Metformin in reproductive health, pregnancy and gynaecological cancer: established and emerging indications

Vanitha N. Sivalingam1, Jenny Myers2, Susie Nicholas3, Adam H. Balen3, and Emma J. Crosbie1,∗

1Institute of Cancer Sciences, Manchester Academic Health Science Centre, University of Manchester, Central Manchester University Hospitals NHS Foundation Trust, Oxford Road, Manchester M13 9WL, UK 2Institute of Human Development, Manchester Academic Health Science Centre, University of Manchester, Central Manchester University Hospitals NHS Foundation Trust, Oxford Road, Manchester M13 9WL, UK 3Leeds Centre for Reproductive Medicine, Leeds Teaching Hospitals, Seacroft Hospital, Leeds LS14 6UH, UK

∗Correspondence address. Institute of Cancer Sciences, Manchester Academic Health Science Centre, University of Manchester, Central Manchester University Hospitals NHS Foundation Trust, Oxford Road, Manchester M13 9WL, UK. Tel: +44-161-701-6942; E-mail: emma.crosbie@manchester.ac.uk

Submitted on February 6, 2014; resubmitted on May 26, 2014; accepted on June 4, 2014

TABLE OF CONTENTS

- Introduction
  - The effect of metformin on insulin resistance
  - Indications for metformin in reproductive health, pregnancy and gynaecological cancer
- Methods
- PCOS
  - PCOS, insulin resistance, metabolic dysfunction and infertility
  - Metformin in the management of anovulatory PCOS
  - Metformin therapy in PCOS and assisted reproduction
  - Metformin therapy during pregnancy in PCOS
- Metformin in pregnancy
  - GDM
  - Obesity and pregnancy
- Metformin as an anti-cancer drug
  - Proof of principle window of opportunity studies
  - Metformin to treat endometrial cancer
  - Metformin to prevent endometrial cancer
  - Epidemiological evidence for metformin as anti-cancer drug
  - Putative anti-cancer mechanisms
  - Metformin to treat ovarian cancer
- Conclusions

BACKGROUND: Metformin is an effective oral anti-hyperglycaemic drug used as first-line medical treatment for type 2 diabetes. It improves systemic hyperglycaemia by reducing hepatic glucose production and enhancing peripheral insulin sensitivity. It also stimulates fat oxidation and reduces fat synthesis and storage. The molecular mechanism of this drug is thought to be secondary to its actions on the mitochondrial respiratory chain.

METHODS: This paper reviews the relevant literature (research articles up to October 2013) on the use of metformin in infertility, polycystic ovary syndrome (PCOS), pregnancy and gynaecological cancers. We present a comprehensive discussion of the evidence supporting the efficacy of metformin in these clinical conditions.
RESULTS: Metformin is used clinically off-label in the management of hirsutism, acne and insulin resistance in PCOS, although the evidence for anti-androgenic effects is inconsistent. Metformin is also used to improve ovulation in women with PCOS both alone and in combination with clomiphene citrate. Trial findings are conflicting but metformin treatment in IVF/ICSI cycles may reduce the risk of ovarian hyperstimulation syndrome and increase live birth rates. Metformin also appears to be effective and safe for the treatment of gestational diabetes mellitus (GDM), particularly for overweight and obese women. Studies have shown that metformin is safe in pregnancy and women with GDM treated with metformin have less weight gain during pregnancy than those treated with insulin. One study with a 2-year follow-up demonstrated that babies born to women treated with metformin also developed less visceral fat, making them less prone to insulin resistance in later life. These findings have sparked interest in the use of metformin for pregnant, obese, non-diabetic women. On-going clinical trials are underway to determine if women treated prophylactically with metformin have a reduced incidence of GDM and demonstrate less weight gain during pregnancy. The hypothesis in these studies is that babies born to obese women on prophylactic metformin will also have better outcomes. Epidemiological studies have linked metformin exposure to a decreased risk of cancer. Pre-clinical experiments report that metformin has a growth-static effect on several cancers, including endometrial cancer, which may be partly due to the effect of metformin on the PI3K/AKT/mTOR signal transduction pathway. A number of on-going early phase clinical trials aim to explore the anti-cancer effects of metformin and investigate its potential as a chemopreventative or adjuvant treatment.

CONCLUSIONS: Obesity is on the rise in developing countries and is strongly linked to several reproductive health problems, including PCOS, GDM and endometrial cancer. Traditional lifestyle measures aimed at weight reduction are challenging to implement and maintain. Metformin may be a valuable alternative to, or adjunct for, modifying the toxic effects of obesity in these populations. This review will appraise the evidence for the use of metformin for the prevention and treatment of adverse health outcomes in obstetrics and gynaecology.

Key words: metformin / endometrial cancer / polycystic ovary syndrome / gestational diabetes / obesity in pregnancy

Introduction

Insulin resistance occurs when insulin-responsive tissues (including liver, skeletal muscle and adipose tissue) become less sensitive to insulin (Lebovitz, 2001). Failure of pancreatic β islet cells to produce sufficient compensatory insulin results in chronic hyperglycaemia and hyperinsulinaemia. Insulin resistance comprises a spectrum of disease ranging from pre-clinical impaired glucose tolerance to overt type 2 diabetes mellitus (T2DM). T2DM is an increasing problem in elderly, overweight populations. Treatment of T2DM aims to normalize glycaemic levels as much as possible as this has been shown specifically to reduce microvascular complications (The Diabetes Control and Complications Trial Research Group, 1993). Lifestyle interventions that reverse or reduce obesity have a beneficial effect on glycaemic control (Pi-Sunyer et al., 2007) but are limited by poor compliance and high relapse rates. Drug therapy is initiated where lifestyle measures fail to achieve glycaemic targets.

Metformin is the preferred and most cost-effective first-line oral therapy for the treatment of T2DM (Nathan et al., 2009; Inzucchi et al., 2012) and is usually well tolerated and not complicated by hypoglycaemia. The most serious side effect, lactic acidosis, is rarely seen in patients with normal renal and hepatic function, occurring in just 3/100 000 patient-years of use (Howlett and Bailey, 1999; Lalau and Race, 2000; Sappleter et al., 2006). In contrast to most oral hypoglycaemic agents, metformin promotes weight loss. The UK Prospective Diabetes Study (UKPDS) found that metformin offers protection from the macrovascular complications of T2DM independently of its hypoglycaemic actions (UKPDS, 1998). Reduced atherogenesis, less oxidative stress and redistribution of visceral adiposity have all been described as potential mediators of this effect (Scarpello and Howlett, 2008). Metformin is also associated with reductions in total cholesterol, low-density lipoprotein-cholesterol and triglyceride levels (Nagi and Yudkin, 1993).

Metformin can have health benefits in non-diabetic patients at risk of the disease. The US Diabetes Prevention programme found metformin reduces the risk of frank diabetes in patients with impaired glucose tolerance by as much as 31% (95% confidence interval (CI) 17–43) when compared with placebo, although lifestyle interventions that achieve weight loss and regular exercise are even more effective (58% reduction in risk, 95% CI 48–66) (Knowler et al., 2002). Other studies have reported similar results (Ramachandran et al., 2006). Metformin can also improve histological and biochemical liver function in non-alcoholic fatty liver disease and non-alcoholic steatohepatitis (NASH), diseases associated with insulin resistance and the metabolic syndrome (Loomba et al., 2009; Nadeau et al., 2009). Improvements in systemic insulin and glucose levels are the likely mechanisms responsible here.

The effect of metformin on insulin resistance

Metformin is a synthetically derived biguanide that takes its origins from Galega officinalis, a medicinal herb used in medieval Europe (Bailey et al., 2008). Taken orally, it is absorbed rapidly across the intestinal epithelium and then conveyed via the portal vein to the liver, where it accumulates. The liver is its primary site of action. Metformin is not metabolized and is excreted unchanged in the urine and bile.

Metformin reduces hepatic glucose production, stimulates insulin-mediated glucose uptake by the liver and skeletal muscle, and reduces substrate availability for gluconeogenesis by lowering serum lipid levels. Its effects on glucose metabolism appear to be secondary to its actions on the mitochondrial respiratory chain. Metformin inhibits the mitochondrial respiratory complex I (El-Mir et al., 2000; Owen et al., 2000), leading to reduced oxidative phosphorylation and ATP production. The resultant increase in AMP: ADP ratio inhibits gluconeogenesis and activates AMP-activated kinase (AMPK) (Fig. 1). AMPK is frequently inactivated in energy-rich environments such as obesity and insulin resistance. Its activation by metformin decreases lipid synthesis, increases fatty acid oxidation and inhibits gluconeogenesis (McGarry and Brown, 1997; Li et al., 2011). Metformin regulates AMPK via an upstream kinase, LKB1, a tumour suppressor gene product that controls cell growth. Metformin
also has AMPK-independent effects on glucose metabolism, since mice deficient in both LKB1 and AMPK show reduced serum glucose levels following treatment with metformin. These effects may be caused by the change in AMP:ATP ratio which modulates hepatic glucose output upstream of AMPK and inhibits cyclic-AMP-protein kinase A signalling.

Indications for metformin in reproductive health, pregnancy and gynaecological cancer

Metformin is currently used for the management of gestational diabetes mellitus (GDM). It is also prescribed off-label in polycystic ovary syndrome (PCOS) to treat insulin resistance and improve outcomes in assisted reproduction. Recently, epidemiological, pre-clinical and early phase clinical trials have suggested that metformin may be effective as an anti-cancer drug in tumours driven by insulin resistance and obesity. This article will summarize the current clinical uses of metformin in pregnancy and reproductive healthcare and discuss the emerging evidence from the research setting for potential novel applications of this ‘old’ drug.

Methods

PubMed and the Cochrane Library were searched for high-quality studies including randomized trials, systematic reviews and meta-analyses between 1 January 2000 and 1 October 2013 for the terms ‘endometrial cancer’, ‘endometrial hyperplasia’, ‘atypical endometrial hyperplasia’, ‘endometrioid’, ‘uterine cancer’, ‘ovarian cancer’, ‘polycystic ovary syndrome’, ‘polycystic ovary’, ‘ovulation induction’, ‘anovulatory infertility’, ‘in-vitro fertilisation’, ‘insulin resistance’, ‘type 2 diabetes’, ‘gestational diabetes’, ‘obesity in pregnancy’, ‘metformin’ and ‘biguanides’. In addition, a hand-search identified older publications that were considered to be important. Pertinent references from selected articles were also included.
PCOS

PCOS, insulin resistance, metabolic dysfunction and infertility

PCOS is the most common endocrine disorder to affect women during their reproductive years (ESHRE/ASRM, 2004). The symptoms of PCOS include menstrual cycle disturbance and features of hyperandrogenism (hirsutism, acne, alopecia), with associated fertility problems, obesity and psychological issues.

In PCOS, both extra-ovarian and intra-ovarian factors lead to dysregulation of normal follicular recruitment and ovulation. There is uncertainty as to the mechanisms that lead to an increased pre-antral follicle population in the polycystic ovary and there appear to be intra-ovarian disturbances in the expression of growth differentiation factor-9, anti-Müllerian hormone and androgen production by the theca cells (Chang and Cook-Andersen, 2013). These are further influenced by disturbances in gonadotrophin secretion (FSH and LH) secondary to abnormalities of the GnRH pulse generator and perturbations of ovarian-pituitary feedback.

LH stimulates androgen production by the ovaries and hypersecretion of LH in PCOS, which is seen predominantly in slim women, occurs secondary to both an increase in the GnRH pulse generator and abnormalities in ovarian-pituitary feedback. Hyperandrogenism is also amplified by hyperinsulinaemia secondary to insulin resistance, which in turn may be promoted by obesity (Cussons et al., 2008). Serine kinase activity leads to phosphorylation of the insulin receptor, blocking insulin signalling, and also phosphorylation of the cytochrome P450c17a enzyme (CYP17) to activate androgen production (Bremer and Miller, 2008). Excess insulin also binds to insulin-like growth factor (IGF)-1 receptors which enhances theca cells androgen production in response to LH stimulation (Bergh et al., 1993). Hyperinsulinaemia inhibits hepatic secretion of insulin-like growth factor binding protein-1 (IGFBP-1), leading to increased bioavailability of IGF-1 and 2, important regulators of ovarian follicular maturation (De Leo et al., 2000). This further augments ovarian androgen production, which contributes to anovulation by promoting follicular atresia. Sex hormone-binding globulin synthesis by the liver is also lowered by hyperinsulinaemia leading to an increase in bioactive serum free-testosterone concentration (Fig. 1). A number of additional defects within the polycystic ovary have been described (Chang and Cook-Andersen, 2013) but are beyond the scope of this review.

PCOS may also be associated with an increased risk of an individual developing T2DM, the metabolic syndrome and endometrial cancer (Cussons et al., 2008). Both obese and non-obese women with PCOS are more insulin-resistant and hyperinsulinaemic than age- and weight-matched women with normal ovaries, suggesting a tendency towards insulin resistance which is independent of obesity (Tsilchorozidou, 2004). Pancreatic beta cell dysfunction has been described in women with PCOS, where there is increased basal secretion of insulin yet an inadequate post-prandial response. This defect may remain even after weight loss, despite an improvement in glucose tolerance (Holte et al., 1995). Women with PCOS who are oligomenorrheic are more likely to be insulin resistant than those with regular cycles, irrespective of their BMI (Robinson et al., 1993).

There are now many studies that have shown that women with PCOS have an increased rate of impaired glucose tolerance, T2DM and metabolic syndrome when compared with weight-matched controls (Moran et al., 2010). Cardiovascular risk factors for ischaemic heart disease and stroke include obesity, insulin resistance, glucose intolerance, diabetes, hypertension, dyslipidaemia (in particular raised serum triglycerides) and various markers of inflammation (Fauser et al., 2012). Lifestyle advice and weight reduction should be the first-line approach for the management of PCOS, and options for treatment then include a range of therapies depending upon the constellation of an individual’s problems. These are too numerous to be elucidated (RCOG, 2010) and this review will focus on the use of metformin.

Metformin in the management of anovulatory PCOS

PCOS accounts for anovulatory infertility in ~80–90% of women. Algorithms for the induction of ovulation are well established (ESHRE/ASRM TEA-SPCWG, 2008) and include the use of clomiphene citrate as first-line therapy followed by either gonadotrophins or laparoscopic ovarian diathermy for those who fail to ovulate with clomiphene citrate (Balen, 2013). There may also be a role for aromatase inhibitors while IVF remains the last resort. Weight loss prior to treatment in the overweight improves the chance of success and decreases the risks of miscarriage, fetal abnormalities and maternal morbidity from GDM and pre-eclampsia (Clark et al., 1995; Fedorcsak et al., 2001; Balen and Anderson, 2007). In the UK, national guidelines suggest that ovulation induction should not be commenced in a woman with a BMI > 35 kg/m² and preferably limited to women with a BMI <30 kg/m² (Balen et al., 2006; Balen and Anderson, 2007).

Insulin-sensitizing agents, such as metformin, were thought to have potential in the management of PCOS, and indeed early studies suggested an improvement in reproductive function and the possibility of benefits to long-term health (Stadtmueller et al., 2002). Metformin may act indirectly by reducing systemic insulin levels and directly within the ovary itself (Diamanti-Kandarakis et al., 2010), with a reduction in CYP17 activity and subsequent androgen production (Nestler and Jakubowicz, 1996; Attia et al., 2001). Metformin therapy may also lead to an increase in IGFBP-1 thereby reducing the availability of IGF-1 (De Leo et al., 2000).

The results of large prospective, randomized studies have, however, failed to demonstrate benefit: significant weight loss is not achieved by metformin and whilst some biochemical parameters may improve this does not translate into a significant benefit in outcomes, whether for the dermatological manifestations of hyperandrogenism (Costello et al., 2007) or the enhancement of fertility (Tang et al., 2012).

The combined effects of lifestyle modification and metformin on obese anovulatory women (BMI > 30 kg/m²) with PCOS were evaluated in a prospective randomized, double blind, placebo-controlled multicentre study (Tang et al., 2006a). All the patients had an individualized assessment by a research diettian in order to set a realistic goal which could be sustained for a long period of time with an average reduction of energy intake of 500 kilo calories per day. As a result, both the metformin-treated and placebo groups managed to lose weight, and the amount of weight reduction did not differ between the two groups. An increase in menstrual cyclicity was observed in those who lost weight but again did not differ between the two arms of the study, reinforcing the notion of weight reduction holding the key to improving reproductive function (Tang et al., 2006a).

Other large studies have explored the use of metformin combined with clomiphene citrate. In a Dutch multicentre trial, 228 women with...
PCOS were randomly allocated to receive either clomiphene citrate plus metformin or clomiphene citrate plus placebo (Moll et al., 2006). The ovulation rate in the metformin group was 64% compared with 72% in the placebo group (non-significant), and furthermore there were no significant differences in either the rates of ongoing pregnancy (40 versus 46%, respectively) or spontaneous miscarriage (12 versus 11%, respectively). The gastrointestinal side effects of metformin led to more women discontinuing therapy than in the control group (16 versus 5%). The pregnancy in polycystic ovary syndrome trial enrolled 626 anovulatory women with PCOS and randomized them to three different treatment arms for a total of six cycles or 30 weeks: (i) metformin 1000 mg twice daily plus placebo, (ii) clomiphene citrate 50 mg/day on Day 3–7 of cycle plus placebo, or (iii) combined metformin 1000 mg twice daily plus clomiphene citrate 50 mg/day (Days 3–7). Overall, live birth rates were 7.2% (15/208), 22.5% (47/209) and 26.8% (56/209), respectively, with the metformin group alone faring significantly worse than the other two groups. Pregnancy loss rates were also higher in the metformin alone group (40.0% versus 22.6% and 25.5%, respectively). The mean BMI of recruited patients was 35 kg/m², so the results of this study may not be applicable to all PCOS patients (Legro et al., 2007).

The most recent Cochrane review included 38 trials with a total of 3495 participants (ranging from 16 to 626 per study), with a median daily dose of metformin of 1500 mg and durations ranging from 4 to 48 weeks. This systematic review concluded that metformin is effective in achieving ovulation in women with PCOS when comparing metformin versus placebo (odds ratio (OR) 1.81, 95% CI 1.13–2.93; 16 RCTs, 1208 participants) as well as metformin and clomiphene citrate versus clomiphene citrate alone (OR 1.74, 95% CI 1.5–2.0; 18 RCTs, 3265 cycles) (Tang et al., 2012). The analysis of pregnancy rates showed a significant treatment effect for metformin and clomiphene citrate (111 RCTs, 1208 participants; OR 1.51, 95% CI 1.17–1.96); however, these benefits were not translated into live birth rates (7 RCTs, 907 participants; OR 1.16, 95% CI 0.85–1.56). Live birth rates were significantly improved in obese women with PCOS taking clomiphene citrate compared with those taking metformin alone (2 RCTs, 500 participants; OR 0.30 95% CI 0.17–0.52) (Legro et al., 2007; Zain et al., 2009). There was also a suggestion that those with clomiphene-resistance might benefit from the combined use of clomiphene citrate with metformin to improve the chance of ovulation, although the numbers studied were relatively small and this did not translate into an increase in live births. This review concluded that the benefit of metformin in women with anovulatory PCOS may be limited and did not demonstrate any benefit from metformin in improving weight loss, insulin sensitivity or lipid profiles (Tang et al., 2012). In contrast, a recent large study from Finland enrolled 329 women to receive metformin (1500–2000 mg/day) or placebo for 3 months prior to fertility treatment, for a further 9 months during fertility treatment, and until the 12th week of gestation if the patient conceived, showed an increase chance of pregnancy from 40.4 to 53.6% (OR 1.61, 95% CI 1.13–2.29), with the greatest benefit seen in obese women (Morin-Papunen et al., 2012). Whilst there was no reduction in miscarriage rate, the live birth rate was significantly increased in those who received metformin (41.9 versus 28.8%, P = 0.014). This latest study keeps the debate open and will have to be incorporated into the updated Cochrane review, which is currently in progress.

**Metformin therapy in PCOS and assisted reproduction**

Although the benefits of metformin in inducing ovulation in PCOS appear to be limited, there is more supportive evidence for its use in women with PCOS who are undergoing IVF. Metformin appears to act as a brake on the response of polycystic ovaries to exogenous stimulation, which para-enthetically may explain its lack of efficacy in ovulation induction. Women with polycystic ovaries are at high risk of developing ovarian hyperstimulation syndrome (OHSS), which may cause serious morbidity and even death from intravascular volume depletion, thrombosis and adult respiratory distress syndrome. Risk reduction strategies include low dose stimulation protocols, freezing embryos for those at serious risk and the use of a GnRH agonist cycles with a GnRH agonist to trigger oocyte maturation (Nastri et al., 2010).

One of the first large RCTs to compare metformin with placebo in IVF cycles in women with PCOS, randomized 101 consecutive cycles using a conventional long GnRH agonist protocol and metformin 850 mg twice daily prior to egg collection. There was no difference in total FSH dose, number of oocytes retrieved or overall fertilization rate; however, there was a significant increase in clinical pregnancy rate beyond 12 weeks (38.5 versus 16.3%, P = 0.023) and a clinically significant reduction in severe OHSS (3.8 versus 20.4%, P = 0.023). Metformin was also shown to attenuate the ovarian secretion of vascular endothelial growth factor, which is thought to be key in the pathophysiology of OHSS (Tang et al., 2006b). Early work by Kjotrod et al. (2004) had found similar benefit; lean women with PCOS had a higher live birth rate. Subsequent work from the same group provided further support. A total of 150 patients were randomized to either 2000 mg/day metformin or placebo for 12 weeks prior to IVF treatment. Implementing intention to treat analysis, the live birth rate was significantly higher in the metformin group (48.6 versus 32.0; 95% CI: 1.1–32.2; P = 0.0383) (Kjotrod et al., 2011). A Cochrane meta-analysis concluded that the main benefit of metformin in the context of IVF therapy for women with PCOS is for the prevention of OHSS (OR 0.27, 95% CI 0.16–0.47) (Tso et al., 2009). The potential benefit of metformin therapy may also stretch into subsequent frozen embryo transfer cycles. A retrospective study found that for those who used metformin during the fresh attempt, the subsequent frozen cycle had a significantly increased live birth rate (28.6 versus 12.3%) (Brewer et al., 2010). This was most significant in those who had all embryos frozen due to OHSS risk, in whom a 9-fold increase in live birth rate was seen (Brewer et al., 2010).

**Metformin therapy during pregnancy in PCOS**

Some early studies, with inferior design, suggested that metformin might reduce the miscarriage rate in women with PCOS although this has since been shown not to be the case (Palomba et al., 2009; Morin-Papunen et al., 2012). Women with PCOS are at increased risks of pregnancy-related complications including GDM, pregnancy-induced hypertension, pre-eclampsia and neonatal morbidity (Boomsma et al., 2006). A large Swedish population-based cohort study found a strong association with pre-eclampsia (adjusted OR 1.45, 95% CI 1.24–1.69) and preterm birth in women with PCOS compared with those without PCOS. The risk of GDM was doubled (Roos et al., 2011). This group concluded that there was an increase in adverse pregnancy events for those
with PCOS not purely explained by increased use of assisted conception. In view of the favourable effects of metformin on thrombotic events in the diabetic population, it would seem feasible that microvascular dysfunction and subsequent downstream events could be improved in PCOS pregnancies with metformin. However, a large Norwegian multicentre RCT found no improvement in these complications with continued use of metformin from late first trimester to delivery (Vanky et al., 2010), although there appeared to be a reduction in late miscarriage and preterm delivery rates, which is now the subject of a large ongoing RCT. Women in the metformin group also gained less weight during pregnancy compared with the placebo group. It is reassuring that metformin has a good safety profile in healthy women and early pregnancy with no evidence of teratogenicity, although the gastrointestinal side effects that occur in ~10% are well recognized (Tang et al., 2012).

In summary, metformin may both reduce the risk of OHSS and increase ongoing pregnancy rates in women with PCOS undergoing IVF, although further research is needed to substantiate these findings.

**Metformin in pregnancy**

**GDM**

The tide has turned quite dramatically over the last decade in favour of metformin for the treatment of diabetes, both pre-existing (T2DM) and newly diagnosed in pregnancy (GDM) since the first study that used metformin in pregnancy in 1979 (Coetzee and Jackson, 1979). In 2001, in a critical appraisal of the published evidence describing treatment options for GDM, Dorman stated ‘It would be a brave, possibly fool-hardy, person to do a controlled trial of metformin’ in diabetic pregnancy (Dorman and Hollis, 2001). Since then three retrospective studies, two non-randomized prospective studies and five RCTs have published pregnancy outcomes for around 1690 women treated with metformin during pregnancy (Ekpebeeh et al., 2007; Rowan et al., 2008, 2011; Tertti et al., 2008; Balani et al., 2009; Goh et al., 2011; Ijas et al., 2011; Gandhi et al., 2012; Niromanesh et al., 2012; Spaulonci et al., 2013). Despite methodological issues with many of these studies and limitations associated with comparison populations due to differences in baseline characteristics, the overwhelming conclusion from these studies is that metformin is safe in pregnancy. There have been no reports of an increase in congenital abnormalities or deleterious effects on fetal growth or short-term neonatal health. In fact several studies have described a number of beneficial effects of metformin (Lautatitzis et al., 2013).

A normal pregnancy is associated with a 50% reduction in insulin sensitivity in the third trimester, which is compensated by a 200–250% increase in the production of insulin in order to maintain a euglycaemic state (Catalano et al., 1991; Kuhl, 1998). Women who are at the highest risk of developing GDM are those with a pre gravid reduction in insulin sensitivity, including obese women, women with family history of T2DM and those who have previously been affected by GDM. A reduction in insulin sensitivity coupled with suppressed pancreatic insulin production in late pregnancy (Buchanan et al., 1990) results in both fasting and post-prandial hyperglycaemia with increased nutrient availability to the fetus (Catalano et al., 2003). The exact mechanisms by which insulin resistance develops in the second half of gestation are not completely understood but are likely attributable to placental hormones (e.g. human placental lactogen, human placental growth hormone) (Beck and Daughaday, 1967; Handwerger and Freemark, 2000) and a number of adipokines including leptin, adiponectin, tumour necrosis factor (TNF)-α, interleukin (IL)-6 and resistin (Barbour et al., 2007). Metformin treatment combats both of these effects by increasing insulin sensitivity and reducing basal hepatic glucose output (Catalano et al., 1991; Stumvoll et al., 1995) (Figs 1 and 2).

One of the most consistent observations from the metformin studies to date is the reduction in maternal weight gain during pregnancy (Rowan et al., 2008; Balani et al., 2009; Niromanesh et al., 2012) which is likely to be associated with long-term health benefits for women. Maternal hyperglycaemia is also much less prevalent in women treated with metformin alone and although 10–46% of women with GDM treated with metformin have required supplemental insulin therapy to achieve optimal glycaemic control, the total dose of insulin in metformin-treated women is lower than in women treated with insulin alone (Lautatitzis et al., 2013). As demonstrated by the recent study performed by Tertti et al., women who require supplemental insulin are older and often require pharmacological treatment earlier in pregnancy. Interestingly, fructosamine was shown to be a useful predictor of the need for supplemental insulin in this study (Tertti et al., 2013). Metformin was well tolerated in all of the studies with around 2–7% unable to tolerate it (Rowan et al., 2008; Balani et al., 2009; Niromanesh et al., 2012). It was also an acceptable treatment in those studies that assessed this element (e.g. (Rowan et al., 2008)). The majority of the studies did not identify a significant difference in short-term neonatal outcomes in women treated with metformin compared with insulin although individual trials were not powered to demonstrate differences in severe perinatal morbidity or perinatal mortality. Neonatal birthweight, frequency of large for gestational age, small for gestational age, preterm deliveries and neonatal unit admissions were not different between groups in the majority of studies (Rowan et al., 2008; Tertti et al., 2008; Goh et al., 2011; Ijas et al., 2011; Niromanesh et al., 2012; Spaulonci et al., 2013). Neonatal hypoglycaemia was reduced in the metformin group in the metformin in GDM (MiG) trial (Rowan et al., 2008) and whilst a reduction in neonatal anthropometric measurements (head, arm and chest circumference) was demonstrated in the study by Niromanesh et al. (2012), this was not shown in the larger MiG trial (Rowan et al., 2008).

A common criticism of RCTs in pregnancy is the assessment of meaningful long-term child health outcomes. This is particularly important for interventions which could have a profound effect on the metabolic programming of the fetus/infant. There is now a wealth of evidence supporting the hypothesis that an adverse in utero environment has lasting effects on the metabolic and future cardiovascular health of the offspring. The children from the MiG trial have been followed up to 2 years (Rowan et al., 2011). Anthropometric assessments were performed on the children that did not identify differences between the groups in central fat measures, total fat mass, percentage body fat, or central-to-peripheral fat, however the children exposed to metformin in utero had larger upper arm circumference, and bigger biceps and subscapular skinfolds. The authors concluded that this would suggest that exposure to metformin in utero leads to a shift in fat deposition from visceral fat stores to subcutaneous sites, which has important implications for the development of insulin resistance in the future (Ali et al., 2011).

**Obesity and pregnancy**

Maternal obesity in pregnancy is a significant and growing health problem. Obese women are at significantly higher risk of a number of pregnancy
complications including miscarriage, GDM, pre-eclampsia and Caesarean delivery (Galtier-Dereure et al., 2000). Many of these complications have been attributed to insulin resistance, metabolic dysfunction, inflammation and oxidative stress affecting the mother and the placenta (Ramsay et al., 2002).

The effect of metformin on inflammation in pregnancy
In addition to its role as an insulin-sensitizing agent, metformin also has anti-inflammatory actions (Dandona et al., 2004; Isoda et al., 2006) which may be relevant to its beneficial effects in pregnancy for both the mother and fetus. Moderation of both metabolic dysfunction and inflammation may improve pregnancy outcomes by altering placental development and function, in addition to reducing the likelihood of vascular endothelial activation (Hattori et al., 2006; Isoda et al., 2006) and potentially reducing insulin resistance in the fetus (Fig. 2). Metformin crosses the placenta and therapeutic levels of the drug have been quantified in cord blood (Hague et al., 2003); therefore it is likely that there is some effect of metformin on placental and fetal metabolism.

Figure 2 Putative mechanisms of action of metformin in pregnancy. Insulin-stimulated binding to IR is defective in GDM, leading to reduced autophosphorylation of the IR tyrosine residue and subsequent activation of IRS-1. Insulin-stimulated glucose uptake in skeletal muscles involves activation of IRS-1 that results in the production of PIP3. PIP3 production is required for activation of AKT and signalling of GLUT4 translocation. Increased inhibitory serine phosphorylation of IRS-1 is noted in women with GDM compared with normal obese non-diabetic subjects accompanied by impaired glucose uptake. Serine phosphorylation of IRS-1 leads to reduced translocation of GLUT4 to the plasma membrane and decreased insulin-stimulated glucose uptake to skeletal muscle. This is also linked with increased activation of JNK and PKC and enhanced activation of the mTOR-p70S6K pathway. Increased phosphorylation of p70S6K is noted in GDM. Insulin resistance in the second trimester is attributed to placental hormones including human placental lactogen, placental growth hormone, TNF-α and IL-6. Adiponectin (an insulin sensitisers) levels are lower in obesity and GDM, contributing to hyperglycaemia and increased activation of the mTOR pathway. Metformin treatment results in reduced systemic hyperglycaemia and hyperinsulinaemia. In addition, metformin causes upstream activation of AMPK, resulting in inhibition of the mTOR pathway. Metformin also has anti-inflammatory actions, which may improve pregnancy outcomes by altering placental development and function. Abbreviations: isotype 4 of glucose transporter (GLUT4), insulin receptor (IR), insulin receptor substrate 1 (IRS1), interleukin-6 (IL-6), mammalian target of rapamycin (mTOR), Jun kinase (JNK), nuclear factor kappa beta (NFκB), p70 ribosomal protein S6 kinase (p70S6K), phosphatidylinositol (3,4,5)-triphosphate (PIP3), protein kinase C (PKC), tumour necrosis factor alpha (TNFα), vascular endothelial growth factor (VEGF).
Pre-clinical models for metformin use in pregnancy
Several animal studies have demonstrated the link between maternal obesity, inflammation and significant and lasting metabolic effects in the offspring, particularly insulin resistance (Ainge et al., 2011). In pregnant rats, a reduction in maternal inflammatory cytokines (IL-6, CCL2, TNFα) has been observed following treatment with metformin. This is particularly relevant as these cytokines have been implicated in the development of insulin resistance and T2DM (Fried et al., 1998; Sartipy and Loskutoff, 2003; Ainge et al., 2011). In addition, in vitro studies of placental cell lines pre-treated with metformin demonstrated a significant reduction in IL-6 production in response to TNFα stimulation (Desai et al., 2013), and placental levels of TNF-α and IL-6 were also significantly reduced in metformin-treated animals compared with obese animals. It has been proposed that obesity contributes to placental inflammation through regulation of the NFκB pathway (Zhu et al., 2010). In the study by Desai et al., inhibition of NFκB reduced IL-6 production in response to TNFα stimulation and metformin treatment suppressed TNFα-induced degradation of IkBα, the natural inhibitor of NFκB nuclear localization and activation (Desai et al., 2013). In other studies, metformin has been shown to reduce proteasome activity which mediates IkBα degradation (Chen et al., 1995) and suppresses TNFα-induced NFκB activation in human vein umbilical endothelial cells (Hattori et al., 2006).

Additional pathways by which metformin may alter inflammatory signals in the placenta include mediators of oxidative stress (Srividhya et al., 2002) and the AMP kinase/mammalian target of rapamycin (mTOR) pathways (Fig. 2) (Nerstedt et al., 2010) although these signalling pathways have not been directly tested in placental models. Animal studies clearly provide an important opportunity to investigate the potential impacts of metformin during pregnancy in the context of both obesity and diabetes.

Current clinical studies in obese pregnant women
A number of studies have investigated the impact of lifestyle interventions on pregnancy outcomes with limited success (Oteng-Ntim et al., 2012); one such study is ongoing in the UK (UPBEAT). Whilst it seems logical that lifestyle interventions can impact favourably on pregnancy outcomes, it also seems likely that pharmacological interventions may be associated with a more significant metabolic effect. At time of writing two UK-based RCTs aim to investigate the effect of metformin on maternal and fetal outcomes in pregnancies complicated by maternal obesity. The ‘Metformin in Obese Non-diabetic Pregnant Women’ (MOP) (NCT01273584) trial is currently recruiting for a sample size of 546 women across seven centres. The primary outcome is a difference in birthweight centile and a number of secondary maternal and fetal outcomes including maternal weight gain, development of GDM and hypertensive complications, post-partum haemorrhage, Caesarean delivery, neonatal hypoglycaemia, jaundice and respiratory distress. The EMPOWAr (Efficacy of Metformin in Pregnant Obese Women: a Randomized controlled trial) (ISRCTN12798435) study is also currently recruiting in several centres in the UK. In addition to evaluating a primary outcome of birthweight z score corresponding to the gestational age and sex-adjusted birthweight centiles, this study will also evaluate a number of mechanistic outcomes. Maternal insulin resistance, hepatic and skeletal insulin sensitivity, maternal and neonatal inflammatory and lipid indices (C-reactive protein, IL-6, leptin, triglycerides and plasmogenic activator inhibitor (PAI)1/PAI2 ratio), placental glucocorticoid receptor expression and maternal brachial arterial endothelial dependent flow mediated dilatation will be assessed. Detailed measurements of neonatal body composition will also be obtained. This study aims to recruit 400 women.

Ultimately, in order to understand the effects of metformin treatment in pregnancy, long-term follow-up studies of the infants and children are necessary. Population studies have already demonstrated that pregestational maternal obesity is associated with a significant increase in fetal abdominal circumference and birthweight (Tanvig et al., 2013). It is hoped that studies such as the ‘Lifestyle in Pregnancy and Offspring—Comparison Between Children Born to Obese Women and Children Born to Normal Weight Women’ will provide valuable information related to the metabolic health of children, although studies such as these are likely to be confounded by other lifestyle factors. In addition to the MiG follow-up study (Rowan et al., 2011), ‘The MiTy Kids Trial’ is a follow-up to the MiTy Trial, which will determine whether treatment with metformin during pregnancy in women with T2DM will lead to a reduction in adiposity and improvement in insulin resistance in the offspring at 2 years of age. However, definitive answers on the long-term effects of metformin treatment in pregnancy will only be determined by larger, longer term studies which follow children for much longer than 2 years.

Metformin as an anti-cancer drug
Several large epidemiological studies first pointed to a role for metformin as an anti-cancer drug. A flurry of prospective observational studies suggested that patients with T2DM taking metformin were not only at lower risk of developing cancer (Libby et al., 2009) but were also less likely to die from it (Evans et al., 2005). These studies sparked excitement from cancer research scientists, who set off to demonstrate the anti-neoplastic effects of metformin in animal and pre-clinical models. Metformin was found to have a growth static effect on breast, prostate, pancreatic, ovarian and endometrial cancer cell lines, amongst others, with demonstrable impact on glucose metabolism and PI3K-Akt-mTOR pathway inhibition (Fig. 1) (Zakhikhan et al., 2006, 2008, 2010; Cantrell et al., 2010; Sarfstein et al., 2013). Metformin has a well-established safety profile and its extensive use in diabetic patients, including those diagnosed with and undergoing treatment for cancer, suggests that it is safe to be used in the anti-cancer setting. This has encouraged bypassing of the traditional drug development paradigm and early translation of laboratory-based research findings to the clinic.

Proof of principle window of opportunity studies
Promising data from pre-clinical research inspired proof of principle intervention studies that tested the effects of metformin in newly diagnosed cancer patients awaiting standard surgical care. This clinical setting was ideal as it allowed single-agent metformin to be tested in an uncontaminated therapeutic period without compromising standard patient care. Most of the early phase clinical studies have compared tumour biopsies taken before and after metformin treatment in the pre-surgical window period between diagnosis and surgery. These so-called ‘window’ studies offer a unique opportunity to test the biological effects of metformin in proof of principle analyses that use surrogate markers of early clinical response as primary end-points. The most widely used primary outcome measure in studies thus far has been the Ki-67
proliferation index, where the proportion of tumour cells actively dividing is measured by immunohistochemistry using antibodies directed against Ki-67. Proliferation is a hallmark of cancer (Hanahan and Weinberg, 2011) and Ki-67 is a nuclear protein that is only expressed by proliferating cells. Ki-67 has been extensively validated as a prognostic and predictive biomarker of clinical response in breast cancer (Dowsett et al., 2007), and subsequently extrapolated for similar uses in different cancer types. Other studies have examined the proportion of tumour cells undergoing programmed cell death, or apoptosis, before and after metformin treatment, as measured by TUNEL (terminal deoxynucleotidyl transferase dUTP nick end labelling). Reduced apoptosis is another classical feature of malignancy.

Most of the work published thus far has focused on breast cancer. Two small, uncontrolled pilot studies (n = 55 and n = 39, respectively) found a significant reduction in Ki-67 expression by breast tumours following the short-term pre-surgical administration of metformin (mean 15.9 days, range 13–21 days) and median 18 days (range 13–40), respectively) (Hadad et al., 2011; Niraula et al., 2012). A larger placebo-controlled trial (n = 200) went on to show that metformin reduced tumour Ki-67 expression (by ~10%) in overweight or insulin-resistant breast cancer patients, but not in the whole metformin-treated population (Bonanni et al., 2012). Niraula et al. additionally reported an increase in tumour apoptosis, as assessed by TUNEL, following short-term administration of metformin (Niraula et al., 2012). A placebo-controlled study was not able to replicate these findings, however, with increased tumour apoptosis seen only in non-insulin-resistant breast cancer patients following subgroup analysis (Cazzanga et al., 2013), findings that were contrary to the conventional hypothesis. Such conflicting results may be explained by small patient numbers and study heterogeneity, particularly with respect to the histological subtypes included in the studies, the duration and dose of metformin treatment received, and the range of BMI and insulin resistance observed at baseline in the different patient populations examined.

Besides breast cancer, an early phase clinical study of prostate cancer treated pre-surgically with metformin has also yielded promising results. In a study involving 24 patients who received neoadjuvant metformin for a median duration of 41 days (range 18–81 days) prior to radical prostatectomy, metformin reduced the Ki-67 proliferation index by an average of 29% compared with the pretreatment biopsy (Joshua et al., 2013). Several studies are also underway to test the pre-surgical administration of metformin in endometrial cancer (NCT01911247, NCT01205672 and ISRCTN81570194). Preliminary findings from one group found a reduction in tumour Ki-67 expression in sixteen obese patients with endometrial cancer following short-term pre-surgical administration of metformin (mean 14.5 days). Metformin treatment was associated with a reduction in Ki-67 proliferation index (mean 19.5%) in 10 of 16 patients (Schuler et al., 2013). Two other groups with similar study designs are actively recruiting and read-outs from these studies are eagerly awaited.

**Metformin to treat endometrial cancer**

Targeting tumour metabolism would seem a rational therapeutic intervention in cancers linked to obesity and insulin resistance. A comprehensive systematic review and meta-analysis of obesity and risk of all cancers found that endometrial cancer is the most strongly associated with obesity (Renehan et al., 2008). Excess body fat increased endometrial cancer risk in a dose-dependent manner, with every 5 kg/m² increase in BMI conferring a 1.6-fold (95% CI 1.5, 1.68) increased risk. This effect was observed even at extremely obese BMIs; indeed, a woman with a BMI of 42 has a near 10-fold increased risk of type I (also known as endometrioid) endometrial cancer than her normal weight counterparts (BMI 20–25 kg/m²) (Crosbie et al., 2010). Insulin resistance is also a recognized risk factor for type I endometrial cancer. Women with T2DM have a 2-fold increased risk of endometrial cancer compared with non-diabetic controls according to a large meta-analysis of 16 studies (relative risk (RR) 2.10, 95% CI 1.75–2.53) (Fnberg et al., 2007). One prospective study of endometrial cancer patients found 30% had T2DM and a further 36% had previously undiagnosed insulin resistance, which was independently and significantly associated with increasing BMI (P < 0.001) (Burzawa et al., 2011). Large case–control studies have also shown that diabetes has a supramultiplicative effect with BMI on endometrial cancer risk (Svalingam and Crosbie, 2013). Using non-diabetic, non-obese women as the referent group, the endometrial cancer risk was 1.4 (95% CI 0.9, 2.4) for non-obese diabetic women, 2.3 (95% CI 1.8, 3.0) for obese non-diabetic women, and 5.1 (95% CI 3.0–8.7) for obese diabetic women (Lucente-forte et al., 2007).

If obesity and insulin resistance are important drivers of endometrial cancer, metformin may inhibit tumour growth both by reducing its nutrient supply (glucose) and by thwarting its growth-stimulatory environment (reduced insulin and IGF levels) (Fig. 1). Thus metformin may be useful post-hysterectomy, in the adjuvant setting, to prevent recurrence and improve long-term survival from endometrial cancer. Animal studies have shown that combining metformin treatment with conventional cytotoxic agents can overcome tumour chemoresistance and inhibit re-emergence of cancer stem cells (Hirsch et al., 2009). Indeed, diabetic patients with breast cancer on metformin were shown to have higher rates of pathological complete response to neoadjuvant chemotherapy than those not on metformin, according to a retrospective study of 2529 patients, including 68 and 87 diabetic patients taking and not taking metformin, respectively (Jiralerspong et al., 2009). While there are currently no studies testing metformin for endometrial cancer in the adjuvant setting, a phase III randomized trial of adjuvant metformin versus placebo in 3649 early-stage non-diabetic breast cancer patients is underway and due to report in 2016 (Goodwin et al., 2011). This study is powered to look at recurrence-free survival as the primary outcome measure.

**Metformin to prevent endometrial cancer**

Besides possible applications in the adjuvant setting, it is logical that metformin, by reducing the carcinogenic effects of obesity and insulin resistance, could be used as a long-term chemopreventative in women at high risk of endometrial cancer. Identification of a high risk group is important, and this may include women with PCOS, morbid obesity, impaired glucose tolerance and/or endometrial hyperplasia. Several case reports and one small randomized open label study have demonstrated resolution of endometrial hyperplasia following treatment with metformin, including two separately reported individual cases of progesterin-resistant atypical hyperplasia (Session et al., 2003; Shen et al., 2008). Metformin treatment in a rat model reverses endometrial hyperplasia (Tas et al., 2013) possibly through a reduction in S6, a downstream kinase of the mTOR pathway (Fig. 2) (Erdemoglu et al., 2009). These data are
encouraging but a definitive chemoprevention trial of metformin in high risk women would require thousands of patients engaged in long-term follow-up if a clinical end-point (e.g. incident endometrial cancer) were used as the primary outcome measure. Initial proof of principle studies are therefore using surrogate markers of early clinical response as primary end-points, specifically effects on insulin signalling and cellular proliferation. One such study plans to recruit 100 obese, insulin-resistant (although not frankly diabetic) post-menopausal women aged 50–65 years to a study that compares the effects of metformin plus lifestyle changes (dietary advice and supervised exercise); placebo plus lifestyle changes; metformin alone; and placebo alone on endometrial Ki-67 expression and biomarkers of endometrial mTOR inhibition and insulin resistance (NCT01697566).

**Epidemiological evidence for metformin as anti-cancer drug**

Despite initial promise, the epidemiological data linking metformin use in T2DM patients to reduced cancer risk are inconsistent. One meta-analysis of eleven retrospective studies, 4042 cancer events and 529 cancer deaths, concluded that metformin reduces cancer risk by one-third (DeCensi et al., 2010). This effect was limited to certain cancer types, specifically pancreatic and hepatocellular cancer, with a non-significant reduction also observed in colon, breast and prostate cancer. A meta-analysis of fourteen RCTs did not concur with these findings, however, reporting no association between metformin use and cancer risk. The authors acknowledge that their findings are limited by the heterogeneity of the included trials, absent cancer data from two, and a relatively short follow-up period (average 4.1 years). Many of the studies involved treatment with several glucose-lowering agents in combination, and it is unclear whether other hypoglycaemics actually increase cancer risk, rather than metformin reducing it (Stevens et al., 2012). Those studies that specifically tested metformin monotherapy did find a non-significant 16% reduction in cancer risk in patients taking metformin, but CIs were wide due to small numbers in the subgroup analyses. A recent case-control analysis derived from the UK-based General Practice Research Database specifically explored the association between metformin use and endometrial cancer risk over a 17-year follow-up period. Comparing 2554 endometrial cancer cases with 529 controls matched on age, clinical stage, grade and adjuvant therapy. The lack of association between metformin use and endometrial cancer risk is consistent with those not using metformin and those without diabetes. This association was significant after adjusting for age, clinical stage, grade and adjuvant therapy. The lack of association between metformin use and overall survival in type 1 endometrial cancers may be explained by fewer deaths in this group (14% of patients with type 1 endometrial cancer died versus 52% of patients with type 2 disease) (Nevadunsky et al., 2013). Another study of similar design found improved recurrence-free and overall survival in metformin users with endometrial cancer, after controlling for age, stage, grade, histology and adjuvant treatment. Non-metformin users had 1.7-fold worse RFS (95% CI, 1.3–2.6, P = 0.01), and were 2.3-fold more likely to die compared with metformin users (95% CI 1.3–4.2, P = 0.005) (Ko et al., 2013).

**Putative anti-cancer mechanisms**

The mechanisms that underlie metformin’s putative anti-cancer activity are incompletely understood. Insulin and IGFs are strongly mitogenic and their stimulatory effect on cancer cell growth and metastasis is well established (Wang et al., 2012; Ferguson et al., 2013). Although normal target tissues (liver, skeletal muscle and adipose tissue) show reduced sensitivity to insulin in the context of hyperinsulinaemia, tumour cells may remain exquisitely sensitive to it and continue to respond to its stimulation (Pollak, 2012). High circulating insulin levels increase hepatic IGF-1 production but reduce IGF binding protein synthesis, resulting in a net increase in circulating, bioavailable IGF-1. Specific interactions between insulin, IGF-1 and their respective receptors trigger a cascade of downstream events that ultimately drive cellular proliferation through activation of the PI3K-AKT-mTOR pathway (Fig. 3) (Pollak, 2008). The PI3K-AKT-mTOR pathway is frequently up-regulated in many cancers and is associated with resistance to chemotherapeutic drugs.

Metformin activates AMPK, a potent inhibitor of the PI3K-AKT-mTOR pathway. This has been demonstrated in numerous pre-clinical cancer models, including endometrial cancer cell lines, where a supratherapeutic concentration of metformin stops cellular proliferation, induces phosphorylation of AMPK, and reduces S6 phosphorylation, a downstream target of the mTOR pathway (Fig. 3). Metformin was shown to induce Gl cell cycle arrest, increase apoptosis and reduce human telomerase reverse transcriptase expression (Cantrell et al., 2010; Sarfstein et al., 2013). These findings were confirmed in a mouse xenograft model where metformin reduced 56 phosphorylation and decreased mean tumour weight (Iglesias et al., 2013).

Recent studies have demonstrated that metformin also inhibits mTOR through AMPK-independent pathways, including the Rag family of GTPases (Kalender et al., 2010) and the hypoxia inducible factor (HIF) target gene, regulated in development and DNA damage response 1 (REDDI) (Ben Sahra et al., 2008, 2011). Iglesias et al. (2013) showed that treating endometrial cancer cells with metformin causes displacement of constitutively active KRAS from the cell membrane resulting in uncoupling of the mitogen-activated protein kinase (MAPK) signalling pathway (Fig. 3) (Iglesias et al., 2013). Additional anti-cancer activity that may be especially relevant in endometrial carcinogenesis includes anti-aromatase activity (Brown et al., 2010), leading to a reduction in circulating oestrogen levels in obese women, and increased progesterone receptor expression by endometrial cancer cells (Xie et al., 2011). Metformin also has anti-angiogenic effects (Liao et al., 2012), directly scavenges free radicals and can block endogenous reactive oxygen species. In an in vitro model, the latter effects significantly reduced DNA damage and mutation rates (Fig. 2) (Algiere et al., 2012), offering an explanation for the reduced risk of cancer seen in metformin users across several epidemiological studies.

**Metformin to treat ovarian cancer**

Whilst the association with obesity is less pronounced than it is for endometrial cancer, a systematic review found ovarian cancer was slightly more common in obese women (BMI > 30 kg/m², OR 1.3, 95% CI 1.1–1.5) (Joly et al., 1974) and a prospective cohort study showed
increased mortality from ovarian cancer amongst overweight (RR 1.16, 95% CI 1.04–1.30) and obese women (RR 1.26, 95% CI 1.07–1.48) compared with normal weight women (Rodriguez et al., 2002). Furthermore, a recent systematic review and meta-analysis of 19 studies found an increased risk of ovarian cancer in diabetic women (RR 1.17, 95% CI 1.02–1.33), which persisted after adjusting for age, BMI, smoking and alcohol intake (RR 1.55, 95% CI 1.11–2.19) (Lee et al., 2013).

The effect of metformin on ovarian cancer risk has been studied in a retrospective case–control analysis, where long-term use of metformin was associated with lower rates of ovarian cancer (OR 0.61, 95% CI 0.3–1.25) (Bodmer et al., 2011). A further case–control analysis found an association between metformin and improved survival from ovarian cancer (5-year disease-specific survival for cases versus controls, 73 versus 44%; $P = 0.002$). When considering epithelial ovarian cancer alone, and after adjusting for age, stage, optimal cytoreduction, serous histology and platinum chemotherapy, women taking metformin had improved 5-year survival rates compared with controls (67 versus 47%; $P = 0.007$) (Kumar et al., 2013).

Proof of principle window studies to assess the effects of metformin treatment prior to definitive surgery are more challenging in the context of ovarian cancer, where diagnosis is suspected but not confirmed in the majority until laparotomy. Taking a biopsy from an apparently early-stage ovarian cancer could result in spillage of malignant cells into the abdominal cavity, upstage the patient and mandate adjuvant chemotherapy, where this may have been avoided. However, a study using metformin in combination with (neo)adjuvant chemotherapy and

---

**Figure 3** Putative mechanisms of action of metformin in obesity and neoplasia. Metformin reduces systemic insulin, IGF-1 and estrogen, thereby decreasing tyrosine kinase receptor signalling to downstream targets such as the AKT/mTORC1 pathway. Intracellularly, metformin activates AMPK and induces phosphorylation of the TSC1/2 and subsequent inhibition of mTORC1. This leads to decreases protein synthesis and cell growth and increases autophagy and apoptosis. AMPK activation also inhibits ACC, decreasing fatty acid synthesis. Inhibition of mTORC1 also occurs through AMPK-independent pathways, including the hypoxia inducible factor gene, REDD1 and K-ras induced uncoupling of the MAPK/ERK pathway. Anti-inflammatory actions have been reported including reduced cytokine levels. Anti-angiogenic effects and reduced endogenous reactive oxygen species are also observed after metformin treatment. Abbreviations: Acetyl-CoA carboxylase (ACC), eIF4E binding protein (4EBP1), estrogen receptor (ER), Insulin receptor (IR), Insulin-like growth factor 1 receptor (IGF1R), Insulin receptor substrate 1 (IRS1), interleukin-6 (IL-6) mitogen-activated protein kinase/extracellular receptor kinase (MAPK/ERK), mammalian target of rapamycin complex 1 (mTORC1), tuberous sclerosis complex (TSC), reactive oxygen species (ROS), regulated in development and DNA damage responses 1 (REDD1), ribosomal protein S6 (S6).
**Table 1** Established and novel indications for metformin in human pregnancy and reproductive health and the potential effects on the common pathophysiological drivers.

<table>
<thead>
<tr>
<th>Pathophysiological driver</th>
<th>Effect of metformin</th>
<th>Clinical impact</th>
<th>Proposed mechanism of action</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregnancy</td>
<td></td>
<td>↓ Maternal weight gain and neonatal hypoglycaemia in GDM and obesity&lt;br&gt;↓ Insulin resistance in the infant of obese and diabetic mothers with possible metabolic reprogramming of the infant</td>
<td>• ↓ Hepatic glucose output and substrate activation of IR/IGF1R&lt;br&gt;• ↑ AMPK activation and mTOR inhibition</td>
<td>Ali et al. (2011), Balani et al. (2009), Catalano et al. (1991), Hague et al. (2003), Lautatzi et al. (2013), Nersted et al. (2010), Niromaneshe et al. (2012), Rowan et al. (2008), Rowan et al. (2011), Stumvoll et al. (1995), Bonanni et al. (2011), Cantrell et al. (2010), Erdemoglu et al. (2009), Hadad et al. (2011), Nirlaula et al. (2012), Session et al. (2003), Shen et al. (2008), Tas et al. (2013), Zakhikhan et al. (2006), Tang et al., (2006a, b), Tso et al. (2009)</td>
</tr>
<tr>
<td>Anti-cancer</td>
<td>Prevention and treatment</td>
<td>• ↓ Tumour growth&lt;br&gt;• ↑ Reverses endometrial pre-cancerous change</td>
<td>• ↓ PI3K/AKT/mTOR pathway activation&lt;br&gt;• ↓ MAPK/ERK pathway activation</td>
<td>Attia et al. (2001), Brewer et al. (2010), De Leo et al. (2000), Diamanti-Kandarakis et al. (2010), Morin-Papunen et al. (2012), Nestler and Jakubowicz (1996), Tang et al. (2006a, b), Ali et al. (2011), Balani et al. (2009), Catalano et al. (1991), Hague et al. (2003), Lautatzi et al. (2013), Nerstedt et al. (2010), Niromaneshe et al. (2012), Rowan et al. (2008), Rowan et al. (2011), Stumvoll et al. (1995), Bonanni et al. (2011), Cantrell et al. (2010), Erdemoglu et al. (2009), Hadad et al. (2011), Nirlaula et al. (2012), Session et al. (2003), Shen et al. (2008), Tas et al. (2013), Zakhikhan et al. (2006), Tang et al., (2006a, b), Tso et al. (2009)</td>
</tr>
<tr>
<td>Inflammation and oxidative stress</td>
<td>Polycystic ovary syndrome</td>
<td>• ↓ Incidence of severe OHSS in women with PCOS undergoing IVF</td>
<td>• ↓ Response of polycystic ovaries to exogenous stimulation&lt;br&gt;• ↓ Secretion of VEGF</td>
<td>Attia et al. (2001), Brewer et al. (2010), De Leo et al. (2000), Diamanti-Kandarakis et al. (2010), Morin-Papunen et al. (2012), Nestler and Jakubowicz (1996), Tang et al. (2006a, b), Ali et al. (2011), Balani et al. (2009), Catalano et al. (1991), Hague et al. (2003), Lautatzi et al. (2013), Nerstedt et al. (2010), Niromaneshe et al. (2012), Rowan et al. (2008), Rowan et al. (2011), Stumvoll et al. (1995), Bonanni et al. (2011), Cantrell et al. (2010), Erdemoglu et al. (2009), Hadad et al. (2011), Nirlaula et al. (2012), Session et al. (2003), Shen et al. (2008), Tas et al. (2013), Zakhikhan et al. (2006), Tang et al., (2006a, b), Tso et al. (2009)</td>
</tr>
<tr>
<td>Pregnancy</td>
<td></td>
<td>• Alters placental development and function&lt;br&gt;• ↓ Vascular endothelial activation&lt;br&gt;• ↑ Improve pregnancy outcomes</td>
<td>• ↓ maternal inflammatory cytokines (IL-6, CCL2, TNFα)&lt;br&gt;• ↓ TNFα induced NFκB activation</td>
<td>Attia et al. (2001), Brewer et al. (2010), De Leo et al. (2000), Diamanti-Kandarakis et al. (2010), Morin-Papunen et al. (2012), Nestler and Jakubowicz (1996), Tang et al. (2006a, b), Ali et al. (2011), Balani et al. (2009), Catalano et al. (1991), Hague et al. (2003), Lautatzi et al. (2013), Nerstedt et al. (2010), Niromaneshe et al. (2012), Rowan et al. (2008), Rowan et al. (2011), Stumvoll et al. (1995), Bonanni et al. (2011), Cantrell et al. (2010), Erdemoglu et al. (2009), Hadad et al. (2011), Nirlaula et al. (2012), Session et al. (2003), Shen et al. (2008), Tas et al. (2013), Zakhikhan et al. (2006), Tang et al., (2006a, b), Tso et al. (2009)</td>
</tr>
<tr>
<td>Anti-cancer</td>
<td>Prevention and treatment</td>
<td>• ↓ Migration and metastasis&lt;br&gt;• ↓ DNA damage and mutation rates</td>
<td>• Anti-angiogenic effects and ↑ REDD1 expression&lt;br&gt;• Blocks production of endogenous reactive oxygen species</td>
<td>Attia et al. (2001), Brewer et al. (2010), De Leo et al. (2000), Diamanti-Kandarakis et al. (2010), Morin-Papunen et al. (2012), Nestler and Jakubowicz (1996), Tang et al. (2006a, b), Ali et al. (2011), Balani et al. (2009), Catalano et al. (1991), Hague et al. (2003), Lautatzi et al. (2013), Nerstedt et al. (2010), Niromaneshe et al. (2012), Rowan et al. (2008), Rowan et al. (2011), Stumvoll et al. (1995), Bonanni et al. (2011), Cantrell et al. (2010), Erdemoglu et al. (2009), Hadad et al. (2011), Nirlaula et al. (2012), Session et al. (2003), Shen et al. (2008), Tas et al. (2013), Zakhikhan et al. (2006), Tang et al., (2006a, b), Tso et al. (2009)</td>
</tr>
<tr>
<td>Cancer stem cells</td>
<td>Anti-cancer drug</td>
<td>• Improves efficacy of existing chemotherapy&lt;br&gt;• Overcomes chemoresistance</td>
<td>• Inhibits re-emergence of cancer stem cells</td>
<td>Attia et al. (2001), Brewer et al. (2010), De Leo et al. (2000), Diamanti-Kandarakis et al. (2010), Morin-Papunen et al. (2012), Nestler and Jakubowicz (1996), Tang et al. (2006a, b), Ali et al. (2011), Balani et al. (2009), Catalano et al. (1991), Hague et al. (2003), Lautatzi et al. (2013), Nerstedt et al. (2010), Niromaneshe et al. (2012), Rowan et al. (2008), Rowan et al. (2011), Stumvoll et al. (1995), Bonanni et al. (2011), Cantrell et al. (2010), Erdemoglu et al. (2009), Hadad et al. (2011), Nirlaula et al. (2012), Session et al. (2003), Shen et al. (2008), Tas et al. (2013), Zakhikhan et al. (2006), Tang et al., (2006a, b), Tso et al. (2009)</td>
</tr>
</tbody>
</table>

Abbreviations: AMP-activated kinase (AMPK), chemokine (CC-motif) ligand 2 (CCL2), cytokine P450c17 enzyme (CYP17), insulin-like growth factor 1 (IGF-1), insulin-like growth factor binding protein-1 (IGFBP-1), insulin-like growth factor receptor 1 (IGFIR), interleukin-6 (IL-6), insulin receptor (IR), mammalian target of rapamycin (mTOR), mitogen-activated protein kinase /extracellular receptor kinase (MAPK/ERK), NF-kappa beta (NFκB), ovarian hyperstimulation syndrome (OHSS), polycystic ovary syndrome (PCOS), phosphatidylinositol 3-kinase (PI3K), protein kinase B (AKT), regulated in development and DNA damage responses 1 (REDD1), tumour necrosis factor alpha (TNFα), type 2 diabetes mellitus (T2DM).
surgical debulking in ovarian cancer is actively recruiting (NCT01579812). The hypothesis is that metformin will act as an anti-cancer stem cell agent, enhancing the efficacy of (neo)adjuvant chemotherapy by preventing the emergence of chemoresistant clones.

Conclusions

Metformin has several established and emerging applications in reproductive healthcare, pregnancy and gynaecological cancer (Table I). Its recognized impact as an insulin sensitizing drug has been exploited in the treatment of GDM and for cardiovascular risk reduction in PCOS and obesity. Whilst its effects on fertility in PCOS is uncertain, it remains a promising candidate for further scrutiny because of its affordability and low toxicity profile. Its extensive safety credentials make it an obvious choice for application in pregnancy, where it can be difficult to establish safety parameters of new drugs. In obese pregnant women, metformin may not only improve maternal outcomes, but also initiate favourable metabolic reprogramming in the fetus. In addition to these indications, epidemiological evidence as well as pre-clinical and early phase clinical trials have indicated a role for metformin in the prevention and treatment of cancer. Its potential in this setting is unproven but extremely promising.

Authors’ roles

All authors contributed to the conception and design of this review and drafted the manuscript. Critical revisions were finalized by V.N.S. and E.J.C. The final version has been approved by all authors.

Funding

V.N.S. is a clinical research fellow who is supported by a Wellbeing of Women/Wellcome Trust Research Training Fellowship. J.M. and E.J.C. are NIHR Clinician Scientists and Senior Lecturers at the University of Manchester. S.N. and A.H.B. are employed by Leeds Teaching Hospitals NHS Trust. No additional funding was required to complete this work.

Conflict of interest

V.N.S., J.M., S.N., A.H.B. and E.J.C. report no conflicts of interest.

References


Asta GR, Rainey WE, Carr BR. Metformin directly inhibits androgen production in human thecal cells. Fertil Steril 2001;76:517 – 524.


Fedorcsak P, Dale PO, Storeng R, Tanbo T, Abyholm T. The impact of obesity and respiratory chain complex I.


