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

Irving L. M. H. AYE, PhD[1,2*], Catherine E. AIKEN, MD, PhD[1], D. Stephen CHARNOCK-JONES, PhD[1,2,] Gordon C. S. SMITH, MD, PhD[1,2]

[1]Department of Obstetrics and Gynaecology, University of Cambridge, NIHR Cambridge, Comprehensive Biomedical Research Centre, Cambridge, United Kingdom

[2]Centre for Trophoblast Research (CTR), Department of Physiology, Development and Neuroscience, University of Cambridge, Cambridge, United Kingdom

Conflict of Interest: GS and DSC-J have received research support from Roche (supply of equipment and reagents for biomarker studies) and GS has been paid to attend an advisory board by Roche.

Financial Support: I L.M.H. Aye is funded by a Next Generation Fellowship from the Centre for Trophoblast Research, University of Cambridge.

Corresponding Author
Dr Irving L. M. H. Aye,
Department of Obstetrics and Gynaecology, University of Cambridge, Box 223 The Rosie Hospital, Cambridge, CB2 0SW, UK.
Tel: +44 (0) 1223 336874, Fax: +44 (0) 1223 215327
Email: ia319@medschl.cam.ac.uk

Word count: Abstract 195; Main Text: 5192

Condensation:
Understanding placental energy metabolism may explain many of the phenotypes that are associated with preeclampsia and provide new insights into future therapeutic strategies.

Short title:
Placental metabolism in preeclampsia

Abstract

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. Lastly, 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.

Keywords:

Preeclampsia; Placenta; Metabolism; Glycolysis; Mitochondria; Fetal Growth Restriction; Reactive Oxygen Species; Epigenetics; Metformin

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" which 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 which may be accompanied by an ischemia-reoxygenation type of injury[3] and placental stress (oxidative, endoplasmic reticulum and inflammatory). The maternal peripheral endothelial activation and systemic inflammatory response are then triggered by placentally-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 placental-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 ATP and beyond

Central carbon metabolism describes the series of reactions that result in the 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, co-factors, and co-substrates 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 ~5 μmol of ATP per gram of tissue per minute from glucose[5,6] equivalent to more than 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 more than 50% of the total ATP consumption[6,7]. 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 1-4 g per day[8], which requires

~366 mg of ATP for hPL protein synthesis[9]. Therefore, high ATP-consuming processes such as protein synthesis[10] and nutrient transport[11,12] are impaired in preeclamptic placentas 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[13]. Glucose metabolism by glycolysis generates pyruvate with a net gain of two ATP molecules.

This pyruvate is transported into the mitochondria and feeds into the tricarboxylic acid (TCA) cycle after oxidation into acetyl-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 one ATP molecule is generated for each acetyl-coA, but the iterative oxidation reactions produce NADH and $FADH_2$, 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, one molecule of NADH and $FADH_2$ produces 2.5 and 1.5 ATP molecules respectively. However, in practice, this is considerably less due to energy consumption by active mitochondrial transport of substrates (e.g. pyruvate, phosphate and ADP) used during mitochondrial metabolism, as well as by mitochondrial proton leak[14].

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[15,16,17]. *In vivo* metabolic tracing using stable isotopes in non-pregnant mice indicates that the contribution of ^{13}C-lactate towards TCA cycle metabolism is greater than ^{13}C-glucose in all tissues except the brain[17]. *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[18]. This naturally raises the question of why so much of placental glucose metabolism is invested in generating lactate under normoxic conditions. Firstly, the reduction of pyruvate to lactate by LDH regenerates 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[15]. 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[19] and reduced placental lactate transport to the fetus is associated with FGR[20].

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 UDP-*N*-acetylglucosamine (UDP-glcNAc), a key substrate for protein glycosylation.

Dihydroxyacetone phosphate (DHAP) interconversion from fructose-bisphosphate provides the glycerol backbone necessary for triglyceride synthesis. Lastly, 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 to the mitochondria. Citrate is exported from the mitochondria into the cytosol and converted into acetyl-coA. While mitochondrial acetyl-coA is used to generate energy, cytosolic acetyl-coA is metabolized into fatty acids or condensed in the 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, i.e. 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 alpha-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]. On the other hand, 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 glutamine 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 significant source of cellular ROS, arising from Complex I and III (**Figure 3**). During normal mitochondrial function, as many as 2% of electrons leak from the ETC and reduce oxygen to superoxide (O_2•-)[27]. O_2•- can be dismutated to produce hydrogen peroxide (H_2O_2), which in turn may be partially reduced to form hydroxyl radicals ($OH^{\bullet}$-). ROS are highly reactive and excessive mitochondrial production causes oxidative damage to macromolecules, and therefore counterbalancing is required. Mitochondria rely on the combined activities of glutathione and thioredoxins to decompose the locally generated ROS. NADPH donates the reducing equivalent for the regeneration of glutathione and thioredoxin, necessary to neutralize (i.e. reduce) ROS.

Considering the crucial role of NAD(P)+ and NAD(P)H in managing oxidative stress as well as providing essential co-factors for metabolic reactions, the maintenance of NAD(P)+/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 which oxidizes NADH to NAD^+.

It is important to consider that while excessive ROS production is undesirable, low ROS concentrations are responsible for a wide variety of physiological processes in the placenta (previously reviewed in [28]). Therefore, the inappropriate suppression of ROS may have detrimental effects on placental development and/or function (discussed further below).

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, co-factors, and co-substrates also function as signaling molecules (**Figure 4**). The signaling function provides a means of communicating the cellular status between 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 AMP-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, co-activators and co-repressors. AMPK is activated by an increase in AMP/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. Additionally, 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[29–33], and both the activity of mTOR and AMPK are altered in placental-related pregnancy complications associated with altered fetal growth, including preeclampsia[10,34–36]. The glycolytic intermediate DHAP activates mTOR through an AMPK independent route, thus allowing cells to respond to glucose availability in a manner independent of cellular energy status[37].

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 2α are hydroxylated by prolyl hydroxylase domain proteins (PHDs). 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 2α accumulation and nuclear translocation where they dimerize with HIF-1β and function as a transcription factor. PHDs are 2-oxoglutarate-dependent dioxygenases (2-OGDD) that catalyze α-KG (also known as 2-oxoglutarate) into succinate and utilize O_2 as a co-substrate[38]. As with other OGDDs, PHDs are inhibited by succinate and fumarate under normoxic conditions[39]. Interestingly, mitochondrial ROS can also inhibit PHDs to activate HIF1 or 2α under normoxia[40,41]. 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[42,43] but may have different transcriptional targets. HIF-1α regulates glycolytic enzymes in several non-placental

cells[44]. HIF-2α promotes sFlt-1 transcription in trophoblast-derived cell lines[42], which may explain why levels of sFlt-1 mRNA do not correlate with HIF-1α protein in normal or preeclamptic placentas[43].

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 co-factors 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[45–47]. 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[48] whereas, the sirtuin class of HDACs requires NAD^+ as a co-factor[49]. 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[50,51], which are intermediates of central energy metabolism. PPP generates NADPH while 3-phosphoglycerate channels metabolites into the serine synthesis pathway (**Figure 1**). Notably, stimulating glycolysis increases SAM production by increasing carbon flux into these pathways[52]. Similarly, demethylation of histones and DNA is coordinately regulated by the same metabolites. The Jumonji C domain-containing histone demethylases (JMJDs) and ten-eleven translocation DNA demethylases (TETs) are 2-OGDD enzymes and as such, they require αKG as a co-substrate 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

While metabolic reprogramming has largely been discussed in the context of pathological 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 (e.g. histiotrophic to hemotrophic nutrition) and cellular demands (e.g. proliferation and differentiation). Due to the obvious constraints, less is known about the mechanisms underpinning human placental metabolism during early pregnancy (i.e. first and second trimester). 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[53]. Glycolysis as well as HBP and PPP enzyme activities are high in the first-trimester[54], suggesting a preference for non-oxidative metabolism. The reliance on these pathways may be necessary to support biosynthetic and signaling functions, and the generation of reducing equivalents NADPH and GSH to protect against ROS-mediated teratogenesis[55]. Despite the low oxygen tension, this environment should not be considered hypoxic, since hypoxia reflects the metabolic state relating to cellular oxygen availability and demand, rather than oxygen tension *per se,* which varies considerably between different tissues[56]. For example, placental ATP:ADP ratio, glucose and lactate concentrations, and HIF-

signaling do not change across gestation[57].

With the onset of the uteroplacental circulation, the oxygen tension rises and oxidative metabolism becomes dominant[58]. The activities of LDH and TCA cycle enzymes increase[59,60] 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 >5-fold increase in mitochondrial DNA, suggesting that OXPHOS activity relative to mitochondrial content becomes less efficient[58]. It is possible that the significantly larger surface area and higher oxygen concentrations in the term placenta means that mitochondrial respiration is not required to proceed at full capacity. Indeed compared to the first-trimester, term placentas have greater spare respiratory capacity[58] (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[61,62]. Differentiation of the trophectoderm (from which all trophoblasts are derived) during mouse embryonic development is controlled by glucose metabolism[63]. 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[63].

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[64]. Low oxygen tension of the early placental microenvironment has been proposed as a requirement for cytotrophoblast proliferation, whereas differentiation is triggered by the rise in oxygen. However, all three 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[65–67]. 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[68,69]. 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, loss of the HDAC Sirt1 in mice results in trophoblast differentiation failure and reduced fetal/placental weights[71].

Dysregulation of metabolic reprogramming in preeclampsia

As 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/or dysfunction. Derangements in energy metabolism and its consequences are commonly

reported in the placentas of women with preeclampsia. However, the various sub-types of preeclampsia show differences in their (in)ability to reprogram their metabolism (**Figure 5**). Mitochondrial dysfunction and oxidative stress are commonly reported in preeclamptic placentas of various sub-types[72,73]. It is still unclear if mitochondrial dysfunction is the cause of oxidative stress or *vice versa*, but these two 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/reoxygenation associated with intermittent placental perfusion secondary to abnormally shallow invasion[76,77]. Paradoxically, prolonged hypoxia without reoxygenation also promotes mitochondrial ROS generation. This occurs because insufficient oxygen is available for reduction by the ETC, the reducing equivalents NADH and $FADH_2$ accumulate, increasing the availability of electrons for the reduction of oxygen to O_2•- and subsequently into H_2O_2. Hypoxia and mitochondrial generated ROS stabilize both HIF-1/2α[41]. This leads to transcription of glycolytic enzymes through HIF-1α[78] whereas HIF-2α increases anti-angiogenic factors including sFlt-1 to be released from the trophoblasts into the maternal circulation leading to maternal endothelial activation[42,79].

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 due 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 as well as the provision of biosynthetic precursors and bioenergetic functions in the placenta. The ensuing decline in placental ATP levels activates AMPK which functions to restore energy balance by reducing ATP-demanding processes including placental nutrient transport and protein synthesis via mTOR inhibition, contributing to placental-related FGR[33]. Protein synthesis occurs in the endoplasmic reticulum (ER), which consumes large amounts of ATP imported from the cytosol and the mitochondria. Significant cross-talk between the mitochondria and the ER membranes exist via direct contact sites called mitochondrial associated membranes (MAM) to signal cellular metabolic status as well as 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[86]. Severe preeclampsia is associated with ER stress[87] which decreases protein synthesis to reduce the demand for ATP[10]. Moreover, the ER stress mediated transcription factor XBP-1s increases the transcription of HBP enzyme to promote cell survival[88],

suggesting that ER stress response may support metabolic reprogramming towards glycolysis. An additional consequence of altered energy metabolism relates to its epigenetic functions. Reductions in trophoblast acetyl-coA synthesis and/or NAD^+ homeostasis may lead to reduced cytotrophoblast proliferation leading to incomplete development of the cytotrophoblastic 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 preeclamptic placentas suggesting differentiation defects[89]. 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 placental-related pregnancy complications[90–92]. Overall pre-eclampsia risk is higher in male fetuses. However, when stratified by sub-type, 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[94]. 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 due to impaired placentation 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 preeclamptic pregnancies that proceed past the first-trimester, the male placentas consistently show greater pathological 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 which escape X-chromosome inactivation (or XCI escapees) resulting in female-biased (over)expression[105,106]. In term placentas, ~15% of the XCI escapees are involved in metabolism[104]. One of these escapees, spermine synthase (SMS) participates in polyamine metabolism which 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[84]. Importantly, female trophoblasts were resistant to polyamine depletion due 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 Vitamin C and E offers protection against multiple forms of ROS.

The initial clinical trials of Vitamin C and E supplementation beginning at mid-pregnancy in women at risk of preeclampsia suggested improved oxidative stress markers[110,111] 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 as well as several other studies[118,119] 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 due 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 due to the possibility that oxidative stress represents one endpoint in a cascade of events related to placental metabolic dysfunction and therefore targeting oxidative stress alone is unlikely to produce significant 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 which is activated in response to oxidative stress. Its activity is suppressed under basal conditions by 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 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 pharmacological 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 and/or E demonstrate higher apoptosis even though oxidative stress was improved[123,124]. 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 primarily due to its effects in reducing hepatic glucose production. The precise mechanism of action of metformin remains unclear as it has been associated with pleiotropic effects in different tissues and has been proposed to have many beneficial effects. As such over 1700 clinical trials have been registered to test the effects of metformin in different diseases (https://clinicaltrials.gov). Due to its anti-hyperglycemic and insulin-sensitizing effects, metformin is prescribed during pregnancy for the treatment of pre-gestational Type 2 diabetes, gestational diabetes and polycystic ovarian syndrome[125]. In a randomized control trial examining the effects of metformin compared with placebo in non-diabetic obese women, metformin had no effect on the primary outcome (birth weight) but reduced preeclampsia incidence by more than 3-fold[126], 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[127,128] compared to other glucose-normalizing therapeutic approaches.

Metformin has profound effects on cellular energy metabolism that may explain some of the observed effects on the preeclamptic placenta (**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 co-factors required to run the TCA cycle[129]. 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 sEng secretion, lessening the impact of placental stress-mediated endothelial dysfunction[130]. The reduction in α-KG by metformin will also inhibit other 2-OGDD enzymes including JMJD histone demethylases[131]. The decline in ATP as a result of reduced OXPHOS activity leads to AMPK activation[132]. In preeclamptic placentas, 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)[133], which functions as a transcription co-activator 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 re-routes metabolic flux into glycolytic pathways through AMPK-dependent and independent mechanisms. AMPK activation stimulates glycolysis by phosphorylating and activating phosphofructokinase[134] and promoting hexokinase II transcription[135]. Independently of AMPK, metformin inhibits gluconeogenesis by inhibiting glucose-6-phosphatase mediated conversion of glucose-6-phosphate to glucose[136]. 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 towards glycolysis (*unpublished observations*). Although metformin reduces mitochondrial ATP production in normal placentas, in preeclamptic placentas 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 as well as 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 birth weight[128] although it is currently unclear if these effects are mediated directly via 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[137] 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)[138,139]. The placenta exhibits the highest tissue levels of GDF15 and secretes large amounts into the maternal circulation[140]. 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[141]. 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 placental-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 up-regulate 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 preeclamptic placenta. However, given the multiple phenotypes associated with targeting energy metabolism, the potential risks must be carefully weighed.

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, i.e. 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 (e.g. 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 (i.e. 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 UDP-*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 as well as under pathological conditions.
8. **Pentose phosphate pathway:** A metabolic pathway that operates in parallel to glycolysis that results in the generation of pentoses (5-carbon sugars), ribose-5-phosphate (a precursor for nucleotide synthesis), and produces NADPH.
9. **Redox:** An oxidation-reduction (redox) reaction involves the transfer of electrons between two species. Reducing equivalents and oxidizing agents play important roles as co-factors 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 $FADH_2$) 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 one of the two 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 (i.e. increased) gene expression.

Acknowledgements

We are grateful to Francesca Gaccioli for her valuable comments. All figures were created with Biorender.com.

REFERENCES:

1. Jauniaux E. Partial moles: From postnatal to prenatal diagnosis. *Placenta*. 1999;20(5-6):379-388.
2. Brosens I, Pijnenborg R, Vercruysse L, Romero R. The "great Obstetrical Syndromes" are associated with disorders of deep placentation. *Am J Obstet Gynecol*. 2011;204(3):193-201.
3. Burton GJ, Woods AW, Jauniaux E, Kingdom JCP. Rheological and Physiological Consequences of Conversion of the Maternal Spiral Arteries for Uteroplacental Blood Flow during Human Pregnancy. *Placenta*. 2009;30(6):473-482.
4. Austdal M, Thomsen LCV, Tangerås LH, et al. Metabolic profiles of placenta in preeclampsia using HR-MAS MRS metabolomics. *Placenta*. 2015;36(12):1455-1462.
5. Bax BE, Bloxam DL. Energy metabolism and glycolysis in human placental trophoblast cells during differentiation. *Biochim Biophys Acta*. 1997;1319:283-292.
6. Carter AM. Placental oxygen consumption. Part I: In vivo studies - A review. *Placenta*. 2000;21(SUPPL.1):S31-S37.
7. Hay WW. Energy and substrate requirements of the placenta and fetus. *Proc Nutr Soc*. 2005;50(2):321-336.
8. Gusseck DJ. Role of nucleic acids in the regulation of human placental lactogen synthesis. *Gynecol Obstet Invest*. 1977;8(3-4):162-182.
9. Stouthamer AH. A theoretical study on the amount of ATP required for synthesis of microbial cell material. *Antonie Van Leeuwenhoek*. 1973;39(1):545-565.
10. Yung HW, Calabrese S, Hynx D, et al. Evidence of placental translation inhibition and endoplasmic reticulum stress in the etiology of human intrauterine growth restriction. *Am J Pathol*. 2008;173(2):451-462.
11. Baumann M, Körner M, Huang X, Wenger F, Surbek D, Albrecht C. Placental ABCA1 and ABCG1 expression in gestational disease: Pre-eclampsia affects ABCA1 levels in syncytiotrophoblasts. *Placenta*. 2013;34(11):1079-1086.
12. Lüscher BP, Marini C, Joerger-Messerli MS, et al. Placental glucose transporter (GLUT)-1 is down-regulated in preeclampsia. *Placenta*. 2017;55:94-99.
13. Gu W, Jones CT, Harding JE. Metabolism of glucose by fetus and placenta of sheep. The effects of normal fluctuations in uterine blood flow. *J Dev Physiol*. 1987;9(4):369-389.
14. Divakaruni AS, Brand MD. The regulation and physiology of mitochondrial proton leak. *Physiology (Bethesda)*. 2011;26(3):192-205.
15. Rabinowitz JD, Enerbäck S. Lactate: the ugly duckling of energy metabolism. *Nat Metab*. 2020;2(7):566-571.
16. Robergs RA, McNulty CR, Minett GM, Holland J, Trajano G. Lactate, not lactic acid, is produced by cellular cytosolic energy catabolism. *Physiology*. 2018;33(1):10-12.
17. Hui S, Ghergurovich JM, Morscher RJ, et al. Glucose feeds the TCA cycle via circulating lactate.

611 *Nature*. 2017;551(7678):115-118.

612 18. Aldoretta PW, Hay WW. Effect of glucose supply on ovine uteroplacental glucose metabolism.
613 *Am J Physiol - Regul Integr Comp Physiol*. 1999;277(4 46-4).

614 19. Burd LI, Jones MD, Simmons MA, Makowski EL, Meschia G, Battaglia FC. Placental production
615 and foetal utilisation of lactate and pyruvate. *Nature*. 1975;254(5502):710-711.

616 20. Settle P, Sibley CP, Doughty IM, et al. Placental Lactate Transporter Activity and Expression in
617 Intrauterine Growth Restriction. *J Soc Gynecol Investig*. 2006;13(5):357-363.

618 21. REMESAR X, AROLA L, PALOU A, ALEMANY M. Activities of Enzymes Involved in Amino-Acid
619 Metabolism in Developing Rat Placenta. *Eur J Biochem*. 1980;110(1):289-293.

620 22. Self JT, Spencer TE, Johnson GA, Hu J, Bazer FW, Wu G. Glutamine Synthesis in the Developing
621 Porcine Placenta. *Biol Reprod*. 2004;70(5):1444-1451.

622 23. Day PEL, Cleal JK, Lofthouse EM, et al. Partitioning of glutamine synthesised by the isolated
623 perfused human placenta between the maternal and fetal circulations. *Placenta*.
624 2013;34(12):1223-1231.

625 24. Roifman M, Niles KM, MacNeil L, et al. Homozygous GLUL deletion is embryonically viable and
626 leads to glutamine synthetase deficiency. *Clin Genet*. October 2020:cge.13844.

627 25. Moores RR, Vaughn PR, Battaglia FC, Fennessey P V., Wilkening RB, Meschia G. Glutamate
628 metabolism in fetus and placenta of late-gestation sheep. *Am J Physiol - Regul Integr Comp*
629 *Physiol*. 1994;267(1 36-1).

630 26. Battaglia FC. Glutamine and glutamate exchange between the fetal liver and the placenta. In:
631 *Journal of Nutrition*. Vol 130. American Institute of Nutrition; 2000:974-977.

632 27. Jastroch M, Divakaruni AS, Mookerjee S, Treberg JR, Brand MD. Mitochondrial proton and
633 electron leaks. *Essays Biochem*. 2010;47:53-67.

634 28. Burton GJ, Jauniaux E. Oxidative stress. *Best Pract Res Clin Obstet Gynaecol*. 2011;25(3):287-
635 299.

636 29. Rosario FJ, Kanai Y, Powell TL, Jansson T. Mammalian target of rapamycin signalling
637 modulates amino acid uptake by regulating transporter cell surface abundance in primary
638 human trophoblast cells. *J Physiol*. 2013;591(3):609-625.

639 30. Roos S, Kanai Y, Prasad PD, Powell TL, Jansson T. Regulation of placental amino acid
640 transporter activity by mammalian target of rapamycin. *Am J Physiol - Cell Physiol*.
641 2009;296(1):142-150.

642 31. Rosario FJ, Gupta MB, Myatt L, et al. Mechanistic Target of Rapamycin Complex 1 Promotes
643 the Expression of Genes Encoding Electron Transport Chain Proteins and Stimulates Oxidative
644 Phosphorylation in Primary Human Trophoblast Cells by Regulating Mitochondrial Biogenesis.
645 *Sci Rep*. 2019;9(1):1-14.

646 32. Gaccioli F, Lager S, Powell TL, Jansson T. Placental transport in response to altered maternal
647 nutrition. *J Dev Orig Health Dis*. 2013;4(2):101-115.

648 33. Jansson T, Aye ILMH, Goberdhan DCI. The emerging role of mTORC1 signaling in placental
649 nutrient-sensing. *Placenta*. 2012;33(SUPPL. 2).

650 34. Roos S, Jansson N, Palmberg I, Säljö K, Powell TL, Jansson T. Mammalian target of rapamycin
651 in the human placenta regulates leucine transport and is down-regulated in restricted fetal

652 growth. *J Physiol*. 2007;582(1):449-459.

653 35. Chen YY, Rosario FJ, Shehab MA, Powell TL, Gupta MB, Jansson T. Increased ubiquitination
654 and reduced plasma membrane trafficking of placental amino acid transporter SNAT-2 in
655 human IUGR. *Clin Sci*. 2015;129(12):1131-1141.

656 36. Jansson N, Rosario FJ, Gaccioli F, et al. Activation of placental mTOR signaling and amino acid
657 transporters in obese women giving birth to large babies. *J Clin Endocrinol Metab*.
658 2013;98(1):105-113.

659 37. Orozco JM, Krawczyk PA, Scaria SM, et al. Dihydroxyacetone phosphate signals glucose
660 availability to mTORC1. *Nat Metab*. 2020;2(9):893-901.

661 38. Kaelin WG. Cancer and altered metabolism: Potential importance of hypoxia-Inducible factor
662 and 2-oxoglutarate-dependent dioxygenases. *Cold Spring Harb Symp Quant Biol*.
663 2011;76:335-345.

664 39. Koivunen P, Hirsilä M, Remes AM, Hassinen IE, Kivirikko KI, Myllyharju J. Inhibition of hypoxia-
665 inducible factor (HIF) hydroxylases by citric acid cycle intermediates: Possible links between
666 cell metabolism and stabilization of HIF. *J Biol Chem*. 2007;282(7):4524-4532.

667 40. Chandel NS, McClintock DS, Feliciano CE, et al. Reactive oxygen species generated at
668 mitochondrial Complex III stabilize hypoxia-inducible factor-1α during hypoxia: A mechanism
669 of O2 sensing. *J Biol Chem*. 2000;275(33):25130-25138. http://www.jbc.org/. Accessed
670 October 11, 2020.

671 41. Guzy RD, Hoyos B, Robin E, et al. Mitochondrial complex III is required for hypoxia-induced
672 ROS production and cellular oxygen sensing. *Cell Metab*. 2005;1(6):401-408.

673 42. Sasagawa T, Nagamatsu T, Morita K, et al. HIF-2α, but not HIF-1α, mediates hypoxia-induced
674 up-regulation of Flt-1 gene expression in placental trophoblasts. *Sci Rep*. 2018;8(1):1-12.

675 43. Korkes HA, De Oliveira L, Sass N, Salahuddin S, Karumanchi SA, Rajakumar A. Relationship
676 between hypoxia and downstream pathogenic pathways in preeclampsia. *Hypertens*
677 *Pregnancy*. 2017;36(2):145-150.

678 44. Hu C-J, Wang L-Y, Chodosh LA, Keith B, Simon MC. Differential Roles of Hypoxia-Inducible
679 Factor 1α (HIF-1α) and HIF-2α in Hypoxic Gene Regulation. *Mol Cell Biol*. 2003;23(24):9361-
680 9374.

681 45. Lozoya OA, Wang T, Grenet D, et al. Mitochondrial acetyl-CoA reversibly regulates locus-
682 specific histone acetylation and gene expression. *Life Sci alliance*. 2019;2(1).

683 46. McDonnell E, Crown SB, Fox DB, et al. Lipids Reprogram Metabolism to Become a Major
684 Carbon Source for Histone Acetylation. *Cell Rep*. 2016;17(6):1463-1472.

685 47. Moussaieff A, Rouleau M, Kitsberg D, et al. Glycolysis-mediated changes in acetyl-CoA and
686 histone acetylation control the early differentiation of embryonic stem cells. *Cell Metab*.
687 2015;21(3):392-402.

688 48. Latham T, MacKay L, Sproul D, et al. Lactate, a product of glycolytic metabolism, inhibits
689 histone deacetylase activity and promotes changes in gene expression. *Nucleic Acids Res*.
690 2012;40(11):4794-4803.

691 49. Cantó C, Menzies KJ, Auwerx J. NAD+ Metabolism and the Control of Energy Homeostasis: A
692 Balancing Act between Mitochondria and the Nucleus. *Cell Metab*. 2015;22(1):31-53.

693 50. Locasale JW. Serine, glycine and one-carbon units: Cancer metabolism in full circle. *Nat Rev*

694 *Cancer*. 2013;13(8):572-583.

695 51. Yang M, Vousden KH. Serine and one-carbon metabolism in cancer. *Nat Rev Cancer*.
696 2016;16(10):650-662.

697 52. Yu W, Wang Z, Zhang K, et al. One-Carbon Metabolism Supports S-Adenosylmethionine and
698 Histone Methylation to Drive Inflammatory Macrophages. *Mol Cell*. 2019;75(6):1147-1160.e5.

699 53. Burton GJ, Watson AL, Hempstock J, Skepper JN, Jauniaux E. Uterine glands provide
700 histiotrophic nutrition for the human fetus during the first trimester of pregnancy. *J Clin*
701 *Endocrinol Metab*. 2002;87(6):2954-2959.

702 54. Diamant YZ, Mayorek N, Neuman S, Shafrir E. Enzymes of glucose and fatty acid metabolism
703 in early and term human placenta. *Am J Obstet Gynecol*. 1975;121(1):58-61.

704 55. Burton GJ, Hempstock J, Jauniaux E. Oxygen, early embryonic metabolism and free radical-
705 mediated embryopathies. *Reprod Biomed Online*. 2003;6(1):84-96.

706 56. Carreau A, Hafny-Rahbi B El, Matejuk A, Grillon C, Kieda C. Why is the partial oxygen pressure
707 of human tissues a crucial parameter? Small molecules and hypoxia. *J Cell Mol Med*.
708 2011;15(6):1239-1253.

709 57. Cindrova-Davies T, Tissot Van Patot M, Gardner L, Jauniaux E, Burton GJ, Charnock-Jones DS.
710 Energy status and HIF signalling in chorionic villi show no evidence of hypoxic stress during
711 human early placental development. *Mol Hum Reprod*. 2014;21(3):296-308.

712 58. Holland OJ, Hickey AJR, Alvsaker A, et al. Changes in mitochondrial respiration in the human
713 placenta over gestation. *Placenta*. 2017;57:102-112.

714 59. Jones CJP, Fox H. An ultrahistochemical study of the placental content of respiratory enzymes
715 in normal and prolonged pregnancies. *Investig Cell Pathol*. 1978;1(3):217-225.

716 60. Scislowski PWD, Zolnierowicz S, Zelewski L. Subcellular distribution of isocitrate
717 dehydrogenase in early and term human placenta. *Biochem J*. 1983;214(2):339-343.

718 61. Miyazawa H, Aulehla A. Revisiting the role of metabolism during development. *Dev*.
719 2018;145(19):dev131110.

720 62. Gándara L, Wappner P. Metabo-Devo: A metabolic perspective of development. *Mech Dev*.
721 2018;154:12-23.

722 63. Chi F, Sharpley MS, Nagaraj R, Roy S Sen, Banerjee U. Glycolysis-Independent Glucose
723 Metabolism Distinguishes TE from ICM Fate during Mammalian Embryogenesis. *Dev Cell*.
724 2020;53(1):9-26.e4.

725 64. Burton GJ, Jauniaux E. The cytotrophoblastic shell and complications of pregnancy. *Placenta*.
726 2017;60:134-139.

727 65. Okae H, Toh H, Sato T, et al. Derivation of Human Trophoblast Stem Cells. *Cell Stem Cell*.
728 2018;22(1):50-63.e6.

729 66. Haider S, Meinhardt G, Saleh L, et al. Self-Renewing Trophoblast Organoids Recapitulate the
730 Developmental Program of the Early Human Placenta. *Stem Cell Reports*. 2018;11(2):537-551.

731 67. Turco MY, Gardner L, Kay RG, et al. Trophoblast organoids as a model for maternal–fetal
732 interactions during human placentation. *Nature*. 2018;564(7735):263-267.

733 68. Mathieu J, Ruohola-Baker H. Metabolic remodeling during the loss and acquisition of
734 pluripotency. *Dev*. 2017;144(4):541-551.

69. Miyazawa H, Aulehla A. Revisiting the role of metabolism during development. *Dev*. 2018;145(19).

70. Kolahi KS, Valent AM, Thornburg KL. Cytotrophoblast, Not Syncytiotrophoblast, Dominates Glycolysis and Oxidative Phosphorylation in Human Term Placenta. *Sci Rep*. 2017;7(1):1-12.

71. Jaju Bhattad G, Jeyarajah MJ, McGill MG, et al. Histone deacetylase 1 and 2 drive differentiation and fusion of progenitor cells in human placental trophoblasts. *Cell Death Dis*. 2020;11(5):311.

72. Roberts JM, Escudero C. The placenta in preeclampsia. *Pregnancy Hypertens*. 2012;2(2):72-83.

73. Marín R, Chiarello DI, Abad C, Rojas D, Toledo F, Sobrevia L. Oxidative stress and mitochondrial dysfunction in early-onset and late-onset preeclampsia. *Biochim Biophys Acta - Mol Basis Dis*. 2020;1866(12):165961.

74. Say RE, Whittaker RG, Turnbull HE, McFarland R, Taylor RW, Turnbull DM. Mitochondrial disease in pregnancy: a systematic review. *Obstet Med*. 2011;4(3):90-94.

75. Torbergsen T, Øian P, Mathiesen E, Borud O. Pre-Eclampsia-A Mitochondrial Disease? *Acta Obstet Gynecol Scand*. 1989;68(2):145-148.

76. Hung TH, Skepper JN, Charnock-Jones DS, Burton GJ. Hypoxia-reoxygenation: A potent inducer of apoptotic changes in the human placenta and possible etiological factor in preeclampsia. *Circ Res*. 2002;90(12):1274-1281.

77. Hung TH, Burton GJ. Hypoxia and reoxygenation: A possible mechanism for placental oxidative stress in preeclampsia. *Taiwan J Obstet Gynecol*. 2006;45(3):189-200.

78. Fukasawa M, Tsuchiya T, Takayama E, et al. Identification and characterization of the hypoxia-responsive element of the human placental 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase gene. *J Biochem*. 2004;136(3):273-277.

79. Clark DE, Smith SK, He Y, et al. A vascular endothelial growth factor antagonist is produced by the human placenta and released into the maternal circulation. *Biol Reprod*. 1998;59(6):1540-1548.

80. Holland OJ, Cuffe JSM, Dekker Nitert M, et al. Placental mitochondrial adaptations in preeclampsia associated with progression to term delivery. *Cell Death Dis*. 2018;9(12):1150.

81. Gómez-Gutiérrez AM, Parra-Sosa BE, Bueno-Sánchez JC. Glycosylation Profile of the Transferrin Receptor in Gestational Iron Deficiency and Early-Onset Severe Preeclampsia. *J Pregnancy*. 2019:9514546.

82. Marini M, Bonaccini L, Thyrion GDZ, Vichi D, Parretti E, Sgambati E. Distribution of sugar residues in human placentas from pregnancies complicated by hypertensive disorders. *Acta Histochem*. 2011;113(8):815-825.

83. Akella NM, Ciraku L, Reginato MJ. Fueling the fire: Emerging role of the hexosamine biosynthetic pathway in cancer. *BMC Biol*. 2019;17(1):1-14.

84. Bloxam DL, Bullen BE, Walters BNJ, Lao TT. Placental glycolysis and energy metabolism in preeclampsia. *Am J Obstet Gynecol*. 1987;157(1):97-101.

85. McIntyre KR, Vincent KMM, Hayward CE, et al. Human placental uptake of glutamine and glutamate is reduced in fetal growth restriction. *Sci Rep*. 2020;10(1):16197.

86. Hayashi T, Rizzuto R, Hajnoczky G, Su TP. MAM: more than just a housekeeper. *Trends Cell Biol*. 2009;19(2):81-88.

87. Yung HW, Colleoni F, Dommett E, et al. Noncanonical mitochondrial unfolded protein response impairs placental oxidative phosphorylation in early-onset preeclampsia. *Proc Natl Acad Sci U S A*. 2019;116(36):18109-18118.

88. Wang Z V., Deng Y, Gao N, et al. Spliced X-box binding protein 1 couples the unfolded protein response to hexosamine biosynthetic pathway. *Cell*. 2014;156(6):1179-1192.

89. Tsang JCH, Vong JSL, Ji L, et al. Integrative single-cell and cell-free plasma RNA transcriptomics elucidates placental cellular dynamics. *Proc Natl Acad Sci U S A*. 2017;114(37):E7786-E7795.

90. Broere-Brown ZA, Adank MC, Benschop L, et al. Fetal sex and maternal pregnancy outcomes: A systematic review and meta-analysis. *Biol Sex Differ*. 2020;11(1):26.

91. Walker MG, Fitzgerald B, Keating S, Ray JG, Windrim R, Kingdom JCP. Sex-specific basis of severe placental dysfunction leading to extreme preterm delivery. *Placenta*. 2012;33(7):568-571.

92. Jaskolka D, Retnakaran R, Zinman B, Kramer CK. Fetal sex and maternal risk of pre-eclampsia/eclampsia: a systematic review and meta-analysis. *BJOG An Int J Obstet Gynaecol*. 2017;124(4):553-560.

93. Schalekamp-Timmermans S, Arends LR, Alsaker E, et al. Fetal sex-specific differences in gestational age at delivery in pre-eclampsia: A meta-analysis. *Int J Epidemiol*. 2017;46(2):632-642.

94. Myatt L, Muralimanoharan S, Maloyan A. Effect of preeclampsia on placental function: Influence of sexual dimorphism, microRNA's and mitochondria. *Adv Exp Med Biol*. 2014;814:133-146.

95. Murji A, Proctor LK, Paterson AD, Chitayat D, Weksberg R, Kingdom J. Male sex bias in placental dysfunction. *Am J Med Genet Part A*. 2012;158 A(4):779-783.

96. Orzack SH, Stubblefield JW, Akmaev VR, et al. The human sex ratio from conception to birth. *Proc Natl Acad Sci U S A*. 2015;112(16):E2102-E2111.

97. Muralimanoharan S, Maloyan A, Myatt L. Evidence of sexual dimorphism in the placental function with severe preeclampsia. *Placenta*. 2013;34(12):1183-1189.

98. Muralimanoharan S, Guo C, Myatt L, Maloyan A. Sexual dimorphism in MIR-210 expression and mitochondrial dysfunction in the placenta with maternal obesity. *Int J Obes*. 2015;39(8):1274-1281.

99. Wang Y, Bucher M, Myatt L. Use of Glucose, Glutamine, and Fatty Acids for Trophoblast Respiration in Lean Women, Women with Obesity, and Women with Gestational Diabetes. *J Clin Endocrinol Metab*. 2019;104(9):4178-4187.

100. Sood R, Zehnder JL, Druzin ML, Brown PO. Gene expression patterns in human placenta. *Proc Natl Acad Sci U S A*. 2006;103(14):5478-5483.

101. Buckberry S, Bianco-Miotto T, Bent SJ, Dekker GA, Roberts CT. Integrative transcriptome metaanalysis reveals widespread sex-biased gene expression at the human fetal-maternal interface. *Mol Hum Reprod*. 2014;20(8):810-819.

102. Gonzalez TL, Sun T, Koeppel AF, et al. Sex differences in the late first trimester human placenta transcriptome. *Biol Sex Differ*. 2018;9(1):1-23.

103. Syrett CM, Sierra I, Berry CL, Beiting D, Anguera MC. Sex-Specific Gene Expression Differences Are Evident in Human Embryonic Stem Cells and during in Vitro Differentiation of Human Placental Progenitor Cells. *Stem Cells Dev*. 2018;27(19):1360-1375.

104. Gong S, Sovio U, Aye IL, et al. Placental polyamine metabolism differs by fetal sex, fetal growth restriction, and preeclampsia. *JCI insight*. 2018;3(13).

105. Balaton BP, Brown CJ. Escape Artists of the X Chromosome. *Trends Genet*. 2016;32(6):348-359.

106. Tukiainen T, Villani AC, Yen A, et al. Landscape of X chromosome inactivation across human tissues. *Nature*. 2017;550(7675):244-248.

107. Aye I, Gong S, Charnock-Jones DS, Smith GCS. 2019 | Scientific Abstracts. In: *Reproductive Sciences*. Vol 26. ; 2019:62A-390A.

108. Avellino G, Charnock-Jones DS, Smith G, Aye IL. SRI 2020: Scientific Abstracts. *Reprod Sci*. 2020;27:1-444.

109. Traber MG, Stevens JF. Vitamins C and E: Beneficial effects from a mechanistic perspective. *Free Radic Biol Med*. 2011;51(5):1000-1013.

110. Chappell LC, Seed PT, Briley A, et al. A longitudinal study of biochemical variables in women at risk of preeclampsia. *Am J Obstet Gynecol*. 2002;187(1):127-136.

111. Chappell LC, Seed PT, Kelly FJ, et al. Vitamin C and E supplementation in women at risk of preeclampsia is associated with changes in indices of oxidative stress and placental function. *Am J Obstet Gynecol*. 2002;187(3):777-784.

112. Chappell LC, Seed PT, Briley AL, et al. Effect of antioxidants on the occurrence of pre-eclampsia in women at increased risk: A randomised trial. *Lancet*. 1999;354(9181):810-816.

113. Rahimi R, Nikfar S, Rezaie A, Abdollahi M. A meta-analysis on the efficacy and safety of combined vitamin C and e supplementation in preeclamptic women vitamin C and e supplements in preeclamptic women. *Hypertens Pregnancy*. 2009;28(4):417-434.

114. Poston L, Briley A, Seed P, Kelly F, Shennan A. Vitamin C and vitamin E in pregnant women at risk for pre-eclampsia (VIP trial): randomised placebo-controlled trial. *Lancet*. 2006;367(9517):1145-1154.

115. Basaran A, Basaran M, Topatan B. Combined vitamin C and e supplementation for the prevention of preeclampsia: A systematic review and meta-analysis. *Obstet Gynecol Surv*. 2010;65(10):653-667.

116. Conde-Agudelo A, Romero R, Kusanovic JP, Hassan SS. Supplementation with vitamins C and e during pregnancy for the prevention of preeclampsia and other adverse maternal and perinatal outcomes: A systematic review and metaanalysis. *Am J Obstet Gynecol*. 2011;204(6):503.e1-503.e12.

117. Rumbold A, Ota E, Hori H, Miyazaki C, Crowther CA. Vitamin E supplementation in pregnancy. *Cochrane Database Syst Rev*. 2015;2016(3).

118. Palacios C, Kostiuk LK, Peña-Rosas JP. Vitamin D supplementation for women during pregnancy. *Cochrane Database Syst Rev*. 2019;2019(7).

119. Ota E, Mori R, Middleton P, et al. Zinc supplementation for improving pregnancy and infant outcome. *Cochrane Database Syst Rev*. 2015;2015(2).

120. Bjelakovic G, Nikolova D, Gluud LL, Simonetti RG, Gluud C. Mortality in randomized trials of antioxidant supplements for primary and secondary prevention: Systematic review and meta-analysis. *J Am Med Assoc*. 2007;297(8):842-857.

121. Nezu M, Souma T, Yu L, et al. Nrf2 inactivation enhances placental angiogenesis in a preeclampsia mouse model and improves maternal and fetal outcomes. *Sci Signal*. 2017;10(479).

122. Ma Q. Role of Nrf2 in oxidative stress and toxicity. *Annu Rev Pharmacol Toxicol*. 2013;53:401-426.

123. Kawashima A, Sekizawa A, Koide K, et al. Vitamin C induces the reduction of oxidative stress and paradoxically stimulates the apoptotic gene expression in extravillous trophoblasts derived from first-trimester tissue. *Reprod Sci*. 2015;22(7):783-790.

124. Hung T-H, Chen S-F, Li M-J, Yeh Y-L, Hsieh T-T. Differential Effects of Concomitant Use of Vitamins C and E on Trophoblast Apoptosis and Autophagy between Normoxia and Hypoxia-Reoxygenation. Dietz HP, ed. *PLoS One*. 2010;5(8):e12202.

125. Nguyen L, Chan SY, Teo AKK. Metformin from mother to unborn child – Are there unwarranted effects? *EBioMedicine*. 2018;35:394-404.

126. Syngelaki A, Nicolaides KH, Balani J, et al. Metformin versus Placebo in Obese Pregnant Women without Diabetes Mellitus. *N Engl J Med*. 2016;374(5):434-443.

127. Tarry-Adkins JL, Aiken CE, Ozanne SE. Comparative impact of pharmacological treatments for gestational diabetes on neonatal anthropometry independent of maternal glycaemic control: A systematic review and meta-analysis. Burden C, ed. *PLoS Med*. 2020;17(5):e1003126.

128. Tarry-Adkins JL, Aiken CE, Ozanne SE. Neonatal, infant, and childhood growth following metformin versus insulin treatment for gestational diabetes: A systematic review and meta-analysis. *PLoS Med*. 2019;16(8):e1002848.

129. Liu X, Romero IL, Litchfield LM, Lengyel E, Locasale JW. Metformin Targets Central Carbon Metabolism and Reveals Mitochondrial Requirements in Human Cancers. *Cell Metab*. 2016;24(5):728-739.

130. Brownfoot FC, Hastie R, Hannan NJ, et al. Metformin as a prevention and treatment for preeclampsia: Effects on soluble fms-like tyrosine kinase 1 and soluble endoglin secretion and endothelial dysfunction. *Am J Obstet Gynecol*. 2016;214(3):356.e1-356.e15.

131. Tanaka Y, Konishi A, Obinata H, Tsuneoka M. Metformin activates KDM2A to reduce rRNA transcription and cell proliferation by dual regulation of AMPK activity and intracellular succinate level. *Sci Rep*. 2019;9(1):1-12.

132. Shaw RJ, Lamia KA, Vasquez D, et al. Medicine: The kinase LKB1 mediates glucose homeostasis in liver and therapeutic effects of metformin. *Science (80-)*. 2005;310(5754):1642-1646.

133. Garcia D, Shaw RJ. AMPK: Mechanisms of Cellular Energy Sensing and Restoration of Metabolic Balance. *Mol Cell*. 2017;66(6):789-800.

134. Marsin AS, Bertrand L, Rider MH, et al. Phosphorylation and activation of heart PFK-2 by AMPK has a role in the stimulation of glycolysis during ischaemia. *Curr Biol*. 2000;10(20):1247-1255.

135. Thomson DM, Herway ST, Fillmore N, et al. AMP-activated protein kinase phosphorylates transcription factors of the CREB family. *J Appl Physiol*. 2008;104(2):429-438.

136. Moonira T, Chachra SS, Ford BE, et al. Metformin lowers glucose 6-phosphate in hepatocytes by activation of glycolysis downstream of glucose phosphorylation. *J Biol Chem*. 2020;295(10):3330-3346.

137. Griss T, Vincent EE, Egnatchik R, et al. Metformin Antagonizes Cancer Cell Proliferation by Suppressing Mitochondrial-Dependent Biosynthesis. Green DR, ed. *PLOS Biol*. 2015;13(12):e1002309.

138. Coll AP, Chen M, Taskar P, et al. GDF15 mediates the effects of metformin on body weight and energy balance. *Nature*. 2020;578(7795):444-448.

139. Day EA, Ford RJ, Smith BK, et al. Metformin-induced increases in GDF15 are important for suppressing appetite and promoting weight loss. *Nat Metab*. 2019;1(12):1202-1208.

140. Moore AG, Brown DA, Fairlie WD, et al. The Transforming Growth Factor-β Superfamily Cytokine Macrophage Inhibitory Cytokine-1 Is Present in High Concentrations in the Serum of Pregnant Women 1. *J Clin Endocrinol Metab*. 2000;85(12):4781-4788.

141. Chen Q, Wang Y, Zhao M, Hyett J, da Silva Costa F, Nie G. Serum levels of GDF15 are reduced in preeclampsia and the reduction is more profound in late-onset than early-onset cases. *Cytokine*. 2016;83:226-230.

Figure legends

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. 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. Co-factors are depicted in blue and orange, biosynthetic precursors are shown in red. Cyt c, cytochrome C; Co Q, coenzyme-Q; TCA, tricarboxylic acid.

Figure 2. Placental-fetal glutamine-glutamate shuttle. Due to the absence of the glutaminase enzyme, placental glutamine metabolism requires the input of fetal liver enzymes to convert glutamine to glutamate which is then re-extracted by the placenta from the fetal circulation. Glutamate is converted back to glutamine or α-ketoglutarate which provides the substrate for TCA anaplerosis. GLUD, glutamate dehydrogenase; GLS, glutaminase; GS, glutamine synthetase; TCA, tricarboxylic acid.

Figure 3. Mitochondrial generation of reactive oxygen species and their scavenging by endogenous antioxidant defenses. 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 TRX_{Red} 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. CoQ, coenzyme Q; Cyt c, cytochrome c; TCA cycle, tricarboxylic acid cycle; O_2•-, superoxide; H_2O_2, hydrogen peroxide; GPx, glutathione peroxidase; GSH, reduced glutathione; GSSG, oxidized glutathione; TPx, thioredoxin peroxidase; TRX_{Red}, reduced thioredoxin; TRX_{Ox}, oxidized thioredoxin.

Figure 4. Signaling functions of metabolic intermediates. Intermediaries of central energy metabolism have diverse non-metabolic signaling roles with important effects on placental physiology and disease. Glycolytic intermediates and ATP:ADP ratio signal towards 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. SIRT-class of HDACs require NAD^+ as co-factors. DNA and histone methylation by TET and JMJD are activated by α-ketoglutarate and inhibited by succinate and fumarate. High α-ketoglutarate to succinate or fumarate ratio enhances PHD activity leading to hydroxylation of HIF-1/2α leading to its ubiquitination and proteasomal degradation. Low α-ketoglutarate to succinate or fumarate ratio, hypoxia and ROS inhibit PHDs leading to HIF-1/2α stabilization and nuclear translocation where it promotes transcription of their respective target genes. Ac, acetyl-group; AMPK, AMP protein kinase; FLT1, fms-like tyrosine kinase 1; HAT, histone acetyltransferase; HDAC, histone deacetylase; HIF, hypoxia-inducible factor; JMJD, jumonji C domain-containing proteins; La,

963 lactyl-group; Me, methyl-group; mTOR, mechanistic target of rapamycin; PHD, prolyl-hydroxylase
964 domain enzymes; ROS, reactive oxygen species; TCA cycle, tricarboxylic acid cycle; TET, ten-eleven
965 translocation enzymes; Ub, ubiquitin.

966

967 **Figure 5. Placental metabolic reprogramming during development and adaptation to**
968 **preeclampsia.** First-trimester placentas utilize glycolysis and its shunt pathways to generate
969 bioenergy and biosynthetic precursors, although they do retain some mitochondrial activity. Mild
970 preeclampsia resulting in term delivery may allow the placenta to adapt to mitochondrial
971 dysfunction by reverting its metabolism to the early developmental state. Failure in metabolic
972 reprogramming may result in the loss of bioenergetic and biosynthetic homeostasis resulting in the
973 severe form of preeclampsia associated with preterm delivery and fetal growth restriction. Solid
974 arrows indicate changes previously reported in literature, and dashed arrows indicate predicted
975 effects. α-KG, α-ketoglutarate; Glu, glutamine; ROS, reactive oxygen species.

976

977 **Figure 6. Metformin targets multiple pathways in energy metabolism.** The key metabolic effects of
978 metformin are a result of complex I inhibition and activation of AMPK. Detailed description of the
979 potential effects are described in the main text. In summary, the outcomes include reduction in
980 mitochondrial-ROS generation, re-routing of metabolism towards glycolysis, restoring mitochondrial
981 biogenesis and inhibition of 2-oxoglutarate dependent dioxygenases such as TET, JMJD and PHD, and
982 activation of AMPK and its downstream effects. CoQ, coenzyme Q; Cyt c, cytochrome c; sFlt-1,
983 soluble fms-like tyrosine kinase-1; G6Pase, glucose-6-phosphatase; HIF, hypoxia-inducible factor; HK,
984 hexokinase; JMJD, Jumonji C domain-containing histone demethylases; PFK2, phosphofructokinase
985 2; PHD, prolyl-hydroxylase domains; PGC1α, peroxisome proliferator-activated receptor-gamma
986 coactivator-1 alpha; ROS, reactive oxygen species; TCA, tricarboxylic acid; TET, ten-eleven
987 translocation DNA demethylases; Ub, ubiquitin.

Glycolysis

Cytosol

Glucose

ATP
ADP

Glucose-6-phosphate

Pentose Phosphate Pathway

6-Phosphogluconolactone
NADP+
NADPH
6-Phosphogluconate
NADP+
NADPH
Ribulose-5-phosphate

Purine Nucleotides

Fructose-6-phosphate
Glucosamine-6-phosphate
ATP
ADP
Fructose-1,6-phosphate
N-acetylglucosamine-6-phosphate
Uridine diphosphate-
N-acetylglucosamine
(UDP-glcNac)
N-linked glycosylation
O-linked glycosylation

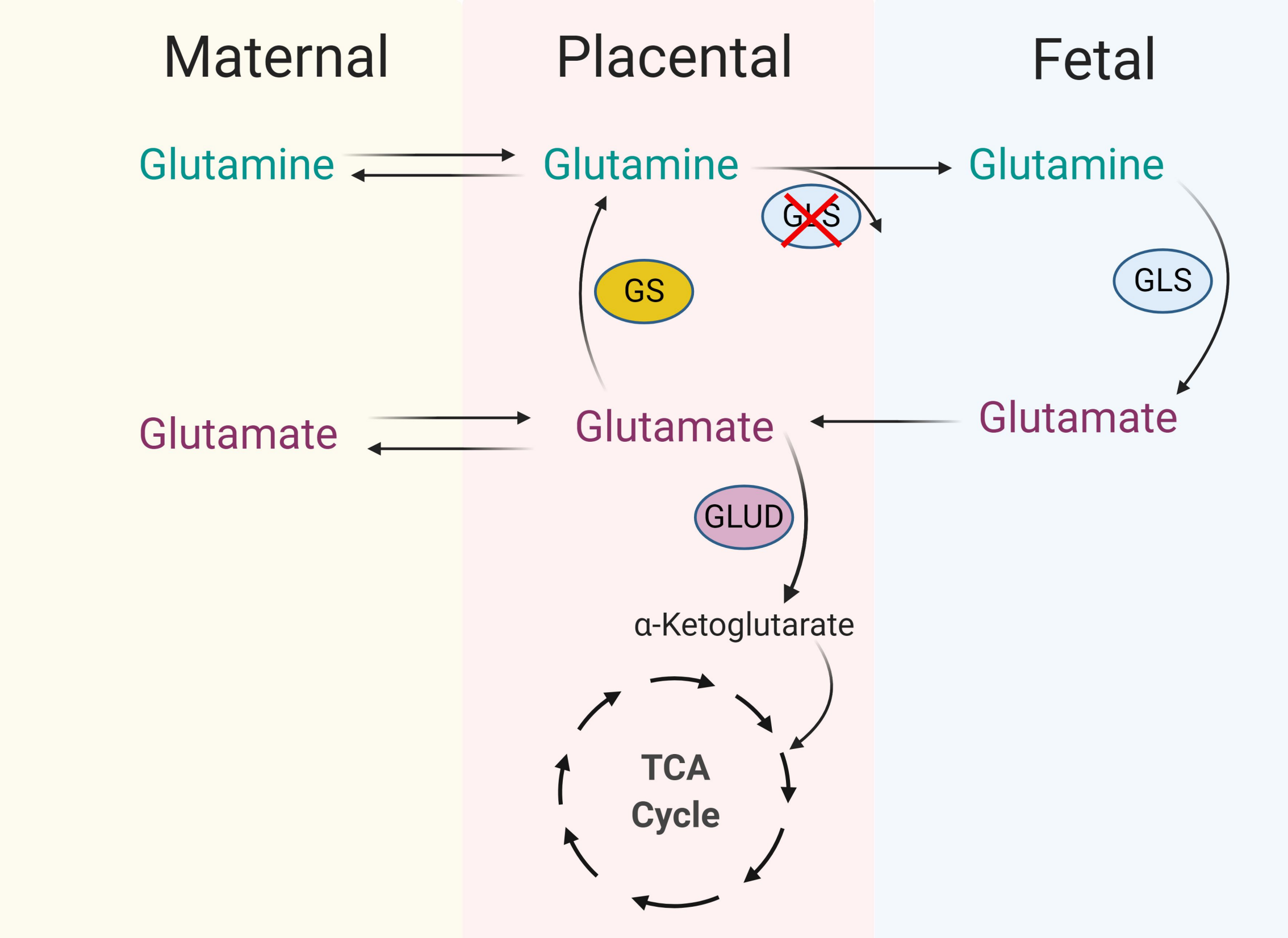
Hexosamine Biosynthetic Pathway

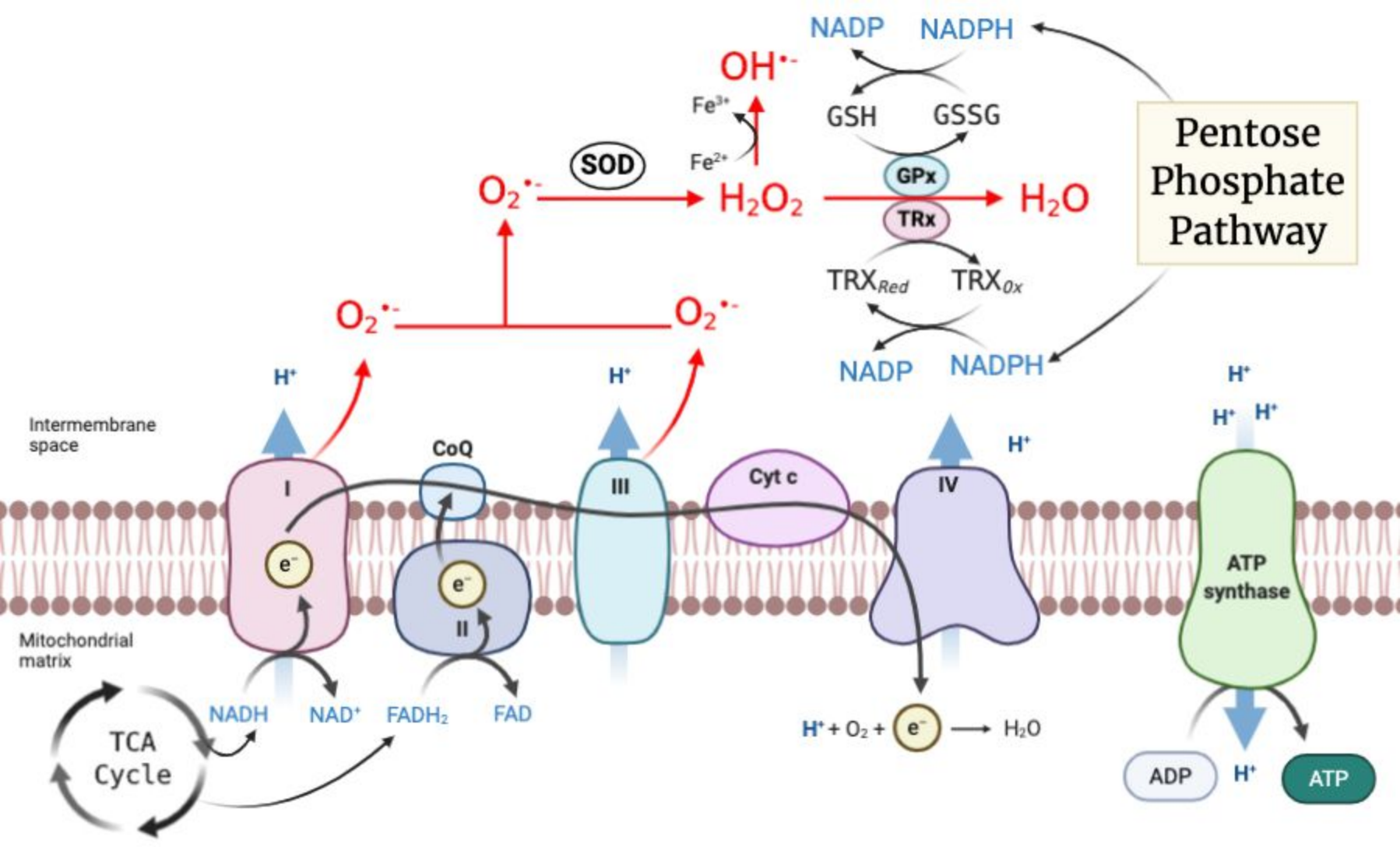
Triglycerides
Glycerol
3-phosphate
Dihydroxyacetone
phosphate
Glyceraldehyde
3-phosphate
NAD+
NADH
1,3-Bisphosphoglycerate
ADP
ATP
3-phosphoserine
3-phosphohydroxypyruvate
3-Phosphoglycerate
2-Phosphoglycerate
Serine
Cysteine
Tetrahydrofolate
5,10-methylene-
tetrahydrofolate
Glycine
Glutathione
Phosphoenolpyruvate
ADP
ATP
One carbon metabolism
Redox balance
HMG-CoA
Cholesterol Synthesis
Steroid Synthesis
NAD+
NADH
Lactate
Pyruvate
Acetyl-CoA
Malonyl-CoA
Fatty Acid Synthesis
Citrate

Mitochondrion

Pyruvate
Acetyl-CoA
Fatty Acid β-oxidation
Oxaloacetate
NADH
NAD+
Citrate
Malate
Isocitrate
NAD+
NADH
TCA Cycle
Fumarate
α-Ketoglutarate
Glutamate
NAD+
NADH
FADH2
FAD
Succinate
Succinyl CoA
ATP
ADP

H+
V
ATP synthase
ATP
ADP
H2O
IV
e-
Cyt c
III
CoQ
II
e-
FAD
+ H+
FADH2
I
e-
NAD+
+ H+
NADH
Outer membrane
Intermembrane space
Inner membrane


Maternal
Placental
Fetal
Glutamine
Glutamine
Glutamine
GLS
GS
GLS
Glutamate
Glutamate
Glutamate
GLUD
α-Ketoglutarate
TCA
Cycle


NADP
NADPH
OH•-
Fe3+
Fe2+
GSH
GSSG
SOD
GPx
O2•-
H2O2
H2O
TRx
TRXRed
TRX0x
Pentose Phosphate Pathway
O2•-
O2•-
NADP
NADPH
H+
H+
H+
H+
H+
Intermembrane space
CoQ
I
III
Cyt c
IV
ATP synthase
e-
e-
II
Mitochondrial matrix
NADH
NAD+
FADH2
FAD
TCA Cycle
H+ + O2 + e- → H2O
ADP
H+
ATP

Kinase signaling

Histone Acetylation/
Lactylation

Glucose

Fructose-1,6-phosphate

Nutrient Transport

mTOR

Dihydroxyacetone phosphate

Glyceraldehyde 3-phosphate

Protein Synthesis

AMPK

ATP

Acetyl-CoA

Pyruvate

Lactate

Ac

HAT

NAD+

SIRT

HDAC

NADH

?

La

Electron Transport Chain

TCA Cycle

α-ketoglutarate

ROS

HIF-1/2α

α-ketoglutarate

Hypoxia

PHDs

Succinate
Fumarate

HIF-1/2α

Ub

Ub

Ub

HIF-1α

Glycolytic genes

HIF-2α

Angiogenic genes
e.g. FLT1

α-ketoglutarate

Me

Me

TET

JMJD

Succinate
Fumarate

HIF transcriptional regulation

DNA / Histone Methylation

Metabolic reprogramming with development and adaptation to preeclampsia stress

Dysregulation of metabolism in preeclampsia leading to severe phenotype

Glucose

Pentose Phosphate Pathway ⇌ Glucose-6-phosphate ⇌ Pentose Phosphate Pathway

Hexosamine Biosynthetic Pathway ⇌ Fructose-6-phosphate ⇌ Hexosamine Biosynthetic Pathway

NAD+ → NADH

NAD+ → NADH

ADP → ATP

ADP → ATP

Lactate ⇌ Pyruvate ⇌ Lactate

Acetyl-CoA

TCA Cycle

α-KG ← Glu

Electron Transport Chain

ROS

ROS

ATP

ATP

Changes in first trimester placenta

Changes with mild preeclampsia

Changes with severe preeclampsia

Glucose
ATP
G6Pase
HK
ADP
Glucose-6-phosphate
Glycolysis
Fructose-6-phosphate
PFK2
Fructose-1,6-phosphate
ADP
ATP
Pyruvate
Citrate
TCA Cycle
α-Ketoglutarate
α-Ketoglutarate
Nutrient Transport
mTOR
Protein Synthesis
AMPK
PGC1α
Mitochondrial Biogenesis
Fatty Acid Synthesis
METFORMIN
sFlt-1
FLT1
HIF-2α
Ub
Ub
Ub
HIF-2α
PHD
ROS
O_2^-
O_2^-
Me
TET
JMJD
Me
NADH
NAD^+ + H^+
I
II
e^-
e^-
CoQ
III
Cyt c
IV
e^- → H_2O
ADP
H^+
ATP
ATP synthase
V
Inner mitochondrial membrane
Intermembrane space
H^+
H^+
H^+
H^+
H^+
H^+