Metformin in obstetrics and gynecology - Evaluation of its activity and possible risks

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ABSTRACT — This review aims at presenting an overview on metformin effects in diabetic and pre-diabetic states as well as in Polycystic Ovary Syndrome. The molecular mechanisms in which metformin is involved are discussed in depth. Particular attention is paid to the metabolic alterations and infertility which are important aspects characterizing PCOS. The part devoted to the molecular mechanisms underlying the metformin activity is a necessary introduction to the following sections and helps explaining its therapeutic activity in disorders where the common denominator is insulin resistance. The review also compares the effects of metformin with those of other therapeutic molecules. An important part is also dedicated to the side effects and limitations in the use of this drug, with a particular attention to the long-term effects. Metformin exerts multiple activities at the molecular level, still partly to be clarified, and induces a number of side effects. For these reasons, it should be administered with caution and always under careful control to patients suffering from diabetic and pre-diabetic states, and from PCOS. Furthermore, wherever possible it would be necessary to prefer the use of safer molecules.

KEYWORDS
Clomiphene citrate, Combined oral contraceptives, Hyperandrogenism, Hyperglycemia, Hyperglucagonemia, Hyperinsulinemia, Infertility, Insulin-resistance, Metformin, Myo-inositol, Polycystic Ovary Syndrome, Pre-diabetic status, Pregnancy rate, Type 2 Diabetes Mellitus.

INTRODUCTION

The intracellular insulin pathway has long been the subject of numerous studies that have helped clarify most of cellular components. These acquired bases allowed to investigate the molecular mechanisms underlying insulin-resistance.

Over the world, people who develop insulin-resistance are continuously rising. In many cases, the insulin-resistance could result in an intermediate condition between the healthy and pathological status of type II diabetes mellitus (T2DM). It is the "pre-diabetic" status, characterized by increase of glucose and insulin levels in bloodstream. There are different pathologies that can generate an insulin-resistance status.

Altered insulin sensitivity observed in T2DM and pre-diabetic syndromes, such as obesity and polycystic ovary syndrome (PCOS), may result from reduced levels of receptor expression or low affinity for insulin[1]. Many alterations of metabolic enzymes and downstream factors in insulin pathway have been highlighted in the muscular and adipose tissues of subjects with T2DM and pre-diabetic states, showing insulin-resistance.

Metformin is a biguanide derived from *Galega officinalis* and acts as insulin sensitizing agent when administered orally. Of note, several data displayed that the intestine is the main site for the response to metformin, so the parameters obtained from plasma are not fit for clinical response evaluation. In fact, metformin given intravenously to healthy individuals does not possess any acute direct hypoglycemic effect, displaying that it needs the intestinal passage[2].

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Metformin administration by oral route is one of the most successful treatments for T2DM. The contrast of insulin resistance progression and/or pancreatic β-cell dysfunction are the key for preventing or delaying pre-diabetic syndromes conversion in T2DM[3]. Although metformin is the oldest drug used for these pathologies, only recently some studies have begun to delineate its mechanism of action[4].

METFORMIN: MECHANISM OF ACTION

Many clinical trials conducted in the early 1990s and the knowledge improvement about action and pharmacokinetics have led metformin to be the first-choice drug in T2DM therapy.

Its pharmacokinetics is mainly determined by the intracellular transport of the active form through some organic cation transporters (OCT). So, cellular internalization is through OCT1, whose polymorphisms can affect its efficiency at hepatocyte level[5].

The main mechanism of action involves the mitochondrial respiration reduction, resulting in adenosine triphosphate (ATP) synthesis decrease. The inhibition occurs at level I of electron transport chain (ETC)[6]. At mitochondrial level, it has been demonstrated that the effect is mediated by the binding between metformin and metal ions[7]. However, the best evidence is based on the direct interaction between metformin and proteins[8]. Recently, the correlation between mitochondrial effect and gluconeogenesis inhibition has been highlighted. Hyperglucagonemia or an altered insulin/glucagon ratio can play a pivotal role in controlling hyperglycemia, as shown in people with T2DM and in diabetic animal models. In fact, it is believed that chronic hyperglucagonemia is partially responsible for the production of hepatic glucose and hyperglycemia in T2DM[9]. Moreover, the decrease in intracellular ATP leads to a reduction in the use of pyruvate in the tricarboxylic acid cycle. Such change causes a shifting from aerobic metabolism to anaerobic metabolism, with increasing glycolysis and lactate accumulation. The molecular basis of metformin action in skeletal muscle involves the subcellular redistribution of GLUT1 proteins from an intracellular compartment to the plasma membrane. Such a recruitment process may form an integral part of the mechanism by which the drug stimulates glucose uptake (and utilization) in skeletal muscle and facilitates lowering of blood glucose in the management of T2DM[10]. In addition, metformin reduces hepatic gluconeogenesis by the inhibition of mitochondrial glycerophosphate dehydrogenase (mGPD)[4].

On the other hand, metformin effect on glucagon-like peptide 1 (GLP-1) secretion and upregulation of its receptor expression goes to support β-cells function, increasing insulin release and hindering glucagon secretion[11,12]. Glucagon inhibition occurs through cyclic adenosine monophosphate (cAMP) signal reduction[13]. Accumulation of cAMP and consequently protein kinase A (PKA) activation are indirectly inhibited by energy imbalance, which increases intracellular AMP concentrations. In fact, AMP binds an inhibitory site on adenylate cyclase, called "site P"[14].

Metformin also plays an inhibitory effect on mitochondrial reactive oxygen species (ROS) production by selectively inverse electronic flow blocking through the complex I of ETC[15]. The change in the cellular energy balance, caused by ETC complex I inhibition through metformin, involves the activation of adenosine monophosphate kinase (AMPK). AMPK is a heterotrimeric protein consisting of a catalytic subunit and two regulatory subunits. Activation occurs after AMP binds to γ subunit regulatory domains, causing conformational changes that inhibit the Thr172 dephosphorylation. Thr172 phosphorylation is required for enzymatic activation and is AMP-mediated through a serine-threonine kinase, called Liver Kinase B1 (LKB1)[16].

LKB1/AMPK pathway controls the key gluconeogenic genes expression, inhibiting CREB regulated transcription coactivator 2 (CRTC2) phosphorylation[17]. In the fasting status, CRTC2 is dephosphorylated and localized in the nucleus, increasing the gluconeogenic genes transcription, such as phosphoenolpyruvate carboxykinase (PEPCK) and glucose 6-phosphatase (G6Pase). So, metformin inhibits gluconeogenesis independently of the insulin/protein kinase B (PKB, also known as Akt signal), using another pathway. However, since with insulin-resistance CRTC2 is O-glycosylated, the metformin-mediated phosphorylation is inhibited[18]. Recently, a possible alternative mechanism has been proposed for gluconeogenesis inhibition with metformin by target of rapamycin C2 (TORC2). Using such pathway, metformin increases Sirtuin 1 (SIRT1) hepatic activity, protein deacetylase NAD^+-dependent, mediated through nicotinamide phosphoribosyltransferase (NAMPT) induction, a restrictive enzyme for NAD^+ biosynthesis, by AMPK activation[19].

A recent genome study has found a locus associated with metformin treatment containing Ataxia-Telangiectasia Mutated (ATM) gene involved in DNA repair and cell cycle control. ATM inhibition significantly decreases AMPK activation by metformin, indicating just how ATM acts AMP upstream and is necessary to mediate the metformin effect[20].

The strong mitochondrial alteration leads to greater vulnerability to metformin treatment in cells closely related to ATP production, like muscle or tumor cells[21].

Although the liver represents the primary target, metformin may act on other tissues like skeletal muscle and endothelium[22,23].

PCOS AND ALTERED METABOLISM

One of most common endocrine disorders is PCOS, a complex disorder affecting up to 10–15% of women of reproductive age in western countries where it represents one of the leading causes of infertility. Polycystic ovarian morphology, hyperandrogenism and ovulatory dysfunction are considered its main clinical features. Hyperinsulinemia, obesity and insulin-resistance are evident especially in women with hyperandrogenism[24]. Despite a huge number of studies and researches, the etiopathogenesis of this syndrome remains partially unknown, though everyone agrees on its multifactorial nature, with genetic and environmental components.

PCOS patients display an increased risk for T2DM, infertility and mood disorders, and probably also for cardiovascular events, ovarian and endometrial cancer[25].

The frequent insulin-resistance incidence in PCOS women is the reason why it represents a risk factor for impaired glucose tolerance (IGT) and T2DM[26]. Until now, only some follow-up studies have been conducted to evaluate IGT conversion rates to T2DM in patients with PCOS[27,28]. These rates are probably underestimated with the population rate in which each year the conversion from IGT to T2DM occurs[29].

In PCOS, hyperglycemia entails increased insulin secretion[30]. In this way, increased insulin compensates peripheral insulin-resistance[31]. Hyperglycemia becomes uncontrollable when β-cells are no longer able to secrete enough insulin to meet physiological needs. Some studies have highlighted the roles of insulin resistance and insulin secretion in the pathogenesis of glucose intolerance in PCOS[32].

An important aspect in this syndrome is that, also in presence of an insulin resistance status, the ovarian granulosa cells remain insulin sensitive. In this context, we may take in consideration alternative pathways within the insulin pathway that contribute to androgen biosynthesis[33]. Indeed, a study showed abnormal glucose metabolism leading to insulin-dependent cells lactate production in ovarian granulosa in women with PCOS[34]. These cells have a physiological response to insulin that leads to androgen biosynthesis, displaying insulin-resistance in glucose metabolism[35]. So, an alteration in insulin signaling transduction for the metabolic actions may be present after receptor binding[36].

Primary hyperinsulinemia observed in PCOS contributes to ovarian hyperandrogenemia[37]. In fact, the pharmacological reduction of insulin levels improves both hyperinsulinemia and hyperandrogenemia, restoring ovulation in women with PCOS. On the other hand, the reduction of androgen levels has no effect on insulin-resistance or hyperinsulinemia. Insulin action increases the androgen-mediated luteinizing hormone (LH) production and increases the free insulin-like growth factor-1 (IGF-1) levels, reducing the IGF-binding proteins production in the liver. In this way, the IGF-1 ovarian action and LH signaling stimulated by gonadotropin-releasing hormone (GnRH) are improved[38]. Some studies display that hyperinsulinemia and insulin resistance are independent by the alteration of GnRH stimulation in PCOS[39]. Until now, it has been impossible to directly associate insulin-resistance development in PCOS patients, as it develops heterogeneously. However, it is known that both PCOS and obesity display deleterious effects on insulin sensibility and metabolic complications[40].

PCOS AND INFERTILITY

A PCOS chronic consequence is the high infertility rate in these women. Infertility can be associated with anovulation, or recurrent implantation failures. Indeed, high circulating levels of androgens, insulin-resistance, and contemporary inflammation, would seem to cause uterine dysfunction, along with the de-regulation of protein expression, necessary for endometrium implantation[41]. Indeed, several clinical studies have displayed that a reduced endometrial proteins expression is present in infertile women[42].

At molecular level, it is well-known that tyrosine phosphorylation of insulin receptor substrate 1 (IRS-1) and its binding to phosphatidylinositol 3-kinase (PI 3-kinase) are key events in the cascade of insulin signalling to allow the physiologic insulin-stimulated glucose transport. Among many "diabetogenic" factors, hyperinsulinemia increase the serine phosphorylation of IRS-1. This reaction hinders the normal activity of IRS-1 and, consequently, induces insulin resistance in the tissues or cells which normally are insulin responsive[43]. This observation suggests that the same factor, which causes phosphorylation in IRS1 Ser and therefore insulin-resistance, could also phosphorylate serine enzymes for ovarian androgens biosynthesis, causing hyperandrogenemia[44]. The close association between hyperinsulinemia and hyperandrogenemia in PCOS[45] is evident in the insulin pathway.

The gene and protein expression of 12-lipoxygenase (12-LOX), an enzyme that synthesizes peroxisome proliferator-activated receptor (PPAR) -gamma ligands, is decreased by chronic hyperandrogenism, detected in several PCOS patients. Metformin can antagonize this adverse effect positively modulating the enzyme that synthesizes PPAR-gamma ligand[46]. Moreover, it was demonstrated that metformin inhibits in mice the lipopolysaccharide (LPS) -induced pulmonary inflammatory responses by upregulating PPAR-gamma activity[47]. A recent study showed that PPAR-gamma expression is sig-

nificantly downregulated in LPS-induced vascular smooth muscle cells (VSMCs). Metformin was able to stimulate, in a concentration-dependent manner, mRNA and protein expression of PPAR-gamma, counteracting the LPS-induced reduction of PPAR-gamma expression[48].

CLINICAL TRIALS WITH METFORMIN: EFFECTS ON METABOLISM, FERTILITY AND PREGNANCY (INCLUDED *IN VITRO* FERTILISATION)

Al-Zubeidi et al[49] carried out a 6-month trial with metformin in adolescents to normalise metabolism. After the treatment they found a significant reduction in weight loss and androgen levels. In this study, the metformin effect was compared to that with oral contraceptive pill (OCP), but this treatment achieved only a weight loss. The decrease in body mass index (BMI) metformin-dependent, could be explained partly due to decreased appetite and caloric intake secondary to gastrointestinal side effects that the drug causes. Positive effects have also been seen on circulating lipid concentrations and insulin-resistance, but no one of the results reached statistical significance, due to reduced sample size[49]. A longer study displayed that, at two years, serum androgen levels were comparable between control group and metformin treated patients. Instead, the effects on insulin-resistance and BMI displayed continuous improvement during the two years of treatment[50].

Furthermore, metformin has been administered for many years and is still used for the treatment of T2DM, prediabetic states and, as off-label drug, PCOS. It is also administered as second-line therapy in PCOS women with oligomenorrhea, since they need endometrial protection (chiefly in women with contraindications to combined oral contraceptives use)[51]. The data are very varied and contradictory with reference to the restoration of ovulatory menses by metformin in PCOS women, reporting ovulatory rates from 23% to 90%[52-57].

Moreover, clinical pregnancy and live-birth rates do not seem to be improved after metformin administration to PCOS women before or during IVF cycles[58-60]. However, metformin appears to reduce the risk of ovarian hyperstimulation syndrome (OHSS)[58,59,61]. Although metformin can decrease the androgen concentrations in serum, the data so fa available do not support its use to cure hirsutism or as first-line treatment for ovulation induction in PCOS patients[62,63].

A metformin daily dose of 2 g was administered from the first trimester to birth in pregnant women with PCOS. Even if no differences were observed in new-borns to birth between the treated and placebo group, after one year women who took metformin displayed to have lost less weight and their infants had a higher weight than those in the placebo group[64].

Some studies reported the correlation between epigenetic changes in fetus, H19-mediated, and metformin treatment in pregnancy. According to Barker's hypothesis, the energy metabolism reprogramming to pregnancy time could have long-term effects on fetus health due to gene reprogramming. Embryos that develop under unfavourable conditions with reduced energy availability are more easily inclined in adulthood to have a metabolic syndrome phenotype[65].

A pharmacogenetics study was carried out in randomized PCOS patients to test the response of the major metformin transporters to 12 months treatment with this drug. Specifically, the authors aimed at investigating the influence of the genetic variations in these transporters (OCT1, MATE1, MATE2-K), in an OCT1 transcriptional regulator (HNF1A) or in a metformin target gene (ATM). Although the results demonstrated an improvement in PCOS patients, this effect was not significant[66].

Some studies were performed to compare metformin with other molecules (synthetic or natural) used in PCOS.

A prospective study displayed that metformin treatment for 6 months did not increase pregnancy rate compared to clomiphene citrate in PCOS women[67]. However, based on a systematic review and meta-analysis[68], clomiphene alone was proved to be more effective than metformin alone for ovulation and live birth rate. The combined administration of the two drugs is better than clomiphene alone as a primary treatment to induce ovulation and to get pregnancy in PCOS women. Concerning the live birth rate, no significant difference between clomiphene and metformin was found. In conclusion, it was suggested that metformin should not be used as a primary ovulation induction agent in women with PCOS in consideration of its side effects and contraindications[68].

According to another systematic review and meta-analysis by Misso et al[69] there is insufficient evidence to prove a difference between metformin and clomiphene with reference to ovulation, pregnancy, live birth, miscarriage and multiple pregnancy rates in women with PCOS and a BMI < 32[69]. Again, there is inadequate evidence to support metformin use in pregnancy to decrease the chance of miscarriage[70].

A meta-analysis by Facchinetti et al[71] evaluated six clinical trials[72-77] where myo-inositol (MI) and metformin treatments were compared in PCOS patients. The study included a total of 355 patients with 178 metformin treated and 177 MI treated. It demonstrated that by the end of the treatment (length: 3–6 months) metformin and MI achieve comparable effects on parameters such as fasting

insulin, homeostatic model assessment for insulin resistance (HOMA-IR) index, testosterone, androstenedione, sex hormone-binding globulin (SHBG), and BMI. However, the authors found a significant heterogeneity among the analysed studies for HOMA-IR index, SHBG, BMI changes. It is important to highlight the absence of adverse effects in the subjects receiving MI at the therapeutic dose in comparison with those in the metformin group. In fact, a clear evidence of an increased risk of adverse events was found in the metformin group compared to that treated with MI.

It necessary to underline that metformin administration in PCOS is not well standardized in clinical practice, and various protocols have been used until now with an extremely variable target doses per day[78].

Just for contrasting results obtained, metformin has been limited to a secondary role. According the Australian guidelines for assessment and management of PCOS[79], metformin may be recommended alone to increase the ovulation and pregnancy rate in PCOS women, when the first line drug has no effect, or in infertile women with low BMI (≤ 30 kg/m^2).

Similarly, the new American guidelines on the role of metformin in infertile patients with PCOS have displayed as the significant results obtained from the previous studies were incorrect. Some limitations are evident in the studies currently available in the literature, which reduce or eliminate the significance of the data shown. The considered samples of women with treated PCOS are extremely heterogeneous and small to evaluate their significance. Moreover, in previous studies, the insulin-resistance was not documented, and the inclusion criteria resulted highly varied. In fact, many studies used several PCOS definitions, not showing that metformin increases the live-birth rate in women with PCOS[70].

METFORMIN: SIDE EFFECTS AND LIMITATIONS IN THE USE

The most common side effects of metformin occur at the gastrointestinal level, such as nausea, vomiting, diarrhea, flatulence, constipation, digestive disorders. They affect approximately 20% of the patients. Metformin decreases intestinal absorption of vitamin B12 in up to 30% of patients and can cause vitamin B12 deficiency in 5% to 10% of patients, leading to megaloblastic anaemia in a small number of cases[80]. Lactic acidosis also was reported, as a very rare complication in otherwise healthy individuals. Metformin exposure of pregnant PCOS women from the first trimester to birth is correlated with a weight increased of the offspring (four years old)[81].

The main contraindications in use of metformin are mostly associated with pre-existing pathological conditions in patients. The typical patient treated with metformin has an insulin-resistance status with hyperglycemia and T2DM. As seen above, the insulin-resistance status is not only manifested in T2DM, but also in pre-diabetic syndromes. As described in detail, metformin metabolic changes favour cellular sensitization to insulin, not restoring the pathway, but the function. Functional restoration is accomplished through interactions between many pathways modified in T2DM patients. The steps forward in understanding of metformin mechanisms of action and safety profiles have permitted the treatments extension to patients with pre-diabetic syndromes[82], even though the potential long-term treatment effects are still study object.

The main limitations on early use of metformin in pre-diabetic patients are due to the probable impairment of its beneficial effects. This is why the metabolic alterations caused by metformin may result then in intracellular accumulation of metabolites and/or deficit in long-term, so in patients with pre-diabetic syndromes.

Lactic acidosis, a rare pathological condition in T2DM patient treatments, could be more easily observed in pre-diabetic patients, becoming a common pathological condition. For early treatment, metformin accumulation may increase over time, with cytotoxic action and increasing lactate concentrations. Similarly, it can be verified for acidosis caused by an increase in ketogenesis.

Additionally, the glycogenosis occurrence could be detected through G6Pase deficiency, condition that occurs in metformin long-term treatment. This pathology, in turn, promotes lactic acidosis in positive feedback, also causing hypoglycemia. The hypoglycemic condition, which has already been verified in slight form in metformin treated T2DM patients, may be more marked in patients with pre-diabetic syndromes with more severe symptoms.

From the other side, in PCOS patients the use of metformin without limitations may cause early negative effects. In fact, in PCOS metformin treatment decreases androgen production, as it reduces NAD^+ biosynthesis by ETC inhibition, which is necessary for key enzymes function in androgen biosynthesis[83]. Moreover, we have to take in consideration that the enzymes to produce ATP in glycolysis are absent in early oocytes and embryos; therefore metformin may compromise oocytes maturation and embryonic development as they are totally dependent on oxidative mitochondrial phosphorylation[84].

These pathological conditions may or may not worsen in PCOS patients or in metformin long-term treatment; clearly, an appropriate diet closely associated with this therapy can help the patient.

EPIGENETIC MODIFICATIONS DUE TO METFORMIN

Progress in understanding genic expression mechanisms has allowed to correlate the transcription of genes or non-coding RNAs (ncRNAs) with epigenetic mechanisms. In this way, some studies have investigated whether metformin could induce changes in methylation and in chromatinic reorganization of genic loci.

H19 is a long non-coding RNA (lncRNA) whose gene is adjacent to insulin-like growth factor-2 (IGF-2)[85]. This gene expression is ruled by genomic imprinting. The imprinting involves silencing by hypermethylation of maternal or paternal allele within the imprinting control region (ICR) located between the two loci[86]. H19 undergoes paternal imprinting, and so the maternal allele expression. In contrast, IGF-2 undergoes maternal imprinting, resulting in the paternal allele expression[87].

H19 is expressed in embryo, particularly in the fetal liver, playing a crucial role in development, cell proliferation and differentiation, with possible functions such as oncogene and/or onco-suppressor. In the fetal liver, it regulates hematopoietic function, suppressing important genes in the Imprinted Gene Network (IGN), like IGF-2 and Dlk1, recruiting epigenetic modifiers[88]. However, after the birth, the liver hematopoietic function is silenced to activate metabolic functions. In addition, it blocks the fetal liver proliferation, inhibiting β-catenin and so Wnt pathway (Wnt is an acronym made from the names Wingless and Int-1), which regulates liver development[89].

Metformin treatment reduces H19 levels increasing methylation[90] and S-adenosylhomocysteine hydrolysis (SAHH) activity. This is the only eukaryotic enzyme able to hydrolyse S-adenosylhomocysteine (SAH), a potent SAM-dependent methyl-transferase inhibitor, like DNA-Methyl transferase (DNMTs). SAHH alterations could affect all DNMTs, as it causes SAH accumulation[91]. Particularly important is DNA (cytosine-5-)-methyltransferase 3 beta (DNMT3B) contribution to metformin-mediated H19 induction. Indeed, in some *in vitro* and *in vivo* studies, metformin-induced AMPK activation has led to an increase in let-7 levels. Some studies have shown how H19 acts as a "sponge" capable to trap the miRNA let-7[92]. The miRNA up-regulation permits the connection with H19, leading to degradation. The downregulation induced by let-7 releases SAHH which in this way can hydrolyze SAH and favour DNMT3B activation. These studies let a link between hypermethylation and metformin treatment by activating the H19-SAHH-DNMT3B signaling. Additionally, it can be supposed that the AMPK initial activation may be due to chronic H19 activation induced through promoter hypermethylation[93].

For hepatic functions, H19 would seem implicated in the pathogenesis of T2DM. With insulin, smooth muscle cells increase H19 expression by 5 times, responding to concentration changes[94]. In addition, it was observed that IGF-2 bi-allelic expression[95] predisposes muscle cells to T2DM[96]. In another study, H19 increase and its hypomethylation degree displayed a significant difference between healthy patients and T2DM patients[97], and the hypomethylation was associated with insulin resistance[98].

The evidence that H19 was able to interact with let-7 permitted to highlight in patient muscles with T2DM a feedback mechanism caused by hyperinsulinemia, where, through KSRP-dependent signalling system, let-7 binds H19, reducing expression levels and leading to degradation. Let-7, having as its target the *Insr* and *Lpl* genes, can inhibit PI3K-mTOR insulin pathway. Indeed, H19 down-regulation in T2DM limits let-7 sequestration, increasing insulin pathway inhibition and promoting insulin-resistance[92].

Metformin is one of drugs that can overcome the placental barrier, get significant concentrations in fetus and therefore can have a direct or indirect effect on it. A correlation between hyperglycemia with consequent glucose intolerance and pharmacological treatment during pregnancy has recently been demonstrated for dexamethasone, which increases Hnf4α factor expression and deregulates PEPCK in fetal liver[99]. Hnf4α encodes for a key factor in differentiation, maturation and liver architecture, and regulates gluconeogenesis genes like PEPCK and G6Pase[100]. Gluconeogenesis is not present to liver level before birth, and so premature expression could contribute to deregulation in glucose metabolism in adulthood (Figure 1).

Only recently, one study correlated metformin therapeutic treatment in women with T2DM, with the H19 up-regulation found in fetal liver as a metformin probable direct effect on fetus. In fetal liver, H19 over-expression revealed significant functional interaction with Hnf4α, which is increased and regulate G6Pase positively. In addition, insulin receptor was inhibited by metformin treatment, altering fetal insulin signalling also. Previous correlations between H19 levels reduction and Hnf4α methylation increase[93] display how H19 can affect it, inhibiting SAHH.

Since Hnf4α intervenes in liver lobular organization, which is crucial for positional heterogeneity[101], the metformin exposure during pregnancy could have deep effects on liver function in adulthood, for example leading to glucose intolerance[102] or T2DM development[103], independently from condition of mother.

On the other hand, H19 chronic reduction inhibits glycogen synthesis and interferes with glucose metabolism. In addition to the liver, also the pancreas displayed to be a H19 ICR differential methylation site in fetuses whose mothers had natural pre-hyperglycemic condition. Hyperglycemia, through let-7 feedback, leads to low H19 and IGF-2 expression, causing glucose intolerance[104]. These data reveal a contradiction in metformin action, which causes positive gluconeogenesis regulation in fetus and negative in adulthood, which could be explained through tissue cell-dependent and developmental status effects.

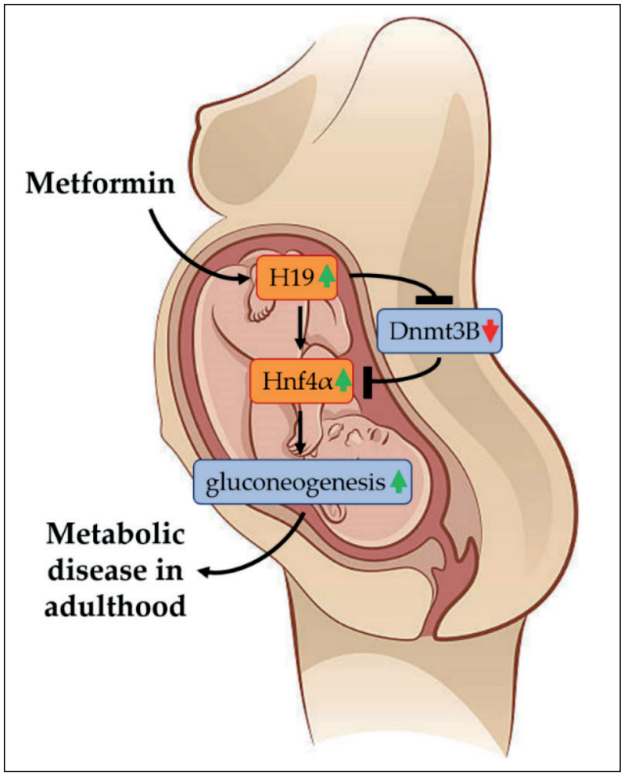

Figure 1. Schematic diagram of epigenetic modifications metformin-induced on offspring.

CONCLUSIONS

The disadvantages established by clinical trials seem to concern metformin intake during pregnancy in patients suffering from obesity, IGT, insulin-resistance, PCOS and T2DM.

The clinical trials on metformin administered to reduce infertility and increase the implantation rate, display a doubtful efficacy. In fact, the data obtained are contradictory, although they support a drastic change in lifestyle and weight loss as favourable agents for increasing fertility.

Therefore, the negative potentials of metformin treatment should be carefully considered in a crucial and sensible period of embryonic development since this drug can exert long-term consequences on metabolism epigenetic reprogramming in adulthood (Table 1).

In consideration that the treatment in patients with pre-diabetic disorders, such as obesity, IGT, insulin-resistance and PCOS begins much earlier than in patients with T2DM, it might be easier to detect the manifestation of the possible intracellular cytotoxic effects.

According to the current available evidence, metformin may act, to long-term, as a promoter of the diabetic syndrome and/or its negative effects in patients with a pre-diabetic syndrome. In fact, unexpectedly, the preventive use of metformin could not block the diabetic syndrome manifestation, but could favour cytotoxic manifestations, some of which are not even evident in diabetic patients. The most worrying effect is certainly the long-term effects in the offspring, still under study.

New researches could definitively clarify this issue. Additional in-depth studies could allow to understand better the mechanisms of action involved in the metabolic alterations associated with long-term treatment in patients with pre-diabetic syndromes.

For these reasons, metformin should be administered with caution and always under careful control. Furthermore, wherever possible it would be necessary to prefer the use of safer molecules.

Table I. Summary table of all metformin effects in PCOS. *statistically not significant result.

	PCOS			
Metabolism	BMI reduction Circulating androgen*	Efficacy dependent on transporters polymorphisms	ATP biosyntesis reduction AMPK activation	Gluconeogenesis inhibition Androgen biosynthesis reduction
Infertility	Implantation failure reduction* Increased endometrial receptivity*	Improved birth rate*	Impaired oocyte maturation	Improved pregnancy rate IVF*
Long-term / Offspring	Increased BMI in offspring Epigenetic alteration in offspring	Early activation of gluconeogenesis Metabolism alteration in adulthood	Zygotes-induced embryos reduction	Poor energy availability
	In vivo		*In vitro*	

Conflicts of interest:
The Authors declare that there are no conflicts of interest.

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