DIABETIC Medicine
DIABETES UK CARE. CONNECT. CAMPAIGN.

The risk of pre-eclampsia in women taking metformin: systematic review and meta-analysis

Journal:	*Diabetic Medicine*
Manuscript ID	DME-2017-00434
Manuscript Type:	Systematic Review or Meta-analysis
Date Submitted by the Author:	27-Jun-2017
Complete List of Authors:	Alqudah, Abdelrahim; Queen's University Belfast, Centre for Experimental Medicine McKinley, Michelle; Queens University Belfast, Centre for Public Health McNally, Ross; Queen's University Belfast, Centre for Experimental Medicine Graham, Una; Queen's University Belfast, Centre for Public Health; Royal Victoria Hospital, Regional Centre for Diabetes and Endocrinology Watson, Chris; Queen's University Belfast, Centre for Experimental Medicine Lyons, Timothy; Queen's University Belfast, Centre for Experimental Medicine; Medical University of South Carolina, Division of Endocrinology and Diabetes McClements, Lana; Centre for Experimental Medicine, School of Medicine, Dentistry and Biomedical Sciences
Keywords:	pregnancy, gestational diabetes, metformin, insulin therapy, other complications

Full title: The risk of pre-eclampsia in women taking metformin: systematic review and meta-analysis

Running head: Metformin and the risk of pre-eclampsia

A ALQUDAH[1], MC MCKINLEY[2], R MCNALLY[1], U GRAHAM[2,3], CJ WATSON[1], TJ LYONS[1,4], and L MCCLEMENTS[1]

[1]Centre for Experimental Medicine; School of Medicine, Dentistry and Biomedical Sciences, Queen's University Belfast
[2]Centre for Public Health; School of Medicine, Dentistry and Biomedical Sciences, Queen's University Belfast
[3]Belfast Health and Social Care Trust; Royal Victoria Hospital, Belfast
[4] Division of Endocrinology and Diabetes, Medical University of South Carolina, Charleston, SC 29425, USA

Corresponding author: Dr Lana McClements; The Wellcome-Wolfson Building, Centre for Experimental Medicine, School of Medicine, Dentistry and Biomedical Sciences, Queen's University Belfast, 97 Lisburn road, Belfast, BT9 7BL; Email: l.mcclements@qub.ac.uk; Telephone number: +44 28 9097 6474. Fax: +44 28 90972451.

Word count: 4,513 words.

Funding: This study was supported by Queen's University Belfast international PhD funding received from The Hashemite University, Jordan.

Conflicts of Interest: No potential conflicts of interest relevant to this article were reported.

Novelty statement:

- This study's novel results demonstrate that metformin ± insulin treatment is linked to the lower incidence of pre-eclampsia than insulin treatment alone in women with GDM or Type 2 DM, which is likely to be age-dependent and associated with reduced weight gain during pregnancy.
- Surprisingly, in other high-risk pregnancies where glucose-lowering agents are not essential, metformin does not appear to be beneficial.
- Current clinical guidelines which stipulate insulin treatment as a first line choice in pregnancies complicated by diabetes should be reviewed in light of these findings and adequately designed RCTs with pre-eclampsia as a primary outcome carried out urgently.

Abstract

AIMS

Hypertensive disorders in pregnancy, particularly pre-eclampsia, are leading causes of maternal or foetal morbidity and mortality, and effective treatments are lacking. The aim of this study was to perform meta-analyses of studies evaluating the risk of pre-eclampsia in high-risk insulin-resistant women taking metformin prior or during pregnancy.

METHODS

Medline, EMBASE, Web of Science and Scopus databases were searched. Both randomised controlled trials [RCTs] and prospective observational studies of metformin treatment vs. placebo/control or insulin, either prior to or during pregnancy, were selected. The main outcome measure was the incidence of pre-eclampsia in each treatment group.

RESULTS

Overall, in nine studies comparing metformin treatment [n=1,281] to placebo/control [n=1,341], no difference in the risk of pre-eclampsia was demonstrated [combined/pooled RR=0.98; 95% CI 0.53-1.82; p=0.95; I^2=54%]. Restricting analysis to five RCTs again showed no significant effect [RR=0.86; 95% CI 0.33-2.26; p=0.76; I^2=66%]. However, a meta-analysis of nine studies comparing metformin [n=1,303] to insulin [n=1,235] showed reduced risk of pre-eclampsia with metformin [RR=0.71; 95% CI 0.53-0.96; p=0.03; I^2=0%], also seen when analyses were restricted to the eight RCTs [n=1,674; RR=0.68; 95% CI 0.48-0.95; p=0.02; I^2=0%]. High levels of heterogeneity were present in studies comparing metformin to placebo/control. Pre-eclampsia was a secondary outcome in most of the studies. Mean weight gain from enrolment to delivery was lower in metformin group [p=0.05, metformin vs. placebo; p=0.004, metformin vs. insulin].

CONCLUSIONS

In studies randomising pregnant women to glucose-lowering therapy, metformin is associated with a lower risk of pre-eclampsia than insulin.

Introduction

Pre-eclampsia is a complication of pregnancy that occurs in the second half of gestation. It is defined as the new onset, after 20 weeks gestation, of hypertension (≥140/90 mmHg) and proteinuria (≥300 mg per 24 h), or in the absence of proteinuria, any of the following: thrombocytopenia (platelets<100,000/μl), impaired liver function, progressive renal insufficiency, pulmonary oedema or cerebral or visual disturbances[1]. Pre-eclampsia is classified according to gestational age at onset: term pre-eclampsia (onset ≥37 weeks), preterm pre-eclampsia (34-37 weeks), and early-onset pre-eclampsia (<34 weeks)[2]. Severe features of pre-eclampsia include blood pressure ≥160/110 mmHg, thrombocytopenia, impaired liver function, progressive renal insufficiency, pulmonary oedema, cerebral or visual disturbances[1]. Pre-eclampsia is the leading cause of maternal and foetal morbidity and mortality: by conservative estimates, it affects 10 million pregnant women worldwide annually, and is responsible for 76,000 maternal and 500,000 infant deaths[3]. In addition to the short-term risks, pre-eclampsia is associated, later in life, with cardiovascular disease and/or Type 2 diabetes mellitus (Type 2 DM) in both mothers and offspring(4,5).

The incidence of pre-eclampsia is ~4-6% in the general population, but is greatly increased by insulin-resistant disorders such as gestational diabetes (GDM), Type 2 DM, polycystic ovary syndrome (PCOS) and obesity(6–8). In women with pre-gestational diabetes, whether Type 1 or Type 2 DM, the risk for pre-eclampsia in increased approximately four-fold(9,10).

There are currently no reliable biomarkers or effective preventative measures, and no treatments for pre-eclampsia other than delivery. The pathogenesis of pre-eclampsia is linked to aberrant angiogenesis and inadequate remodelling of the spiral uterine artery, later leading to the development of an ischemic placenta; however, understanding of underlying mechanisms is inadequate, impeding the rational for development of preventative and treatment strategies.

A few therapeutic approaches have been explored. Low dose aspirin (75 mg daily) may reduce the incidence of pre-eclampsia by up to 25% if taken before 16 weeks gestation(11), and is recommended from 12 weeks gestation for women who have one or more of the following risk factors: history of pre-eclampsia, multifoetal gestation, chronic hypertension, diabetes (Type 1 or 2 DM), renal disease or autoimmune disease (e.g. systemic lupus erythematous, antiphospholipid syndrome). Anti-oxidant supplements have also been assessed, but so far failed to show benefit in the prevention of pre-eclampsia(10).

Metformin can be safely used in pregnancy[12] enabling investigation of its effects on pregnancy outcomes in women who are at higher risk such as obese women or women with GDM, Type 2 DM, or PCOS[13–16]. Metformin reduces insulin resistance, and mitigates endothelial dysfunction and hyperglycaemia, factors which have been associated with pre-eclampsia[17,18]. Metformin is an AMPK activator, and reduced AMPK pathway activity has also been implicated in the pathogenesis of pre-eclampsia[19,20]. It is established that the circulating anti-angiogenic factor, fms-like tyrosine kinase 1 (sFlt-1) is significantly increased in pregnancies complicated by pre-eclampsia, and recently, it was demonstrated that metformin can reduce sFlt-1 secretion from placental tissue and placental explants[21]. Metformin therefore warrants investigation as a preventive treatment for pre-eclampsia.

The aim of this systematic review and meta-analysis is to evaluate evidence concerning the efficacy of metformin compared to placebo/control or insulin in reducing the incidence of pre-eclampsia in high-risk pregnant women using randomised controlled trials (RCTs) and prospective observational studies or RCTs alone.

Methods

Data sources and searches

A systematic literature search was conducted using Medline (1946), Embase (1974), Web of Science, and Scopus databases for eligible studies from inception until November 2016. Filters were not used for the type of study or language, however only studies in human populations were included. In collaboration with the subject librarian (RF) at the Medical Library, Queen's University Belfast, the following terms and keywords were used: a) "Metformin or Glucophage", b) "Pre-eclampsia or Pre-eclamp* or Preeclampsia or Preeclamp*" and c) "Gestational hypertension or Pregnancy-induced hypertension". Combinations of a) AND b) or a) AND c) were also used.

Study selection

Only those studies that met all of the following criteria were considered: 1) original study; 2) RCT or prospective observational study/cohort study (CS); 3) women took metformin before pregnancy and/or during pregnancy, and 4) women were followed throughout the pregnancy and pregnancy outcomes were recorded. We only included studies in which pre-eclampsia was diagnosed based on the following criteria: at least two consecutive blood pressure measures ≥140/90 mm Hg with proteinuria (≥0.3 g per 24 hours or 2+ on dipstick testing), with documented onset after gestational week 20. Three studies were included that defined and diagnosed pre-eclampsia in women with new onset hypertension in the absence of proteinuria but with one of the following: haematological involvement, liver involvement, neurological involvement, pulmonary oedema, foetal growth restriction or placental abruption[13,14,22].

As depicted in the PRISMA flow chart (Fig. 1), the database search and literature screening yielded 364 studies. After removing duplicates, two reviewers (AA and LM) screened titles

and abstracts of remaining 321 articles. Following initial screening 293 articles were excluded because they did not meet the inclusion criteria and were outside of the scope of the review. Two reviewers (AA and LM) assessed full text of remaining 28 articles. Three were excluded because they repeated findings from another included study. Three studies did not clearly define pre-eclampsia. Three studies were excluded because corresponding authors, contacted for needed clarification, provided no responses[23–25]. One observational study was excluded because of poor matching of BMI in the treatment groups[26]. This yielded total number of 18 studies. Selected studies compared treatment with metformin to or healthy control (n=5)[22,27–30] or placebo (n=4)[14,16,31,32] or insulin (n=10)[15,22,31,33–39] (one study, with three treatment arms, was included both in metformin vs. control and metformin vs. insulin analysis[22]). The groups of women recruited into selected studies included women with GDM (n=7)[13,15,22,35–38], Type 2 DM (n=1)[34], PCOS (n=6)[16,28–30,32,40], obese women (n=2)[14,31], and women with both GDM and Type 2 DM (n=2)[37,39].

Data Extraction

The following data were extracted from the 18 studies selected: study characteristics (author, year of publication, country), population characteristics (age, body mass index (BMI) at enrolment, blood pressure at baseline, weight change during pregnancy, glycaemic control), treatment design (number of women on metformin or placebo///insulin, dose of metformin and duration of treatment) and outcomes (primary and secondary outcomes) Table 1.

Quality Assessment

Quality assessment of the included studies was independently performed by two reviewers (AA and LM) using Critical Appraisal Skills Programme [CASP, c/o Better Value Healthcare

Ltd, Oxford] tools specifically designed for RCTs and CSs. Assessment was based on the eleven criteria, with one point being awarded for each if met in the study. The eleven criteria were: 1) the aim of the study, 2) randomisation or appropriateness of the method used, 3) blinding, 4) patient recruitment and baseline characteristics, 5) equal treatment of the groups, 6) follow-up of the women, 7) the significance of the results, 8) the precision of the results, 9) ability to apply results locally, 10) primary and secondary outcomes, and 11) whether benefit is worth the harm and costs or whether the results fit other available evidence. The scores were compared between the two reviewers (AA and LM) and any significant differences discussed individually.

Assessing the risk of bias was only possible for RCTs. We used RevMan 5.3 (Cochrane, UK) software which automatically generated a panel representing overall risk of bias for each study based on A) random sequence generation, B) allocation and concealment, C) blinding of participants and personnel, D) blinding outcome assessment, E) incomplete outcome data, F) selective reporting and G) other bias.

Data synthesis and analysis

Risk ratio (RR) and accompanying standard errors were extracted from each study in relation to pre-eclampsia. In each, unadjusted estimates were recorded. A meta-analysis was performed to obtain pooled RR for pre-eclampsia in pregnant women treated with metformin compared with placebo/control or insulin. Patient clinical characteristics which have shown a positive association with pre-eclampsia (e.g. age, BMI and blood pressure at enrolment; mean fasting blood glucose (FBG) from enrolment to delivery; HbA1c at 36-37 weeks; GDM incidence; mean weight gain from enrolment to delivery) where available, were used to perform a meta-analysis to obtain pooled standard mean differences in pregnant women treated with metformin compared with placebo/control or insulin. Therefore, two separate

analyses were performed to compare effects of metformin vs. placebo/control and metformin vs insulin on the incidence of pre-eclampsia. In our analyses, any patient on metformin who subsequently needed insulin to maintain good glycaemic control during the course of the pregnancy was included in the metformin arm.

Random effects models were used to combine estimates to account for any heterogeneity present in the studies. Heterogeneity among studies was tested using a Chi-squared test and measured using the I-squared statistic. RevMan 5.3 software (The Nordic Cochrane Centre, The Cochrane Collaboration, Copenhagen, Denmark) and Stata 12 software (StataCorp LP, College Station, TX) were used to carry out these analyses. First, both RCTs and CSs were included in the meta-analyses. Following this, only RCTs were included in the meta-analyses. Publication bias was assessed by funnel plots representing the log RR against the standard error[41].

Results

Study characteristics

Characteristics of the 18 selected studies are described in detail in Table 1. Fourteen RCTs[13–16,30–39] and four CSs[22,28,29,40] studies were included. The baseline age range for all the women (n=3,374) was 16-46 years; most of the studies included women less than 35 years old. The majority of women were overweight (BMI: 25-30 kg/m², 35%) or (BMI>30 kg/m², 64%); only 1% had normal BMI at enrollment. Metformin treatment was initiated before pregnancy in three studies[28,29,40], all in women with PCOS, in whom the aim was to enhance fertility. Pre-eclampsia was reported as a primary outcome in three studies[16,29,40] and as a secondary outcome in fifteen[13–15,22,28,30–39]. All studies were published in or after the year 2000. The studies were carried out worldwide: Australia (n=1); Brazil (n=1); Finland (n=2); Ghana (n=1); Italy (n=1), Iran (n=2); New Zealand (n=2); Norway (n=2); Pakistan (n=2); UK (n=2); USA (n=3).

Meta-analysis of pre-eclampsia incidence

A meta-analysis of nine studies[14,16,22,28–32,40] comparing the effects of metformin vs. control (placebo//healthy pregnancy) included 2,622 pregnant women (1,281 in the metformin group, and 1,341 in the control group; Fig. 2a). The incidence of pre-eclampsia was equal in both groups with a RR in the treatment arm of 0.98 (95% CI 0.53-1.82; p=0.95). There was significant heterogeneity among these nine studies (I^2=54%, p=0.03). Three observational studies compared women with PCOS taking metformin with healthy controls which yielded similar pre-eclampsia incidence therefore suggesting possible benefit of metformin in PCOS cohort of pregnant women[28,29,40]. Due to a difference in study design

between RCTs and observational studies, we also performed meta-analyses using the more rigorous RCTs only, in order to reduce heterogenity. A meta-analysis of metformin vs. placebo/control included five RCT studies[14,16,30–32] with a total of 1,220 pregnant women (611 on metformin treatment and 609 on placebo/control; Fig. 2b). Again, there was no diffeence in the incidence of pre-eclampsia between pregnant women taking metformin vs. placebo/control group (RR=0.86, 95% CI 0.33-2.26; p=0.76), and despite exclusion of observational studies there was still significant heterogeneity among the studies (I^2=66%, p=0.02).

When the effects of metformin vs. insulin were compared, following exclusion of one RCT[38] in which a high risk of bias was dicovered (RevMan 5.3 risk of bias assessment), an overall meta-analysis of nine studies[13,15,22,33–37,39] was performed. This meta-analysis included 2,538 pregnant women (1,303 on metformin treatment and 1,235 on insulin treatment) demonstrated a reduction in pre-eclampsia incidence associated with metformin (RR=0.71; 95% CI 0.53-0.96, p=0.03; Fig. 2c). There was no heterogeneity among these studies in relation to pre-eclampsia incidence (I^2=0%). A meta-analysis of RCTs only that compared the use of metformin vs. insulin during pregnancy in eight studies[13,15,33–37,39] including 1,674 pregnant women (838 on metformin treatment, and 836 on insulin), found that the incidence of pre-eclampsia was lower by over 30%, in the metformin group (RR=0.68; 95% CI 0.48-0.95, p=0.02; Fig. 2d) with no heterogeneity between the studies in relation to pre-eclampsia incidence (I^2=0%). All meta-analyses performed, associated forest and funnel plots, and the risk of bias assessment for RCTs are summarized in Fig. 2.

Meta-analyses of clinical characteristics associated with pre-eclampsia

Considering that advanced age, BMI, blood pressure, GDM, glycemic control in women with diabetes and weight gain during pregnancy have all been strongly and independently associated with pre-eclampsia[2,9,17,42–44], we evaluated differences in these factors between metformin and control or insulin groups. BMI has been strongly linked to pre-eclampsia with the incidence typically doubling with each 6 kg/m^2 increase in pre-pregnancy BMI[17]. Advanced age, higher blood pressure at enrollement and GDM are all independently associated with an increase in the incidence of pre-eclampsia[2,43,45]. Furthermore, good glycemic control in both GDM and Type 2 DM has also been linked to reduced incidence of pre-eclampsia[42]. Mean weight gain from enrollment to delivery was recorded in three studies comparing metformin to placebo[14,16,31] and seven studies comparing metformin to insulin[13,15,33–37].

Metformin vs. placebo/control. A borderline difference in age (p=0.07, Fig. 3a) was observed between metformin and placebo/control treatment groups when both RCTs and CSs were combined in the meta-analysis. Restricting meta-analysis to RCTs only, showed no difference in age between the groups (p=0.21; Fig. 3b). Similarly to age, a meta-analysis including both RCTs and CSs showed significant difference in BMI between metformin and placebo/control groups (p=0.006, Fig. 3c). This was not surprising as three CSs compared PCOS population taking metformin to healthy controls[28,29,40]. High heterogeneity was present between the studies in relation to age and BMI. However when meta-analysis was restricted to four RCTs[14,16,31,32] only, there was no difference in BMI between metformin and placebo groups (p=0.23, Fig. 3d) and heterogeneity disappeared (I^2=0%). One RCT did not report individual baseline parameters in each group but commented that these were homogenous between metformin and placebo group[30].

In relation to the baseline blood pressure, no difference in systolic or diastolic blood pressure was found between metformin and placebo group when three studies[16,31,32] which

recorded these parameters were included in the meta-analysis (systolic, p=0.78, Fig. 3e; diastolic, p=0.62; Fig. 3f).

Furthermore, in terms of the incidence of dysglycaemia in PCOS and obese women, no difference was found in the number of GDM cases between metformin and placebo/control group when we included both RCTs and CSs (n=1236 in metformin group and n=1417 in placebo/control group; RR=0.93, 95% CI 0.71–1.2, p=0.57; Fig. 4a) or when we restricted analysis to RCTs only (n=522 in metformin group and n=499 in placebo group; RR=0.89, 95% CI 0.69-1.16, p=0.4; Fig. 4b). Heterogeneity between the studies was small (I^2=27%; when RCTs and CSs were combined) or absent (I^2=0%, RCTs only).

When meta-analysis using pooled mean weight gain from three RCTs[14,16,31] comparing metformin to placebo was performed, borderline significance was achieved in favour of metformin (p=0.05; Fig. 4c). Heterogeneity amongst the studies was very high (I^2=93%) therefore it is difficult to interpret this finding.

Metformin vs. insulin. No difference was detected in age between metformin and insulin groups [p=0.58, Fig. 5a]. A meta-analysis of seven RCTs comparing BMI at enrollment showed no statistically significant difference between metformin and insulin groups (p=0.63; Fig. 5b).

In terms of glycaemic control we investigated differences in mean FBG and HbA1c between metformin and insulin groups from enrollment until week 36-37. The mean FBG was recorded in six studies[13,15,34,36,37,39] whereas HbA1c was recorded in five studies[13,15,33,36,37]. There were no significant differences between metformin and insulin treatment groups in relation to FBG (p=0.36; Fig. 5c) and HbA1c (p=0.73, mmol/mol, Fig. 5d; p=0.75, %, Supplementary Fig. S1). It is important to note that the percentage of women in metfomin group who subsequently received insulin ranged from 14-85% (Supplementary

Table S1). The two biggest studies reported that, between them, 46% of women in the metformin groups received supplementary insulin[13,22].

Furthermore, there was substantial variation in the way that weight gain was recorded, so that mean weight gain could only be included from four out of seven studies which included metformin and insulin groups, in the meta-analysis[13,33,36,37]. These studies recorded weight gain from enrolment to delivery. A meta-analysis performed using pooled mean weight gain from entry demonstrated that women on metformin were less likely to gain weight during pregnancy than women on insulin (p=0.004; Fig. 5e).

Unfortunately, we were unable to obtain baseline blood pressure data in these studies. None of the studies had pre-eclampsia as a primary outcome.

High heterogeneity was reported within the studies in relation to the following clinical parameters that were included in the meta-analyses: Age (I^2=82%), BMI (I^2=80%), FBG (I^2= 66%), HbA1c (I^2=80%) and mean weight gain (I^2=78%).

Discussion

In this systematic review, we analysed and critically appraised clinical studies which compared the use of metformin treatment with placebo/control or insulin treatment in pregnant women with insulin-resistant disorders such as PCOS, obesity, Type 2 DM and GDM. Such women are at higher risk of developing complications of pregnancy including pre-eclampsia. We performed two different meta-analyses: 1) including RCTs and prospective observational studies or CSs, and 2) including RCTs only. We also carried out two separate analyses comparing metformin to placebo/control and metformin to insulin.

Metformin vs. placebo/control. The results obtained suggest that there is no difference in the incidence of pre-eclampsia between women given metformin vs. placebo/control. We recognize that in the RCTs, the presence of a placebo arm implies that a hypoglycaemic agent was not clinically mandated (e.g. PCOS and obese cohorts), and therefore eligible participants are likely to have lesser risk factors than those requiring randomisation to metformin vs. insulin (Type 2 DM and GDM cohorts). Limitations of some studies included comparison of metformin-treated women with PCOS to healthy pregnant controls[28,29,40]. This was reflected in the meta-analysis of clinical characteristics which found age and BMI to be higher in metformin vs. placebo/control groups when both RCTs and CSs were included in the analysis. Higher age and BMI are confounding factors for the risk of pre-eclampsia. However, when RCTs were only included in the analysis, there was no difference in age or BMI between metformin and placebo group. Another limitation was that pre-eclampsia was the primary outcome in only three of nine studies, two observational[29,40] and one RCT[16]. The heterogeneity between studies was also high.

Only two studies comparing metformin to placebo recruited obese women with BMI> 30 kg/m^2 and without diabetes, but interestingly, although of similar size, the two reached opposite conclusions[14,31]. In one, the number of women with pre-eclampsia was significantly lower in metformin group (odds ratio [OR] = 0.24; p=0.001)[14] whereas in the other, although statistical significance was not reached, a higher incidence of pre-eclampsia was reported in metformin group (OR=2.39; p=0.21)[31]. The baseline characteristics of the participants in both studies were very similar except that one study included all white women[31] whereas the other study included all racial groups and therefore was more representative of the general population[14]. In the latter study lower incidence of pre-eclampsia was observed in metformin group compared to placebo. In both pre-eclampsia was recorded as a secondary outcome.

Furthermore, the incidence of GDM was not different between metformin and placebo/control groups whether RCTs and CS were combined or when the analysis was restricted to RCTs alone suggesting that metformin did not have a significant effect on preventing GDM. Women who develop GDM have higher incidence of pre-eclampsia[17] therefore it is possible that metformin in these cohorts of women was unable to prevent GDM and, as a result, no difference in pre-eclampsia incidence was observed. On the other hand, weight gain, which was only reported in three RCTs[14,16,31], was borderline significant in favour of metformin. Therefore, the effect of metformin on weight gain appears to be more pronounced in people with DM vs. without DM.

Despite the fact that metformin activates the AMPK pathway, an effect which has been shown to inhibit processes directly relevant to the pathogenesis of pre-eclampsia such as irregular angiogenesis, endothelial dysfunction and inappropriate placental development[19–21], it did not demonstrate superiority over placebo/control in reducing the incidence of pre-eclampsia in this meta-analysis. Interestingly, Vanky and colleagues reported that severe pregnancy complications, which included pre-term delivery before 32 weeks, severe pre-eclampsia or serious post-partum events occurred only in placebo group (placebo, 7/22 vs. metformin, 0/18, p=0.01)[32]. Therefore, the effect of metformin vs. placebo on severe pre-eclampsia should be investigated in the future.

Interestingly, clinical studies which assessed cardiovascular effects of metformin in people without Type 2 DM showed little or no effect on the markers of cardiovascular disease[46]; the Diabetes Prevention Program also demonstrated no beneficial effect of metformin in reducing the incidence of hypertension in people without Type 2 DM[47]. Conversely, in people with Type 2 DM the cardiovascular benefits of metformin were well substantiated in the UKPDS trial[48]. This differential effect of metformin in people with vs. without DM

could also be relevant to pre-eclampsia, a disease of cardiovascular system, characterised by hypertension and proteinuria.

Metformin vs. Insulin. The comparison between metformin and insulin demonstrated a reduction in RR of pre-eclampsia in favour of metformin whether RCTs and CS were combined or when the analysis was restricted to RCTs alone. This result is convincing considering there was no heterogeneity between the studies. Nevertheless, in these studies, a common weakness was that neither the investigators nor the participants were blinded because of the different routes of administration of the study drugs. Most of the risk factors for pre-eclampsia, such as age, BMI and glycaemic control were similar between groups at the start or throughout the trial; weight gain after enrolment was significantly lower in metformin group. Weight gain has been linked to an increased risk of pre-eclampsia[49]. Other possibilities for bias included a high risk for random sequence generation, and allocation concealment which was present in three studies[15,34,38].

Considering all studies, on average 45% of the women in the metformin group needed supplementary insulin (Supplementary Table S1). When we carried out meta-analysis comparing metformin alone vs. insulin alone, which included seven studies [five RCTs; Supplementary Fig. S2a,b], the incidence of pre-eclampsia remained lower in the metformin group but significance was lost (p=0.18, RCTs and CSs; p=0.21; RCTs only). Administration of aspirin was not reported in any of the studies included. Nevertheless, most of these studies are relatively small, and therefore there may still be justification for a larger study with pre-eclampsia as a primary outcome, to address the question definitively.

Overall, even though metformin ± insulin vs. insulin alone was associated with a lower risk for pre-eclampsia, it is unclear whether this is because insulin itself might increase the risk of pre-eclampsia, perhaps in part by causing the weight gain, or whether this is a beneficial

effect of metformin. A large population-based register data in Finland[45] compared pregnancy outcomes in women with GDM who were either under or over 35 years old vs. women without GDM in the same age groups. Women with GDM were treated with diet or insulin. We calculated RR for pre-eclampsia, based on the data presented in the paper, between women with GDM treated on diet vs. insulin treatment in both age groups. This showed that in women with GDM who were less than 35 years old, insulin [238/2845] increased the risk of pre-eclampsia (RR=1.19; CI 1.04 – 1.36; p=0.0092) compared to diet [1161/19422]; no difference was found in the prevalence of pre-eclampsia between diet and insulin group in women with GDM who were more than 35 years old. Most of the women (>90%) in our meta-analysis were less than 35 years old, which suggests that metformin could only have marginal effect on preventing the risk of pre-eclampsia however it is still a better option than insulin alone in terms of the risk of pre-eclampsia and possibly other pregnancy complications. Further trials are needed to explore the incidence of pre-eclampsia between insulin and diet interventions. Perhaps perspective studies comparing insulin treatment to diet in women with GDM or Type 2 DM could address this question. It is possible that metformin could have advantages over insulin in pregnant women who require a hypoglycaemic agent; these advantages could be even more pronounced in women over the age of 35 according to the findings by Lamminpää and colleagues[45]. Nevertheless, these women might still need insulin supplementation in the later stages of pregnancy to control hyperglycaemia. In women on metformin ± insulin, the weight gain is less than in women on insulin alone, and it is likely that the dose of insulin may be lower when metformin is used: both factors are potentially beneficial in relation to pre-eclampsia. In contrast, in the studies comparing metformin with placebo or no treatment, hypoglycaemic intervention was either optional or not needed: in these women, the data show no evidence in favour of metformin in reducing risk for pre-eclampsia. It is important to explore further the effects of metformin vs.

placebo/control on the early or severe type of pre-eclampsia characterised by onset of pre-eclampsia before 34 weeks gestation or blood pressure ≥160/110 mmHg, respectively. It is possible, as suggested by Myatt and colleagues, that there are different phenotypes of pre-eclampsia and that this is the reason why large clinical studies have failed to validate findings observed in smaller studies. Therefore correct stratification of high-risk women according to age, presence of diabetes, blood pressure or BMI is important and this could determine the most appropriate preventative treatment. Also, women with GDM or Type 2 DM during pregnancy are frequently given or swapped to insulin instead of metformin. These women are at high risk of pre-eclampsia, it is possible, based on this review, and other published data, that metformin ± supplementary insulin would be a better option during pregnancy in these women than insulin alone.

Clearly, in this systematic review we could not include (and did not find) any studies of pregnancy in Type 1 DM women. These women also have a four-fold increased risk of developing pre-eclampsia, similar to women with Type 2 DM[9,10]. Considering that metformin in addition to insulin appears beneficial compared to insulin alone, future randomised double-blind placebo-controlled trials investigating the ability of metformin, in addition to insulin, in prevention of pre-eclampsia in pregnant women with Type 1 DM could be valuable. In the current analysis, with a pre-eclampsia rate of 20% in the insulin group and 14% in the metformin group (estimated based upon a 30% reduction in metformin group observed in this meta-analysis), 650 women with pre-gestational Type 1 DM would need to be recruited in each group to have over 80% power to detect this difference as statistically significant.

Conclusion

In pregnant women requiring hypoglycaemic treatment, metformin alone or metformin in combination with insulin is associated with less weight gain and a lower incidence of pre-eclampsia than insulin alone. This suggests that metformin ± supplementary insulin treatment is linked to more favourable pregnancy outcomes such as reduced risk of pre-eclampsia than insulin alone. This effect is likely to be age-dependent and associated with reduced weight gain during pregnancy. In other high-risk pregnancies where glucose-lowering agents are not essential, we did not find a case for prescribing metformin. Considering that metformin can safely be used in pregnancy, adequately designed and powered RCTs which have pre-eclampsia as a primary outcome should be carried out in the future in GDM or Type 2 DM, and perhaps in Type 1 DM pregnancies.

Funding: Funding was received from the Hashemite University, Jordan.

Conflict of interest: None reported.

Acknowledgements. The authors thank Dr Chris Cardwell, Centre for Public Health, Queen's University Belfast for his help with RevMan 5.3 software, providing power calculation and for reading the draft and providing feedback. The guarantor for this manuscript is Dr Lana McClements.

References

1. American College of Obstetricians and Gynecologists, Task Force on Hypertension in Pregnancy. Hypertension in Pregnancy. Obstet Gynecol. 2013;122:1122–31.

2. Kenny LC, Black MA, Poston L, Taylor R, Myers JE, Baker PN, et al. Early Pregnancy Prediction of Preeclampsia in Nulliparous Women, Combining Clinical Risk and BiomarkersNovelty and Significance. Hypertension. 2014;64:644-52

3. Khan KS, Wojdyla D, Say L, Gülmezoglu AM, Van Look PF. WHO analysis of causes of maternal death: a systematic review. Lancet. 2006;367:1066–74.

4. Manten GTR, Sikkema MJ, Voorbij HAM, Visser GHA, Bruinse HW, Franx A. Risk factors for cardiovascular disease in women with a history of pregnancy complicated by preeclampsia or intrauterine growth restriction. Hypertens pregnancy. 2007;26:39–50.

5. Libby G, Murphy DJ, McEwan NF, Greene SA, Forsyth JS, Chien PW, et al. Pre-eclampsia and the later development of type 2 diabetes in mothers and their children: an intergenerational study from the Walker cohort. Diabetologia. 2007;50:523–30.

6. Metzger BE, Lowe LP, Dyer AR, Trimble ER, Chaovarindr U, Coustan DR, et al. Hyperglycemia and adverse pregnancy outcomes. N Engl J Med. 2008;358:1991–2002.

7. Palomba S, de Wilde MA, Falbo A, Koster MPH, La Sala GB, Fauser BCJM. Pregnancy complications in women with polycystic ovary syndrome. Hum Reprod Update. 2015;21:575–92.

8. Abell SK, Boyle JA, de Courten B, Soldatos G, Wallace EM, Zoungas S, et al. Impact of type 2 diabetes, obesity and glycaemic control on pregnancy outcomes. Aust New Zeal J Obstet Gynaecol. 2016; doi: 10.1111/ajo.12521. [Epub ahead of print].

9. Yu Y, Jenkins AJ, Nankervis AJ, Hanssen KF, Scholz H, Henriksen T, et al. Anti-angiogenic factors and pre-eclampsia in type 1 diabetic women. Diabetologia. 2009;52:160–8.

10. McCance DR, Holmes VA, Maresh MJA, Patterson CC, Walker JD, Pearson DWM, et al. Vitamins C and E for prevention of pre-eclampsia in women with type 1 diabetes [DAPIT]: a randomised placebo-controlled trial. Lancet. 2010;376:259–66.

11. Henderson JT, Whitlock EP, O'Connor E, Senger CA, Thompson JH, Rowland MG, et al. Low-Dose Aspirin for Prevention of Morbidity and Mortality From Preeclampsia: A Systematic Evidence Review for the U.S. Preventive Services Task Force. Ann Intern Med. American College of Physicians; 2014;160:695.

12. Cassina M, Dona M, Di Gianantonio E, Litta P, Clementi M. First-trimester exposure to metformin and risk of birth defects: a systematic review and meta-analysis. Hum Reprod Update. 2014;20:656–69.

13. Rowan JA, Hague WM, Gao W, Battin MR, Moore MP, MiG Trial Investigators. Metformin versus Insulin for the Treatment of Gestational Diabetes. N Engl J Med. 2008;358:2003–15.

14. Syngelaki A, Nicolaides KH, Balani J, Hyer S, Akolekar R, Kotecha R, et al. Metformin versus Placebo in Obese Pregnant Women without Diabetes Mellitus. N Engl J Med. 2016;374:434–43.

15. Ainuddin J, Karim N, Hasan AA, Naqvi SA. Metformin versus insulin treatment in gestational diabetes in pregnancy in a developing country: a randomized control trial. Diabetes Res Clin Pract. 2015;107:290–9. (a)

16. Vanky E, Stridsklev S, Heimstad R, Romundstad P, Skogøy K, Kleggetveit O, et al. Metformin versus placebo from first trimester to delivery in polycystic ovary syndrome: a randomized, controlled multicenter study. J Clin Endocrinol Metab. 2010;95:E448-55.

17. Catalano PM, McIntyre HD, Cruickshank JK, McCance DR, Dyer AR, Metzger BE, et al. The hyperglycemia and adverse pregnancy outcome study: associations of GDM and obesity with pregnancy outcomes. Diabetes Care. 2012;35:780–6.

18. Agatisa PK, Ness RB, Roberts JM, Costantino JP, Kuller LH, McLaughlin MK. Impairment of endothelial function in women with a history of preeclampsia: an indicator of cardiovascular risk. Am J Physiol Heart Circ Physiol. 2004;286:H1389-93.

19. Skeffington KL, Higgins JS, Mahmoud AD, Evans AM, Sferruzzi-Perri AN, Fowden AL, et al. Hypoxia, AMPK activation and uterine artery vasoreactivity. J Physiol. 2015

20. Banek CT, Bauer AJ, Needham KM, Dreyer HC, Gilbert JS. AICAR administration ameliorates hypertension and angiogenic imbalance in a model of preeclampsia in the rat. Am J Physiol Heart Circ Physiol. 2013;304:H1159-65.

21. Brownfoot FC, Hastie R, Hannan NJ, Cannon P, Tuohey L, Parry LJ, et al. Metformin as a prevention and treatment for preeclampsia: effects on soluble fms-like tyrosine kinase 1 and soluble endoglin secretion and endothelial dysfunction. Am J Obstet Gynecol. 2016;214:356.e1-356.e15.

22. Goh JEL, Sadler L, Rowan J. Metformin for gestational diabetes in routine clinical practice. Diabet Med. 2011;28:1082–7.

23. Ibrahim MI, Hamdy A, Shafik A, Taha S, Anwar M, Faris M. The role of adding metformin in insulin-resistant diabetic pregnant women: a randomized controlled trial. Arch Gynecol Obstet. 2014;289:959–65.

24. Mesdaghinia E, Samimi M, Homaei Z, Saberi F, Moosavi SGA, Yaribakht M. Comparison of newborn outcomes in women with gestational diabetes mellitus treated with metformin or insulin: A randomised blinded trial. International Journal of Preventive Medicine. 2013:327–33.

25. Khattab S, Mohsen IA, Aboul Foutouh I, Ashmawi HS, Mohsen MN, van Wely M, et al. Can metformin reduce the incidence of gestational diabetes mellitus in pregnant women with polycystic ovary syndrome? Prospective cohort study. Gynecol Endocrinol. 2011;27:789–93.

26. Hellmuth E, Damm P, Mølsted-Pedersen L. Oral hypoglycaemic agents in 118 diabetic pregnancies. Diabet Med. 2000;17:507–11.

27. Glueck CJ, Pranikoff J, Aregawi D, Wang P. Prevention of gestational diabetes by metformin plus in patients with polycystic ovary syndrome. Fertil Steril. 2008;89:625–34.

28. Glueck CJ, Goldenberg N, Pranikoff J, Khan Z, Padda J, Wang P. Effects of metformin- intervention before and throughout pregnancy on obstetric and neonatal outcomes in patients with polycystic ovary syndrome. Curr Med Res Opin. 2013;29:55–62.

29. De Leo V, Musacchio MC, Piomboni P, Di Sabatino A, Morgante G. The administration of metformin during pregnancy reduces polycystic ovary syndrome related gestational complications. Eur J Obstet Gynecol Reprod Biol. 2011;157:63–6.

30. Jamal A, Milani F, Al-Yasin A. Evaluation of the effect of metformin and aspirin on utero placental circulation of pregnant women with PCOS. Iran J Reprod Med. 2012;10:265–70.

31. Chiswick C, Reynolds RM, Denison F, Drake AJ, Forbes S, Newby DE, et al. Effect of metformin on maternal and fetal outcomes in obese pregnant women [EMPOWaR]: a randomised, double-blind, placebo-controlled trial. lancet Diabetes Endocrinol. 2015;3:778–86.

32. Vanky E, Salvesen KA, Heimstad R, Fougner KJ, Romundstad P, Carlsen SM. Metformin reduces pregnancy complications without affecting androgen levels in pregnant polycystic ovary syndrome women: results of a randomized study. Hum Reprod. 2004;19:1734–40.

33. Tertti K, Ekblad U, Koskinen P, Vahlberg T, Rönnemaa T. Metformin vs. insulin in gestational diabetes. A randomized study characterizing metformin patients needing additional insulin. Diabetes, Obes Metab. 2013;15:246–51.

34. Ainuddin JA, Karim N, Zaheer S, Ali SS, Hasan AA. Metformin Treatment in Type 2 Diabetes in Pregnancy: An Active Controlled, Parallel-Group, Randomized, Open Label Study in Patients with Type 2 Diabetes in Pregnancy. J Diabetes Res. 2015:1–11. (b)

35. Ijäs H, Vääräsmäki M, Morin-Papunen L, Keravuo R, Ebeling T, Saarela T, et al. Metformin should be considered in the treatment of gestational diabetes: a prospective randomised study. BJOG An Int J Obstet Gynaecol. 2011;118:880–5.

36. Niromanesh S, Alavi A, Sharbaf FR, Amjadi N, Moosavi S, Akbari S. Metformin compared with insulin in the management of gestational diabetes mellitus: A randomized clinical trial. Diabetes Res Clin Pract. 2012;98:422–9.

37. Hickman M, McBride R, Boggess K, Strauss R. Metformin Compared with Insulin in the Treatment of Pregnant Women with Overt Diabetes: A Randomized Controlled Trial. Am J Perinatol. 2012;30:483–90.

38. Spaulonci CP, Bernardes LS, Trindade TC, Zugaib M, Francisco RPV. Randomized trial of metformin vs insulin in the management of gestational diabetes. Am J Obstet Gynecol. 2013;209:34.e1-7.

39. Beyuo T, Obed SA, Adjepong-Yamoah KK, Bugyei KA, Oppong SA, Marfoh K. Metformin versus insulin in the management of pre-gestational diabetes mellitus in pregnancy and gestational diabetes mellitus at the Korle Bu Teaching Hospital:A randomized clinical trial. PLoS One. 2015;10:e0125712.

40. Glueck CJ, Goldenberg N, Pranikoff J, Loftspring M, Sieve L, Wang P. Height, weight, and motor-social development during the first 18 months of life in 126 infants born to 109 mothers with polycystic ovary syndrome who conceived on and continued metformin through pregnancy. Hum Reprod. 2004;19:1323–30.

41. Sterne JA, Egger M. Funnel plots for detecting bias in meta-analysis: guidelines on choice of axis. J Clin Epidemiol. 2001;54:1046–55.

42. Yogev, Chen, Hod, Coustan, Oats, McIntyre, et al. Hyperglycemia and Adverse Pregnancy Outcome [HAPO] study: preeclampsia. Am J Obstet Gynecol. 2010;202:255.e1-7.

43. North RA, McCowan LME, Dekker GA, Poston L, Chan EHY, Stewart AW, et al. Clinical risk prediction for pre-eclampsia in nulliparous women: development of model in international prospective cohort. BMJ. 2011;342:d1875.

44. Montoro MN, Kjos SL, Chandler M, Peters RK, Xiang AH, Buchanan TA. Insulin Resistance and Preeclampsia in Gestational Diabetes Mellitus. Diabetes Care. 2005;28:1995-2000.

45. Lamminpää R, Vehviläinen-Julkunen K, Gissler M, Selander T, Heinonen S. Pregnancy outcomes in women aged 35 years or older with gestational diabetes – a registry-based study in Finland. J Matern Neonatal Med. 2016;29:55–9.

46. Preiss D, Lloyd SM, Ford I, McMurray JJ, Holman RR, Welsh P, et al. Metformin for non-diabetic patients with coronary heart disease [the CAMERA study]: a randomised controlled trial. Lancet Diabetes Endocrinol. 2014;2:116–24.

47. Ratner R, Goldberg R, Haffner S, Marcovina S, Orchard T, Fowler S, Temprosa M; Diabetes Prevention Program Research Group. Impact of Intensive Lifestyle and Metformin Therapy on Cardiovascular Disease Risk Factors in the Diabetes Prevention Program. Diabetes Care. 2005;28:888-94.

48. Holman RR, Paul SK, Bethel MA, Matthews DR, Neil HAW. 10-Year Follow-up of Intensive Glucose Control in Type 2 Diabetes. N Engl J Med. 2008;359:1577–89.

49. Myatt Leslie, Redman Christopher W., Staff Anne Cathrine HS, Wilson Melissa L., Laivuori Hannele, Poston Lucilla RJM;, [COLAB] for the GPC. Strategy for Standardization of Preeclampsia Research Study Design. Hypertension. 2014;63:1293–301.

Table 1. Study characteristics and outcomes measured.

Figure 1. PRISMA Guidelines flow diagram.

Figure 2. Meta-analysis comparing the risk ratio of pre-eclampsia in metformin vs. non-metformin treatment group. [A] A meta-analysis including both RCTs and cohort studies [CSs] comparing metformin to placebo/control. [B] A meta-analysis including both RCTs and CSs comparing metformin to insulin. [C] A meta-analysis of only RCTs comparing metformin to placebo/control. [D] A meta-analysis of only RCTs comparing metfomin to insulin. Random effects models were used to combine estimates and analyses were carried out using RevMan 5.3 software; risk ratio was calculated; the overall effect was measured using Z-test with p values less than 0.05 being statistically significant. Heterogeneity was calculated using Chi^2 test and measured by I^2 statistic.

Figure 3. Meta-analyses of age, BMI and blood pressure in metformin vs. placebo treatment group. [A] A meta-analysis of both RCTs and CSs comparing age between metformin and placebo/control group. [B] A meta-anaylsis of RCTs comparing age between metformin and placebo group. [C] A meta-analysis of body mass index [BMI] at enrolement, RCTs and CS combined. [D] A meta-analysis of body mass index [BMI] at enrolement, RCTs only. Meta-analysis of systolic [E] and diastolic [F] blood pressure at baseline between metformin and placebo group. Random effects models were used to combine estimates and analyses were carried out using RevMan 5.3 software; standard mean difference or risk ratio was calculated; the overall effect was measured using Z-test with p values less than 0.05 being statistically significant. Heterogeneity was calculated using Chi^2 test and measured by I^2 statistic.

Figure 4. Meta-analyses of the incidence of GDM and weight gain in metformin vs. placebo treatment group. [A] A meta-analysis comparing the risk ratio of gestational diabetes [GDM] between metformin and placebo/control group, RCTs and CS combined. [B] A meta-analysis comparing the risk ratio of GDM between metformin and placebo/control group, RCTs only. [C] A meta-analysis of weight gain between enrolment and delivery. Random effects models were used to combine estimates and analyses were carried out using RevMan 5.3 software; standard mean difference or risk ratio was calculated; the overall effect was measured using Z-test with p values less than 0.05 being statistically significant. Heterogeneity was calculated using Chi^2 test and measured by I^2 statistic.

Figure 5. Meta-analyses of patient clinical risk factors for pre-eclampsia in metformin vs. insulin treatment group, RCTs only. [A] A meta-analysis of age. [B] A meta-analysis of BMI at enrolment. [C] A meta-analysis of mean fasting blood glucose [FBG] from enrolment to delivery. [D] A meta-analysis of glycated haemoglobin [HbA1c, mmol/mol] recorded between 36 and 37 weeks. [E] A meta-analysis of weight gain between enrolment and delivery. Random effects models were used to combine estimates and analyses were carried out using RevMan 5.3 software; standard mean difference was calculated; the overall effect was measured using Z-test with p values less than 0.05 being statistically significant. Heterogeneity was calculated using Chi^2 test and measured by I^2 statistic.

Supplementary Table 1. Percentage of patients in metformin group who were supplemented with additional insulin.

Supplementary Fig. 1. Meta-analyses of HbA1c [%] in metformin vs. insulin treatment group, RCTs only. A meta-analysis of glycated haemoglobin [HbA1c] recorded between 36 and 37 weeks. Random effects models were used to combine estimates and analyses were carried out using RevMan 5.3 software; standard mean difference was calculated; the overall effect was measured using Z-test with p values less than 0.05 being statistically significant. Heterogeneity was calculated using Chi^2 test and measured by I^2 statistic.

Supplementary Fig. 2. A meta-analysis comparing the risk ratio of pre-eclampsia in metformin only vs. insulin treatment group. [A] A meta-analysis including both randomised controlled trials [RCTs] and cohort studies [CSs] comparing metformin only treatment to insulin only treatment. [B] A meta-analysis including only RCTs comparing metformin only vs. insulin only treatment.

Table 1: Study characteristics and outcomes measured.

Author(year)/ Country	Trial type	Patient cohort	Age (range)	BMI (kg/m^2)	No. of patients on metformin ± insulin	No. of patients on non-metformin treatment	Dose of metformin	Duration of the treatment	Primary outcomes	Secondary outcomes
Glueck et al. 2004/USA[48]	CS	PCOS	28-38	26-42	97	252 (control*)	1500-2550 mg/day	Pre-conception until delivery	Pre-eclampsia	GDM
De Leo et al. 2011/Italy[37]	CS	PCOS	26-38	26-30	98	110 (control*)	1700-3000 mg/day	3–4 months before infertility treatment was	Miscarriage/GDM/ Pre-eclampsia/PIH	N/A

								initiated until 37-week gestation.		
Glueck et al. 2013/USA[36]	CS	PCOS	28-34	29.4-39.3	76	156 (control*)	2000-2550 mg/day	On average 6.8 months before conception until delivery	Miscarriage	GDM/ Pre-eclampsia
Goh et al. 2014/ New Zealand[30]	CS	GDM	N/A	N/A	465 metformin	399 (insulin)	2500-3000 mg/day	From 25-29 weeks gestation until delivery	Caesarean delivery/ Preterm birth	PIH/ Pre-eclampsia

Vanky et al. 2004/Norway[40]	RCT	Pregnant women with PCOS	24.4-32.8	23.3-37.9	18	22 (placebo)	850-1700 mg/day	From 8 weeks gestation until delivery	Dehydroepiandrosterone sulfate (DHEAS), androstenedione, testosterone, SHBG and free testosterone index (FTI)	GDM/ Pre-eclampsia
Rowan et al. 2008/ New Zealand & Australia[18]	RCT	GDM	28.1-38.9	26.8-43.4	363	370 (insulin)	500-2500 mg/day	From 20-33 weeks gestation until delivery.	Neonatal complications	PIH/ Pre-eclampsia
Ijas et al. 2010/Finland[43]	RCT	GDM	25.6-37.8	25.4-36.2	47	50 (insulin)	750-2250 mg/day	From 26-34 weeks gestation until delivery	Macrosomia	Neonatal complication/ Pre-eclampsia

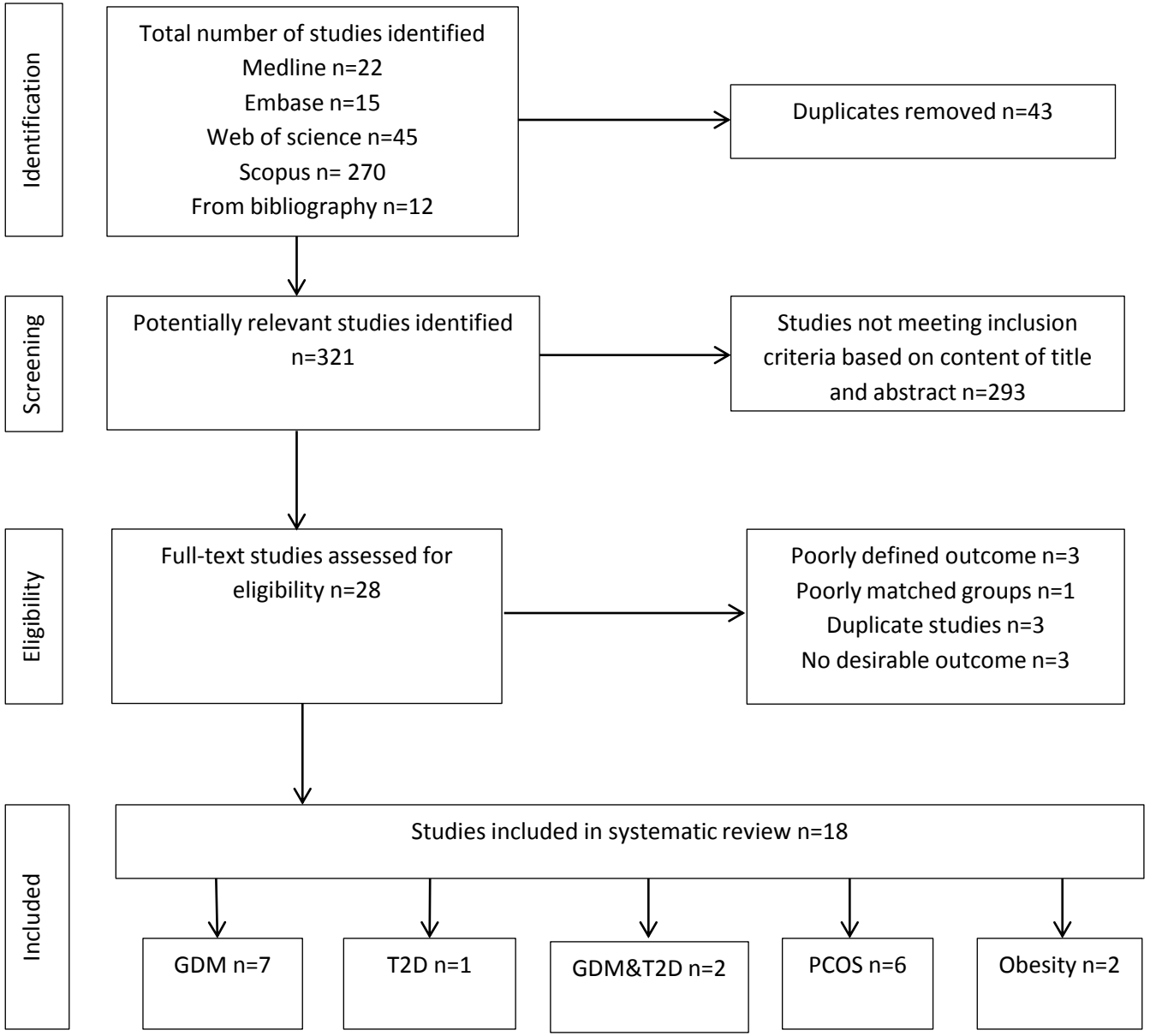
Vanky et al. 2010/Norway[21]	RCT	PCOS	25.2-34	22.5-36.5	135	135 (placebo)	2000 mg/day	From 5-12 weeks gestation until delivery	GDM/ Pre-eclampsia	N/A
Niromanesh et al. 2012/Iran[44]	RCT	GDM	25.2-36.2	24.1-32.1	80	80 (insulin)	1000-2500 mg/day	From 20-34 weeks gestation until delivery	Maternal glycemic control/ Birth weight	PIH/ Pre-eclampsia
Hickman et al. 2012/USA[45]	RCT	T2D/GDM	26-37	27-41	14	14 (insulin)	500-2500 mg/day	From 10-22 weeks gestation until delivery	Glycemic control	Maternal and neonatal outcomes/ Pre-eclampsia
Jamal et al.	RCT	PCOS	18-40	N/A	35	35	2000	From 6-12	Mean Uterine Artery/	Pre-

2012/Iran[38]						(control**)	mg/day	weeks gestation until delivery	Pulsatility Index	eclampsia
Spaulonci et al. 2013/Brazil[46]	RCT	GDM	25.9-37.9	27.2-36.7	46	46 (insulin)	1700-2550 mg/day	From 26-34 weeks gestation until delivery	Glycemic control	Pre-eclampsia
Tertti et al. 2013/Finland[41]	RCT	GDM	26.9-36.9	23.5-35.3	110	107 (insulin)	500-2000 mg/day	From 22–34 weeks gestation until delivery	Mean birth weight	PIH/ Preeclampsia
Ainuddin et al. 2015	RCT	GDM	27-35	N/A	75	75 (insulin)	500-2500 mg/day	From 20-36 weeks	Mean birth weight	PIH/ Pre-

a/Pakistan[20]								gestation until delivery		eclampsia
Ainuddin et al. 2015 b/Pakistan[42]	RCT	T2D	28.9-34.6	28-42	106	100 (insulin)	500-2500 mg/day	From the first trimester to delivery	Perinatal death/ Birth weight	PIH/ Pre-eclampsia
Chiswick et al. 2015/UK[39]	RCT	Obese	>16	>30	221	222 (placebo)	500-2500 mg/day	From 12-16 weeks gestation until delivery.	Median birth-weight Z score (IQR)	Maternal insulin resistance/ Pre-eclampsia
Beyuo et al. 2015/Ghana[47]	RCT	T2D/GDM	28.5-38.17	26.52-40.42	43	40 (insulin)	500-2500 mg/day	From 20-30 weeks gestation until	Glycemic control	Pre-eclampsia

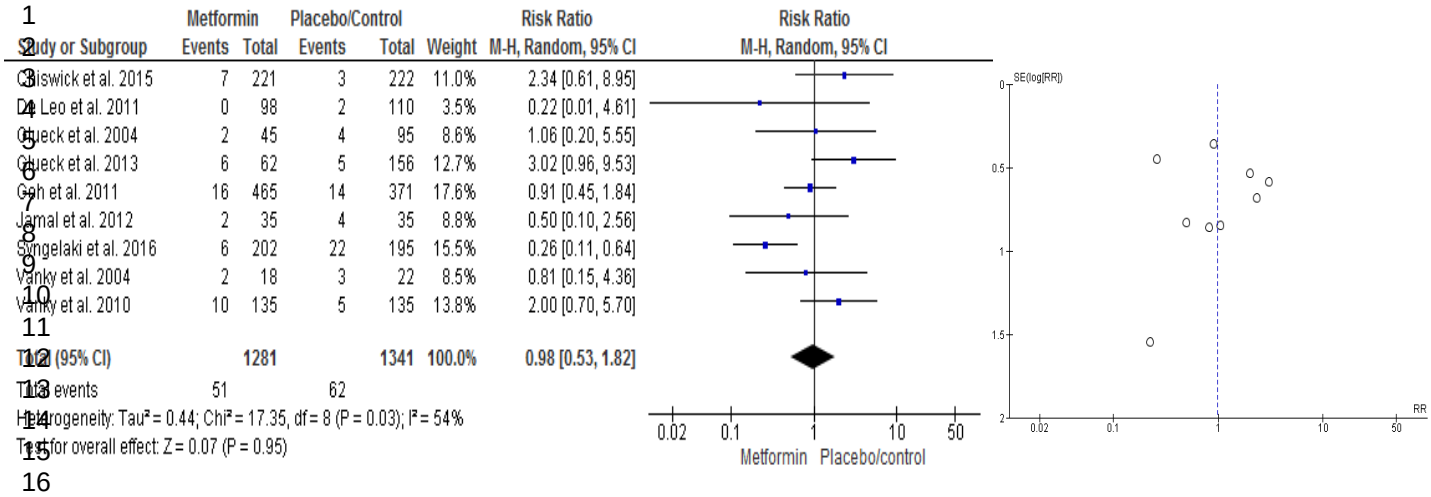
								delivery		
Syngelaki et al. 2016/UK[19]	RCT	Obese	27.3-36.2	36.5-36.2	202	198 (placebo)	3000 mg/day	From 12-18 weeks gestation until delivery	Median birth-weight Z score (IQR)	PIH/ Pre-eclampsia

CS: cohort study; RCT: randomized controlled trial; T2D: Type 2 diabetes; GDM: gestational diabetes mellitus; PCOS: Polycystic ovary syndrome; BMI: body mass index; PIH: pregnancy induced hypertension; control: healthy control group; control**: no intervention*


Identification
Total number of studies identified
Medline n=22
Embase n=15
Web of science n=45
Scopus n= 270
From bibliography n=12
Duplicates removed n=43
Screening
Potentially relevant studies identified n=321
Studies not meeting inclusion criteria based on content of title and abstract n=293
Eligibility
Full-text studies assessed for eligibility n=28
Poorly defined outcome n=3
Poorly matched groups n=1
Duplicate studies n=3
No desirable outcome n=3
Included
Studies included in systematic review n=18
GDM n=7
T2D n=1
GDM&T2D n=2
PCOS n=6
Obesity n=2

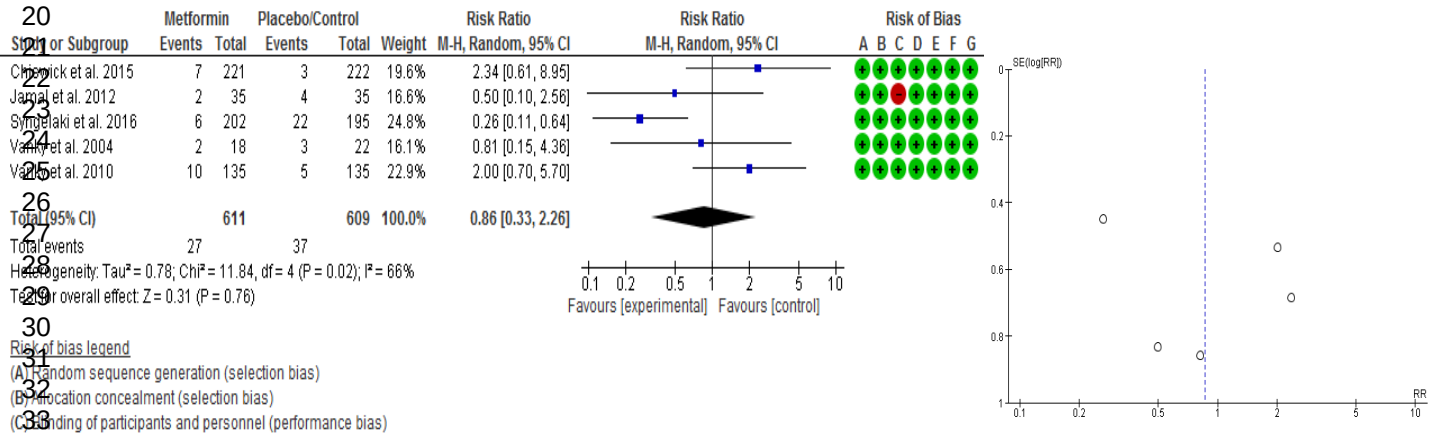
a

Metformin vs. Placebo/Control; RCTs and CS



b

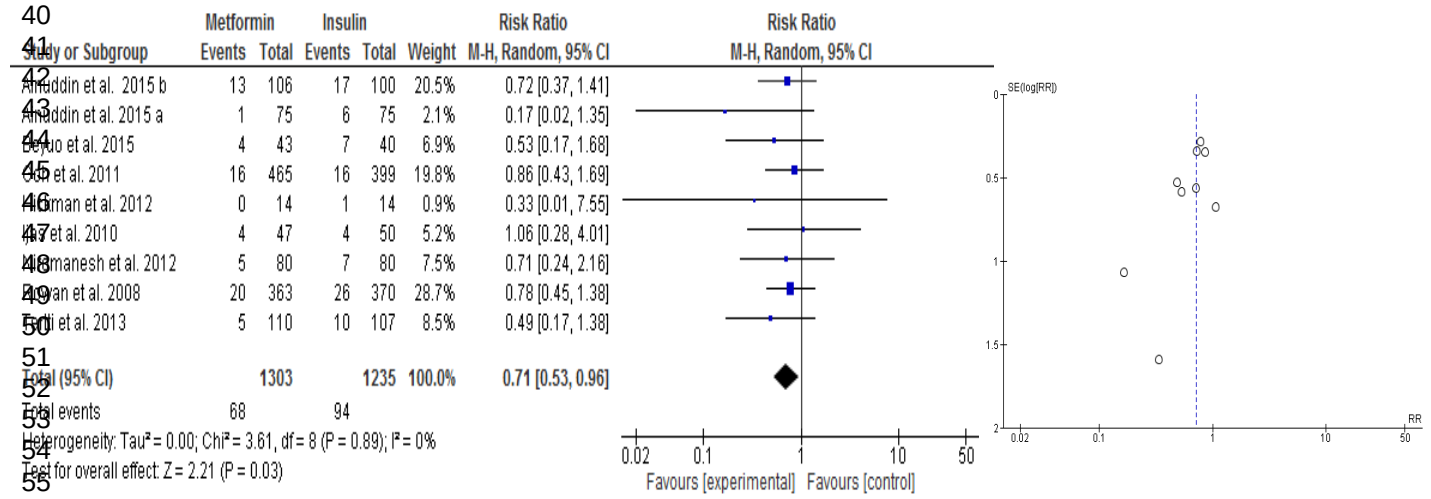
Metformin vs. Placebo/Control; RCTs only



Risk of bias legend
(A) Random sequence generation (selection bias)
(B) Allocation concealment (selection bias)
(C) Blinding of participants and personnel (performance bias)
(D) Blinding of outcome assessment (detection bias)
(E) Incomplete outcome data (attrition bias)
(F) Selective reporting (reporting bias)
(G) Other bias

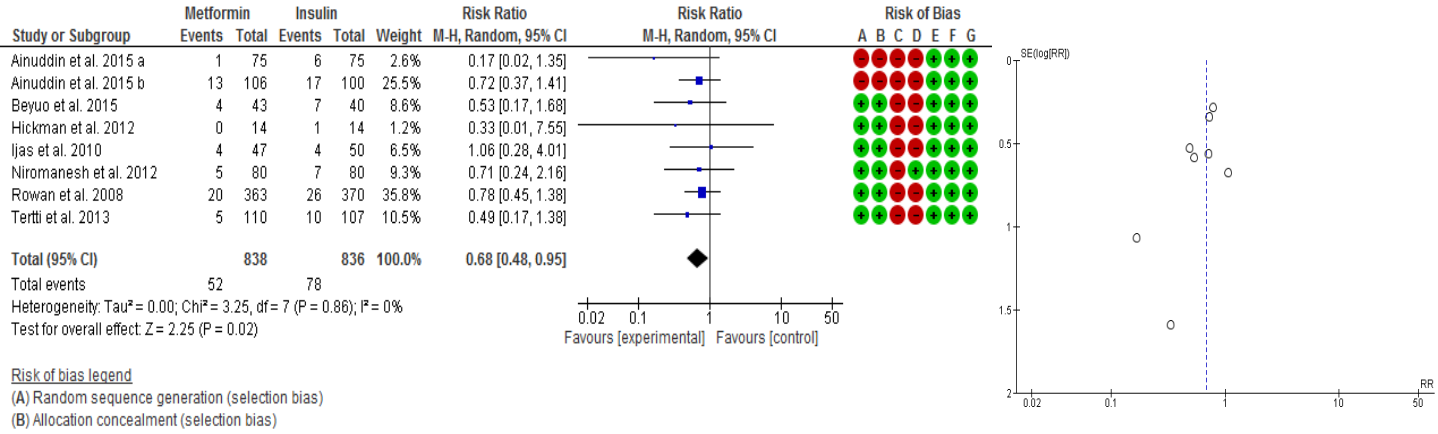
c

Metformin vs. Insulin; RCTs and CS



d

Metformin vs. Insulin; RCTs only



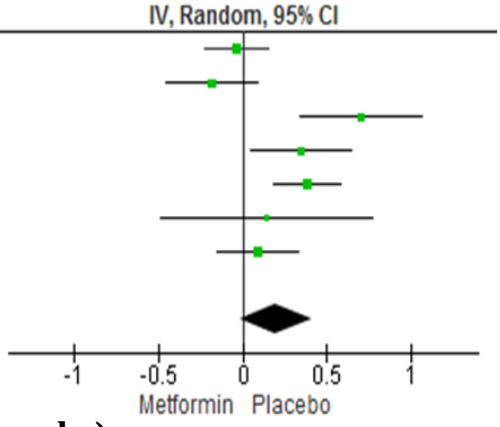
Risk of bias legend
(A) Random sequence generation (selection bias)
(B) Allocation concealment (selection bias)
(C) Blinding of participants and personnel (performance bias)
(D) Blinding of outcome assessment (detection bias)
(E) Incomplete outcome data (attrition bias)
(F) Selective reporting (reporting bias)
(G) Other bias

a

Age (metformin vs. placebo/control, RCTs and CS)

Study or Subgroup	Metformin Mean	SD	Total	Placebo Mean	SD	Total	Weight	Std. Mean Difference IV, Random, 95% CI
Chiswick et al. 2015	28.7	5.8	226	28.9	5.1	223	17.4%	-0.04 [-0.22, 0.15]
De Leo et al. 2011	32	6	98	33	5	110	15.1%	-0.18 [-0.45, 0.09]
Glueck et al. 2004	33	5	45	29	6	95	12.6%	0.70 [0.33, 1.06]
Glueck et al. 2013	32	5	62	30	6	156	14.4%	0.35 [0.05, 0.64]
Syngelaki et al. 2016	31.7	3	202	30.6	2.7	198	17.1%	0.38 [0.19, 0.58]
Vanky et al. 2004	28.9	4.8	18	28.3	3.7	22	7.3%	0.14 [-0.48, 0.76]
Vanky et al. 2010	29.6	4.4	135	29.2	4.4	138	16.0%	0.09 [-0.15, 0.33]
Total (95% CI)			**786**			**942**	**100.0%**	**0.19 [-0.02, 0.41]**

Heterogeneity: Tau² = 0.06; Chi² = 25.54, df = 6 (P = 0.0003); I² = 77%
Test for overall effect: Z = 1.80 (P = 0.07)

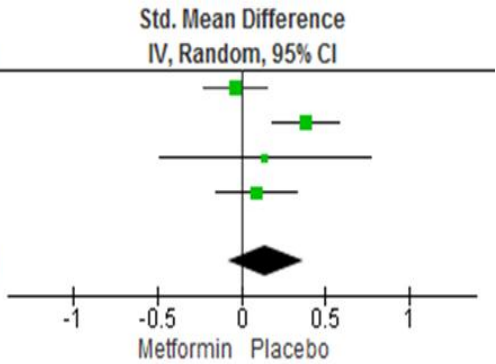


b

Age (metformin vs. placebo, RCTs only)

Study or Subgroup	Metformin Mean	SD	Total	Placebo Mean	SD	Total	Weight	Std. Mean Difference IV, Random, 95% CI
Chiswick et al. 2015	28.7	5.8	226	28.9	5.1	223	31.6%	-0.04 [-0.22, 0.15]
Syngelaki et al. 2016	31.7	3	202	30.6	2.7	198	30.7%	0.38 [0.19, 0.58]
Vanky et al. 2004	28.9	4.8	18	28.3	3.7	22	9.9%	0.14 [-0.48, 0.76]
Vanky et al. 2010	29.6	4.4	135	29.2	4.4	138	27.8%	0.09 [-0.15, 0.33]
Total (95% CI)			**581**			**581**	**100.0%**	**0.15 [-0.08, 0.37]**

Heterogeneity: Tau² = 0.03; Chi² = 9.53, df = 3 (P = 0.02); I² = 69%
Test for overall effect: Z = 1.26 (P = 0.21)

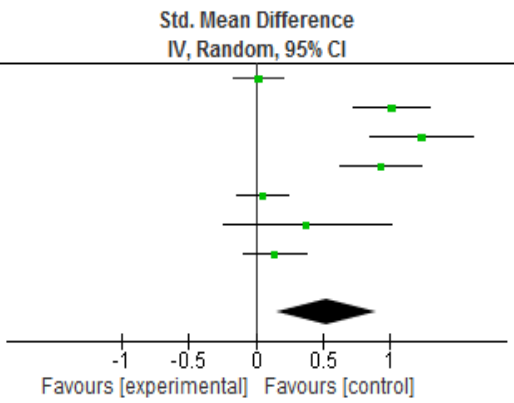


c

BMI (metformin vs. placebo/control; RCTs and CSs)

Study or Subgroup	Metformin Mean	SD	Total	Placebo/control Mean	SD	Total	Weight	Std. Mean Difference IV, Random, 95% CI
Chiswick et al. 2015	37.8	4.9	221	37.7	5.6	222	15.5%	0.02 [-0.17, 0.21]
De Leo et al. 2011	28.35	2.15	98	26.6	1.2	110	14.7%	1.02 [0.73, 1.31]
Glueck et al. 2004	33.8	7.8	45	25.6	5.9	95	13.8%	1.24 [0.86, 1.63]
Glueck et al. 2013	33.3	7.4	62	26.9	6.6	156	14.5%	0.93 [0.63, 1.24]
Syngelaki et al. 2016	39	1.87	202	38.9	2	195	15.4%	0.05 [-0.15, 0.25]
Vanky et al. 2004	32.1	6.1	18	29.3	8	22	11.1%	0.38 [-0.25, 1.01]
Vanky et al. 2010	29.5	7	135	28.5	7.2	135	15.1%	0.14 [-0.10, 0.38]
Total (95% CI)			**781**			**935**	**100.0%**	**0.53 [0.16, 0.90]**

Heterogeneity: Tau² = 0.23; Chi² = 78.86, df = 6 (P < 0.00001); I² = 92%
Test for overall effect: Z = 2.77 (P = 0.006)

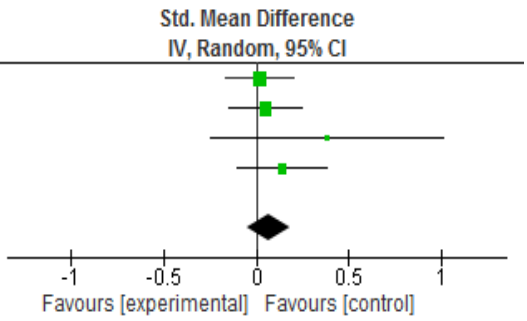


d

BMI (metformin vs. placebo; RCTs only)

Study or Subgroup	Metformin Mean	SD	Total	Placebo/control Mean	SD	Total	Weight	Std. Mean Difference IV, Random, 95% CI
Chiswick et al. 2015	37.8	4.9	221	37.7	5.6	222	38.6%	0.02 [-0.17, 0.21]
Syngelaki et al. 2016	39	1.87	202	38.9	2	195	34.6%	0.05 [-0.15, 0.25]
Vanky et al. 2004	32.1	6.1	18	29.3	8	22	3.4%	0.38 [-0.25, 1.01]
Vanky et al. 2010	29.5	7	135	28.5	7.2	135	23.5%	0.14 [-0.10, 0.38]
Total (95% CI)			**576**			**574**	**100.0%**	**0.07 [-0.04, 0.19]**

Heterogeneity: Tau² = 0.00; Chi² = 1.59, df = 3 (P = 0.66); I² = 0%
Test for overall effect: Z = 1.20 (P = 0.23)

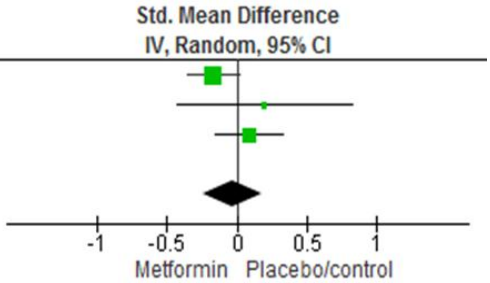


e

Systolic blood pressure (metformin vs. placebo; RCTs only)

Study or Subgroup	Metformin Mean	SD	Total	Placebo/control Mean	SD	Total	Weight	Std. Mean Difference IV, Random, 95% CI
Chiswick et al. 2015	117.6	10.8	226	119.4	10.4	223	49.8%	-0.17 [-0.35, 0.02]
Vanky et al. 2004	120	14	18	117	16	22	10.1%	0.19 [-0.43, 0.82]
Vanky et al. 2010	119	12	135	118	11	138	40.1%	0.09 [-0.15, 0.32]
Total (95% CI)			**379**			**383**	**100.0%**	**-0.03 [-0.24, 0.18]**

Heterogeneity: Tau² = 0.01; Chi² = 3.44, df = 2 (P = 0.18); I² = 42%
Test for overall effect: Z = 0.28 (P = 0.78)

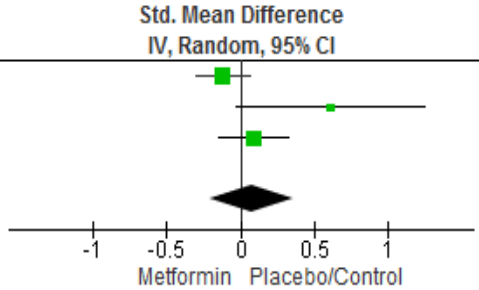


f

Diastolic blood pressure (metformin vs. placebo; RCTs only)

Study or Subgroup	Metformin Mean	SD	Total	Placebo/Control Mean	SD	Total	Weight	Std. Mean Difference IV, Random, 95% CI
Chiswick et al. 2015	68	7.8	226	68.9	7.3	223	45.2%	-0.12 [-0.30, 0.07]
Vanky et al. 2004	78	9	18	72	10	22	14.6%	0.61 [-0.02, 1.25]
Vanky et al. 2010	74	12	135	73	10	138	40.2%	0.09 [-0.15, 0.33]
Total (95% CI)			**379**			**383**	**100.0%**	**0.07 [-0.21, 0.36]**

Heterogeneity: Tau² = 0.04; Chi² = 5.69, df = 2 (P = 0.06); I² = 65%
Test for overall effect: Z = 0.50 (P = 0.62)



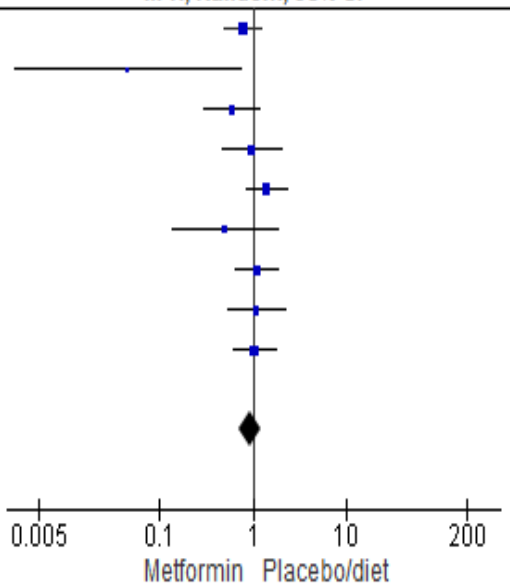
a

GDM (metformin vs. placebo/control; RCTs and CSs)

Study or Subgroup	Metformin Events	Metformin Total	placebo/diet Events	placebo/diet Total	Weight	Risk Ratio M-H, Random, 95% CI
Chiswick et al. 2015	26	142	36	153	18.8%	0.78 [0.50, 1.22]
De Leo et al. 2011	0	98	12	110	0.8%	0.04 [0.00, 0.75]
Glueck et al. 2004	9	95	40	251	10.9%	0.59 [0.30, 1.18]
Glueck et al. 2013	8	56	23	156	9.6%	0.97 [0.46, 2.04]
Koh et al. 2011	37	465	21	371	15.9%	1.41 [0.84, 2.36]
Jamal et al. 2012	3	35	6	35	3.7%	0.50 [0.14, 1.84]
Syngelaki et al. 2016	25	202	22	195	15.2%	1.10 [0.64, 1.88]
Vanky et al. 2004	8	18	9	22	10.1%	1.09 [0.53, 2.23]
Vanky et al. 2010	22	125	21	124	15.0%	1.04 [0.60, 1.79]
Total (95% CI)		**1236**		**1417**	**100.0%**	**0.93 [0.71, 1.20]**
Total events	138		190			

Heterogeneity: Tau² = 0.04; Chi² = 11.01, df = 8 (P = 0.20); I² = 27%

Test for overall effect: Z = 0.57 (P = 0.57)



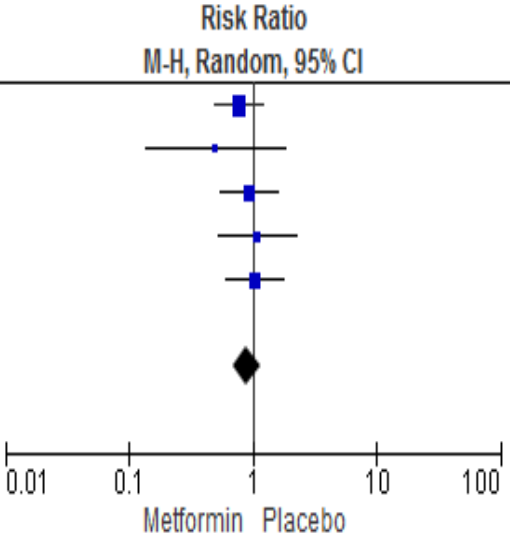
b

GDM (metformin vs. placebo; RCTs only)

Study or Subgroup	Metformin Events	Metformin Total	Placebo Events	Placebo Total	Weight	Risk Ratio M-H, Random, 95% CI
Chiswick et al. 2015	26	142	36	153	34.5%	0.78 [0.50, 1.22]
Jamal et al. 2012	3	35	6	35	4.1%	0.50 [0.14, 1.84]
Syngelaki et al. 2016	25	202	22	165	24.4%	0.93 [0.54, 1.58]
Vanky et al. 2004	8	18	9	22	13.4%	1.09 [0.53, 2.23]
Vanky et al. 2010	22	125	21	124	23.6%	1.04 [0.60, 1.79]
Total (95% CI)		**522**		**499**	**100.0%**	**0.89 [0.69, 1.16]**
Total events	84		94			

Heterogeneity: Tau² = 0.00; Chi² = 1.73, df = 4 (P = 0.79); I² = 0%

Test for overall effect: Z = 0.84 (P = 0.40)



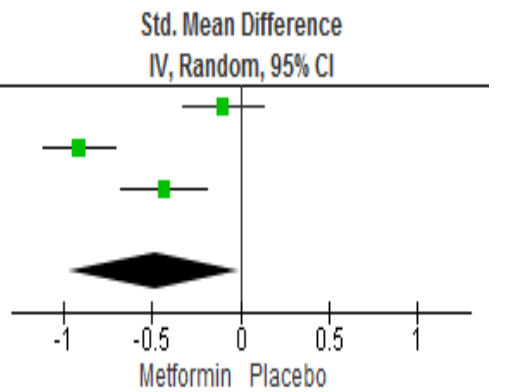
c

Mean weight gain (metformin vs. placebo; RCTs only)

Study or Subgroup	Metformin Mean	Metformin SD	Metformin Total	Placebo Mean	Placebo SD	Placebo Total	Weight	Std. Mean Difference IV, Random, 95% CI
Chiswick et al. 2015	6.7	6	143	7.23	4.9	156	33.3%	-0.10 [-0.32, 0.13]
Syngelaki et al. 2016	4.28	2.1	202	6.3	2.3	198	33.7%	-0.92 [-1.12, -0.71]
Vanky et al. 2010	7	4.4	135	9.2	5.6	138	33.0%	-0.44 [-0.68, -0.19]
Total (95% CI)			**480**			**492**	**100.0%**	**-0.48 [-0.97, -0.00]**

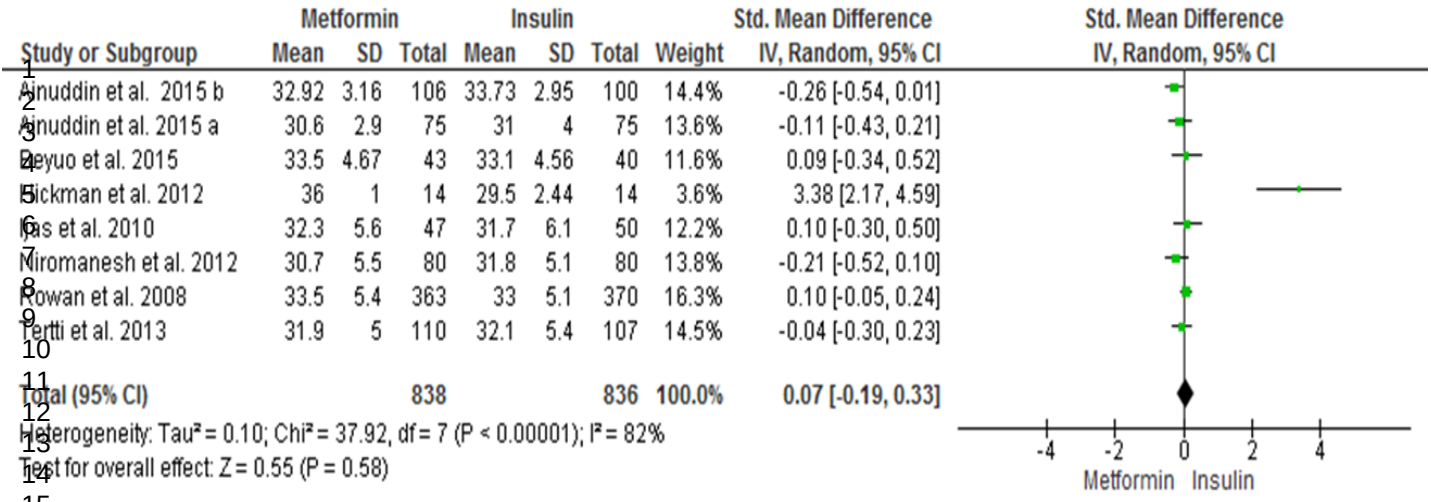
Heterogeneity: Tau² = 0.17; Chi² = 27.98, df = 2 (P < 0.00001); I² = 93%

Test for overall effect: Z = 1.96 (P = 0.05)



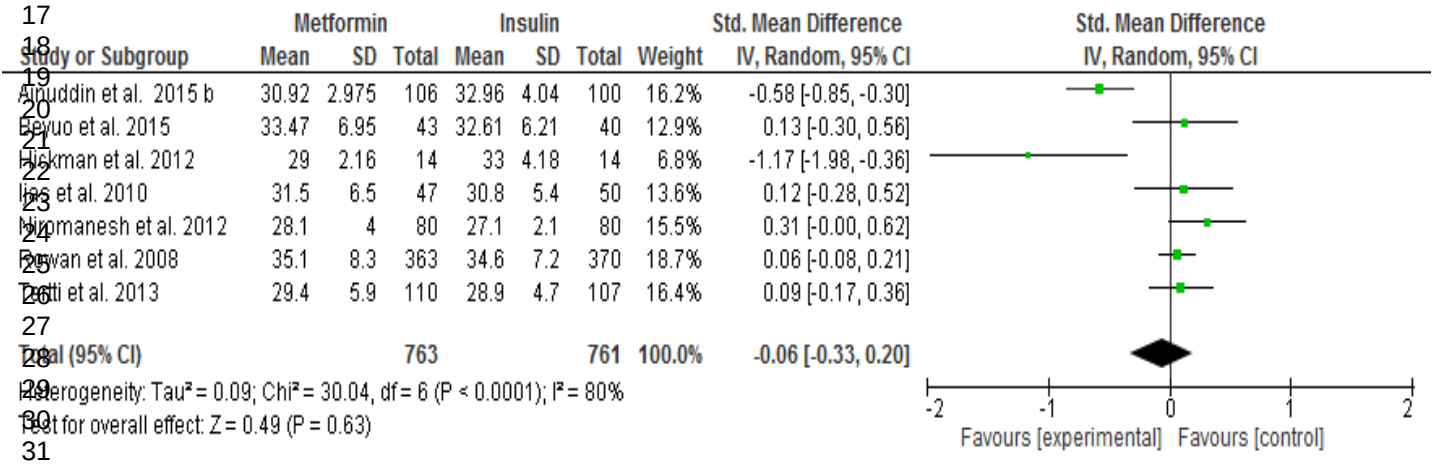
a

Age (metformin vs. insulin; RCTs only)



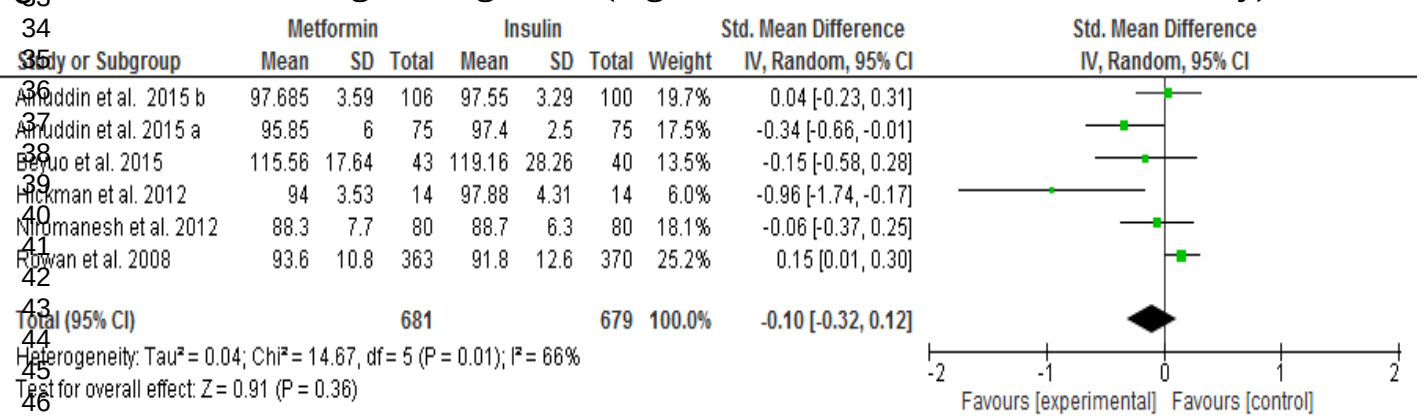
b

BMI (kg/m^2;metformin vs. insulin; RCTs only)



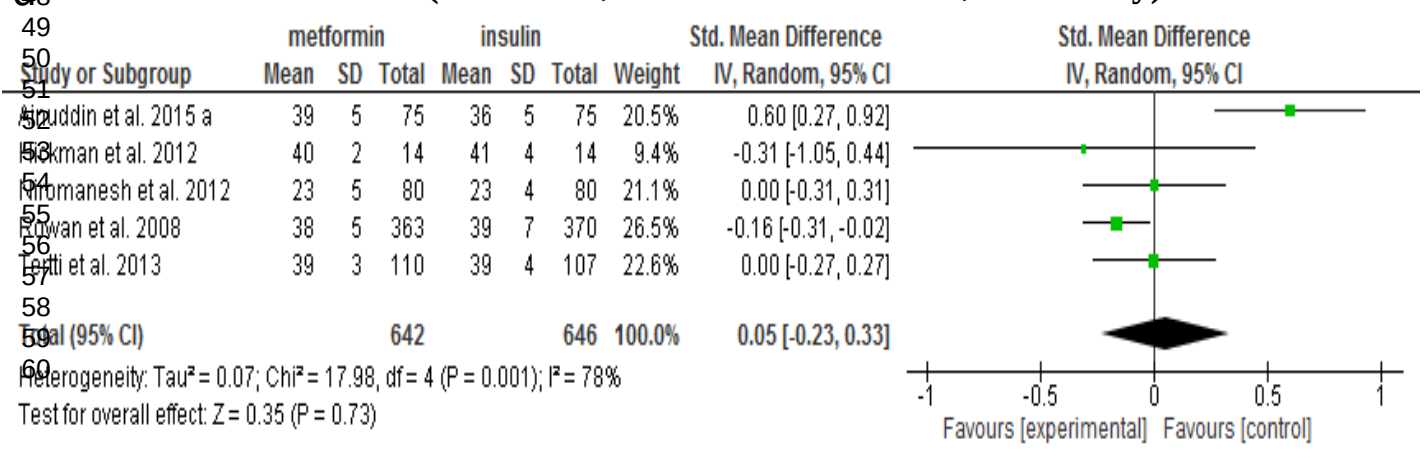
c

Fasting blood glucose (mg/dl; metformin vs. insulin; RCTs only)



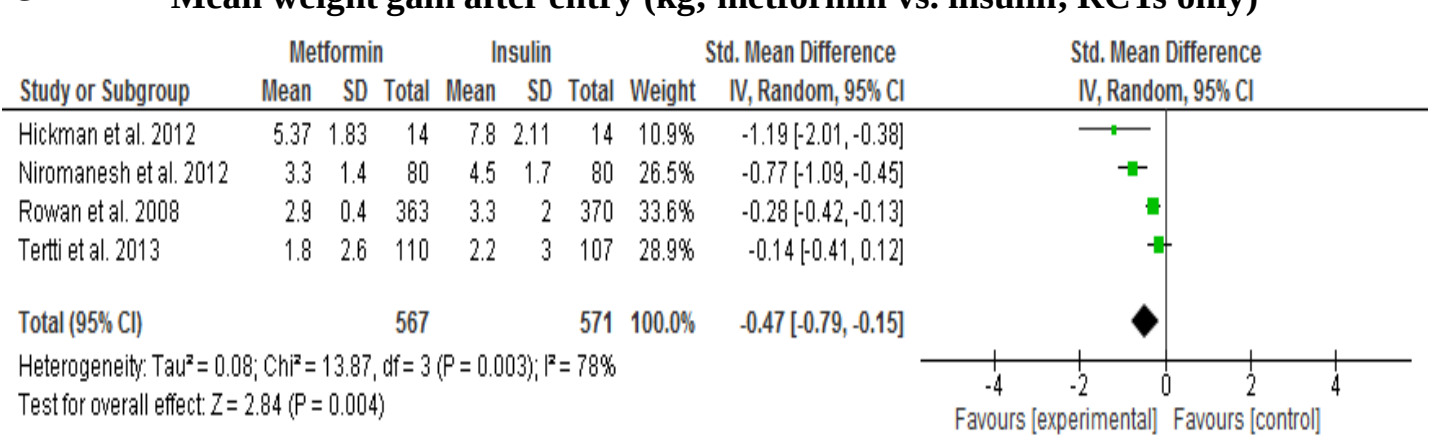
d

HbA1c (mmol/mol; metformin vs. insulin; RCTs only)

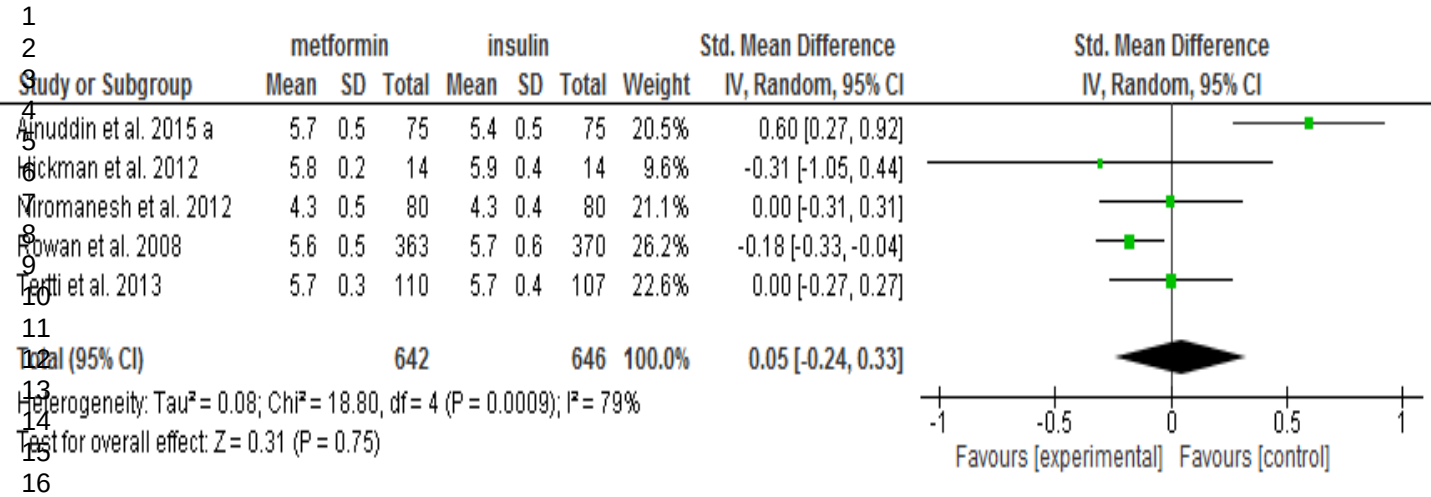


e

Mean weight gain after entry (kg; metformin vs. insulin; RCTs only)



HbA1c (%; metformin vs. insulin; RCTs only)



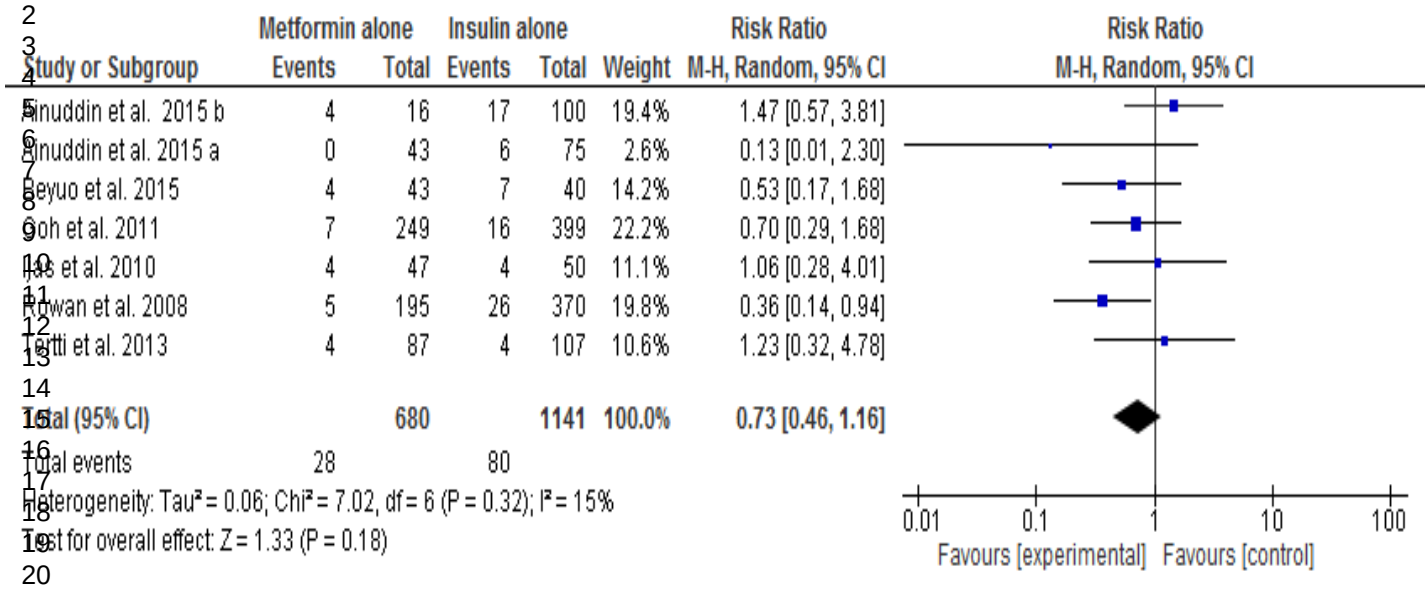
Supplementary Fig. S1. Meta-analyses of HbA1c (%) in metformin vs. insulin treatment group, RCTs only. A meta-analysis of glycated haemoglobin (HbA1c) recorded between 36 and 37 weeks. Random effects models were used to combine estimates and analyses were carried out using RevMan 5.3 software; standard mean difference was calculated; the overall effect was measured using Z-test with p values less than 0.05 being statistically significant. Heterogeneity was calculated using Chi^2 test and measured by I^2 statistic.

Table S1. Percentage of patients in metformin group who were supplemented with additional insulin

Study	No. of patients on metformin alone	No. of patients on metformin+insulin	Total	Percentage of patients needed additional insulin
Ainuddin et al. 2015 a	43	32	75	43%
Ainuddin et al. 2015 b	16	90	106	85%
Goh et al. 2011	249	216	465	46%
Hickman et al. 2012	8	6	14	43%
Ijas et al. 2011	32	15	47	32%
Niromanesh et al. 2012	69	11	80	14%
Rowan et al. 2008	195	168	363	46%
Tertti et al. 2013	87	23	110	21%
Total	**699**	**561**	**1260**	**45%**

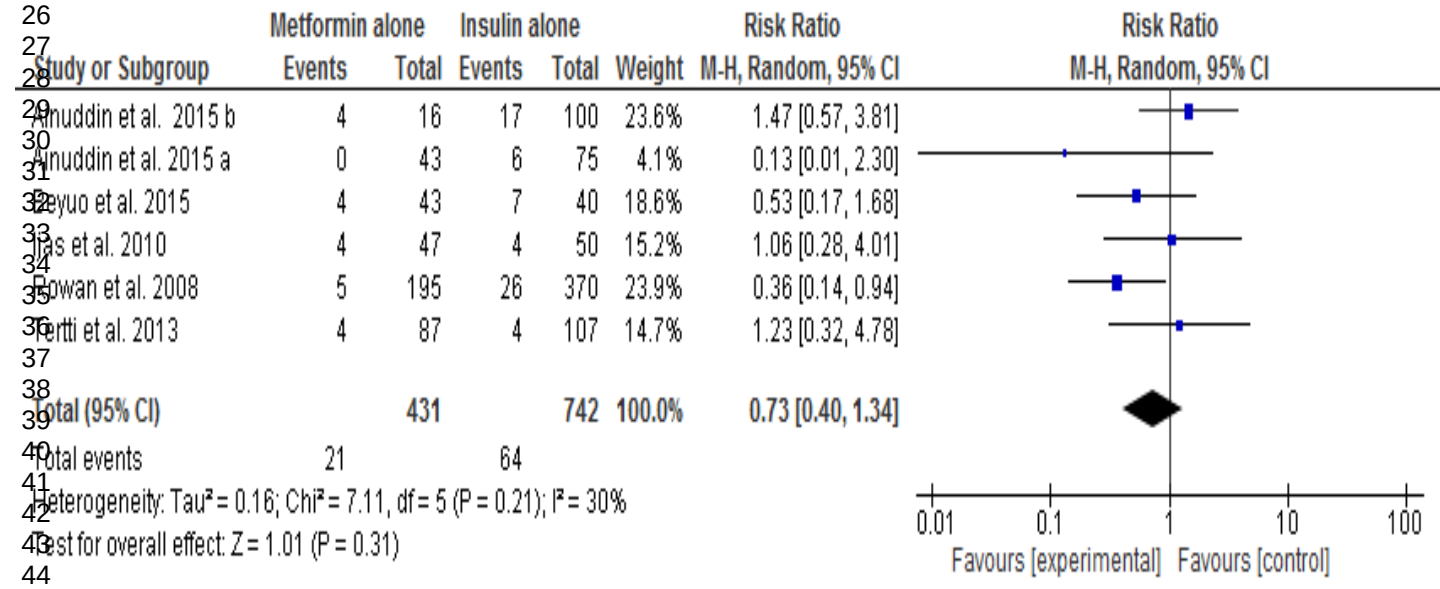
a

Metformin only vs insulin only (RCTs and CSs)



b

Metformin only vs insulin only (RCTs only)



Supplementary Figure S2: A meta-analysis comparing the risk ratio of pre-eclampsia in metformin only vs. insulin treatment group. (A) A meta-analysis including both randomised controlled trials (RCTs) and cohort studies (CSs) comparing metformin only treatment to insulin only treatment. (B) A meta-analysis including only RCTs comparing metformin only vs. insulin only treatment.