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Metformin, the aspirin of the 21st century: its role in gestational diabetes, prevention of preeclampsia and cancer, and the promotion of longevity

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Abstract

Metformin is everywhere. Originally introduced in clinical practice as an anti-diabetic agent, its role as a therapeutic agent is expanding to include treatment of pre-diabetes, gestational diabetes, polycystic ovarian disease, and more recently, experimental studies, as well as observations in randomized clinical trials, suggest that metformin could have a place in the treatment or prevention of preeclampsia. This article provides a brief overview of the history of metformin in the treatment of diabetes, reviews the results of meta-analyses of metformin in gestational diabetes, and the treatment of obese non-diabetic pregnant women to prevent macrosomia. We highlight the results of a randomized clinical trial in which metformin administration in early pregnancy did not reduce the frequency of large-for-gestational-age infants (primary endpoint), but did decrease the frequency of preeclampsia (a secondary endpoint). The mechanisms by which metformin may prevent preeclampsia include a reduction in the production of anti-angiogenic factors (soluble vascular endothelial growth factor receptor-1 and soluble endoglin), and improving endothelial dysfunction, probably through an effect on the mitochondria. Another potential mechanism whereby metformin may play a role in the prevention of preeclampsia is its ability to modify cellular homeostasis and energy disposition, mediated by mTOR. Metformin has

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a molecular weight of 129 Dalton, and therefore, readily crosses the placenta. There is considerable evidence suggesting that this agent is safe during pregnancy. New literature on the role of metformin in the prevention of cancer, a chemotherapeutic adjuvant, and in prolonging life and protecting against aging, is briefly reviewed. Herein we discuss the mechanisms of action and potential benefits of metformin.

Suddenly, it's metformin time. Want to live longer and be healthier(1–6)? Take metformin(3, 4). Don't want to get cancer? Take metformin(7–10). Do you have polycystic ovary syndrome(11–14), congestive heart failure(15), chronic liver disease(15), chronic kidney disease(15), multiple sclerosis(16, 17), renal tubulointerstitial fibrosis(18), or nonalcoholic fatty liver disease(19)— metformin (Figure 1).

The good news for obstetricians is that preeclampsia has the potential to be added to this list (20). This could be quite important as preeclampsia affects about 5-7% of pregnancies worldwide, is a leading cause of maternal and perinatal morbidity and mortality, and imposes substantial costs on the healthcare system(20). We felt it was time to review how metformin works and what it can offer obstetricians for treating pregnancy disorders, other than diabetes and obesity, now and into the future.

Metformin: from the Pharaohs to the Present

Metformin (dimethylbiguanide) is a constituent of many herbal remedies, and the Ebers Papyrus, written in 1500 B.C.E.(21), (Figure 2) records its use in Egypt since the time of the Pharaohs. In Europe, herbal remedies derived from the plant *Galega officinalis* (Figure 3) that contained metformin have been prescribed to treat polyuria and other symptoms of diabetes since the Middle Ages(21–24), but it was not until the early 1900s that guanidine was identified as responsible for the hypoglycemic effects of *G. officinalis* extracts(25, 26).

Guanidine was too toxic for clinical use, and isoamylene guanidine (Galegine) was used as an anti-diabetic agent in the 1920s until the development of metformin and phenformin(27, 28). Phenformin was withdrawn from clinical use because it caused lactic acidosis(29), and although metformin did not have this side-effect, its use, as well as the use of other biguanide derivatives to treat diabetes, was displaced by insulin, which was purified and synthesized in 1921 and used clinically to treat diabetes in humans the following year(30, 31).

Nevertheless, research with biguanides continued, as they were effective in treating malaria, and the hypoglycemic effects of the anti-malarial agent chloroguanidine hydrochloride eventually paved the way for the development of metformin for the treatment of diabetes by Professor Jean Sterne at the Hopital Laennec in Paris, who coined the name “glucophage” (“glucose eater”) for metformin (Figure 4)(32). Two unexpected side-effects of some biguanides—lactic acidosis and increased cardiac mortality—then caused the biguanides to be withdrawn from clinical use in the United States(22, 33–38); however, metformin was relatively safe and, after 20 years of clinical use in Europe, metformin was approved by the FDA in 1995 for the treatment of diabetes in the United States. A joint consensus statement by the American Diabetes Association (ADA) and the European Association for the Study

of Diabetes (EASD) now recommends metformin as initial oral therapy for patients with type 2 diabetes(39, 40). Recently, the Professional Practice Committee of the American Diabetes Association has recommended the use of metformin for patients with prediabetes (fasting glucose 100-125 mg/dl, 2-hr post-load glucose 140-199 mg/dl, or A1C 5.7-6.4%), especially in those who are younger than 60 years old, have a BMI >35 kg/m², or have a history of gestational diabetes(41–43).

Several mechanisms of action are considered responsible for this effect, including: 1) a decreased in hepatic glucose production by suppression of gluconeogenesis(44, 45); 2) an increased insulin suppression of endogenous glucose production by the liver(44, 45); 3) reduction of glucose absorption by the gastro-intestinal tract(44, 45); By far the most important mechanism is the reduction in hepatic glucose production that is considered mediated by the activation of the global energy sensor in cells, adenosine monophosphate-activated protein kinase (AMPK) (Figure 1).(46)

Metformin was mostly used in non-pregnant diabetic patients until Coetzee and Jackson reported its use in the late 1970s with pregnant diabetics from South Africa(47–50), after which metformin became the treatment of choice for gestational diabetes(51–68) and, in the 1990s, for type 2 diabetes (69, 70) because of its ease of administration and high compliance rate.

Important results from the meta-analyses: metformin reduces the frequency of gestational hypertension in gestational diabetes

In 2013, the efficacy and safety of metformin in the management of gestational diabetes had been compared to that of insulin in five randomized clinical trials(56, 71–74). Gui et al.(75) published a systematic review and meta-analysis in which metformin was shown to be superior to insulin in reducing maternal weight gain during pregnancy as well as in the frequency of gestational hypertension (Figure 5a,); however, metformin did not change the frequency of large-for-gestational-age (LGA) or small-for-gestational-age (SGA) fetuses or of hypoglycemia and preeclampsia(75). Two subsequent meta-analyses confirmed metformin's effect on maternal weight gain during pregnancy and on gestational hypertension(76, 77); furthermore, Butalia et al.(77) reported that, compared to insulin, metformin significantly decreased the frequency of neonatal hypoglycemia, LGA neonates, and admissions to a neonatal intensive care unit (Figure 5b,)(56, 72–74, 77–83). Gui et al. (75) suggested that metformin reduced the rate of gestational hypertension because of its effects on endothelial function and its decrease in the production of reactive oxygen species, the two mechanisms implicated in the pathophysiology of preeclampsia(84–94).

Two trials from the UK report conflicting results of treating obese, non-diabetic, pregnant women with metformin

In a randomized controlled clinical trial called “Efficacy of Metformin in Pregnant Obese Women” (EMPOWaR), the effect of metformin was compared to placebo in non-diabetic, obese (defined here as a BMI>30kg/m²), pregnant women(95). Women randomized to receiving metformin began treatment between 12-16 weeks of gestation with a starting dose of 500 mg that was increased, as necessary, to a maximum tolerable dose not exceeding

2,500 mg. In this trial, metformin had no significant effect on birth weight, maternal weight gain during gestation, preeclampsia, or combined adverse pregnancy outcomes, including miscarriage, termination of pregnancy, or fetal or neonatal death(95).

The treatment of obese (defined here as a BMI>35 kg/m²), non-diabetic, pregnant women with metformin was compared to placebo in another randomized clinical trial in the UK — the Fetal Medicine Foundation trial(96). In this trial, women randomized to receiving metformin started treatment between 12-18 weeks of gestation at a dose of 1g/day that was increased by 0.5g/week to a maximum dose of 3g/day. The goal was to reduce the rate of LGA infants(96): the primary outcome was a reduction of median neonatal birthweight by 0.3 standard deviations, which would represent a 50% reduction in the incidence of LGA neonates. Although metformin did not reduce the frequency of LGA neonates, it significantly reduced the frequency of preeclampsia and maternal weight gain, although not the rate of gestational diabetes(96). The finding that metformin decreased the frequency of preeclampsia was consistent with the findings reported in a meta-analysis by Gui et al.(75) and Feng and Yang(76).

Trial design and execution may explain the contradictory results of the two UK trials?

Differences in trial design, execution and compliance are the most likely explanations for the contradictory results obtained in these two randomized clinical trials from the United Kingdom. In the Fetal Medicine Foundation trial, women had a higher BMI (>35 kg/m² vs. >30 kg/m²), were treated with a higher starting and maximum dose of metformin, and were more compliant than women in the EMPOWaR trial(95, 96). In the Fetal Medicine Foundation trial, almost 80% of women took at least 50% of the total number of tablets prescribed, and 91% of those prescribed more than 2.5 g/day did so(96). By contrast, in the EMPOWaR trial, compliance was defined as ingestion of at least one tablet for at least 29% of the days between randomization and delivery, and only 67% of women fulfilled this criterion(95). Suboptimal compliance in randomized trials is well known to cause negative results(97–101). It is also noteworthy that only 13% (443/3329) of eligible patients consented to participate in the EMPOWaR study, whereas 47% (400/844) of eligible women were recruited to the Fetal Medicine Foundation study, making the latter group more representative of women who meet the study's eligibility criteria and to whom the study's results apply.

Mechanisms by which metformin may prevent preeclampsia

The role of angiogenic and antiangiogenic factors in the genesis of preeclampsia

For more than 100 years, preeclampsia was thought to be caused by the release of “toxic factors” from an ischemic placenta, hence, the name “toxemia” (see references(102–117) for a review). The most widely known ‘toxins’ at this time are sFlt-1 (or sVEGFR-1) and soluble endoglin(118–142).

It has been hypothesized that sVEGFR-1 is produced in preeclampsia because the placenta is ischemic or hypoxic, and sVEGFR-1 antagonizes angiogenic molecules, such as VEGF and placental growth factor (PlGF)(119, 143–150). Soluble endoglin is a cell surface co-receptor

for transforming growth factor (TGF)- β 1, which blocks TGF- β 1-mediated activation of endothelial nitric oxide synthase (eNOS) and promotes vasorelaxation(124). There is excessive production of sVEGFR-1 and soluble endoglin in the uterus of preeclamptic patients(121) proportional to the severity of the disease (151–153) that causes maternal plasma concentrations of sVEGFR-1 and soluble endoglin to increase before preeclampsia is diagnosed, making them potential biomarkers for the disease(111, 120, 128, 129, 132, 133, 137, 138, 154–179). The increase of sVEGFR-1 causes a parallel decrease of maternal plasma concentrations of PIGF in preeclampsia(119, 143–150).

The administration of sFlt-1 (or sVEGFR-1) and/or soluble endoglin to animals produces changes characteristic of preeclampsia. For example, if sVEGFR-1 is administered to rats using an adenovirus vector, the animals develop hypertension, proteinuria, and glomerular endotheliosis(180). Additionally, if sVEGFR1 and soluble endoglin are given to pregnant rats, the animals not only develop preeclampsia but also liver dysfunction, thrombocytopenia, and intrauterine growth restriction(124); similar results have been obtained in mice(141). This condition is indistinguishable from the HELLP syndrome observed in humans(181–184).

Although the emphasis on the toxic factors produced by an ischemic placenta has been on the balance between angiogenic and anti-angiogenic factors, evidence now suggests that cytokines, such as TNF- α and IL-10, are altered in early and late preeclampsia, and the changes correlate with the type of histopathological changes in the placenta(185). The importance of ischemic placental disease, not limited to preeclampsia, has been the subject of several recent studies, i.e., preterm labor, preterm PROM, fetal growth restriction, fetal death, and other complications of pregnancy(186–189).

Brownfoot et al.(20) from the Translational Obstetrics Group of the Department of Obstetrics and Gynecology, Mercy Hospital for Women, at the University of Melbourne, Heidelberg, Victoria, Australia, reported that metformin reduced the production of soluble fms-like tyrosine kinase-1 (sFlt-1, also known as soluble vascular endothelial growth factor receptor 1 or sVEGFR-1) and soluble endoglin (sENG) in a dose-dependent manner by endothelial cells, villous trophoblast, and villous explants (Figure 6); the report also suggested that metformin regulates these anti-angiogenic factors at the level of the mitochondria. Metformin also decreased the expression of vascular cell adhesion molecule 1 (VCAM1), expressed by endothelial cells that are dysfunctional or have been stimulated by incubation with tumor necrosis factor alpha (TNF- α), a cytokine increased in the circulation of patients with preeclampsia(190–195).

However, the most persuasive evidence that metformin has a vascular effect is the finding that it reverses the impairment of vascular relaxation induced by incubating the maternal blood vessels obtained from the omentum at the time of cesarean delivery with placental conditioned media of patients with preeclampsia(20). Metformin also abrogated the reduction of angiogenic sprouting induced in human omental vessel explants by incubation with VEGFR-1 (Figure 7)(20). Overall, Brownfoot et al.'s findings suggest that metformin may have a role in the prevention of preeclampsia through its effect on cell metabolism, on

the anti-angiogenic state, and, most likely, on other processes associated with this obstetrical syndrome(20).

Preeclampsia as a mitochondrial disorder and the effect of metformin on mitochondrial function

Torbergsen et al.(196) first suggested that mitochondria might be involved in the pathogenesis of preeclampsia based upon the high frequency of preeclampsia in a family suffering from mitochondrial dysfunction. Six of 10 women with a mitochondrial disease had at least one pregnancy complicated by preeclampsia or eclampsia, whereas none had been hypertensive in the non-pregnant state. Torbergsen et al. suggested that these women developed preeclampsia because their dysfunctional mitochondria could not meet the increased energy demands of pregnancy, and the failure of adequate cellular energy production led to an accumulation of ADP, which is able to mediate most of the changes seen in preeclampsia(89, 197) ADP is a vasoconstrictor, causes platelet aggregation, and breaks down to uric acid.

In 1990, Shanklin and Sibai(198) reported that mitochondria from small vessels, myometrial smooth muscle, myometrial interstitial cells, circulating leukocytes, epidermal and dermal cells, and hepatocytes in women with preeclampsia all had morphologic changes that were quite different from those seen in normal pregnancies(198). The tissues of women with preeclampsia showed central disruption in mitochondrial morphology and changes in the Golgi apparatus, endoplasmic reticulum, and small, unidentified microvesicles, and these mitochondrial changes were not limited to uterine tissues, thus implying that mitochondrial dysfunction was a systemic disorder. That same year, Berkowitz et al.(199) reported a case of abnormal mitochondrial morphology observed in an endomyocardial biopsy obtained from a 20-year-old nulliparous woman, known to have mitochondrial myopathy, who developed severe preeclampsia(199).

Since that time, the following findings have further implicated mitochondria in the pathogenesis of preeclampsia:

1. Comparative proteomics analysis of placental mitochondria in normal patients and those with preeclampsia has shown up-regulation of four proteins and down-regulation of 22 proteins. Using bioinformatic tools, differentially expressed proteins in this study were identified as participating in many critical processes of preeclampsia, such as reactive oxygen species generation, apoptosis, fatty acid oxidation, respiratory chain function, and the tricarboxylic acid cycle(200);
2. the median maternal whole blood mitochondrial DNA copy number was higher in women with preeclampsia than in those who experienced a normal pregnancy ($p<0.001$)(201), suggesting that an influx of mitochondrial DNA into the maternal circulation may act as a danger signal/alarmin responsible for the sterile (absence of infection) intravascular inflammatory processes of this condition(202, 203);
3. the placentas of patients with preeclampsia(204), as well as those from pregnancies with preeclampsia and SGA(205), overexpress the microRNA

(miR)-210, which has, as potential targets, regulation of transcription of the innate immune response. Additionally, it was demonstrated that miR-210 was induced by hypoxia, and RNA interference knockdown resulted in autophagosomal and siderosomal iron accumulation, implicating siderosis or interstitial trophoblasts as mechanisms in preeclampsia and SGA(206); and

4. aside from its effects on sterile inflammation and iron metabolism, miR-210 also modulates mitochondrial function. The laboratory of Professor Leslie Myatt provided evidence that placental mitochondrial dysfunction is mediated, at least in part, by miR-210 by demonstrating that mitochondrial complexes I, III, and IV decreased in preeclampsia, along with a decrease in the iron-sulfur cluster scaffold homologue (ISCU)(207). Importantly, transfection of cells with miR-210 resulted in a significant reduction in oxygen consumption by mitochondria, mitochondrial respiratory deficiency, and production of ROS(207).

Recently, Professor Gennady Sukhikh's Research Institute in Moscow has reported the following findings, indicating that mitochondrial functional changes in the placenta occur in both early- and late-onset preeclampsia when compared to normal pregnancy(208):

Women with early-onset preeclampsia had 1) a two-fold increase in the mRNA expression of *OPA* (optic atrophy)-1 and a three-fold increase in OPA-1 protein expression (cleaved and uncleaved forms) in patients with early-onset preeclampsia. This gene is involved in mitochondrial fusion and in the cristae structure of the inner mitochondrial membrane, a fine-tuned process crucial for mitochondrial quality control; 2) a five-fold decrease in the mitochondrial transcription factor (TFAM)-A; 3) a 1.5-fold increase of the relative placental mitochondrial DNA copy number; and 4) increased mitochondrial respiration in the presence of Complex I substrates(208).

An increase in the P/O ratio (a measure for how much ATP is synthesized per 2 electrons transferred to oxygen) was observed in both early and late preeclampsia. Thus, early-onset preeclampsia is associated with mitochondrial activation, up-regulation of OPA-1, active DNA replication (resulting from a high respiration rate), and TFAM down-regulation, while both early and late preeclampsia are associated with an elevated P/O ratio(208).

Collectively, this evidence, coupled with the conduction of workshop recently held by the International Federation of Placental Associations on the role of mitochondria in placental function(209), suggests that mitochondrial dysfunction plays a major role in the pathogenesis of preeclampsia.

Brownfoot et al.(20) found that metformin can improve endothelial dysfunction, reduce the expression of vascular adhesion molecule 1 mRNA induced by TNF α , and improve whole-blood vessel angiogenesis impaired by sFlt-1 or sVEGFR-1. Given the evidence that metformin acts through the mitochondrial electron transport chain (ETC) by inhibiting complex I(46, 210–212) (Figure 8), Brownfoot et al.(20) investigated whether the effects on sFlt-1 and soluble endoglin production are regulated through the mitochondrial electron transport chain.

The reduction in the secretion of anti-angiogenic factors by metformin was reversed when succinate (whose metabolism via complex II bypasses complex I) was used as an electron source(20). However, succinate is also an inhibitor of HIF-1 α (213), which has been implicated in the exaggerated production of sFlt-1 and in the pathophysiology of preeclampsia(214, 215); therefore, succinate could also be acting by inhibiting HIF-1 α , rather than the mitochondrial electron transport chain. However, Brownfoot et al.(20) demonstrated that other electron transport chain inhibitors (antimycin and rotenone) blocked secretion of sFlt-1 and soluble endoglin, suggesting that the electron transport chain is the major effector of the benefits of metformin. Indeed, succinate in the absence of metformin appears to have no effect(20). The role that ROS and the electron transport chain play in the genesis of preeclampsia could be parsed both pharmacologically and genetically(216–219). The changes in the mitochondrial function in preeclampsia suggest there are perturbations in the cellular energy balance of patients with this syndrome that affect cell growth and division (especially in the placenta and the fetus); all are modified by metformin.

The biological basis for the effects of metformin on fetal growth

Nutrient sensing – key for the survival of all living forms

“Cell growth and division are the two most fundamental features of life”(220). All organisms must be able to detect nutrient levels in their environment to coordinate growth and development. This is true of bacteria that must choose whether to grow, remain stationary, and, in the case of motile bacteria, determine in which direction to move depending upon the availability of nutrients. Bacteria evolved specific chemoreceptors for this purpose, which coordinate information received from the environment with specialized structures, such as flagella, to move toward nutrients. The evolutionary history of nutrient-sensing pathways from bacteria to humans is displayed in Figure 9(221).

The evolution from prokaryotes (a two-compartment system) to eukaryotes (Figure 10) created unique opportunities for storage: eukaryotes have a third compartment that allows intracellular storage – here, nutrient sensing can also occur (see Figure 10)(221). Nutrient sensing became more complex in metazoans, as they were required to maintain homeostasis of different tissues and organs. Indeed, over the course of millions of years, specific pathways have evolved for glucose, amino-acids, and energy, and metazoans eventually evolved the endocrine and paracrine systems to meet their requirements for nutrient sensing. The coordinated actions of hormones such as insulin, leptin, and ghrelin regulate the organism’s response to the presence or absence of nutrients, modulate anabolic and catabolic processes, and control feeding behavior by signaling the brain(222–224).

Fetal-placental nutrient sensing

Nutrient sensing is a crucial requirement for fetal development. The key structure in the placenta responsible for nutrient sensing is the syncytiotrophoblast, which covers the villous tree and is in direct contact with the maternal blood in the intervillous space. In this strategic location, the nutrient-sensing mechanisms of the syncytiotrophoblast can detect changes in maternal blood composition, enabling the placenta to constantly monitor it, not only regarding the nutritional status of maternal circulation but also for any threat it contains

to the fetus (i.e. microorganisms). Thus, it is easy to envision that the syncytiotrophoblast and other components of the placenta contain the nutrient-sensing systems that allow the fetus to regulate its own growth by extracting nutrition from the maternal blood.

Disorders in nutrient-sensing pathways may lead to fetal growth disorders, and studies by Professors Powell and Jansson have provided unique insights into nutrient sensing by the human placenta(225–227). For example, down-regulation of mechanistic target of rapamycin (mTOR) in the placenta has been reported in SGA/growth-restricted fetuses (see Figure 11)(226). Whether this represents a primary defect in the sensing mechanisms or an adaptive response by the feto-placental unit remains to be determined. Similarly, when the placenta senses an excess of nutrients (as in obesity, diabetes, or other metabolic disorders) mTOR activation and fetal growth acceleration may be expected to occur.

Nutrient sensing occurs through several pathways(225), the most important of which are:

1. the AMPK pathway, which is a global energy sensor in cells;
2. glycogen synthase 3 (GSK-3), which acts as a glucose sensor;
3. the hexose-amino signaling pathway, which depends on the availability of nutrients such as glucose, glutamine, and acetyl-CoA;
4. the amino-acid response signal transduction pathway, which is activated under conditions essential amino-acids deficiency or imbalance; and
5. the mechanistic target of rapamycin complex 1 (mTORC1) pathway, which integrates nutrient and growth factor signaling.

Each of these nutritient-sensing pathways is present in the human placenta(225–227). There are also changes in these pathways in cases of fetal growth restriction caused by maternal starvation or impaired placentation where there is down-regulation of placental nutrient transport(225, 226).

The mechanistic target of rapamycin (mTOR) translates maternal nutritional status via the placenta to the fetus

Rapamycin is an anti-fungal agent produced by *Streptomyces higroscopicus*(228) that inhibits the growth of *Candida albicans* and that was first discovered in soil on Easter Island (Rapa Nui). The story of its discovery is similar to that of penicillin: its isolation from *Aspergillus penicillinum* and its capacity to inhibit bacterial growth. On the other hand, rapamycin's popularity is due to its powerful immunosuppressive properties, which made it a drug of choice for patients with renal transplants(229), and not its antimicrobial properties.

Rapamycin can also prolong the life cycle of many species as diverse as worms and mice, and its signaling system has been implicated as the master regulator of cellular growth and metabolism. A family of molecules that are the mechanistic target of the rapamycin (mTOR) complex has been attributed a major role in the biology of cell metabolism, growth, longevity, and even preterm birth(230–236).

Rapamycin acts by interacting with a family of TOR molecules that is highly-conserved functionally and evolutionarily from yeast to humans, and it contains two multiprotein complexes that are functionally and structurally different: the first, mTORC1, is rapamycin-sensitive; and the second, mTORC2, is rapamycin-resistant(237). Both complexes control cell growth and metabolism in response to the availability of nutrients, energy, and growth factors. Upon activation, the serine/threonine kinases activate a cascade of intracellular processes involving the mitochondria and the nucleus of the cell to promote cellular growth and, to a certain extent, aging. Rapamycin prolongs lifespan by inhibiting the effects of mTOR. (See references(238, 239) for an in-depth discussion of this topic.)

mTOR is a major human nutrient-sensing receptor in the placenta(225). This signaling system is highly expressed in the syncytiotrophoblast, and its activity is regulated by glucose and amino-acid concentrations(226, 227, 240).

mTOR has key properties in the placenta(225, 226) that include its activation by insulin, IGF-1, and leptin, and its inhibition by cortisol. mTOR regulates two key amino-acid transport systems (A and L). The activation of mTOR is positively correlated with the first-trimester maternal body mass index, linking maternal over-nutrition and nutrient-sensing by the placenta; while its expression is down-regulated when fetal growth is restricted (in both animal models and humans). The mTOR Akt-mTOR-HIF-1 α signaling pathway also affects placental angiogenesis by its ability to increase VEGF and endoglin expression in response to hypoxia in a trophoblast cell line(241).

Safety of metformin during pregnancy

Metformin has a molecular weight of 129 dalton, and it crosses the placenta in direct diffusion without affecting the facilitated transfer of glucose(242). Indeed, an *ex vivo* dually perfused human placental lobule demonstrated the rapid transfer of metformin from maternal to fetal circulation with a lag time of 1.7 ± 0.28 minutes similarly observed in women with normal pregnancies and those diagnosed with gestational diabetes mellitus(242). *In vivo* studies reported the detection of metformin in the umbilical cord blood of neonates from women with polycystic ovarian syndrome who were treated with metformin throughout gestation(243, 244). Similar concentrations of metformin in the umbilical artery and umbilical vein suggest negligible metformin metabolism by the fetus (244). Several meta-analyses that studied the teratogenic effect of metformin on embryonic development found that this drug carries no increased risk for congenital malformations(245, 246) and is currently classified as category B in the United States and as category C in Australia (247, 248). Similarly, no excess of fetal or neonatal complications could be demonstrated when the administration of metformin was compared to glyburide and insulin(249). In addition, a study that investigated the neurodevelopmental effect at two years of age could not identify a significant difference between children exposed *in utero* to metformin and those exposed to insulin(250). Moreover, there were no differences in fat measurements, total fat mass, and percentage of body fat(251). Those who were exposed to metformin during fetal life had a larger upper arm circumference and bigger subscapular skin folds and biceps, suggesting a better fat distribution than children exposed to insulin(251).

Maternal side-effects reported with the use of metformin are mainly gastro-intestinal, i.e., nausea and diarrhea(248). The rate of hypoglycemia is lower than that reported with insulin(79). In addition, rare side-effects such as mild erythema and decreased vitamin B₁₂ absorption have been associated with long-term administration(252).

A role for metformin in cancer and aging

Originally introduced for the treatment of diabetes, metformin is now gaining attention as a potential anti-cancer agent (7–10) (Figure 1). The first observation that metformin might reduce the risk of cancer was made in a population-based case-control study of type 2 diabetic patients treated with metformin(253). A cohort study of type 2 diabetic patients newly treated with metformin later followed in which the frequency of cancer was significantly lower in patients receiving metformin than in the controls who had never received metformin, after adjusting for body mass index, hemoglobin A1C, smoking and the use of other drugs (254): this finding has subsequently been confirmed in several other studies(7–10). In a meta-analysis(255), metformin-treated diabetic patients had a 31% reduction in the incidence of cancer and a 34% reduction in cancer mortality after adjusting for body mass index(255). This epidemiologic evidence has coalesced with experimental work in animals(256–263). Animal experiments have elucidated the mechanisms underlying these epidemiological findings, i.e., suppression of cancer stem cells(264); inhibition of epithelial-to-mesenchymal transition(265), (which is implicated in metastasis); and interference with glucose(266), protein(267, 268), and lipid(269) metabolism of the neoplastic cells. There is further evidence that metformin may also have an adjunctive effect in patients receiving chemotherapy(270), and there are now more than 100 ongoing trials registered in clinicaltrials.gov studying the role of metformin in cancer treatment (see references(45, 271, 272) for a review of this topic and the mechanisms of action underlying these effects of metformin).

Finally, an unbiased search for genes that extend lifespan has identified a disproportionate number of genes that function in mitochondrial metabolism(273), similar observations were also found by targeting mitochondrial genes(274). Not surprisingly, drugs that inhibit mitochondrial function were examined in this context, and metformin was shown to extend longevity in worms(2) and mice(3) (although not in *Drosophila*(275)). Metformin has now been found to target several age-related pathways(3, 276, 277), but the mechanisms by which metformin extends lifespan are far from clear. A randomized clinical trial, TAME (“Targeting aging with metformin”)(6), has been planned to test the effect of metformin on the time to the new occurrence of a composite outcome that includes cardiovascular events, cancer, dementia, and mortality as an endpoint in 3,000 subjects who are 65–79 years of age(6).

Metformin exerts many of its growth inhibitory and anti-neoplastic effects through the nuclear pore complex and acyl-CoA dehydrogenase family member-10 (ACAD10)(278). Nucleocytoplasmic shuttling regulates mTORC activity, an effect that can be reversed by RNA silencing. Biguanides inhibit growth by suppressing mitochondrial respiration, which limits the transit of the complex RagA-RagC GTPase heterodimer through the nuclear pore complex(278). By preventing access to the nucleus, RagC becomes incapable of stimulating

mTORC1 and, therefore, stimulates cellular growth (Figure 12)(278). Wu et al. proposed that the nuclear pore complex and ACAD10 are involved in insulin action and regulation of blood glucose concentration(278). Whether these mechanisms are also responsible for the anti-diabetic actions of metformin and its ability to improve insulin sensitivity is unknown at this time.

Conclusion

Metformin, long known to be an herbal medicine, has evolved from its use as a popular treatment for diabetes into a drug with a significantly wider array of beneficial effects ranging from cancer treatment to extending longevity, as well as, in our field, gestational hypertension and preeclampsia in obese women. Current evidence suggests that metformin's wide-ranging beneficial effects are mediated by at least two primary mechanisms: suppression of intracellular metabolic activity of mitochondria and the cellular nutrient-sensing system mediated by mTOR.

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Figure 1. Different effects of metformin and its signaling pathways

Metformin reduces insulin resistance, secretion, glucose blood levels, inflammation, and angiogenesis as well as reduction in cell growth and metabolism that mediates its anti-tumor activity. These effects are regulated by both AMPK-dependent or -independent mechanisms that lead to the inhibition of mTOR signaling.

(Abbreviations: ACC, acetyl-CoA carboxylase; AMPK, 5' adenosine monophosphate-activated protein kinase; IGF, Insulin-like growth factor; EGF, Epidermal growth factor; FAS, fatty acid synthase; PAI-1, plasminogen-activator inhibitor-1; PI3K, Phosphatidylinositol-4,5-bisphosphate 3-kinase; TSC2, tuberous sclerosis 2; mTOR, mechanistic target of rapamycin; VEGF, vascular endothelial growth factor)

Reproduced with permission from Viollet B, Guigas B, Sanz Garcia N, Leclerc J, Foretz M, Andreelli F. Cellular and molecular mechanisms of metformin: an overview. Clin Sci (Lond). 2012; 122:253-70 and <http://diabetesmanager.pbworks.com/f/1255202482/metformin.JPG>

Figure 2. Ebers papyrus

This document, 20 meters long, contains a collection of medical texts considered to be the most comprehensive account of the practice in Egyptian medicine. Its encyclopedic content addresses not only multiple illnesses, e.g., treatment for diabetes, but also crocodile bites, mental illness, and treatment for death (half an onion and froth of a beer...). The papyrus was purchased by the Chief of Egyptology (Georg Ebers) at the University of Leipzig in Germany where it currently resides. The story goes that the papyrus was discovered between the legs of a mummy.

Adapted from <http://spheresoflight.com.au/axismundi/content/images/ebers-papyrus-colonnes1-2.jpg>.

Figure 3. *Galega officinalis*

This plant, also known as goat's rue, French lilac, or Italian fitch, was used for many years to treat the symptoms of diabetes. In 1920, the anti-diabetic class of drugs called biguanides, originating from this plant, was introduced for the treatment of diabetes.

Adapted from <http://www.naturalmedicinefacts.info/plant/galega-officinalis.html>.

Figure 4. Professor Jean Sterne at the Hospital Laennec, Paris, France

Introduction of metformin (“glucophage”) into clinical medicine.

Reproduced with permission from Bailey CJ, Day C. Metformin: its botanical background.

Practical Diabetes International. 2004;21(3):115-117.

Figure 5. Results of the meta-analyses comparing the efficacy of treatment with metformin vs. insulin in women with gestational diabetes

- a.** The panels present the beneficial effects of metformin vs. insulin in women with gestational diabetes that indicate a reduction in: 1) maternal weight gain during pregnancy; 2) gestational hypertension. Reproduced with permission from Gui J, Liu Q, Feng L. Metformin vs insulin in the management of gestational diabetes: a meta-analysis. PLoS One. 2013 May 27; 8:e64585.
- b.** The panels present the beneficial effects of metformin vs. insulin in women with gestational diabetes that indicate a reduction in: 1) neonatal hypoglycemia; 2) large-for-gestational-age neonates; and 3) admissions to the neonatal intensive care unit. Reproduced with permission from Butalia S, Gutierrez L, Lodha A, Aitken E, Zakariasen A, Donovan L. Short- and long-term outcomes of metformin compared with insulin alone in pregnancy: a systematic review and meta-analysis. Diabet Med. 2017; 34:27-36. (Abbreviations: CI, confidence interval; IV, inverse variance; M-H, Mantel-Haenszel).

Figure 6. Effect of metformin on soluble fms-like tyrosine kinase-1 (sFlt1)/soluble vascular endothelial growth factor receptor-1 (sVEGFR-1) secretion, soluble endoglin, and isoforms *e15a* and *i13* expression in endothelial cells and placental tissue

Metformin reduced in a dose-dependent manner sFlt1 from (A) endothelial cells, (B) villous cytotrophoblast cells, and (C) preterm preeclamptic placental villous explants. Metformin also reduced endothelial cell expression of (D) the sFlt-1 *i13* isoform, (E) villous cytotrophoblast cells, and (F) preterm preeclamptic placental villous explant messenger RNA expression of sFlt-1 *e15a*. Metformin reduced soluble endoglin secretion from (G) endothelial cells and (H) villous cytotrophoblast cells, but it did not change soluble endoglin secretion from (I) preterm preeclamptic placental villous explants. (The single asterisk indicates $P < 0.05$; the double asterisk indicates $P < 0.01$; the triple asterisk indicates $P < 0.0001$; and the quadruple asterisk indicates $P < 0.00001$).

(Abbreviations: mM, millimolar; sENG, soluble endoglin; sFlt-1, Soluble fms-like tyrosine kinase-1).

Modified with permission from Brownfoot FC, Hastie R, Hannan NJ, Cannon P, Tuohey L, Parry LJ, 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; 214:356.e1-356.e15.

Figure 7. Effect of soluble fms-like tyrosine kinase-1 (sFlt1)/soluble vascular endothelial growth factor receptor-1 (sVEGFR-1) and metformin on angiogenesis

Omental vessel rings cultured with sFlt1/sVEGFR-1 reduced the vessel outgrowth (white arrow, middle panel). This effect was resolved when metformin (1 mmol/L) was added to the culture media (right panel). (Abbreviations: sFlt-1, Soluble fms-like tyrosine kinase-1)

Reproduced with permission from Brownfoot FC, Hastie R, Hannan NJ, Cannon P, Tuohey L, Parry LJ, 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; 214:356.e1-356.e15.

Figure 8. Effect of metformin on the mitochondrial respiratory transport chain complex 1
Metformin crosses the plasma membrane of the cell by passive diffusion, and the mitochondria is its main intracellular target. Metformin inhibits mitochondrial respiratory transport chain complex 1 and induces a decrease in the reduced form of nicotinamide adenosine dinucleotide (NADH) oxidation, proton pumping across the inner mitochondrial membrane, and the oxygen consumption rate, leading to a reduction of adenosine triphosphate (ATP) synthesis. (Abbreviations: ADP, Adenosine pyrophosphate; ATP, Adenosine triphosphate; Cyt c, cytochrome complex; FAD, flavin adenine dinucleotide; H^+ , hydrogen ion; H_2O , water; NAD, Nicotinamide Adenine Dinucleotide; OCT, organic cation transporter)

Reproduced with permission from Viollet B, Guigas B, Sanz Garcia N, Leclerc J, Foretz M, Andreelli F. Cellular and molecular mechanisms of metformin: an overview. *Clin Sci (Lond)*. 2012; 122:253-70.

Figure 9. Nutrient-sensing pathways in the evolution of species from unicellular to multicellular organisms

Unicellular organisms have two distinct nutrient pathways (PII and chemoreceptors), while fungi evolved three distinct nutrient-sensing pathways (SPS, Snf3/Rgt2, and MEP2). are denoted, followed by the sensing pathways that are conserved from yeast to man. Blue bars indicate the presence of the nutrient-sensing pathways used by different organisms.

(Abbreviations: AMPK, 5' adenosine monophosphate-activated protein kinase; GCN2, general control nonderepressible 2; MEP, 2-C-methyl-D-erythritol 4-phosphate; SPS, Ssy1-Ptr3-Ssy5; TOR, target of rapamycin)

Reproduced with permission from Chantranupong L, Wolfson RL, Sabatini DM. Nutrient-sensing mechanisms across evolution. *Cell*. 2015; 161:67-83.

Figure 10. Mechanisms of nutrient-sensing within unicellular and multicellular organisms

Panel A: Prokaryotes can sense amino acids through a variety of sensors present in the cytosol and extracellular compartments. Panel B: Similarly, yeast cells sense extracellular amino acids via plasma membrane transporters and cytosolic sensors. Eukaryote cells have another potential compartment, such as a vacuole, where sensing may occur. Panel C: In mammalian cells, sensing may occur via cell membrane transporters in the cytosol and within the lysosome. (Abbreviations: mTORC1, mechanistic target of rapamycin complex 1;)

Reproduced with permission from Chantranupong L, Wolfson RL, Sabatini DM. Nutrient-sensing mechanisms across evolution. *Cell*. 2015; 161:67-83.

Figure 11. Effect of maternal nutrition on the placental nutritional sensing system and fetal growth

The placenta plays a critical role in modulating maternal-fetal resource allocation, thereby affecting fetal growth and the long-term health of the offspring. Maternal under-nutrition decreases circulating levels of IGF-I, leptin, and insulin and increases maternal serum adiponectin concentrations, leading to low fetal nutrient availability. Maternal over-nutrition is associated with increased insulin, Insulin-like growth factor-1 (IGF-1), and leptin concentrations in the maternal circulation and decreased levels of circulating levels of adiponectin, leading to fetal overgrowth. The placenta integrates maternal and fetal nutritional signals with information from intrinsic nutrient sensors such as mammalian target of rapamycin (mTOR) signaling.

(Abbreviations: IGF-1, Insulin-like growth factor-1; mTOR, mechanistic target of rapamycin) Modified with permission from Jansson T, Powell TL. Role of placental nutrient sensing in developmental programming. *Clin Obstet Gynecol.* 2013; 56:591-601; and Jansson T, Aye IL, Gonderhan DC. The emerging role of mTORC1 signaling in placental nutrient-sensing. *Placenta.* 2012; 33 Suppl 2: e23-e29 and <https://clipartfest.com/download/2ccb316331956e87398d72be2d6d14e49c1a9be8.html>

Figure 12. Metformin suppresses cell growth and promotes longevity

Metformin slows *Caenorhabditis elegans* (roundworm) growth by inhibiting the mitochondrial electron transport chain, which limits the transit of the RagC protein through the nuclear pore complex resulting in a reduced activity of the mechanistic target of rapamycin complex 1 (mTORC1). The metformin-induced inhibition of mTORC1 leads to the upregulation of the transcription factor Skn-1/Nrf-2 (a regulator of antioxidant genes) and the expression of acyl-CoA dehydrogenase family member-10 (ACAD10) gene. (Abbreviations: ACAD10, acyl-CoA dehydrogenase family member-10; mTORC1, mechanistic target of rapamycin complex 1; NPC, nuclear pore complex; RagC, Ras-related GTP binding C; RNAi, RNA interference; Skn-1/Nrf-2, protein skinhead-1/nuclear-factor-erythroid-related factor-2).

Reproduced with permission from Wu L, Zhou B, Oshiro-Rapley N, Li M, Paulo JA, Webster CM, Mou F, Kacergis MC, Talkowski ME, Carr CE, Gygi SP, Zheng B, Soukas AA. An ancient, unified mechanism for metformin growth inhibition in *C. elegans* and cancer. *Cell*. 2016; 167:1705-1718.e13.

Table 1

Association between prepregnancy body mass index and the risk of mild and severe preeclampsia and gestational hypertension according to ethnicity in a multinomial regression model (n= 35,422 for preeclampsia Caucasian women and n=36,936 for gestational hypertension).

Ethnicity	Body mass index (kg/me)	Preeclampsia		Gestational hypertension	
		Mild	Severe	Mild	Severe
Caucasian	17	0.7 (0.6 - 0.8)	0.8 (0.5 - 1.1)	0.7 (0.6 - 0.8)	0.4 (0.2 - 0.7)
	20	Reference	Reference	Reference	Reference
	25	1.8 (1.5-2.1)	1.7 (1.1-2.5)	1.8 (1.6-2.0)	3.6 (2.0-6.5)
	30	3.0 (2.3-3.8)	3.4 (2.1-5.6)	3.0 (2.6-3.5)	8.8 (4.4-17.6)
	35	4.9 (3.5-)	7.6 (4.2-13.7)	4.9 (4.0-6.1)	17.4 (8.5-35.9)
African American	17	1.0 (0.7-1.3)	1.4 (0.8-2.3)	1.0 (0.8-1.2)	1.4 (0.6-3.6)
	20	1.4 (1.1-1.6)	1.6 (1.1-2.3)	1.2 (1.0-1.3)	1.9 (1.0- 3.7)
	25	2.2 (1.8-2.7)	2.1 (1.4-3.2)	1.5 (1.4-1.7)	3.0 (1.6- 5.8)
	30	3.1 (2.5-3.9)	3.2 (2.1-5.0)	2.2 (1.9-2.5)	4.9 (2.5-9.6)
	35	4.2 (3.1-5.7)	5.3 (2.9-9.7)	3.3 (2.7 -4.0)	8.0 (3.7-17.5)

Values displayed are adjusted Odds ratio and (95% Confidence interval). Odds ratio was adjusted for maternal age, smoking status, marital status, socio-economic status, parity and maternal height.

Table modified from Bodnar LM, Catov JM, Klebanoff MA, Ness RB, Roberts JM. Epidemiology 2007;18(2):234-239.

Table 2

Summary of Metabolic changes in Pregnancy and Preeclampsia

	Normal Pregnancy		Preeclampsia	
	Early	Late	Early	Late
Maternal metabolism	Anabolic	Catabolic	Anabolic	Catabolic
Glucose intolerance	~	↓	~↓	↓↓
Insulin sensitivity	~	↓	~↓	↓↓
Free fatty acids	↑	↑↑	↑↑	↑↑↑
Triglycerides	↑	↑↑	↑↑↑	↑↑↑
Cholesterol	~	↑	~↑	↑

~ Similar compared to non-pregnant controls.

↑, ↑↑, ↑↑↑: elevated (relative degree increasing by number of arrows) compared to non-pregnancy controls.

↓, ↓↓: lower compared to non-pregnant controls.

Adapted from von Versen-Hoeynck FM and Powers RW. *Frontiers in Bioscience* 2007;12:2457-2470 and Jeyabalan, A., C. A. Hubel, et al. (2014). Metabolic syndrome and preeclampsia. Chesley's hypertensive disorders in pregnancy. R. N. Taylor, J. M. Roberts, F. G. Cunningham and M. D. Lindheimer, Elsevier: 133-160.