidence from 11 case-control studies and concluded that reduced SCFA concentrations, particularly butyrate, are consistently associated with preeclampsia, although notable heterogeneity exists across studies in terms of sampling matrices, analytical methods and population characteristics. All 11 human studies included in the review were case-control or cross-sectional in design. No prospective cohort study yet has examined SCFAs levels before HDP onset, to the best of our knowledge. Therefore, while reduced SCFAs, particularly butyrate, are consistently associated with preeclampsia (7/7 studies reporting lower levels and 6 studies with P<0.05), causality cannot be inferred from the current evidence. Kaihara *et al* (53) performed untargeted plasma metabolomic profiling on 160 women (40 women with gestational hypertension, 40 women with preeclampsia without severe features, 40 women with preeclampsia with severe features and 40 women normotensive controls) and identified distinct metabolic signatures, with butyrate and its derivatives indicating significant progressive reduction across increasing disease severity (P<0.001 for trend), further supporting the potential of SCFAs as components of multi-metabolite diagnostic panels. Despite the consistency of these findings, key limitations warrant consideration, including cross-sectional designs that preclude determination of whether SCFA alterations precede or follow disease onset, variable adjustment for confounders such as dietary intake and medication use and the predominance of studies from Asian populations limiting generalizability.

Molecular mechanisms of SCFAs action in HDP pathophysiology. Hu *et al* (54) conducted a prospective cohort study including 50 preeclamptic and 50 normotensive pregnant women, measuring maternal serum acetate and analyzing fetal thymic tissue. The study reported that serum acetate was significantly reduced by 48% in preeclampsia (median, 17.3 vs. 33.2 μM, P<0.001) and that fetal thymic regulatory T cell

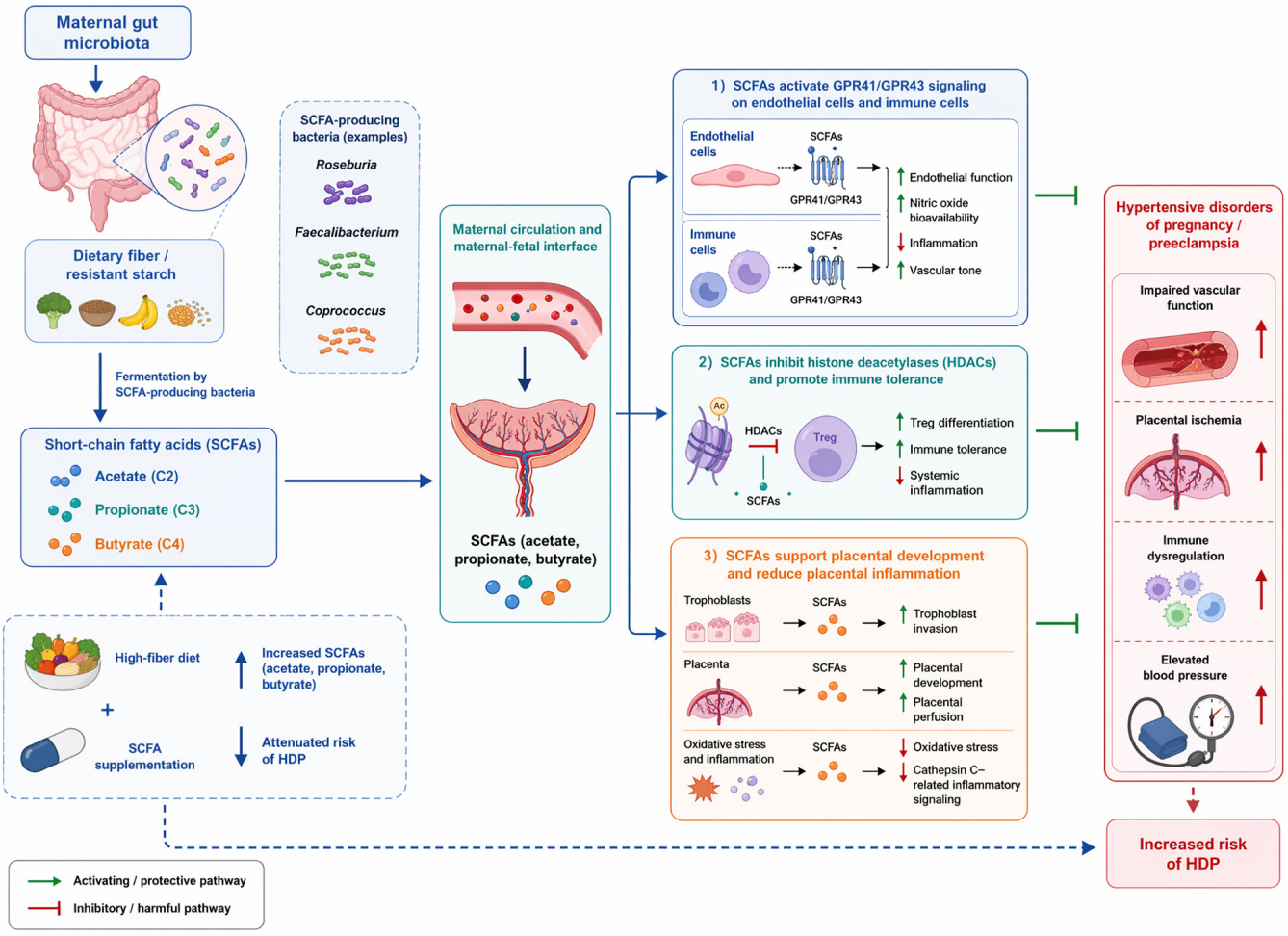

Figure 2. Biosynthesis, gestational dynamics and molecular mechanisms of SCFAs in HDP. SCFAs (acetate, propionate and butyrate) are produced by gut bacterial fermentation of dietary fiber. In normal pregnancy, SCFA levels change dynamically across gestation; in HDP, butyrate is consistently reduced. SCFAs signal via GPCRs (for example, GPR43) and inhibit HDACs, leading to enhanced Treg development, reduced inflammation and improved vascular function. GPCR, G protein-coupled receptor; HDAC, histone deacetylase; HDP, hypertensive disorders of pregnancy; NO, nitric oxide; SCFAs, short-chain fatty acids; Treg, regulatory T cell; GPR41/43, GPCRs 41/43.

(Treg) numbers were significantly reduced by 62% (P<0.001) and correlated positively with maternal acetate levels (r=0.71; P<0.001), establishing a direct association between reduced SCFA availability and impaired immune tolerance in HDP. This immunological perspective was markedly advanced in a previous study by Jin *et al* (55), which used a gut dysbiosis mouse model (antibiotic-treated) and macrophage-trophoblast co-cultures to demonstrate that butyrate (1 mM) significantly increased the proportion of M2 macrophages from 18 to 52% (P<0.001) and significantly enhanced trophoblast invasion by 2.3-fold (P<0.01) via GPR43-mediated signaling; *in vivo*, butyrate supplementation (200 mg/kg/day) to pregnant mice with experimental preeclampsia significantly lowered blood pressure by 28 mmHg (P<0.001) and reduced fetal loss from 45 to 12%, providing causal evidence for SCFA-mediated protection. Cui *et al* (11) comprehensively reviewed the multifaceted roles of SCFAs in preeclampsia regulation, emphasizing that these metabolites simultaneously influence blood pressure control, endothelial function, inflammatory responses and placental development through integrated mechanisms. A recent study by Lu *et al* (56) demonstrated the gut microbiota-SCFAs-cathepsin C pathway as a novel therapeutic target in preeclampsia. The study used a rat model (n=48) and reported that butyrate supplementation (100 mg/kg/day) significantly reduced serum cathepsin C levels by 52% (P<0.001), decreased blood pressure from 152±8 to 122±6 mmHg and normalized proteinuria, revealing that SCFAs regulate lysosomal enzyme activity and subsequent inflammatory mediator release from immune cells. Mackay and Marques (57) provided an accompanying perspective emphasizing that dysbiosis in preeclampsia is amenable to treatment with SCFAs, highlighting the therapeutic potential of targeting the gut microbiota-SCFAs-cathepsin C pathway.

Preclinical functional validation of SCFAs in HDP. The functional consequences of SCFA alterations in HDP have been investigated using *in vitro* cellular models and *in vivo* animal studies, providing causal evidence complementing observational human data. Yong *et al* (58) induced preeclampsia in pregnant rats (n=60) using L-N^G-nitro arginine methyl ester (50 mg/kg/day) and treated them with sodium butyrate (100 mg/kg/day) or vehicle. Butyrate-treated rats had significantly lower systolic blood pressure (128±6 vs. 167±9 mmHg in the untreated PE model group, P<0.001), reduced urinary

protein (118±21 vs. 342±45 mg/24 h in the untreated PE model group, P<0.001) and improved gut microbiota composition (increased *Bifidobacterium* from 2.1 to 7.8%; P=0.003), suggesting a positive feedback loop between exogenous SCFA supplementation and endogenous microbial metabolic capacity. This intervention approach was further validated in Ishimwe *et al* (59), which supplemented Dahl salt-sensitive rats (n=32) with 1,3-butanediol (2% in drinking water) for 2 weeks before mating and throughout pregnancy. Treated rats had suppressed the superimposed preeclampsia-like phenotype, with a significant 34 mmHg reduction in blood pressure (P<0.001) and a 56% reduction in placental oxidative stress markers, indicating that metabolic interventions targeting SCFA-associated pathways may be effective even when initiated prior to conception. The relevance of these findings to human physiology is supported by Alhasan *et al* (60), which used a rat model of superimposed preeclampsia (n=24) and performed fecal 16S rRNA sequencing and SCFA profiling at disease onset. At gestation day 14, the fecal gut microbiome of diseased rats showed lower α diversity and significant differences in β diversity compared with controls, with depletion of bacteria from the families Ruminococcaceae and Oscillospiraceae, as well as the genera *Blautia* and *Faecalibacterium*, while acetate, propionate and valerate were increased (60), closely recapitulating the metabolic disturbances observed in affected women. Beckers *et al* (61) extended these observations to the blood pressure high/5 (BPH/5) mouse model of superimposed preeclampsia, demonstrating that pregnant BPH/5 mice (n=12) had a significant 45% reduction in fecal butyrate (P=0.004) compared with normotensive C57BL/6 mice and that these changes were pregnancy-specific, as non-pregnant BPH/5 mice had normal butyrate levels, further confirming the translational relevance of these animal models. Mechanistic insights from these preclinical studies revealed that SCFAs supplementation exerts antihypertensive effects through multiple pathways, including enhancement of endothelial nitric oxide bioavailability, suppression of systemic inflammation, improvement of placental blood flow and modulation of immune cell populations toward tolerogenic phenotypes.

SCFAs as mediators associating environmental factors with HDP susceptibility. Beyond their direct pathophysiological roles, SCFAs function as key mechanistic mediators associating maternal environmental factors with HDP susceptibility, providing a biologically plausible explanation for how dietary, lifestyle and other factors influence disease risk. Maternal dietary fiber intake represents the primary determinant of SCFAs production, as these metabolites are generated through bacterial fermentation of indigestible carbohydrates. Schwartz *et al* (62) conducted a cross-sectional study of 158 third-trimester pregnant women, using food frequency questionnaires and 16S rRNA sequencing, and reported that each 10 g/day increase in dietary fiber was associated with a significant 12% increase in gut microbial richness (P=0.01) and a 15% increase in the abundance of SCFA-producing bacteria (*Roseburia* and *Faecalibacterium*), establishing the nutritional foundation for SCFA-mediated effects on maternal health. The causal relationship between SCFAs-associated metabolic pathways and HDP has been strengthened by MR studies, such as Wu *et al* (32), which used GWAS data to demonstrate that the genus *Ruminococcaceae* (OR=1.21; 95% CI, 1.08-1.36) and the butyrate production pathway (OR=0.78; 95% CI, 0.66-0.92) exhibited notable causal associations with HDP, supporting a protective role for SCFA biosynthesis. Yang *et al* (63) subsequently performed two-sample MR analysis of 5,979 preeclampsia cases and 229,377 controls, identifying that genetically predicted higher levels of the fatty acid butyrate (inverse-variance weighted OR=0.85 per SD increase; 95% CI, 0.76-0.95) were causally associated with reduced preeclampsia risk, providing genetic evidence supporting the role of SCFA-associated metabolic pathways in disease pathogenesis. The integration of environmental, microbial and metabolic factors is exemplified in a previous study by Fan *et al* (64), which fed BPH/5 mice (n=30) either a control or a high-fiber diet (10% inulin) from early pregnancy and reported that the high-fiber group had a significant 2.8-fold increase in fecal butyrate (P<0.001), a 24 mmHg lower blood pressure (P=0.002) and reduced placental sFlt-1 expression by 51%, illustrating the potential for dietary interventions to modify SCFA-associated pathways and influence disease outcomes. A study by Lu *et al* (65) using a rat model (n=48) demonstrated that probiotic-fermented buffalo milk (containing propionate-producing bacteria; 5 ml/day) significantly increased serum propionate by 3.1-fold (P<0.001) and reduced blood pressure by 22 mmHg (P<0.001) via the phenylalanine metabolic pathway, further supporting the feasibility of microbiome-targeted interventions to enhance SCFA production and improve pregnancy outcomes. Collectively, these findings position SCFAs as central nodes in a complex network connecting maternal diet, gut microbial ecology, metabolic function and HDP pathogenesis, with notable implications for the development of preventive and therapeutic strategies targeting these modifiable pathways in the future.

4. Gut microbial tryptophan metabolites in the pathogenesis of HDP

Tryptophan metabolism represents a key interface between maternal nutrition, gut microbial activity and host physiology, with emerging evidence implicating this pathway in HDP pathogenesis (66-71). Fig. 3 illustrates the dual routes of tryptophan metabolism (the host enzymatic kynurenine pathway and the microbial indole pathway) and their convergence on the AhR as a central regulatory hub.

Metabolic pathways and maternal-fetal distribution of tryptophan metabolites. Tryptophan is metabolized through three principal pathways: The kynurenine pathway catalyzed by IDO and tryptophan 2,3-dioxygenase, the serotonin pathway and the direct microbial conversion to indole derivatives. The kynurenine pathway (producing kynurenine, KYNA) is primarily host-derived, whereas indole derivatives (for example, indole-3-lactic acid, and indole-3-propionic acid) are exclusively of microbial origin. The kynurenine pathway accounts for ~95% of dietary tryptophan degradation and generates metabolites with diverse immunological and vascular effects (66). In pregnancy, the placental expression and activity of IDO are dynamically regulated, with IDO

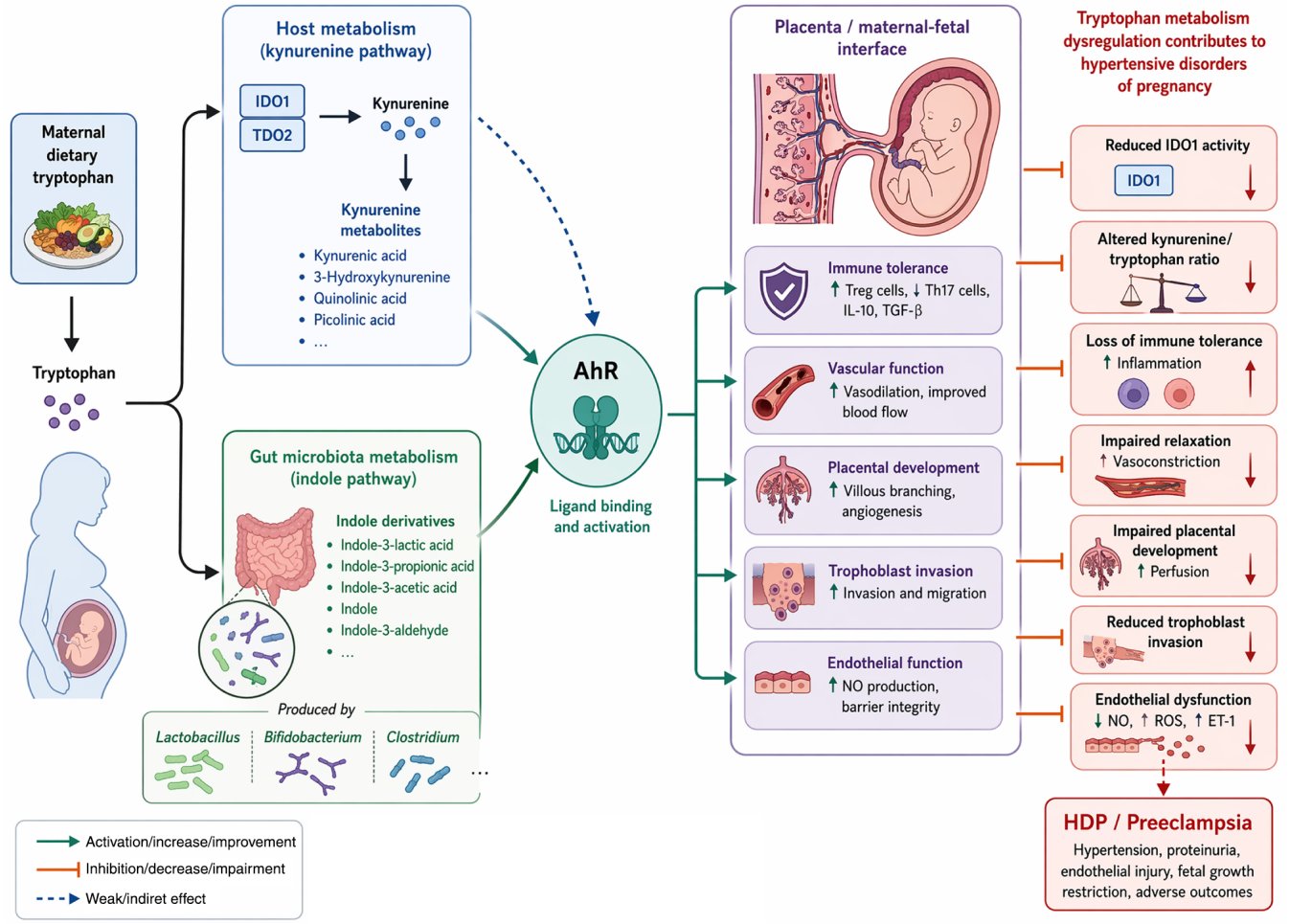

Figure 3. Metabolic pathways of tryptophan and AhR signaling in HDP. Tryptophan is metabolized via the host kynurenine pathway (IDO-dependent) and the microbial indole pathway (producing ILA and IPA). Both routes generate ligands that activate the AhR, which regulates trophoblast migration, immune tolerance and vascular homeostasis. Dysregulation of this pathway is implicated in preeclampsia. HDP, hypertensive disorders of pregnancy; AhR, aryl hydrocarbon receptor; IDO, indoleamine 2,3-dioxygenase; ILA, indole-3-lactic acid; IPA, indole-3-propionic acid; NO, nitric oxide; Treg, regulatory T cell.

serving a key role in maintaining maternal-fetal immune tolerance by depleting tryptophan and generating immunosuppressive kynurenines (66). These two metabolic routes converge on the aryl hydrocarbon receptor (AhR), which serves as a central regulatory hub integrating signals from tryptophan-derived metabolites at the maternal-fetal interface (Fig. 3). Zardoya-Laguardia *et al* (67) performed *ex vivo* wire myography on placental arteries from normotensive (n=15), preeclamptic (n=15) and intrauterine growth restriction (n=15) pregnancies, and reported that endothelial IDO-1 expression was significantly reduced by 67% in preeclampsia (P<0.001) and that blood vessels in these patients exhibited significantly impaired tryptophan-induced relaxation (maximum relaxation, 32±6% vs. 78±8% in controls; P<0.001), establishing a direct association between impaired tryptophan metabolism and placental vascular dysfunction. The distribution of tryptophan metabolites across the maternal-fetal interface exhibits compartment-specific patterns, for example, Zhao *et al* (68) measured tryptophan and its metabolites in paired maternal and fetal cord blood from 30 normotensive and 30 preeclamptic pregnancies, and reported that the fetal-to-maternal ratio of kynurenine was 1.8-fold higher in preeclamptic pregnancies compared with that in normotensive controls (median, 1.32 vs. 0.73; P=0.004), indicating altered transplacental handling of these metabolites. Keaton *et al* (69) further characterized altered tryptophan catabolism in placentas from women with preeclampsia, using LC-MS to measure tryptophan metabolites in placental tissue from 28 preeclamptic and 28 control women, and reported that the kynurenine/tryptophan ratio (a marker of IDO activity) was significantly increased by 42% in preeclampsia (P=0.002), while serotonin was significantly reduced by 39% (P=0.01), identifying specific disruptions in this metabolic pathway that may contribute to disease pathogenesis. Broekhuizen *et al* (70) subsequently demonstrated that L-tryptophan (100 μM) induced vasodilation of isolated placental arteries (n=20 per group), with a significant maximal relaxation of 52±8% in the normotensive group but 78±6% in the group with preeclamptic vessels (P=0.01), indicating adaptive upregulation of tryptophan-mediated vasodilation in the diseased state. A recent comprehensive review by Karahoda *et al* (71) synthesized current understanding of the placental tryptophan pathway across gestation, emphasizing its implications for pregnancy outcomes and identifying key knowledge gaps regarding the regulation of this system in complicated pregnancies.

Clinical associations between tryptophan metabolites and HDP. A prospective cohort study (n=1,057) reported that first-trimester host-derived kynurenine pathway metabolites are associated with subsequent preeclampsia risk, with an adjusted OR of 2.3 (95% CI, 1.4-3.8) for KYNA ≤25th percentile, providing notable evidence for temporality (41). By contrast, majority of indole derivatives (microbial origin) have been studied only in cross-sectional designs or preclinical models. A systematic review by van Zundert *et al* (9) concluded that while alterations in the kynurenine pathway are consistently observed in HDP, causal relationships have not been established yet due to the predominance of cross-sectional studies and lack of longitudinal data. Multiple clinical studies have established notable associations between circulating tryptophan metabolite concentrations and HDP risk, severity and adverse pregnancy outcomes. Jasim *et al* (40) performed a cross-sectional study of 80 preeclamptic and 80 normotensive pregnant women, measuring serum tryptophan metabolites and insulin resistance markers, and reported that kynurenine was significantly elevated by 34% in preeclampsia (P<0.001) and correlated positively with Homeostatic Model Assessment of Insulin Resistance (r=0.52; P<0.001) (72), suggesting that metabolic dysfunction may associate altered tryptophan metabolism with hypertensive complications. Jääskeläinen *et al* (73) employed non-targeted LC-MS profiling to reveal elevated levels of carnitine precursors and trimethylated compounds, including tryptophan-associated metabolites, in the cord blood plasma of preeclamptic infants (n=25) compared with controls (n=25), with a significant 2.4-fold increase in indole-3-acetic acid (P=0.002), indicating that fetal exposure to altered tryptophan metabolism occurs *in utero*. Ferranti *et al* (74) conducted a pilot study examining early pregnancy serum metabolite profiles associated with HDP in African American women (n=40; 20 cases and 20 controls) and identified tryptophan pathway metabolites (kynurenine and indole-3-lactic acid) among the discriminating features (AUC=0.79 for the panel), suggesting that these metabolites may serve as early risk markers for HDP. Recently, Broekhuizen *et al* (75) analyzed umbilical cord blood from 100 pregnancies (50 preeclamptic women and 50 controls) and reported that kynurenine was significantly elevated by 28% (P=0.01) and KYNA significantly reduced by 35% (P=0.003) in the preeclampsia group, providing evidence for fetal programming effects mediated through this metabolic axis. Despite the consistency of these associations, notable limitations include the predominance of cross-sectional designs, variable adjustment for potential confounders such as dietary tryptophan intake and medication use and heterogeneity in the specific metabolites measured and analytical platforms employed.

AhR signaling as a mechanistic hub. The AhR has emerged as the central regulatory hub through which microbial indole derivatives exert their effects on maternal vascular function, immune tolerance and placental development. AhR is a ligand-activated transcription factor that responds to diverse endogenous and exogenous ligands, including several tryptophan metabolites generated by both host enzymes and gut bacteria. Li *et al* (76) systematically reviewed 45 studies on AhR in endothelial function and concluded that AhR activation by tryptophan metabolites (for example, kynurenine and indole-3-lactic acid) promotes angiogenesis through upregulation of VEGF and hypoxia-inducible factor-1α, establishing a potential mechanistic foundation in understanding how this receptor influences placental vascular development. Zhao *et al* (77,78) have made notable contributions to understanding AhR signaling in HDP pathogenesis, demonstrating in a rat model (n=24) that administration of the endogenous AhR ligand 2-(1'H-indole-3'-carbonyl)-thiazole-4-carboxylic acid methyl ester (ITE) (2,3,7,8-tetrachlorodibenzo-p-dioxin analog; 200 μg/kg/day from gestation day 7-18) induced preeclampsia-like phenotypes: Systolic blood pressure significantly increased from 104±5 to 149±8 mmHg (P<0.001), proteinuria significantly increased 4.2-fold (P<0.001) and placental transcriptomic analysis revealed 1,287 differentially expressed genes enriched in inflammatory and angiogenic pathways, identifying AhR as a key mediator of disease. Zhao *et al* (79) further demonstrated that AhR activation in human umbilical vein endothelial cells (n=6; independent donors) with ITE (1 μM; 24 h) dysregulated endothelial functions: Tube formation was significantly reduced by 48% (P=0.002) and the expression level of endothelial nitric oxide synthase (eNOS) was significantly decreased by 62% (P<0.001), providing mechanistic insights into how this pathway may contribute to the generalized endothelial dysfunction characteristic of preeclampsia. Wei *et al* (17) provided key evidence that indole-3-lactic acid, a tryptophan metabolite produced by gut bacteria, alleviates sFlt-1-induced preeclampsia-like phenotypes through activation of AhR signaling, as described in section 1. Wei *et al* (18) subsequently demonstrated that indole-3-lactic acid (10 μM; 24 h) significantly increased trophoblast migration (scratch wound assay, 78% closure vs. 34% in controls; P<0.001) and invasion (Transwell, 2.3-fold; P<0.001) in HTR-8/SVneo cells via the AhR/versican (VCAN) pathway, further elaborating the mechanistic basis for beneficial effects on placental development. Xodo *et al* (80) examined the AhR network in placental tissues from pregnancies complicated by preeclampsia (n=30) compared with controls (n=30) and reported that AhR protein expression significantly increased 2.1-fold (P=0.008) while its downstream target CYP1A1 was significantly reduced by 54% (P=0.02), indicating a dysfunctional signaling system in affected women. Kim *et al* (81) investigated the role of AhR in vascular factors associated with preeclampsia using a smoking mouse model (n=20) and reported that cigarette smoke extract (1 mg/kg/day) activated placental AhR (CYP1A1 significantly increased 4.5-fold; P<0.001) and significantly increased sFlt-1 production by 67% (P=0.002), revealing complex interactions between exogenous ligands and endogenous signaling pathways. Jiang *et al* (82) recently provided genetic evidence supporting the importance of this pathway. The study performed genotyping of the AhR gene (rs2066853) in 500 preeclamptic and 500 control women, and reported that the G allele was significantly associated with a 1.4-fold increased risk (P=0.02) and reduced AhR transcriptional activity by 34% in reporter assays, demonstrating functional implications of AhR gene polymorphism in preeclampsia susceptibility. Marbrey *et al* (83) proposed that AhR-activated placental adrenomedullin may explain the paradoxical protective effects of smoking against preeclampsia. The study

demonstrated that in placental explants (n=12), the AhR agonist 6-formylindolo(3,2-b)carbazole (10 nM) significantly increased adrenomedullin secretion by 3.1-fold (P<0.001) and that adrenomedullin, in turn, significantly reduced sFlt-1 release by 48% (P=0.005), further highlighting the complexity of AhR signaling in pregnancy. Collectively, these studies established AhR as a master regulator integrating diverse environmental and metabolic signals into coordinated cellular responses relevant to HDP pathogenesis.

Functional evidence in placental dysfunction, vascular injury and immune imbalance. The functional consequences of altered tryptophan metabolism in HDP have been investigated using *in vitro* cellular models and *in vivo* animal studies, providing mechanistic insights that complement observational human data. Worton *et al* (84) performed wire myography on isolated omental and placental arteries from normotensive women (n=24) and women with preeclampsia (n=24). The study reported that kynurenine (100 μM) induced relaxation of 78±8% in normotensive arteries and 82±6% in preeclamptic arteries (P=0.45 for between-group comparison), identifying a direct vasodilatory effect of this tryptophan metabolite that is preserved in the diseased state. Worton *et al* (85) further explored the therapeutic potential of targeting the kynurenine pathway in treating the maternal features of preeclampsia. The study reviewed 32 preclinical and clinical studies and concluded that kynurenine pathway modulators (for example, IDO inhibitors and kynurenine aminotransferase activators) represent a novel target for pharmacological intervention, although safety in pregnancy remains to be investigated in the future. Broekhuizen *et al* (86) comprehensively reviewed the function of the kynurenine pathway in the placenta, synthesizing evidence from 48 studies and proposing that KYNA, through GPR35 activation, may protect against placental ischemia; however, elevated kynurenine in preeclampsia could be either adaptive or maladaptive. Marsden (87) provided an accompanying perspective emphasizing the importance of tryptophan metabolism in pregnancy and the therapeutic implications of targeting this pathway. Gumusoglu *et al* (88) investigated the serotonin-immune axis in preeclampsia. The study measured serotonin and inflammatory cytokine levels in serum from 50 preeclamptic and 50 control women and reported that serotonin was significantly reduced by 45% (P<0.001) and correlated negatively with IL-6 (r=-0.61; P<0.001), revealing complex interactions between serotonergic signaling and inflammatory processes. Gumusoglu *et al* (89) also demonstrated that low IDO activity (measured by serum kynurenine/tryptophan ratio ≤0.035) was significantly associated with a 3.1-fold increased risk of both preeclampsia and postpartum depression (P=0.004), linking tryptophan metabolism with both psychiatric and obstetric outcomes. Using the Rgs2 knockout model, Gumusoglu *et al* (90) reported that $Rgs2^{-/-}$ mice (n=12) had a significant 52% reduction in placental serotonin (P<0.001) and a significant 2.7-fold increase in sFlt-1 (P=0.002), identifying anti-angiogenic mechanisms and serotonergic dysfunction relevant to psycho-obstetric risk, further elaborating the mechanistic associations between tryptophan metabolites and pregnancy complications. Prescott *et al* (91) conducted an integrative review examining tryptophan as a biomarker of pregnancy-associated immune expression and modulation. The review synthesized data from 38 studies and concluded that the kynurenine/tryptophan ratio is elevated in preeclampsia, intrauterine growth restriction and preterm birth, suggesting that tryptophan is a general marker of inflammatory stress rather than a HDP-specific signature. Kudo and Sugimoto (15) comprehensively reviewed the role of the placental enzyme IDO in normal and abnormal human pregnancy. The review summarized that IDO expression in syncytiotrophoblasts increases with gestational age and is reduced by 40-60% in preeclamptic placentas, emphasizing its key functions in immune regulation and vascular homeostasis. A previous study by Dupont *et al* (92) used a combination of human samples (n=60) and a mouse model (n=24) to reveal that impaired renal reserve (measured by creatinine clearance) was associated with a significant 2.3-fold increase in serum kynurenine (P=0.005) and a significant 2.1-fold increase in sFlt-1 (P=0.003), identifying a novel mechanism associating renal function, tryptophan metabolism and placental dysfunction. These functional studies collectively demonstrated that tryptophan metabolites influence multiple aspects of HDP pathophysiology, including vascular tone regulation, immune cell function, trophoblast biology and endothelial integrity, through mechanisms that converge on AhR signaling and other downstream effectors.

Interplay with classic HDP pathogenic pathways. Tryptophan metabolites do not function in isolation but rather interact extensively with other classic HDP pathogenic pathways, including the renin-angiotensin-aldosterone system, oxidative stress, inflammatory cascades and angiogenic factor balance. The interplay between tryptophan metabolism and oxidative stress is particularly notable, as both pathways are markedly altered in preeclampsia and exhibit bidirectional regulatory relationships. Chiarello *et al* (93) comprehensively reviewed oxidative stress in normal pregnancy vs. preeclampsia (n=212 studies). The review concluded that reactive oxygen species are increased 2- to 3-fold in preeclampsia and that tryptophan metabolites, particularly kynurenine, can both generate and scavenge free radicals, suggesting bidirectional regulatory relationships. The relationship between tryptophan metabolism and angiogenic balance is exemplified in a previous study by Wei *et al* (17), which demonstrated that indole-3-lactic acid alleviates sFlt-1-induced preeclampsia-like phenotypes, as aforementioned. Hsu and Tain (94) recently reviewed melatonin as a redox modulator in developmental programming, highlighting that tryptophan-derived melatonin reduces oxidative stress markers by 40-60% in preclinical models of pregnancy hypertension and that melatonin supplementation may have therapeutic potential for hypertensive disorders of pregnancy. A comprehensive multi-omics analysis by Marić *et al* (95) used machine learning on data from 2,200 pregnant women (800 preeclamptic women and 1,400 controls) and reported that tryptophan metabolites contributed to 24% of the predictive signal in the final model, with kynurenine and indole-3-lactic acid identified among the top 15 features, enabling early prediction of preeclampsia as early as gestational week 12 (AUC=0.92 in validation cohort). These interconnected pathways create a complex regulatory network

in which perturbations in tryptophan metabolism can propagate through multiple systems to influence overall pregnancy outcomes, suggesting that therapeutic strategies targeting this pathway may have e effects on HDP pathophysiology.

5. Synergistic and antagonistic crosstalk between SCFAs and tryptophan microbial metabolites in HDP pathogenesis

Based on available evidence, three potential modes of interaction are proposed: i) Synergistic amplification: SCFAs inhibit HDACs, increasing chromatin accessibility, while tryptophan metabolites activate AhR and HDAC inhibition may potentiate AhR-driven transcription; ii) competitive antagonism: SCFAs via GPR43 promote anti-inflammatory M2 macrophage polarization, whereas AhR signaling can be pro-inflammatory and high SCFA availability may override AhR-mediated inflammation; and iii) sequential signaling: AhR activation (by indole-3-lactic acid) primes trophoblast migration, while SCFAs (butyrate) support metabolic adaptation and immune evasion. These three modes collectively constitute the specific biochemical crosstalk model proposed in the present review. Direct experimental validation of these three proposed modes of interaction is currently lacking, and the framework thus remains hypothetical at present. The following subsections provide the experimental and clinical evidence that informs these three proposed modes. Specifically, the mechanistic evidence for synergistic amplification is detailed below in the subsection on synergistic protective effects and the evidence for competitive antagonism is detailed in the subsection on antagonistic interactions. Table SI summarizes the key evidence for metabolic interactions between SCFAs and tryptophan pathways in HDP. Direct evidence for SCFAs-tryptophan crosstalk in HDP is hypothesis-generating rather than definitive. No study has measured both metabolite classes in the same cohort to formally test for statistical interactions, to the best of our knowledge. The proposed crosstalk is supported by separate lines of preclinical and observational research; however, prospective studies are warranted in the future.

Shared core regulatory networks. Converging evidence from preclinical and clinical studies indicated that SCFAs and tryptophan metabolites interface through overlapping signaling pathways and shared downstream effectors. These shared nodes provide the molecular basis for potential synergistic or competitive interactions between the two metabolite classes. Using the BPH/5 mouse model (n=10 per group), Beckers *et al* (61) performed integrated microbiome (16S rRNA) and metabolome (LC-MS) analyses and reported that pregnancy-specific shifts in both compartments were correlated [Procrustes analysis, M^2 (a goodness-of-fit statistic with lower values indicating greater concordance between the two datasets)=0.34; P=0.02], with notable alterations in SCFA profiles (butyrate reduced 45%) accompanied by changes in tryptophan metabolites (indole-3-lactic acid reduced 38%), suggesting coordinated dysregulation of these pathways through shared regulatory nodes. This coordinated dysregulation suggests that perturbations in one metabolic pathway may propagate to the other through shared regulatory nodes. Indeed, the AhR has been established as a central hub integrating signals from tryptopha-derived metabolites (17,18), while SCFAs modulate immune cell polarization and trophoblast function via GPR43-mediated signaling (55), collectively highlighting the convergence of these two metabolite classes on shared regulatory networks at the maternal-fetal interface. The convergence of SCFAs and tryptophan metabolite signaling on common transcription factors (for example, AhR and NF-κB) and immune cell populations (Tregs and macrophages) supports the existence of integrated regulatory networks at the maternal-fetal interface.

Synergistic protective effects on vascular and immune homeostasis. Direct mechanistic evidence for synergistic amplification derives from a previous study by Jin *et al* (55), which demonstrated that butyrate acts as a HDAC inhibitor, increasing chromatin accessibility at AhR target gene promoters (for example, CYP1A1) and enhancing AhR recruitment in the presence of tryptophan-derived agonists. This effect is independent of GPR41/43 and was confirmed in human intestinal explants. Xiong *et al* (96) further demonstrated that butyrate upregulates gene expression via HDAC inhibition, providing a mechanistic basis for SCFA-mediated potentiation of AhR-driven transcription. These findings are consistent with the proposed 'synergistic amplification' mode, where SCFA-mediated HDAC inhibition may enhance AhR-driven transcription. In a diagnostic study of 95 preeclamptic and 98 healthy pregnant women, Li *et al* (52) measured plasma SCFAs and reported that a panel of acetate and butyrate levels distinguished preeclampsia from healthy pregnancy with an AUC of 0.94 (95% CI, 0.89-0.98). This clinical association supports the biological plausibility that SCFAs, via HDAC inhibition, may enhance AhR-driven protective transcriptional programs. Complementing these findings, Marić *et al* (95) used machine learning on multi-omics data from 2,200 pregnant women and reported that tryptophan metabolites accounted for 24% of the predictive signal for preeclampsia, with kynurenine and indole-3-lactic acid among the top 15 features. The convergence of SCFA- and tryptophan-based predictive signatures suggests that these metabolite classes may act through shared downstream pathways. The synergistic potential of these metabolite classes is further supported by multi-omics analyses in Cao *et al* (97), which prospectively profiled serum metabolites in 800 patients with suspected preeclampsia and identified a 12-metabolite panel (including butyrate, indole-3-lactic acid and 10 other markers) that predicted progression to preeclampsia with an AUC of 0.91 (95% CI, 0.87-0.95) when measured at 20-24 weeks. While direct evidence for SCFAs-tryptophan synergy in pregnancy remains limited, studies in other systems demonstrated that microbial metabolites can cooperatively enhance host defense mechanisms (98-100). The concept that combinations of microbial metabolites may produce greater effects compared with the sum of their individual contributions warrants systematic investigation in the context of HDP in the future.

Antagonistic interactions and compensatory metabolic regulation. Direct mechanistic evidence for competitive antagonism is provided in the study by Zhao *et al* (78), which demonstrated that AhR activation induces endothelial dysfunction and elevates blood pressure in a rat model of preeclampsia-like phenotype. By contrast, SCFAs, particularly

butyrate, promote anti-inflammatory M2 macrophage polarization via GPR43 activation and enhance Treg development via HDAC inhibition (54,55). These opposing functional outcomes on vascular and immune homeostasis support the 'competitive antagonism' hypothesis, wherein high SCFA availability may override AhR-mediated pro-inflammatory signals. In a rat model of preeclampsia (n=24), Xu *et al* (101) performed metabolomic profiling on maternal brain, placenta and fetal tissues and reported that pathways for SCFAs (butyrate) and tryptophan metabolites (kynurenine) exhibited opposite directional changes: Butyrate significantly decreased by 44% in maternal brain (P=0.002) while kynurenine significantly increased by 67% (P<0.001), suggesting a compensatory metabolic shift. Metabolomic profiling by Yutao *et al* (102) in a rat preeclampsia model (n=30) treated with aspirin (20 mg/kg/day) reported that aspirin simultaneously and significantly increased butyrate by 52% (P<0.001) and decreased kynurenine by 38% (P=0.003), demonstrating that therapeutic interventions can modulate multiple metabolic pathways in opposite directions. Jääskeläinen *et al* (73) identified elevated levels of carnitine precursors and trimethylated compounds, alongside tryptophan-associated metabolites, in the cord blood plasma of preeclamptic infants, suggesting that fetal exposure to metabolic disturbances involves coordinated shifts across multiple pathways, with certain metabolites (butyrate) decreased and others (indole-3-acetate) increased. Understanding these compensatory responses may provide insights into why single-metabolite interventions often yield inconsistent results and why combined approaches may be more effective for preventing or treating HDP.

Modulation by maternal dietary and genetic factors. The crosstalk between SCFAs and tryptophan metabolism is dynamically modulated by maternal dietary intake and genetic predisposition. This population-specific variation highlights the importance of gene-environment interactions in shaping metabolic phenotypes. Luo *et al* (103) demonstrated that human chorionic gonadotropin (hCG; 100 IU/ml) stimulated IL-4-induced-1 (IL4I1) expression in human endometrial stromal cells (n=6 donors) by 3.2-fold (P<0.001) and that IL4I1, through AhR, promoted decidualization (prolactin secretion increased 2.8-fold; P<0.001), revealing a hormonal association with tryptophan metabolism that may interact with SCFA signaling. Dietary factors represent the most readily modifiable determinants of microbial metabolite production; however, the specific dietary patterns that optimally support both SCFAs and tryptophan metabolism during pregnancy remain poorly defined. Kuang *et al* (104) emphasized the importance of understanding metabolic pathways and disease signatures in response to environmental exposures. The study used a murine model to demonstrate that exposure to tribromobisphenol A altered both SCFA and tryptophan metabolic pathways, with a 34% significant reduction in butyrate and a 41% significant reduction in indole-3-lactic acid (both P<0.01), a principle that extends to dietary and pharmacological interventions targeting the maternal gut microbiota.

Combined predictive value for early risk stratification. The integration of SCFAs and tryptophan metabolite measurements may enhance early prediction and risk stratification for HDP beyond what can be achieved with either class alone. Escorcia Mora *et al* (105) reviewed 86 studies on the gut microbiota in female reproductive health and concluded that endometrial signaling pathways (for example, AhR and GPR43) may be influenced by microbial metabolites including SCFAs and tryptophan derivatives, suggesting that combined measurement could improve prediction of implantation success and pregnancy outcomes. Song *et al* (106) investigated the metabolic role of the CD73/adenosine signaling pathway in trophoblast cells (HTR-8/SVneo; n=6; independent experiments) and reported that adenosine (10 μM) significantly increased trophoblast migration by 2.1-fold (P<0.001) and that this effect was augmented by butyrate (1 mM) and indole-3-lactic acid (10 μM) in an additive manner (3.4-fold increase; P<0.001), revealing complex interactions that could serve as nodes for metabolite integration. The multi-omics approach introduced by Marić *et al* (95) demonstrated that combining multiple classes of metabolites with clinical variables markedly improves predictive accuracy for preeclampsia (AUC increased from 0.76 with clinical variables alone to 0.92 after including metabolite data; P<0.001). As Cao *et al* (97) confirmed in a large-scale prospective study (n=800), metabolite-based signatures offer potential in identifying at-risk women before clinical manifestation of disease, with the SCFA-tryptophan panel achieving a negative predictive value of 96% at 20 weeks. Future efforts to develop clinically useful biomarker panels should consider including representatives from both SCFAs and tryptophan pathways to capture the full spectrum of microbial metabolite contributions to HDP pathogenesis.

6. Clinical translation potential of targeting SCFAs and tryptophan microbial metabolism for HDP prevention and management

The growing understanding of microbial metabolite involvement in HDP has opened novel avenues for clinical translation, ranging from early biomarker development to targeted therapeutic interventions. However, it should be noted that the majority of all translational studies discussed below have been conducted in preeclampsia; data on gestational hypertension or eclampsia are lacking and findings may not be directly generalizable to these other HDP subtypes. SCFAs and tryptophan-derived metabolites offer promising opportunities for non-invasive risk stratification, dietary and probiotic-based prevention strategies and novel pharmacological approaches. However, the path from preclinical identification to clinical implementation requires validation across diverse populations and careful consideration of safety in pregnancy. Table SII summarizes the current clinical translation landscape for SCFAs and tryptophan metabolite-based approaches in HDP.

Microbial metabolites as non-invasive early predictive biomarkers. Multiple clinical studies have demonstrated the potential of SCFAs and tryptophan metabolites as early predictive biomarkers for HDP. Chang *et al* (50) quantified fecal SCFAs in 35 preeclamptic and 35 normotensive women and reported that butyrate was significantly reduced by 54% (P<0.001) and propionate by 41% (P<0.001), with these reductions correlating with systolic blood pressure (r=-0.61;

P<0.001). This observation established a potential foundation for the use of fecal SCFAs as non-invasive biomarkers. Building on this evidence, Li *et al* (52) extended these findings to plasma. The study demonstrated that a panel of acetate and butyrate achieved an AUC of 0.94 in distinguishing preeclampsia from healthy pregnancy, indicating that circulating SCFAs also have diagnostic potential for HDP. Collectively, studies have further demonstrated the utility of combining SCFA and tryptophan metabolite measurements for early risk stratification, including prospective profiling in at-risk pregnancies, multi-metabolite discrimination in diverse populations and detection of fetal exposure to altered tryptophan metabolism (73,74,97). Powell *et al* (107) further demonstrated the utility of metabolic profiling in diagnosing pregnancy complications, in a case-control study (n=150) that a panel of 16 metabolites (including butyrate and kynurenine) discriminated preeclampsia from other complications with an AUC of 0.84 (95% CI, 0.77-0.91). Thus, heterogeneity in study populations, sample matrices and analytical platforms warrants standardization and validation in prospective cohorts before clinical use in the future.

Key appraisal of biomarker specificity and technical artifacts. Despite promising diagnostic accuracy in case-control studies, two major challenges limit clinical translation. First, circulating levels of SCFAs and kynurenine pathway metabolites are not disease-specific; they are notably influenced by diet, renal function, systemic inflammation and gut permeability, all of which are altered in HDP (9,62). Thus, observed differences between preeclamptic and normotensive women may reflect secondary changes rather than a unique disease signature. Second, measurement of plasma butyrate is technically problematic: Butyrate can be generated *ex vivo* by residual erythrocytes if blood samples are not processed with immediate centrifugation and plasma separation, a condition rarely achievable in routine clinical settings (108). This factor may have contributed to the heterogeneity across studies (14). Therefore, while metabolite-based signatures are promising, pre-analytical standardization and validation for disease-specificity in prospective cohorts are warranted before clinical implementation in the future.

Dietary intervention strategies for primary prevention. Dietary modification represents the most accessible approach in modulating microbial metabolite production and potentially reducing HDP risk. Both human observational and preclinical evidence support the potential of dietary fiber to enhance SCFA production and improve HDP-related outcomes (62,64). Gomez-Arango *et al* (109) provided clinical evidence associating increased blood pressure with altered gut microbiota composition and reduced butyrate production in early pregnancy. The study demonstrated in a cohort of 80 pregnant women that each 1 log reduction in fecal butyrate was associated with a 4.2 mmHg increase in systolic blood pressure (P=0.02), suggesting that dietary strategies to enhance butyrate production may have preventive potential for HDP (109). The importance of nutritional modulation extends to tryptophan metabolism, for example, Wu *et al* (110) reviewed 78 studies and concluded that dietary nutrients (tryptophan, fiber and polyphenols) mediate intestinal host defense peptide expression, a mechanism potentially relevant to HDP pathogenesis. Whitmore *et al* (111) further explored nutritional modulation of host defense peptide synthesis as a novel host-directed antimicrobial therapeutic strategy, highlighting that butyrate and tryptophan metabolites (indole) synergistically increase defensin expression by 3- to 5-fold in intestinal epithelial cell models, with implications for pregnancy-associated inflammatory conditions. However, intervention trials specifically targeting HDP prevention through dietary modulation of microbial metabolites are urgently needed to translate these preclinical and observational findings into clinical practice.

Probiotics, prebiotics and microbiome-targeted approaches. Microbiome-targeted interventions, including probiotics and prebiotics, offer the potential to restore beneficial microbial metabolite production in at-risk women. Wang *et al* (112) conducted a randomized controlled crossover trial (n=40; healthy young adults) comparing a vegetarian diet with and without lean red meat (100 g/day for 4 weeks). The study reported that red meat addition significantly increased fecal butyrate by 28% (P=0.01) and significantly decreased TMAO by 31% (P=0.02), providing proof-of-concept that dietary interventions can reshape microbial communities relevant to metabolite production. In the context of host defense enhancement, Liu *et al* (113) treated IPEC-J2 cells and neonatal piglets (n=12) with *Lactobacillus reuteri* I5007 (10^9 CFU/day) and reported that the probiotic increased intestinal host defense peptide expression [porcine β-defensin (pBD)-2 increased 4.3-fold; P<0.001], suggesting that probiotic supplementation may influence immune-associated metabolite pathways. Xiong *et al* (96) demonstrated that butyrate (1 mM) upregulated endogenous host defense peptides (pBD-1 and pBD-2) by 2.5- to 3.8-fold in porcine intestinal epithelial cells via HDAC inhibition and that oral butyrate (100 mg/kg) administered to piglets (n=24) reduced bacterial translocation and significantly improved survival from 65 to 90% (P=0.04), providing mechanistic support for SCFA-based interventions. The potential of microbiome-targeted approaches in pregnancy is further supported by Zong *et al* (114), which comprehensively reviewed the association between gut microbiota and preeclampsia (n=78 studies), discussing microbiome changes, mechanisms and potential treatments. Nevertheless, the safety and efficacy of probiotic interventions during pregnancy require further evaluation, and only a limited number of studies have specifically examined their impact on HDP outcomes, including a few clinical trials (115) and preclinical investigations (116).

Preclinical progress of targeted therapeutic agents. Notable preclinical progress has been made in developing targeted therapeutic agents that modulate SCFAs signaling and tryptophan-AhR pathways. Yong *et al* (58) demonstrated that sodium butyrate administration to pregnant rats with experimentally induced preeclampsia alleviated hypertensive and proteinuric phenotypes while simultaneously improving gut microbiota composition and enhancing SCFAs metabolite production. Santillan *et al* (117) provided genetic evidence that pregnant mice lacking IDO (n=10) exhibited preeclampsia phenotypes (systolic blood pressure, 148±6 vs. 112±5 mmHg in wild-type; P<0.001; proteinuria, 245±38 vs. 78±12 mg/dl; P<0.001), supporting the importance of tryptophan metabolism in disease

pathogenesis. Additional therapeutic candidates include: i) Isorhynchophylline, which Wang *et al* (118) investigated in a rat preeclampsia model (n=40) and reported that isorhynchophylline (20 mg/kg/day) significantly reduced blood pressure by 28 mmHg (P<0.001) and increased trophoblast migration by 2.2-fold via PI3K/AKT/mTOR-mediated oxidative stress inhibition; and ii) pravastatin, which Toghi *et al* (119) evaluated in a rat model of gestational hypertension (n=30) and reported that pravastatin (5 mg/kg/day) significantly prevented the increase in MMP-2 activity (reduction, 54%; P=0.01) and oxidative stress (malondialdehyde reduced 38%; P=0.02), while significantly enhancing endothelium-derived nitric oxide-dependent vasodilation by 62% (P=0.005). Despite this promising preclinical landscape, translation to human pregnancy warrants careful consideration of fetal safety, optimal dosing and appropriate timing of intervention in future.

Risk-benefit considerations for AhR-targeted therapies in pregnancy. Although AhR activation by indole-3-lactic acid or other ligands exhibits therapeutic potential in preclinical models, the translational path faces notable safety concerns. AhR is a pleiotropic transcription factor with key roles in embryonic development, including organogenesis of the liver, immune system and vasculature. Pharmacological AhR modulation during pregnancy carries a notable risk of teratogenicity and developmental disruption, as evidenced by dioxin-like compounds, such as 2,3,7,8-tetrachlorodibenzo-p-dioxin and dioxin-like polychlorinated biphenyls, that cause birth defects via AhR overactivation (120). Previous studies have revealed that AhR activation in pregnancy can induce preeclampsia-like phenotypes and dysregulate placental and endothelial functions (78,83), underscoring that AhR modulation is not without hazard. No specific reproductive toxicology studies have been performed for indole-3-lactic acid or other AhR-targeting agents, to the best of our knowledge. Thus, any future therapeutic development would require gestational stage-specific delivery (for example, targeting only the maternal-fetal interface without systemic exposure) and extensive preclinical safety evaluation, making clinical application a distant prospect.

Registered and completed clinical trials. The clinical translation of microbial metabolite-based approaches for HDP management remains in its early stages, with limited registered trials specifically targeting these pathways (121-125). Several studies have examined associated interventions, including the safety of various medications during pregnancy. Fareed *et al* (121) conducted a systematic review and meta-analysis of montelukast use in pregnancy (11 studies; 1,234 exposed pregnancies) and found that montelukast exposure during pregnancy was not associated with a significantly increased risk of major congenital malformations (pooled OR, 1.04; 95% CI, 0.85-1.27; I^2=0%), preterm birth or low birth weight compared with unexposed pregnancies. Subgroup analyses by trimester of exposure and by asthma severity yielded consistent null findings, and the GRADE assessment indicated moderate certainty for the primary outcome. These data provide a framework for evaluating medication safety in pregnancy and support the general safety profile of montelukast when used for asthma treatment during gestation, although the small number of included studies and the observational nature of the evidence preclude definitive conclusions. Petersen *et al* (122) comprehensively assessed the risks and benefits of psychotropic medication in pregnancy using cohort studies based on UK electronic primary care health records (n=45,000 pregnancies). The study reported that antidepressant exposure was associated with a small increase in preterm birth (OR=1.2; 95% CI, 1.1-1.3); however, no increase in major malformations was observed, establishing notable safety benchmarks. Sibiude *et al* (123) examined liver enzyme elevation in pregnant women receiving antiretroviral therapy in the National Agency for Research on AIDS and Viral Hepatitis-French Perinatal Cohort (n=10,000). The study reported that 3% had grade 2 or higher liver enzyme elevations according to the Division of AIDS Toxicity Grading Scale (Grade 2: 2.51-5xULN; Grade 3: 5.1-10xULN; Grade 4: >10xULN) (126), highlighting the importance of monitoring metabolic effects of medications during pregnancy. Regan *et al* (124) investigated coronavirus disease 2019 (COVID-19) vaccination around the time of conception and risk of placenta-mediated adverse pregnancy outcomes (n=120,000 pregnancies) and reported no increased risk (hazard ratio=0.96; 95% CI, 0.89-1.04), providing safety data relevant to future intervention trials. Vesco *et al* (125) evaluated obstetric complications and birth outcomes after antenatal COVID-19 vaccination (n=80,000 vaccinated vs. 120,000 unvaccinated) and reported no differences in preterm birth (7.2 vs. 7.4%; P=0.41) or small-for-gestational age (8.1 vs. 8.3%; P=0.38), further contributing to the evidence base for intervention safety in pregnancy. While these studies do not directly target microbial metabolites, the aforementioned findings established key frameworks in evaluating intervention safety in pregnant populations. Specific clinical trials examining SCFAs or tryptophan-based interventions for HDP prevention or management are warranted to translate preclinical potential into clinical practice in the future.

7. Current limitations, controversies and unresolved scientific questions in the field

Current clinical evidence associating microbial metabolites with HDP is predominantly derived from observational studies with inherent methodological constraints (2,8,14). Cross-sectional designs, which characterize majority of studies, preclude determination of whether metabolite alterations precede or follow disease onset, creating notable ambiguity for causal inference (2,7,9). Wu *et al* (127) employed a nested case-control (300 cases and 300 controls) and MR approach to address temporal relationships, demonstrating notable causal associations between specific microbial taxa and HDP (for example, genus *Lactobacillus*, OR=0.74; 95% CI, 0.62-0.89); however, this design cannot fully exclude reverse causation because the genetic instruments for microbiota were derived from non-pregnant populations. Xiong *et al* (128) similarly applied MR to investigate the causal role of the intestinal microbiome in preeclampsia development, using GWAS data for 211 taxa and 5,976 preeclampsia cases, and reported that the genus *Bifidobacterium* was causally associated with reduced risk (OR=0.83; 95% CI, 0.73-0.94), but acknowledged the limitations of instrument strength (F-statistic, <10 for several instruments) and potential horizontal pleiotropy.

Yang *et al* (63) conducted two-sample MR analysis revealing causal relationships between lipid and fatty acid metabolism and preeclampsia; however, the study emphasized that genetic instruments explained only 4-8% of variance in metabolite levels and residual confounding remains possible. Dunlop *et al* (129) markedly reviewed the maternal microbiome and pregnancy outcomes, highlighting notable heterogeneity in study populations (sample sizes, 20-500), sampling protocols (stool vs. rectal swab and different storage conditions) and analytical pipelines (16S vs. metagenomics, different variable regions and different databases), which markedly limit cross-study comparability and meta-analytic efforts.

A fundamental controversy that persists in the field concerns the directionality of the relationship between microbial metabolites and HDP pathophysiology. While observational studies consistently reported altered SCFAs and tryptophan metabolite profiles in affected women (40,50), whether these changes represent causative factors or epiphenomena secondary to disease-associated alterations in diet, intestinal permeability or immune function remain to be elucidated. The reverse causation scenario, whereby systemic endothelial dysfunction, placental ischemia and the intense inflammatory state of HDP drive gut dysbiosis and altered metabolite production, is equally plausible and is supported by emerging experimental evidence (9,50). Specifically, a case-control study by Chang *et al* (50) compared 35 preeclamptic women with 35 normotensive controls and reported that reduced fecal SCFAs, particularly butyrate and propionate, associated with elevated systolic blood pressure, suggesting a bidirectional relationship between microbial metabolites and disease pathology. These findings are consistent with the broader recognition that systemic inflammation, a hallmark of HDP pathophysiology, can itself drive gut dysbiosis, increased intestinal permeability and subsequent metabolic alterations, creating a self-reinforcing cycle rather than a unidirectional causal chain (9).

Beckers and Sones (130) markedly examined the maternal microbiome and preeclampsia (n=59 studies), noting that cross-sectional associations cannot distinguish cause from consequence and that intervention studies are warranted to establish directionality in the future. Wang *et al* (131) investigated gut microbiota-derived TMAO as a potential target for preeclampsia treatment, demonstrating in a case-control study (n=100) that TMAO levels were significantly elevated by 58% in preeclamptic women ($P<0.001$) and that TMAO inhibition (using 3,3-dimethyl-1-butanol and 1% in drinking water) attenuated disease phenotypes in a rat model (blood pressure significantly reduced by 29 mmHg; $P<0.001$); however, the translational relevance of these findings to human pregnancy remains to be elucidated. The bidirectional nature of this association is further complicated by the recognition that pregnancy itself induces notable physiological remodeling of the maternal gut microbiota, making it challenging to disentangle disease-specific alterations from gestational stage-dependent changes. Gare *et al* (132) conducted a scoping review on periodontal conditions and preeclampsia (n=32 studies), highlighting the complexity of establishing causality in conditions where multiple interacting factors converge and noted that intervention trials targeting periodontal disease failed to reduce preeclampsia risk (pooled OR=0.96; 95% CI, 0.81-1.14), cautioning against oversimplified causal models.

Beyond diet and medication, other major unmeasured confounders further limit causal inference. Gestational weight gain trajectory independently alters gut microbiota composition and SCFA production (39,49); excessive or inadequate weight gain may confound associations between microbial metabolites and HDP. Physical activity patterns modulate both SCFA levels (via enhanced fermentation) and blood pressure (62); however, these patterns are rarely quantified in HDP microbiome studies, to the best of our knowledge. Similarly, sleep chronobiology and circadian disruption affect gut microbial composition and metabolite profiles (10), and are known to influence inflammatory and vascular pathways relevant to HDP (11). To the best of our knowledge, the absence of these variables in the majority of all published studies represents a key source of residual confounding that undermines the interpretability of observed associations. To address the confounding effect of diet, future observational studies should prospectively collect detailed dietary intake data (for example, 24-h dietary recalls or validated food frequency questionnaires) and explicitly adjust for dietary fiber and tryptophan consumption in multivariable models. These adjustments would potentially help distinguish metabolite alterations attributable to HDP pathophysiology from those driven by dietary differences.

Notable technical challenges complicate the accurate detection and quantification of microbial-derived metabolites in pregnant populations. Distinguishing between metabolites of microbial origin and those derived from host metabolism represents a fundamental analytical hurdle, as several metabolites can be generated through both routes. Wagner *et al* (133) demonstrated that prenatal complications are associated with postnatal airway host responses and microbiota in preterm infants (n=150), highlighting the complexity of distinguishing microbial contributions from host responses in biological samples, as 31% of detected bacterial genera were also present in environmental controls. The need for absolute quantification rather than relative abundance measures is increasingly recognized (44,46,47); however, standardized protocols for metabolite extraction, derivatization and calibration across different biological matrices remain lacking. Saadaoui *et al* (134) reviewed the bidirectional relationship between oral microbiome and pregnancy (78 studies), emphasizing that sampling site, timing (gestational week) and processing methods (DNA extraction kit and primer choice) notably influence detection outcomes, with up to 50% variation in α-diversity attributable to methodological choices. Moumne *et al* (135) discussed implications of the vaginal microbiome for maternal health, noting that contamination during sample collection and processing remains a persistent concern that can introduce both false-positive and false-negative findings and that negative controls (blank swabs and extraction blanks) are included in only 12% of studies.

Temporal and spatial heterogeneity of metabolite actions. The present review considers the maternal-fetal interface as a relatively uniform compartment, which represents a major simplification. This simplification reflects the current state of the literature, as majority of existing studies have analyzed whole placental tissue, maternal serum or mixed

cell populations without cell-type or gestational-stage resolution (9,50,54,55). In reality, the cellular constituents of this interface, including maternal vascular endothelial cells, decidual immune cells (macrophages, T and NK cells), extravillous trophoblasts and syncytiotrophoblasts, are likely to exhibit distinct and cell-type-specific responses to SCFAs and tryptophan metabolites. For example, butyrate modulates macrophage polarization via GPR43 (55) while also affecting Treg development (54); however, direct comparisons across cell types are lacking. Furthermore, the functional roles of these metabolites are not static across gestation. Placentation and spiral artery remodeling occur predominantly in the first and early second trimester, whereas fetal growth and immune tolerance maintenance dominate the third trimester. Several studies examining SCFA or tryptophan metabolite effects at different gestational stages are virtually absent (9,14,71), to the best of our knowledge. Thus, the current evidence cannot distinguish whether metabolite alterations are drivers of early placental dysfunction or consequences of late-gestation disease pathology. Future research should adopt spatially resolved (cell-type-specific) and temporally resolved (gestational stage-specific) approaches to deconstruct host-microbiota metabolite interactions at the maternal-fetal interface.

Limitations of MR studies in the context of HDP and microbial metabolites. Several MR studies have been cited as providing genetic evidence supporting causal relationships between gut microbiota or metabolite pathways and HDP (23,32,63,127,128). However, a key and often overlooked limitation is that the genetic instruments for metabolites used in these MR analyses are derived from GWAS conducted in non-pregnant populations, typically middle-aged or elderly adults of European ethnicity. Their validity as proxies for metabolite exposure during pregnancy, a state of notable physiological and metabolic remodeling, is unverified. Pregnancy induces major changes in gut microbiota composition, intestinal permeability, immune function and hormonal milieu, all of which could markedly alter the genetic regulation of metabolite levels (9,71). Furthermore, MR studies assume that genetic variants affect the outcome exclusively through the exposure of interest (no pleiotropy), an assumption that is difficult to satisfy when the exposure (for example, SCFAs) itself is influenced by diet, medication and other environmental factors that change markedly during pregnancy (7,10). Specifically for gut microbiota-metabolite traits, horizontal pleiotropy (where a genetic variant influences the outcome through pathways independent of the exposure) and weak instrument bias (when genetic variants explain only a small fraction of exposure variance) are major concerns that can lead to false causal inferences (7,10). Thus, while MR provides suggestive evidence, it does not offer definitive causal inference in the context of HDP and findings should be interpreted with caution. GWAS of the maternal metabolome during pregnancy particularly are warranted to generate pregnancy-specific genetic instruments in the future.

Key translational barriers limit the extrapolation of findings from preclinical animal models to human HDP pathophysiology. While rodent models have provided notable mechanistic insights into SCFAs and tryptophan metabolite actions (13,67,70), key differences in placental structure, gestational timing and maternal-fetal metabolic exchange between rodents and humans constrain the direct applicability of these findings. Mate *et al* (136) reviewed the relationships between lifestyle, maternal nutrition and healthy pregnancy (n=112 studies), emphasizing that animal models cannot fully recapitulate the complexity of human gene-environment interactions and the multifactorial nature of HDP. Enninga and Garovic (137) discussed a novel mechanism of increased preeclampsia risk after kidney donation, highlighting that animal models of pre-existing conditions may not accurately reflect the metabolic adaptations of healthy human pregnancy.

Key knowledge gaps persist regarding the transmission of microbial metabolites from mother to fetus and the potential intergenerational effects of HDP-associated metabolic disturbances. Whether SCFAs and tryptophan metabolites cross the placenta and reach biologically relevant concentrations in fetal circulation remains incompletely characterized. As demonstrated by Broekhuizen *et al* (75), altered tryptophan metabolism in HDP leads to measurable changes in fetal circulation, but the mechanisms governing trans-placental transfer and the direct effects on fetal development remain poorly understood. However, the mechanisms governing transplacental transfer, the role of placental metabolism in modifying these compounds and the direct effects on fetal development remain poorly understood. Ma *et al* (12) comprehensively reviewed the gut-placenta axis in preeclampsia, identifying key knowledge gaps regarding the regulatory networks associating maternal gut microbiota with placental function and fetal outcomes. The potential in intergenerational programming through microbial metabolite-mediated epigenetic modifications represents an emerging frontier with notable implications in understanding the developmental origins of health and disease. Resolving these unanswered questions will require integrated multi-omics approaches, longitudinal mother-offspring cohort studies with extended follow-up and mechanistic investigations in models that permit direct assessment of fetal programming effects in the future. To improve study quality and cross-study comparability, future studies should consider: i) Standardized gestational age at sampling; ii) medication and dietary records; iii) batch correction; and iv) absolute quantification and negative controls for contamination.

8. Conclusions

Gut microbial metabolites, particularly SCFAs and tryptophan derivatives, serve key roles in HDP by modulating vascular function, immune tolerance and placental development through convergent signaling pathways. Their synergistic and antagonistic crosstalk underscores the integrated nature of host-microbiota interactions. Advancing clinical translation warrants standardized methodologies, prospective validation of predictive biomarkers and further evaluation of targeted interventions to potentially improve maternal and offspring outcomes in the future.

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Authors' contributions

FG, LW, GT and LS made notable contributions to the conception and design of the present review. All authors performed the literature search, data synthesis and interpretation. FG and LS drafted the manuscript, and all authors notably revised the manuscript for key intellectual content. All authors agreed to be accountable for all aspects of the work. All authors read and approved the final manuscript. Data authentication is not applicable.

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Competing interests

The authors declare that they have no competing interests.

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