Placental energy metabolism in health and disease—significance of development and implications for preeclampsia

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The placenta is a highly metabolically active organ fulfilling the bioenergetic and biosynthetic needs to support its own rapid growth and that of the fetus. Placental metabolic dysfunction is a common occurrence in preeclampsia although its causal relationship to the pathophysiology is unclear. At the outset, this may simply be seen as an “engine out of fuel.” However, placental metabolism plays a vital role beyond energy production and is linked to physiological and developmental processes. In this review, we discuss the metabolic basis for placental dysfunction and propose that the alterations in energy metabolism may explain many of the placental phenotypes of preeclampsia such as reduced placental and fetal growth, redox imbalance, oxidative stress, altered epigenetic and gene expression profiles, and the functional consequences of these aberrations. We propose that placental metabolic reprogramming reflects the dynamic physiological state allowing the tissue to adapt to developmental changes and respond to preeclampsia stress, whereas the inability to reprogram placental metabolism may result in severe preeclampsia phenotypes. Finally, we discuss common tested and novel therapeutic strategies for treating placental dysfunction in preeclampsia and their impact on placental energy metabolism as possible explanations into their potential benefits or harm.

Key words: epigenetics, fetal growth restriction, glycolysis, metabolism, metformin, mitochondria, placenta, preeclampsia, reactive oxygen species

Introduction
The placenta plays a vital role in the development and severity of preeclampsia. It has long been established that the presence of the placenta and not the fetus is necessary for preeclampsia. For example, molar pregnancies are susceptible to preeclampsia and the syndrome resolves on removal of the placenta.1

The prevailing hypothesis for the cause of preeclampsia centers on defective placentation and placental dysfunction. As such, preeclampsia shares common pathophysiology with other “disorders of placentation” often referred to as the “great obstetrical syndromes” that include spontaneous miscarriage, placental abruption, and fetal growth restriction (FGR).2 Defective placentation in preeclampsia is characterized by abnormal trophoblast invasion and remodeling of the spiral arteries by extravillous trophoblast. Deficient spiral artery remodeling leads to a failure to establish an appropriate uteroplacental blood supply and therefore is thought to give rise to trophoblast damage that may be accompanied by an ischemia-reoxygenation type of injury3 and placental stress (oxidative, endoplasmic reticulum [ER], and inflammatory). The maternal peripheral endothelial activation and systemic inflammatory response are then triggered by placently released factors associated with placental stress.

Perturbations in placental metabolism and oxidative stress are universally observed in preeclampsia, although the cause-and-effect relationship is not clear. Placental energy metabolism intermediates are inversely correlated with levels of placenta-released soluble fms-like tyrosine kinase 1 (sFlt-1),4 suggesting that the deficiency in energy metabolism correlates with preeclampsia severity. In this review, we provide an overview of our understanding of the placental central energy metabolic pathways and their multifaceted contributions to cellular processes. We highlight the emerging role of metabolic intermediates as cell signaling and epigenetic modifiers and the significance of these links during placental development and implications for preeclampsia.

Central Carbon Metabolism—Contributions to Adenosine Triphosphate and Beyond
Central carbon metabolism describes the series of reactions that result in the

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transformation of nutrients into compounds containing high-energy phosphate bonds such as adenosine triphosphate (ATP). An overview of the metabolic pathways contributing to ATP generation is shown in Figure 1. In addition to fulfilling the bioenergetic functions of the cell, the metabolic intermediates, cofactors, and cosubstrates generated by these reactions also provide biosynthetic precursors, balance reducing equivalents, and orchestrate the management of reactive oxygen species (ROS). Moreover, there is growing evidence of a role for these metabolic intermediates in regulating signal transduction and gene control through transcriptional and epigenetic processes.

Bioenergetics

The placenta produces approximately 5 µmol of ATP per gram of tissue per minute from glucose, which is equivalent to >2.5 kg of ATP per day in a term placenta. This metabolic activity is required to meet the high ATP demand of many energetically demanding tasks, such as nutrient transport and protein synthesis which constitute >50% of the total ATP consumption. To support maternal cardiometabolic adaptations to pregnancy, the placenta secretes large quantities of hormones into the maternal circulation, a process that requires considerable ATP input. For example, human placental lactogen (hPL) production by the term placenta reaches 1 to 4 g/d, which requires approximately 366 mg of ATP for hPL protein synthesis. Therefore, high ATP-consuming processes such as protein synthesis and nutrient transport are impaired in placentas with pre-eclampsia with ensuing FGR.

Glucose is the major nutrient source for energy generation in the placenta. Approximately 50% of the glucose taken up from the maternal circulation is oxidized in the placenta, and only 20% transferred to the fetus with the remainder metabolized into lactate. Glucose metabolism by glycolysis generates pyruvate with a net gain of 2 ATP molecules. This pyruvate is transported into the mitochondria and feeds into the tricarboxylic acid (TCA) cycle after oxidation into acetyl coenzyme A (acytetyl-CoA) either as citrate or oxaloacetate. In addition to glucose, fatty acids and amino acids provide alternative fuel sources to feed into the TCA cycle via their conversion into the metabolic intermediate acetyl-CoA. In the TCA cycle, only 1 ATP molecule is generated for each acetyl-CoA, but the iterative oxidation reactions produce reduced nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide (FADH2), which function as electron carriers to establish the proton gradient that drives ATP production through oxidative phosphorylation (OXPHOS) in the electron transport chain (ETC). Theoretically, 1 molecule of NADH and FADH2 produces 2.5 and 1.5 ATP molecules, respectively. However, in practice, this is considerably less owing to energy consumption by active mitochondrial transport of substrates (eg, pyruvate, phosphate, and adenosine diphosphate [ADP]) used during mitochondrial metabolism and by mitochondrial proton leak.

Under aerobic conditions, pyruvate metabolism provides the link between glycolysis and the TCA cycle. Lactate is also reversibly converted from pyruvate by lactate dehydrogenase (LDH), and this reaction was traditionally believed to occur only under anaerobic conditions resulting in the removal of lactate into the blood. However, this long-held view of lactate as a metabolic waste product has since been revised. In vivo metabolic tracing using stable isotopes in nonpregnant mice indicates that the contribution of 13C-lactate toward TCA cycle metabolism is greater than 13C-glucose in all tissues except the brain. In vivo studies of the human fetal-placental metabolism are not possible, but studies in pregnant ewes using radioactive tracers indicate that 30% of the glucose from the maternal circulation is converted into lactate by the placenta. This naturally raises the question of why so much of placental glucose metabolism is invested in generating lactate under normoxic conditions. First, the reduction of pyruvate to lactate by LDH regenerates nicotinamide adenine dinucleotide (NAD+), allowing glycolytic flux to be maintained. In the absence of lactate, glycolysis must be tightly coupled with the TCA cycle, such that every molecule of NADH and pyruvate produced by glycolysis is cleared by mitochondrial metabolism. Thus, the production of lactate uncouples these pathways so that they can occur independently, and it serves as a universal metabolic fuel source feeding into both the placenta and the fetus. Lactate produced by the placenta accounts for as much as 25% of fetal oxidative metabolism in sheep, and reduced placental lactate transport to the fetus is associated with FGR.

Biosynthetic processes

The intermediates of energy metabolism are also essential for the biosynthesis of nucleotides, fatty acids, cholesterol, and amino acids to form biomass (Figure 1). Glycolysis acts as a metabolic hub connecting with its branched pathways to generate biosynthetic precursors. Glucose 6-phosphate can be diverted into the pentose phosphate pathway (PPP) to generate ribose 5-phosphate, a nucleotide precursor. Fructose 6-phosphate branches off into the hexosamine biosynthetic pathway (HBP) to generate uridine diphosphate N-acetylg glucosamine (UDP-glCNAC), a key substrate for protein glycosylation. Dihydroxyacetone phosphate (DHAP) interconversion from fructose bisphosphate provides the glycerol backbone necessary for triglyceride synthesis. Finally, 3-phosphoglycerate can be used for serine and glycine synthesis, providing a source of methyl groups for one-carbon metabolic pathways that generate purines and glutathione.

TCA cycle intermediates are also biosynthetic precursors. When these metabolites are transported to the cytosol, they exhibit different metabolic functions compared with the mitochondria. Citrate is exported from the mitochondria into the cytosol and converted into acetyl-CoA. Although mitochondrial acetyl-CoA is used to generate energy, cytosolic acetyl-CoA is metabolized into fatty acids or condensed in the
FIGURE 1
Central carbon metabolism and its contribution to bioenergetic and biosynthetic processes

Key metabolic pathways involved in the generation of ATP and biosynthetic precursors from nutrients. The description of the metabolic pathways is discussed in the main text. The glycolytic shunt pathways: pentose phosphate pathway and hexosamine biosynthetic pathway are shaded in yellow and pink, respectively. Cofactors are depicted in blue and orange, and biosynthetic precursors are shown in red.

acetyl-CoA, acetyl coenzyme A; ADP, adenosine diphosphate; ATP, adenosine triphosphate; CoQ, coenzyme Q; Cyt c, cytochrome C; HMG-CoA, 3-hydroxy-3-methyl-glutaryl-coenzyme A; malonyl-CoA, malonyl coenzyme A; NAD+, nicotinamide adenine dinucleotide; NADH, reduced nicotinamide adenine dinucleotide; TCA, tricarboxylic acid.

mevalonate pathway to produce cholesterol and subsequently steroids. The use of TCA cycle metabolites in biosynthetic pathways requires that carbon be resupplied to the cycle and intermediate pools maintained. This is achieved through anaplerosis, that is, the influx of metabolic intermediates into pathways to replace those used for biosynthesis. These anaplerotic pathways replenish TCA cycle metabolites at sites other than acetyl-CoA. The mitochondrial export of citrate results in a decline in α-ketoglutarate (α-KG), which is compensated for by glutaminolysis. In most tissues, this involves the extracellular uptake of glutamine and its conversion into glutamate by glutaminase (GLS), and subsequent metabolism into α-KG. However, the placenta and the fetus coordinate a system of partitioning glutamate and glutamine between the different units (Figure 2). The placenta lacks GLS activity,21,22 and therefore, the majority of the glutamine taken up by the placenta is transferred to the fetus and accounts for up to 80% of the fetal glutamine, the remainder of which is derived from de novo fetal synthesis.23 The fetal reliance on placental glutamine delivery may explain why neonates with deficiency in glutamine synthetase (GS), which synthesizes glutamine from glutamate, survive in utero development but die shortly after birth.24 In contrast, there is no net placental transfer of glutamate from the mother to the fetus. In fact, glutamate is transferred from the fetus to the placenta. Fetal glutamine is metabolized by the fetal liver into glutamate, and up to 90% of this is taken back up by the placenta.25,26 Placental glutamate is then converted back into glutamate by GS or metabolized into α-KG by glutamate dehydrogenase forming the anaplerotic reactions to replenish the TCA cycle.23 This pathway highlights the importance of placental and fetal interrelationships in regulating key aspects of placental metabolism.

Redox homeostasis and reactive oxygen species
Mitochondrial ETC is a major source of cellular ROS, arising from complexes I and III (Figure 3). During normal mitochondrial function, as many as 2% of electrons leak from the ETC and reduce oxygen to superoxide (O2•−).27 O2•− can be dismutated to produce hydrogen peroxide (H2O2), which in turn may be partially reduced to form hydroxyl radicals (OH−). ROS are highly reactive and excessive mitochondrial production causes oxidative damage to macromolecules, and therefore, counterbalancing is required. The mitochondria rely on the combined activities of glutathione and thioredoxins to decompose the locally generated ROS. Nicotinamide adenine dinucleotide phosphate (NADPH) donates the reducing equivalent for the regeneration of glutathione and thioredoxin, necessary to neutralize (ie, reduce) ROS.

Considering the crucial role of NAD(P)+ and NAD(P)H in managing oxidative stress and providing essential cofactors for metabolic reactions, the maintenance of NAD(P)+ and NAD(P)H balance is critical to cellular homeostasis. Glycolysis consumes NAD+, which can be resupplied by LDH conversion of pyruvate to lactate, with oxidation of NADH in the process. The PPP also produces NADPH, providing the reducing equivalents for the biosynthesis of lipids, cholesterol, and nucleotides. In the mitochondria, the reduction of NAD+ to NADH during isocitrate and α-KG oxidation is resupplied by OXPHOS by complexes I and II that oxidizes NADH to NAD+.

It is important to consider that although excessive ROS production is undesirable, low ROS concentrations are responsible for a wide variety of physiological processes in the placenta.28 Therefore, the inappropriate suppression of ROS may have detrimental effects on...
Metabolites in the Control of Cell Signaling and Gene Regulation

Perturbations in cellular energy metabolism have additional consequences beyond bioenergetics and biosynthesis. This is because energy metabolism intermediates, cofactors, and cosubstrates also function as signaling molecules (Figure 4). The signaling function provides a means of communicating the cellular status among different organelles and allows for metabolic pathways to be integrated to cellular function.

Metabolic control of signal transduction

Cellular ATP levels are directly sensed through the adenosine monophosphate activated protein kinase (AMPK). AMPK plays a key role as a master regulator of energy homeostasis by directly phosphorylating metabolic enzymes or by phosphorylating transcription factors, coactivators, and corepressors. AMPK is activated by an increase in AMP to ATP ratio indicating a decline in energy levels. In turn, AMPK phosphorylates metabolic enzymes to switch on catabolic pathways that generate ATP such as glycolysis and fatty acid β-oxidation. In addition, AMPK represses ATP-consuming processes including protein translation by inhibition of the mechanistic target of rapamycin (mTOR). The mTOR signaling pathway integrates inputs from upstream extracellular growth factor signals and intracellular metabolites to regulate cell growth and metabolism. In the placenta, mTOR activity regulates mitochondrial metabolism and nutrient transfer, and both the activities of mTOR and AMPK are altered in placenta-related pregnancy complications associated with altered fetal growth, including preeclampsia. The glycolytic intermediate DHAP activates mTOR through an AMPK independent route, thus allowing cells to respond to placental development and function (discussed in the section “Antioxidants to diminish placental oxidative stress”).

The mitochondrial reactive oxygen species are formed from the leakage of electrons from the electron transport chain complex I and complex III. $O_2•−$ is generated by the addition of an electron to molecular oxygen. $O_2•−$ is dismutated into $H_2O_2$ by SOD. $H_2O_2$ forms hydroxyl radical in the presence of $Fe^{2+}$ or is reduced to $H_2O$ by GPx and TPx in the presence of their reducing equivalents GSH and TRXRed respectively. The reducing capacity of GPx and Prx is dependent on the supply of NADPH from the pentose phosphate pathway. Complexes I and II require NADH and FADH$_2$ which are supplied by the TCA cycle.

\[\begin{align*}
    \text{CoQ} & : \text{coenzyme Q} \\
    \text{Cyt c} & : \text{cytochrome c} \\
    Fe^{2+} & : \text{ferrous ion} \\
    \text{FADH}_2 & : \text{flavin adenine dinucleotide} \\
    \text{Fe^{2+}} & : \text{ferrous ion} \\
    \text{FADH}_2 & : \text{flavin adenine dinucleotide} \\
    \text{NADP} & : \text{nicotinamide adenine dinucleotide phosphate} \\
    \text{GSH} & : \text{reduced glutathione} \\
    \text{GSSG} & : \text{oxidized glutathione} \\
    \text{H}_2\text{O}_2 & : \text{hydrogen peroxide} \\
    \text{NADPH} & : \text{reduced nicotinamide adenine dinucleotide phosphate} \\
    \text{O}_2^{•−} & : \text{superoxide} \\
    \text{Prx} & : \text{peroxiredoxin} \\
    \text{SOD} & : \text{superoxide dismutase} \\
    \text{TCA} & : \text{tricarboxylic acid} \\
    \text{TPx} & : \text{thioredoxin peroxidase} \\
    \text{TRXRed} & : \text{reduced thioredoxin} \\
    \text{TRXOx} & : \text{oxidized thioredoxin}.
\end{align*}\]

Intermediaries of central energy metabolism have diverse nonmetabolic signaling roles with important effects on placental physiology and disease. Glycolytic intermediates and ATP-to-ADP ratio signal toward the cellular energy and nutrient sensors AMPK and mTOR, respectively. AMPK is a protein kinase that can inhibit protein synthesis by directly phosphorylating and inhibiting translation elongation proteins or by inhibiting mTOR-dependent protein synthesis. mTOR is also a protein kinase that phosphorylates key proteins regulating protein synthesis and amino acid transport in the placenta. Acetyl-CoA and lactate provide rate-limiting substrates for acetylation and lactylation of histones. Lactate also inhibits HDACs. The SIRT class of HDACs requires NAD\(^+\) as cofactors. DNA and histone methylation by TET and JMJD are activated by \(\alpha\)-ketoglutarate and inhibited by succinate and fumarate. High \(\alpha\)-ketoglutarate to succinate or fumarate ratio enhances PHD activity leading to hydroxylation of HIF-1 and HIF-2\(\alpha\) leading to its ubiquitination and proteasomal degradation. Low \(\alpha\)-ketoglutarate to succinate or fumarate ratio, hypoxia, and ROS inhibit PHDs leading to HIF-1 and HIF-2\(\alpha\) stabilization and nuclear translocation where it promotes transcription of their respective target genes.

\(\text{Ac, acetyl group; acetyl-CoA, acetyl coenzyme A; ADP, adenosine diphosphate; AMPK, AMP-activated protein kinase; ATP, adenosine triphosphate; Flt-1, fms-like tyrosine kinase 1; HAT, histone acetyltransferase; HDAC, histone deacetylase; HIF, hypoxia-inducible factor; JMJD, Jumonji C domain—containing histone demethylase; L, lactic group; Me, methyl group; mTOR, mechanistic target of rapamycin; NAD\(^+\), nicotinamide adenine dinucleotide; PHD, prolyl hydroxylase domain; ROS, reactive oxygen species; SIRT, sirtuin; TCA, tricarboxylic acid; TET, ten-eleven translocation; Ub, ubiquitin.}\)

Transcriptional and epigenetic regulation of gene expression by central energy metabolism

Hypoxia-inducible factors (HIFs) are well characterized for their role in altering gene transcription to match oxygen demand with availability. Under normoxia, the proline residues of HIF-1 and HIF-2α are hydroxylated by prolyl hydroxylase domain (PHD) proteins. This allows HIFs to be recognized by a ubiquitin ligase targeting them for proteasomal degradation. Under hypoxic conditions, PHD activity is impaired resulting in HIF-1 and HIF-2α accumulation and nuclear translocation where they dimerize with HIF-1β and function as a transcription factor. PHDs are 2-oxoglutarate-dependent dioxygenases (2-OGDDs) that catalyze α-KG (also known as 2-oxoglutarate) into succinate and use O₂ as a cosubstrate. As with other OGDDs, PHDs are inhibited by succinate and fumarate under normoxic conditions. Interestingly, mitochondrial ROS can also inhibit PHDs to activate HIF-1 or HIF-2α under normoxia. Therefore, HIF activity is governed as much by the cellular metabolic state as by the oxygen tension. In the placenta, both HIF-1α and HIF-2α are stabilized under hypoxia but may have different transcriptional targets. HIF-1α regulates glycolytic enzymes in several non-placental cells. HIF-2α promotes sFlt-1 transcription in trophoblast-derived cell lines, which explain why levels of sFlt-1 messenger mRNA do not correlate with HIF-1α protein in normal or placenta with preeclampsia.

Transcription is intimately associated with a permissive chromatin environment that is facilitated by specific histone modifications. Nearly all chromatin-modifying enzymes rely on substrates and cofactors generated from central energy metabolism. Histone acetylation promotes an open chromatin state and thus gene transcription. Acetyl-CoA is the rate-limiting substrate for histone acetylation, and the regulation of acetyl-CoA metabolism profoundly influences histone acetylation. In the reversal to this process, acetyl groups on histones are removed by histone deacetylases (HDACs). Deacetylation reactions are also metabolically sensitive. Lactate is a weak inhibitor of global HDAC activity, whereas the sirtuin (SIRT) class of HDACs requires NAD⁺ as a cofactor. Interestingly, lactate also functions as an epigenetic modifier through histone lactylation promoting gene expression. Hence, histone acetylation and lactylation provide a mechanism by which glycolytic and oxidative metabolism intermediates are uncoupled from energy metabolism and function in the regulation of gene expression.

S-adenosyl methionine (SAM) is a substrate for the methylation of histones and DNA. SAM generation by one-carbon metabolism requires NADPH and serine, which are intermediates of central energy metabolism. PPP generates NADPH whereas 3-phosphoglycerate channels metabolites into the serine synthesis pathway (Figure 1). Notably, stimulating glycolysis increases SAM production by increasing carbon flux into these pathways. Similarly, demethylation of histones and DNA is coordinately regulated by the same metabolites. The Junonji C domain–containing histone demethylases (JMJDs) and ten-eleven translocation DNA demethylases are 2-OGDD enzymes, and as such, they require αKG as a cosubstrate and are inhibited by succinate and fumarate, intermediates downstream in the TCA cycle. Therefore, the balance of TCA cycle reactions can affect the level of DNA and histone methylation and thus influence gene expression.

Reprogramming of Placental Metabolism During Development

Although metabolic reprogramming has largely been discussed in the context of pathologic states, it is clear that such reprogramming occurs in physiological settings. This is best appreciated in the context of placental development where metabolic reprogramming reflects changes in the requirements of bioenergy and biosynthetic precursors in response to the changing extracellular environment (eg, histiotrophic to hematrophic nutrition) and cellular demands (eg, proliferation and differentiation). Owing to the obvious constraints, less is known about the mechanisms underpinning human placental metabolism during early pregnancy (ie, first and second trimesters). However, we may infer these mechanisms based on metabolite and enzyme activity measurements. Early placental development takes place in an environment of low oxygen tension and is supported by secretions from the endometrial gland that are rich in carbohydrates. Glycolysis and HBP and PPP enzyme activities are high in the first trimester, suggesting a preference for nonoxidative metabolism. The reliance on these pathways may be necessary to support biosynthetic and signaling functions, and the generation of reducing equivalents NADPH and reduced glutathione (GSH) to protect against ROS-mediated teratogenesis. Despite the low oxygen tension, this environment should not be considered hypoxic, because hypoxia reflects the metabolic state relating to cellular oxygen availability and demand, rather than oxygen tension per se, which varies considerably among different tissues. For example, placental ATP-to-ADP ratio, glucose and lactate concentrations, and HIF-signaling do not change across gestation.

With the onset of the uteroplacental circulation, the oxygen tension rises and oxidative metabolism becomes dominant. The activities of LDH and TCA cycle enzymes increase to meet the greater biosynthetic requirements associated with the rapid growth of the placenta and the fetus. Interestingly, placental bioenergetics (as determined by OXPHOS activity) does not change substantially between the first trimester and term, despite the >5-fold increase in mitochondrial DNA, suggesting that OXPHOS activity relative to mitochondrial content becomes less efficient. It is possible that the considerably larger surface area and higher oxygen concentrations in the term placenta mean that mitochondrial respiration is not required to proceed at full capacity. Indeed, compared with the first-trimester placentas, term placentas have greater spare respiratory capacity.
(defined as the differences between maximal and basal respiration) which may be important for buffering the effects of acute stress such as labor.

Metabolism is not just a product of developmental programs; metabolic pathways also strongly influence signaling and epigenetic mechanisms associated with development.\(^{52,63}\) The differentiation of the trophectoderm (from which all trophoblasts are derived) during mouse embryonic development is controlled by glucose metabolism.\(^{64}\) However, this process is not associated with its bioenergetic function. Instead, glucose is metabolized into the PPP and HBP to provide nucleotide precursors and glycosylation substrates for post-translational modification and activation of developmental transcription factors.\(^{64}\)

In the first-trimester placenta, rapid cytotrophoblast proliferation is required to build a sufficient pool of progenitor cells for syncytiotrophoblast and extravillous trophoblast differentiation. An abundant cytotrophoblast pool may also be necessary to support the development of a durable cytotrophoblast shell that forms a primitive barrier at the maternal-fetal interface.\(^{65}\) Low oxygen tension of the early placent al microenvironment has been proposed as a requirement for cytotrophoblast proliferation, whereas differentiation is triggered by the rise in oxygen. However, all 3 trophoblast types are present in the placenta before the onset of uteroplacental circulation and thus the surge in oxygen. Moreover, studies using human cytotrophoblast stem cells and organoid models demonstrate continuous self-renewal under atmospheric oxygen concentrations.\(^{66–68}\) We propose an alternative hypothesis whereby the metabolic state, rather than oxygen per se, regulates trophoblast fate. A common characteristic of progenitor cells (including cytotrophoblasts) is that they require high levels of histone acetylation to maintain an open chromatin state, whereas differentiation is associated with a rapid decline in global histone acetylation.\(^{60,69}\) The metabolic support for histone acetylation is achieved through high glycolytic activity generating pyruvate and subsequent oxidation into acetyl-CoA. At the same time, consumption of NAD\(^+\) during glycolysis reduces NAD\(^+\)-dependent HDAC activity, thus also favoring histone acetylation. Consistent with this hypothesis, cytotrophoblasts exhibit higher glycolytic metabolism than their differentiated syncytiotrophoblasts,\(^{70}\) and higher histone acetylation levels.\(^{71}\) Moreover, the loss of the HDAC SIRT1 in mice results in trophoblast differentiation failure and reduced fetal and placental weights.\(^{71}\)

**Dysregulation of Metabolic Reprogramming in Preeclampsia**

Because metabolic reprogramming is a necessary component of physiology, the inability of the placenta to alter its metabolism to the changing environment may underlie abnormal placental development and dysfunction. Derangements in energy metabolism and its consequences are commonly reported in the placentas of women with preeclampsia. However, the various subtypes of preeclampsia show differences in their ability or inability to reprogram their metabolism (Figure 5). Mitochondrial dysfunction and oxidative stress are commonly reported in placentas of preeclampsia of various subtypes.\(^{72,73}\) It is still unclear whether mitochondrial dysfunction is the cause of oxidative stress or vice versa, but these 2 events are likely interrelated and may compound each other. Interestingly, women with known pathogenic mitochondrial DNA mutations entering pregnancy are highly likely to develop preeclampsia.\(^{74,75}\) Although these cases are very rare, such “experiments of nature” underline the importance of mitochondria in the development of preeclampsia.

ROS are triggered by hypoxia and reoxygenation associated with intermittent placental perfusion secondary to abnormally shallow invasion.\(^{76,77}\) Perversely, prolonged hypoxia without reoxygenation also promotes mitochondrial ROS generation. This occurs because insufficient oxygen is available for reduction by the ETC and the reducing equivalents NADH and FADH\(_2\) accumulate, increasing the availability of electrons for the reduction of oxygen to O\(_2\bullet–\) and subsequently into H\(_2\)O\(_2\). Hypoxia and mitochondria-generated ROS stabilize both HIF-1 and HIF-2α.\(^{71}\) This leads to the transcription of glycolytic enzymes through HIF-1α\(^{78}\) whereas HIF-2α increases antiangiogenic factors including sFlt-1 to be released from the trophoblasts into the maternal circulation leading to maternal endothelial activation.\(^{52,79}\)

Varying degrees of mitochondrial dysfunction have been reported in different preeclampsia subtypes and may be proposed as the initial stimulus for altered energy metabolism. However, the degree of alterations or inability to adapt sufficiently in energy metabolism may exacerbate the disease. In less severe forms of preeclampsia associated with term delivery, mitochondrial function adapts by upregulating OXPHOS and antioxidant activity.\(^{80}\) Failure to adapt may result in mitochondrial dysfunction placing greater reliance on glycolysis to maintain the bioenergetic requirements but may lead to reduced net ATP production owing to the lower efficiency of glycolysis. This can also result in greater flux into the HBP\(^{81,82}\) which promotes cell survival through UDP-glcNAc—dependent glycosylation and inhibition of apoptotic proteins.\(^{83}\)

In severe preeclampsia associated with preterm delivery and FGR, glycolytic function is also impaired, suggesting a failure in reprogramming of metabolism. In these placentas, glycolytic enzyme activities are decreased resulting in lower production of pyruvate and lactate.\(^{84}\) The latter of which provides fuel for fetal oxidation. Anaplerotic flux into the TCA cycle via the placental-fetal glutamine-glutamate shuttle is also dysregulated in FGR-associated placentas,\(^{85}\) which would affect the fetal amino acid supply and the provision of biosynthetic precursors and bioenergetic functions in the placenta. The ensuing decline in placental ATP levels activates AMPK that functions to restore energy balance by reducing ATP-demanding processes including placental nutrient transport and protein synthesis via mTOR inhibition, contributing to placenta-related FGR.\(^{33}\) Protein synthesis occurs in the ER, which consumes large amounts of ATP imported from the cytosol and the mitochondria. Considerable cross-talk
between the mitochondria and the ER membranes exists via direct contact sites called mitochondrial associated membranes (MAMs) to signal cellular metabolic status and stress. For example, to meet the energy demands for protein synthesis, calcium signaling by the MAM stimulates TCA cycle enzymes leading to enhanced ATP production via OXPHOS. Severe preeclampsia is associated with ER stress which decreases protein synthesis to reduce the demand for ATP. Moreover, the ER stress-mediated transcription factor XBP-1s increases the transcription of HBP enzyme to promote cell survival, suggesting that ER stress response may support metabolic reprogramming toward glycolysis. An additional consequence of altered energy metabolism relates to its epigenetic functions. Reductions in trophoblast acetyl-CoA synthesis or NAD+ homeostasis may lead to reduced cytrophoblast proliferation leading to the incomplete development of the cytrophoblastic shell and a reduction in the source of extravillous trophoblasts for adequate trophoblast invasion. Indeed, a single-cell transcriptomic study indicated altered extravillous trophoblast transcript signatures in placentas with preeclampsia suggesting differentiation defects. However, the mechanistic relationship between metabolism and trophoblast differentiation in preeclampsia remains to be investigated.

Collectively, these studies suggest that impaired placental energy metabolism in preeclampsia may have widespread effects on cellular processes beyond ATP production and may lead to alterations in biosynthetic precursors, oxidative stress, and transcriptional and epigenetic modifications.

**Sex Differences in Placental Metabolism May Underlie Preeclampsia Severity**

Fetal sex differences are increasingly recognized as an important determinant of the incidence and outcome in placenta-related pregnancy complications. Overall, preeclampsia risk is higher in male fetuses. However, when stratified by...
subtype, term preeclampsia was associated with male fetus whereas preterm preeclampsia is more common when the fetus was female.90,93 One hypothesis that has been proposed to explain these differences relates to early placental development.92 Male embryos are more susceptible to suboptimal implantation and abnormal placental development.95 Therefore, pregnancies with a male embryo that are susceptible to develop preeclampsia owing to impaired placentalation may already have miscarried in the first trimester. This is consistent with the higher rates of first-trimester miscarriage in male embryos.96 In those pregnancies with preeclampsia that proceed past the first trimester, the male placentas consistently show greater pathologic features such as inflammatory and oxidative stress.94,97

Fetal sex differences in placental metabolism may underlie some of the effects of preeclampsia pathophysiology. Placental sex—dependent alterations in oxidative metabolism have been reported in several pregnancy complications including preeclampsia.97–99 These studies indicate that the male placenta demonstrates a lower capacity to reprogram their metabolism in response to changes in nutrient source or stress stimuli.

The human placental transcriptome exhibits profound sex differences throughout gestation.100–104 These sex differences may be explained by genes that escape X chromosome inactivation (XCI) resulting in female-biased expression or overexpression.105,106 In term placentas, approximately 15% of the XCI escapees are involved in metabolism.104 One of these escapees, spermine synthase (SMS), participates in polyamine metabolism that is dysregulated in preeclampsia.104 Although polyamine metabolism is not directly associated with energy metabolism, our preliminary findings show that polyamine metabolites strongly correlate with TCA cycle intermediates in the placenta.107 Moreover, polyamine depletion decreased both glycolytic and oxidative metabolism resulting in reduced TCA cycle intermediates and OXPHOS activity,107 which recapitulates the metabolic phenotypes of placental dysfunction in severe preeclampsia.104 Importantly, female trophoblasts were resistant to polyamine depletion owing to the higher SMS expression associated with XCI escape. Moreover, the decrease in glycolysis and oxidative metabolism with polyamine depletion led to reduced acetyl-CoA availability and decreased histone acetylation resulting in widespread changes in gene expression.108 These findings suggest that fetal sex differences in placental metabolism have far-reaching effects beyond bioenergetics and affect epigenetic regulation of placental function.

**Placental Energy Metabolism as a Target for the Treatment of Preeclampsia**

There is no universally accepted treatment for preeclampsia. The current standard of care is aimed at resolving the maternal symptoms, and delivery remains the only cure. Given the central role of the placenta in preeclampsia pathophysiology, treatments aimed at resolving placental dysfunction are warranted. We briefly review the role of antioxidants and metformin as therapeutic strategies for the prevention or treatment of preeclampsia and examine their potential implications on placental energy metabolism and provide possible explanations for their effectiveness.

**Antioxidants to diminish placental oxidative stress**

Based on the evidence that preeclampsia is commonly associated with maternal and placental oxidative stress, several clinical studies have examined the effectiveness of antioxidants, and in particular vitamins C and E, to prevent or ameliorate the course of preeclampsia. Vitamins C and E are readily available over-the-counter supplements with potent antioxidant properties. Vitamin C is a water-soluble antioxidant that scavenges free radicals, whereas vitamin E is a lipid-soluble peroxyl radical scavenger.109 Therefore, the combined use of vitamins C and E offers protection against multiple forms of ROS.

The initial clinical trials of vitamins C and E supplementation beginning at midpregnancy in women at risk of preeclampsia suggested improved oxidative stress markers110,111 and clinical outcomes.112 However, larger clinical trials and several meta-analyses failed to show any benefits and even demonstrated some harm including reduced fetal growth, preterm birth, and stillbirth.112–117 Taken together, these trials and several other studies118,119 targeting oxidative stress have failed to improve preeclampsia outcome. The exact reasons and mechanisms as to why these studies have failed to produce the expected beneficial effects remain largely unknown but several explanations can be posited. These include the failure to translate the beneficial in vitro effects to in vivo findings owing to poor bioavailability and pharmacokinetic profiles, heterogeneity in the types of antioxidants and doses, and the lack of an appropriate preclinical animal model for preeclampsia. The discrepancies may also be caused by the possibility that oxidative stress represents 1 endpoint in a cascade of events related to placental metabolic dysfunction, and therefore, targeting oxidative stress alone is unlikely to produce substantial overall benefits. It is also interesting to note that in addition to pregnancy disorders, antioxidant supplementation has failed to deliver the expected benefits for many other diseases unrelated to pregnancy and may even increase mortality.120

The evidence for antioxidants to cause harm reinforces the notion that ROS play an important physiological role during pregnancy and that undue suppression of ROS may have detrimental effects. This was elegantly demonstrated in mice where experimental induction of a master transcriptional regulator of the cellular antioxidant system paradoxically led to adverse pregnancy outcomes.121 Nuclear factor erythroid 2—related factor 2 (Nrf2) is a transcription factor that is activated in response to oxidative stress. Its activity is suppressed under basal conditions by the binding of Kelch-like ECH—associated protein 1 (KEAP1) which facilitates Nrf2 degradation in the proteasome.122 However, on exposure to ROS, KEAP1 is oxidized which causes Nrf2 release into the nucleus where it
FIGURE 6
Metformin targets multiple pathways in energy metabolism

- **Glycolysis**
  - Glucose to Glucose-6-phosphate
  - Fructose-6-phosphate to Fructose-1,6-phosphate

- **AMPK**
  - Activated by ADP
  - Regulates mTOR and nutrient transport

- **TCA Cycle**
  - Pyruvate to Citrate
  - α-Ketoglutarate

- **Mitochondrial Biogenesis**
  - PGC1α

- **METFORMIN**
  - Targets sFlt-1, FLT1, HIF-2α, PHD, ROS, NADH, NAD⁺, ATP

- **Oxygen**
  - Oxidative phosphorylation

- **Protein Synthesis**
  - mTOR

- **Energy Metabolism**
  - ATP
  - ADP

- **Inner mitochondrial membrane**
  - CoQ
  - Cyt c

- **Intermembrane space**
  - H⁺
binds to antioxidant response elements in promoter regions of numerous antioxidant genes initiating their transcription. Hence, knockout of Nrf2 in mice does not produce any obvious phenotypes. However, genetic or pharmacologic induction of Nrf2 in a mouse preeclampsia model worsened FGR and decreased placental size, despite reductions in oxidative damage. Similarly, human placental trophoblasts and explants treated with pharmacologically relevant concentrations of vitamin C or E demonstrate higher apoptosis even though oxidative stress was improved. Clearly, targeting of ROS remains an important therapeutic strategy but this needs to be finely balanced to avoid inhibiting the low physiological concentrations of ROS that are required for physiological functions.

**Metformin targets multiple pathways of placental energy metabolism**

Metformin is commonly prescribed for the management of type 2 diabetes mellitus primarily owing to its effects in reducing hepatic glucose production. The precise mechanism of action of metformin remains unclear because it has been associated with pleiotropic effects in different tissues and has been proposed to have many beneficial effects. As such, more than 1700 clinical trials have been registered to test the effects of metformin in different diseases (https://clinicaltrials.gov). Owing to its anti-hyperglycemic and insulin-sensitizing effects, metformin is prescribed during pregnancy for the treatment of pregestational type 2 diabetes mellitus, gestational diabetes, and polycystic ovarian syndrome. In a randomized control trial examining the effects of metformin compared with placebo in nondiabetic obese women, metformin had no effect on the primary outcome (birthweight) but reduced preeclampsia incidence by more than 3-fold, thus prompting subsequent studies investigating its effectiveness for preeclampsia. However, meta-analyses of metformin have raised the concern that it may increase the risk of small-for-gestational-age infants compared with other glucose-normalizing therapeutic approaches.

Metformin has profound effects on cellular energy metabolism that may explain some of the observed effects on the placenta with preeclampsia (Figure 6). Metformin inhibits complex I activity resulting in reduced OXPHOS but decreases mitochondrial ROS generation in the process. Complex I inhibition also prevents NADH oxidation decreasing the availability of essential cofactors required to run the TCA cycle. The decrease in the TCA cycle metabolite α-KG may explain the decrease in HIF-2α stabilization by PHDs in metformin-treated primary trophoblasts and suppression of sFlt-1 and soluble endoglin secretion, lessening the impact of placental stress—mediated endothelial dysfunction. The reduction in α-KG by metformin will also inhibit other 2-OGDD enzymes including JMJDs. The decline in ATP as a result of reduced OXPHOS activity leads to AMPK activation. In placental with preeclampsia, AMPK activation by metformin may have beneficial adaptive effects by removing damaged mitochondria by mitophagy. Moreover, AMPK activates peroxisome proliferator—activated receptor gamma coactivator 1-alpha (PGC-1α), which functions as a transcription coactivator that interacts with a range of transcription factors. PGC-1α plays a prominent role in regulating the transcription of nuclear-encoded mitochondrial proteins promoting mitochondrial biogenesis. Therefore, AMPK activation by metformin may have dual effects on the mitochondria by removing damaged mitochondria concomitant with mitochondrial biogenesis to restore mitochondrial function.

Metformin reroutes metabolic flux into glycolytic pathways through AMPK-dependent and independent mechanisms. AMPK activation stimulates glycolysis by phosphorylating and activating phosphofructokinase and promoting hexokinase II transcription. Independently of AMPK, metformin inhibits gluconeogenesis by inhibiting glucose 6-phosphatase—mediated conversion of glucose 6-phosphate to glucose. Given that gluconeogenesis and glycolysis are regulated in a reciprocal manner to prevent concurrent activity of the opposing pathways, metformin inhibition of glucose 6-phosphatase further supports glycolytic activity. The subsequent increase in glucose 6-phosphate and fructose 6-phosphate may also promote flux into the PPP and the HBP, respectively. These putative effects of metformin are consistent with our preliminary findings in primary human trophoblasts, where metformin treatment at concentrations typically achieved in pregnant women reprogrammed metabolism from OXPHOS toward glycolysis (unpublished data). Although metformin reduces mitochondrial ATP production in normal placentas, in placentas with preeclampsia where mitochondrial dysfunction is prevalent, this effect of metformin may be advantageous because it promotes the reliance on glycolysis over mitochondrial metabolism to restore energy homeostasis and supporting...
survival through increased flux into the PPP and the HBP.

The use of metformin in pregnancy is not without risks. Metformin crosses the placenta and metformin treatment is associated with reduced birthweight, although it is currently unclear whether these effects are mediated directly through placental or fetal shifts in cellular energy metabolism or indirectly via alterations in fetal glucose homeostasis. In the placenta, AMPK activation by metformin may inhibit mTOR-mediated nutrient transport and protein synthesis. Fetal exposure to metformin could lead to reductions in TCA cycle intermediates such as citrate which are substrates for lipogenesis and biomass production contributing to the small-for-gestational-age phenotype.

Recent studies suggest that metformin mediates additional metabolic effects independent of glucose homeostasis through increased levels of the hormone growth/differentiation factor 15 (GDF15). The placenta exhibits the highest tissue levels of GDF15 and secretes large amounts into the maternal circulation. The physiological significance of placental GDF15 secretion is currently unclear, although it is interesting to note that preeclampsia is associated with a decline in maternal serum GDF15 levels.

Future studies investigating the role of GDF15 in the placenta and the effects of metformin may reveal novel insights into placental metabolic dysfunction.

**Conclusions**

It is now evident that metabolism is much more than a “housekeeping” process and fulfills regulatory roles in physiology. However, a major future task will be to establish clear causal relationships between metabolism and placental developmental programs and to determine whether these links are misaligned during placenta-related pregnancy complications including preeclampsia.

Reprogramming of energy metabolism is a hallmark of placental development but may also underpin the ability to respond to the pathophysiology underlying preeclampsia. For instance, placental mitochondrial dysfunction is a common observation in preeclampsia but the inability to upregulate glycolysis is associated with increased severity. It is currently unclear what factors influence placental metabolic flexibility and stress response, but emerging evidence suggests that fetal sex may play an important role. Future therapies aimed at altering energy metabolism may provide an alternative or add-on strategies for the treatment of the placenta with preeclampsia. However, given the multiple phenotypes associated with targeting energy metabolism, the potential risks must be carefully weighed.

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**GLOSSARY OF TERMS**

1. Anaplerosis: Metabolic pathways that result in the replenishing of metabolic intermediates (especially tricarboxylic acid cycle intermediates) to replace those metabolites that have been extracted for biosynthetic processes. The reverse process, that is, the removal of metabolic intermediates from a metabolic cycle, is referred to as cataplerosis.
2. Bioenergetic metabolism: Cellular processes that lead to the transformation of nutrients (eg, glucose, amino acids, and fatty acids) into energy-rich metabolites, usually in the form of ATP. In this review, bioenergetic metabolism refers collectively to the metabolic pathways, glycolysis, tricarboxylic acid cycle, and oxidative phosphorylation.
3. Biosynthetic processes: Cellular processes by which substrates are converted into more complex macromolecules such as proteins, lipids, and nucleotides, which can be used for building cellular organelles and biomass.
4. Central carbon metabolism: A series of metabolic pathways that result in the flow of carbon atoms from nutrients into pathways generating reducing equivalents for energy production and biosynthetic precursors. In eukaryotes, this refers to glycolysis, tricarboxylic acid cycle, and the pentose phosphate pathway.
5. Glycolysis: Metabolic pathway that converts glucose into pyruvate or lactate. The true end product of glycolysis (ie, pyruvate or lactate) is currently a matter of debate.
6. Hexosamine biosynthetic pathway: A metabolic pathway that operates in parallel to glycolysis and results in the production of uridine diphosphate N-acetylglucosamine, a key substrate for protein glycosylation reactions.
7. Metabolic reprogramming: Refers to the ability of cells to alter their metabolism allowing them to adapt to changing internal and environmental conditions. It is important to note that metabolic reprogramming occurs under normal physiological and pathologic conditions.
8. Pentose phosphate pathway: A metabolic pathway that operates in parallel to glycolysis that results in the generation of pentoses (5-carbon sugars) and ribose 5-phosphate (a precursor for nucleotide synthesis) and produces NADPH.
9. Redox: An oxidation-reduction (redox) reaction involves the transfer of electrons between 2 species. Reducing equivalents and oxidizing agents play important roles as cofactors for numerous enzymes involved in energy metabolism and epigenetics. An imbalance in the redox state may result in oxidative stress.
10. TCA cycle: Tricarboxylic acid cycle (also known as the citric acid cycle or Krebs cycle) is a series of chemical reactions that result in the release of stored energy through the oxidation of acetyl-CoA derived from glucose, amino acids, and fatty acids. The TCA cycle is both a major bioenergetic and a biosynthetic pathway. As a bioenergetic pathway, the TCA cycle generates reduced coenzymes (NADH and FADH2) that are used in the electron transport chain for ATP synthesis. As a biosynthetic pathway, the TCA cycle intermediates can be used in the biosynthesis of macromolecules.
11. XCI and XCI escape: X chromosome inactivation (XCI) is a process whereby 1 of the 2 X chromosomes is silenced to balance gene dosage between XX females and XY males. XCI escape genes are specific genes that escape XCI silencing resulting in the expression from the inactivated X chromosome. XCI escape can result in female-biased (ie, increased) gene expression.

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