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DIABETIC Medicine

SYSTEMATIC REVIEW

Fetal and post-natal outcomes in offspring after intrauterine metformin exposure: A systematic review and meta-analysis of animal experiments

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Abstract

Aims: The impact of maternal metformin use during pregnancy on fetal, infant, childhood and adolescent growth, development, and health remains unclear. Our objective was to systematically review the available evidence from animal experiments on the effects of intrauterine metformin exposure on offspring's anthropometric, cardiovascular and metabolic outcomes.

Methods: A systematic search was conducted in PUBMED and EMBASE from inception (searched on 12th April 2023). We extracted original, controlled animal studies that investigated the effects of maternal metformin use during pregnancy on offspring anthropometric, cardiovascular and metabolic measurements. Subsequently, risk of bias was assessed and meta-analyses using the standardized mean difference and a random effects model were conducted for all outcomes containing data from 3

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or more studies. Subgroup analyses were planned for species, strain, sex and type of model in the case of 10 comparisons or more per subgroup.

Results: We included 37 articles ($n = 3133$ offspring from $n = 716$ litters, containing $n = 51$ comparisons) in this review, mostly (95%) on rodent models and 5% pig models. Follow-up of offspring ranged from birth to 2 years of age. Thirty four of the included articles could be included in the meta-analysis. No significant effects in the overall meta-analysis of metformin on any of the anthropometric, cardiovascular and metabolic offspring outcome measures were identified. Between-studies heterogeneity was high, and risk of bias was unclear in most studies as a consequence of poor reporting of essential methodological details.

Conclusion: This systematic review was unable to establish effects of metformin treatment during pregnancy on anthropometric, cardiovascular and metabolic outcomes in non-human offspring. Heterogeneity between studies was high and reporting of methodological details often limited. This highlights a need for additional high-quality research both in humans and model systems to allow firm conclusions to be established. Future research should include focus on the effects of metformin in older offspring age groups, and on outcomes which have gone uninvestigated to date.

KEYWORDS

animal, metformin, pregnancy, rodents

1 | INTRODUCTION

Metformin is increasingly being used in pregnancy for the treatment of type 2 diabetes mellitus (T2DM) and gestational diabetes mellitus (GDM), and a range of other indications due to its favourable effects on perinatal outcomes such as reduction in number of birthweights defined as large for gestational age, neonatal intensive care admission, neonatal hypoglycaemia, reduced risk of preeclampsia and reduction in gestational weight gain compared with other treatment modalities.[1–11]

There is robust evidence that metformin does not increase the risk of congenital anomalies in humans.[12,13] However, the fact that metformin crosses the placenta and circulates up to therapeutic concentrations in the fetus has led to debate on the effects of metformin in the offspring regarding endocrine and metabolic effects, which may only become evident in the offspring in later life.[14–19] Since metformin decreases hepatic glucose production and increases peripheral glucose utilization by increasing insulin sensitivity, it has been hypothesized that metformin may lead to permanently altered fetal endocrine and metabolic setpoints with possible effects for post-natal growth, adiposity and metabolic health.[20] Although evidence in humans is conflicting,[17,21–24] meta-analyses of randomized controlled trials suggested that maternal metformin treatment during pregnancy is associated with offspring adiposity by mid-childhood compared with placebo or insulin.[23,24] Large and longer term clinical studies are needed to determine the impact of maternal metformin treatment on human offspring growth and cardiometabolic outcome.

What's new?

- This systematic review was unable to establish effects of metformin treatment during pregnancy in non-human offspring
- This highlights a need for additional high quality research both in humans and model systems to allow firm conclusions to be established.
- Future research should include focus on the effects of metformin in older offspring age groups, and on outcomes which have gone uninvestigated to date

Human evidence on metformin exposure on offspring outcomes is hampered by many factors including confounding by selective attrition in trial evidence, confounding by indication in observational data, and limited data in childhood and adulthood because of incomplete follow-up. In contrast, animal models allow for carefully controlled environments in both pre- and post-natal conditions, as well as longer term follow-up and mechanistic understanding of potential underlying effects. Animal models could therefore be useful in informing the discussion on metformin's effects on offspring outcomes. Our

objective was therefore to systematically aggregate the available evidence provided by animal experiments on the effects of intrauterine metformin exposure on offspring's anthropometry, cardiovascular and metabolic outcomes.

2 | METHODS

This review was conducted and reported according to the guidelines outlined in the Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) statement.[25] Furthermore, this review was conducted in collaboration with the Meta-Research team of the Radboudumc (Meta-Research team–Radboudumc).

2.1 | Study protocol

The review protocol (version 1.0) was registered at PROSPERO with number CRD42021260833 on the 6th of August 2021 and can be accessed via the website: https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42021260833. No adjustments to the protocol were made during the execution of this review.

2.2 | Literature search

E.v.H., D.R. performed a systematic search with help of C.H. in PUBMED and OVID EMBASE from inception to 12th April 2023 (final update) using both controlled terms (i.e. MESH) and title abstract words. We searched for the following concepts: (1) Animal studies,[26,27] (2) metformin use, (3) maternal exposure in pregnancy. The bibliographic records retrieved were imported and de-duplicated in Rayyan. The complete search strategy is presented in Data S1.

2.3 | Selection process

Two reviewers (E.v.H. and D.R.) independently screened all identified studies for eligibility using Rayyan.[28] We first screened titles and abstracts of all unique studies in duplo. Secondly, we performed eligibility screening of the full text of studies initially deemed eligible after title and abstract screening. Disagreements were resolved by discussion or by consulting a third reviewer (R.P.). There were no language restrictions. The results from each step of the review process are shown in the PRISMA flow diagram (Figure 1).

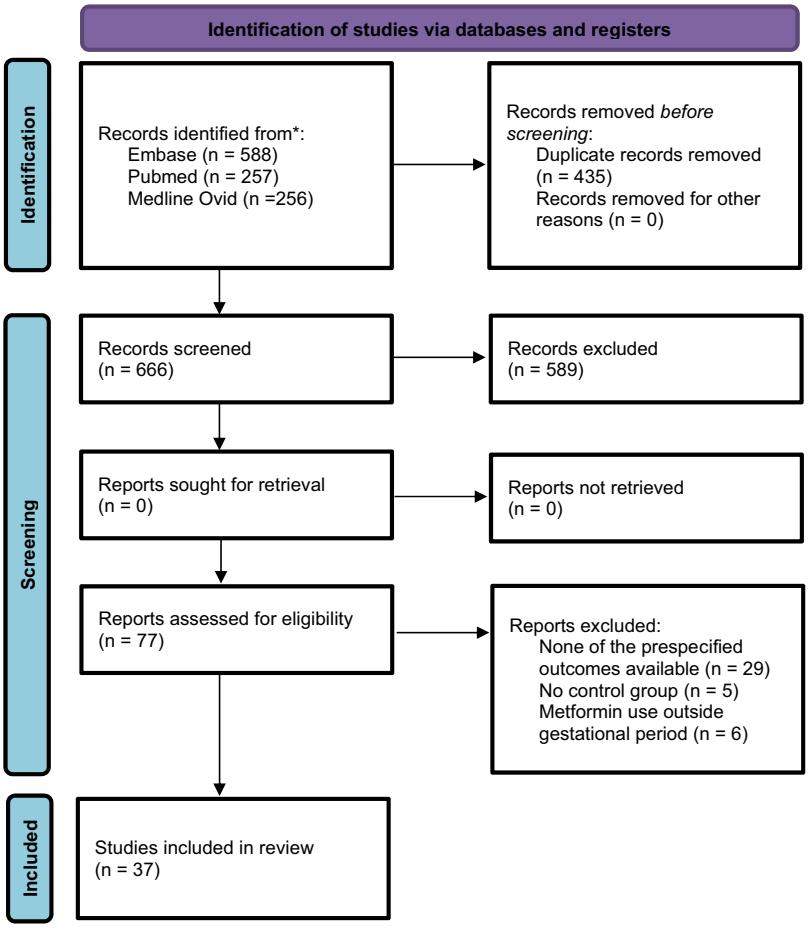

FIGURE 1 PRISMA flowchart.

TABLE 1 Characteristics of studies included in meta-analysis.

Author (year)	Species, strain	Comorbidity, how induced	Type of model	Route of administration metformin	Dose	Frequency	Gestational age of start metformin/ control exposure
Albaghdadi AJ, Hewitt MA, et al (2017)	Mice, RCS-10	Obesity, diet	High-fat diet	Oral	50 mg/kg/day	Once a day	-
Alzamendi A, Del Zotto H, et al (2012)	Rats, Sprague–Dawley	None	Normal diet	Oral	50 mg/kg/day	Once a day	E0.5
		Metabolic syndrome, diet	Fructose-rich diet (tap water with 10% fructose)	Oral	50 mg/kg/day	Once a day	E0.5
Alvarez D, Ceballo K, et al (2018)	Rats, Sprague–Dawley	Obesity, diet	High-fat diet	Oral	160–200 mg/kg/day	Daily (in drinking water)	1 week before mating
Deng J, Mueller M, et al (2019)	Mice, CD-1		Normal diet	Oral	250 mg/kg	Once a day	E0.5
Desai N, Roman A, et al (2013)	Rats, Wistar	Obesity, diet	High-fat diet	Oral	300 mg/kg	Once a day	E1
Forcato S, Montagnini B, et al (2019)	Rats, Wistar	None	Normal diet	Oral	293 mg/kg	Once a day	GD0
			Normal diet	Oral	293 mg/kg	Once a day	GD0
Forcato S, Novi S, et al (2017)	Rats, Wistar	None	Normal diet	Oral	293 mg/kg	Once a day	GD0
			Normal diet	Oral	293 mg/kg	Once a day	GD0
Garbarino VR, Santos TA, et al (2019)	Mice, C57BL/6J	None	Normal diet	Oral	0.5 mg/mL (5.8 mL a day)—250 mg/kg/d begin of experiment, 130 mg/kg/d at peak weight of pregnancy	Once a day	Day before males were introduced
			Normal diet	oral	0.5 mg/mL (5.8 mL a day)—250 mg/kg/d begin of experiment, 130 mg/kg/d at peak weight of pregnancy	once a day	day before males were introduces
Garcia-Contreras C, Vasquez-Gomez, et al (2020)	Sow, Iberian	IUGR, diet	Diet-induced intra uterine growth restricted	Oral (top dressing)	850 mg	Once a day	Day 35 of gestation
			Diet-induced intra uterine growth restricted	Oral	850 mg	Once a day	Day 35 of gestation
Garcia-Contreras C, Vasquez-Gomez, et al (2019)	Sow, Iberian	IUGR, diet	Diet-induced intra uterine growth restricted	Oral	850 mg	once a day	day 35 of gestation
Grace MR, Dotters-Katz SK, et al (2019)	Mice, FVB	None	Normal diet	Oral	2,5 mg/mL	Once a day	E0.5
		Obesity, diet	High-fat diet	oral	2,5 mg/mL	Once day	E0.5
Gregg BE, Botezatu N, et al (2018)	Mice, C57Bl6	None	Normal diet	Oral	5 mg/mL	Weekly	E0.5
Gregg B, Elghazi L, et al (2014)	Mice, C57B16	None	Normal diet	oral	5 mg/mL	weekly	E0.5
Hu J, Zhang J, et al (2019)	Rats, Sprague–Dawley	PE, LPS	LPS	Injection	300 mg/kg	Daily	GD11
Huang L, Yue P, et al (2018)	Mice, C57BL/6J	Diabetes, Streptozotocin	Streptozotocin	Oral	200 mg/kg	Daily	GD11
Huang S-W, Ou Y-C, et al (2021)	Rats, Sprague–Dawley	Obesity, diet	High-fat diet	oral	500 mg/kg	daily (in drinking water)	GD0
Hufnagel A, Fernandez-Twinn DS, et al (2021)	Mice, C57BL/6J	Obesity, diet	High-fat diet	Oral (in milk)	255 mg/kg	Daily	1 week before mating

Duration of exposure	Number dams metformin group	Number of offspring metformin group	Control	Number dams control group	Number of offspring control group	Duration of follow-up	Sex offspring
-	3	17	Vehicle	3	3	Birth	Both
E20	6	46	Tap water	6	46	Birth	Both
E20	6	46	Tap water with fructose 10% w/v)	6	46	Birth	Both
Until PND 21	4	4	HFD + water	4	5	PND60	Female
E18 (until E18.5)	7	63	Vehicle (tap water)	7	48	Birth	Both
E19	9	106	HFD alone	9	110	Birth	Both
21 days until GD21 (without lactation)	11	xx	Drinking water by gavage	11	xx	PND 121	Female
42 days until LD21 (with lactation)	9	xx	Drinking water by gavage	9	xx	PND 121	Female
21 days until GD21 (without lactation)	12	xx	Drinking water by gavage	12	xx	PND 110	Male
42 days until LD21 (with lactation)	11	xx	Drinking water by gavage	11	xx	PND 110	Male
Until delivery	xx	8	Water	xx	16	6–7 weeks	Female
until PND25	xx	8	water	xx	10	6–7 weeks	Male
30 days after delivery	[9]	34	No top dressing	[6]	26	PND30	Male
30 days after delivery	xx	38	No top dressing	xx	21	PND 30	Female
65 days during pregnancy (until day 100)	[3]	23	vehicle	[3]	24	Birth (near to term)	Both
17 days (E17.5)	5	5	Water	5	5	E17.5	Both
17 days (E17.5)	5	45	Water	5	55	E17.5	Both
E18 (until E18.5)	16	xx	Vehicle	16	xx	2 years	Both
until E14	14	27	only water	16	30	birth	Both
8 days (until GD18)	12	xx	LPS	12	xx	Birth	Both
8 days (until GD18)	10	xx	No oral gavage	10	xx	Birth	Both
21 days (GD21)	6	xx	water	6	xx	birth	Both
25,5 days (until E18.5)	13	xx	Only milk	13	xx	Birth	Male

(Continues)

TABLE 1 (Continued)

Author (year)	Species, strain	Comorbidity, how induced	Type of model	Route of administration metformin	Dose	Frequency	Gestational age of start metformin/ control exposure
			High-fat diet	Oral (in milk)	256 mg/kg	Daily	1 week before mating
Jiang S, Teague AP, et al (2020)	Mice	Diabetes, diet	High-fat diet	Oral	2 mg/mL	Daily	6–8 weeks before mating
Kassab B, Hussein H	Rats, Sprague–Dawley	None	?	Intra gastric infusion	250 mg/kg	Daily	E11
Lawal SK, Adeniji AA, et al (2019)	Rats, Sprague–Dawley	Diabetes, Streptozotocin	Streptozotocin	Oral canulla	36.43 mg/lg	Daily	E4
Lu Y, Jia Y et al (2021)	Mice, C57BL/6J	Diabetes, diet	High-fat diet	Oral gavage	300 mg/kg/d	Daily	E0
			High-fat diet	Oral gavage	300 mg/kg/d	Daily	E0
Novi DRBS, Forcato S, et al (2017)	Rats, Wistar	None	Normal diet	Oral gavage	293 mg/kg	Daily	E0
			Normal diet	Oral gavage	293 mg/kg	Daily	E0
Novi DRBS, Vidigal CB, et al (2020)	Rats, Wistar	None	Normal diet	Oral gavage	293 mg/kg	Daily	E0
			Normal diet	oral gavage	293 mg/kg	daily	E0
Nüsken E, Turnwald E, et al (2019)	Mice, C57BL/6J	Obesity, diet	High-fat diet	oral	380 mg/kg	Daily	E0.5
Osinubi AAA, Medubi LJ, et al (2018)	Rats, Sprague–Dawley	Diabetes, Streptozotocin	Streptozotocin	Oral	36.43 mg/kg	Daily	E2
Salomäki H, Heinäniemi M, et al (2014)	Mice, C57BL/6J	Metabolically Challenged pregnancy, diet	High-fat diet	Oral	300 mg/kg	Daily	E0.5
Salomäki H, Vähätalo LH, et al (2013)	Mice, C57BL/6J		Normal diet	Oral	300 mg/kg	Daily	E0.5
Salomäki-Myftari H,Vähätalo LH et al (2016)	Mice, OE-NPYDβH	Genetic model of obesity	Normal diet	Oral gavage	300 mg/kg	Daily	E0.5
Schoonejans JM, Blackmore HL, et al (2021)	Mice, C57BL/6J	Obesity, diet	High-fat diet	Oral	300 mg/kg	Daily	1 week pre-mating
Schoonejans JM, Blackmore HL et al (2022)	Mice, C57BL/6J	Obesity, diet	High-fat diet	Oral	300 mg/kg	Daily	1 week pre-mating
			High-fat diet	Oral	300 mg/kg	Daily	1 week pre-mating
Song A-Q, Sun L-R, et al (2016)	Rats, Sprague–Dawley	Diabetes, streptozotocin	Streptozotocin	Intra gastric infusion	300 mg/kg	Daily	E9
Song L, Cui J et al (2022)	Rats, Sprague–Dawley	None	High-fat diet	?	300 mg/kg/d	Daily	E0.5
			High-fat diet	?	300 mg/kg/d	Daily	E0.5
Sun X, Tavenier A, et al (2018)	Mice, Faah−/−	None, premature delivery	Normal diet	Oral gavage	1 mg/kg	Day 8, 10 and 12	Day 8
	Mice, WT	None, premature delivery	Normal diet	Oral gavage	1 mg/kg	Day 8, 10 and 12	Day 8
Tong JF, Yan F, et al (2011)	Mice, C57BL/6J	Obesity, diet	Obese	Oral	350 mg/kg	Day	
Vidigal CB, Novi DRBS, et al (2018)	Rats, Wistar	None	Normal diet	Oral	293 kg/mg/day	Daily	GD0
Vora NL, Grace MR, et al (2019)	Mice, FVB	None	Normal diet	Oral	2,5 mg/mL	Daily	E0.5
		High-fat diet model	High-fat diet	Oral	2,5 mg/mL	Daily	E0.5
Wang F, Cao G, et al (2019)	Mice, CD-1	Preeclampsia, diet	High-fat diet	Oral	20 mg/kg	Daily	E0.5

Duration of exposure	Number dams metformin group	Number of offspring metformin group	Control	Number dams control group	Number of offspring control group	Duration of follow-up	Sex offspring
25,5 days (until E18.5)	13	xx	Only milk	14	xx	Birth	Female
Throughout lactation	xx	19	HFD alone	xx	17	Until weaning (day 23)	Male
19 days (until E20)	?	?	Water	xx	xx	Birth	Both
13 days (until E17)	5	31	Distilled water (0.5 mL)	5	29	Birth	Both
?	10	80	Water	10	80	8 weeks	Male
?	10	80	Water	10	80	8 weeks	Female
20 days (until E20)	11	120	Water oral gavage	10	125	PND75-80	Both
41 days (until PND21)	10	109	Water oral gavage	10	127	PND75-80	Both
20 days (until E20)	8	80	Water oral gavage	7	70	PND75-80	Both
41 days (until PND21)	7	70	water oral gavage	8	80	PND75-80	Both
18 days	xx	xx	Water	xx	xx	Birth	Both
(17 days (until E19)	5	40	Water	5	47	Birth	Both
17 days (until E17.5)	6	48	Water	6	48	17 weeks	Both
17 days (until E17.5)	6	36	Water	7	49	20 weeks	Both
18 days (until E18.5)	6	23	Vehicle	7	70	7 months	Both
25 days (until E18.5)	6	36	HFD with milk	13	78	8 weeks	Both
18 days (until 18.5)	9	xx	High-fat diet	9	xx	12 months	Male
18 days (until 18.5)	11	xx	High-fat diet	10	xx	12 months	Female
?	7	10	Intragastric infusion saline	7	10	?	Both
PND 21	7	xx	High-fat diet + water	8	xx	16 weeks	Male
PND 21	7	xx	High-fat diet + water	8	xx	16 weeks	Female
3 gifts in 5 days	5	45	Vehicle	5	50	Birth	Both
3 gifts in 5 days	5	50	Vehicle	5	50	Birth	Both
throughout pregnancy and lactation'	6	36	Only water	6	36	PND60	Both
21 days (until GD21)	xx	12	Vehicle (tap water)	xx	11	PND75-780	Both
17 days (unti E17.5)	5	20	Only water	5	45	Birth	Both
17 days (until E17.5)	5	45	Only water	5	45	Birth	Both
18 days (until 18.5)	12	164	HFD alone	13	175	Birth	Both

2.4 | Eligibility criteria

Animal studies were eligible if they compared at least one of our prespecified outcomes in offspring born to females with metformin use during pregnancy to offspring born to females without metformin use during pregnancy. Offspring outcomes included birth weight, body length at birth, weight at last time point identified in the individual experiments, body fat percentage, glucose concentration, insulin concentration, glucose concentration at 60 min after a glucose tolerance test, the largest difference between groups in glucose concentrations during a glucose tolerance test and insulin tolerance, placental weight, placental size, systolic blood pressure, diastolic blood pressure, mean arterial pressure, triglycerides, total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL) and corticosterone/cortisol concentrations. All of the above outcomes were eligible for inclusion in our systematic review if they were measured in the offspring.

Reasons for exclusion were (1) not a full, original article or primary study (e.g. reviews were not eligible) (2) not an animal study, (3) no metformin exposure (4) no pregnant animals were exposed, (5) metformin exposure only in pre-conception or during lactation (without intrauterine exposure) (6) no correct control group (e.g. no control group or interventions interfering with primary effect of metformin use, and lastly (7) none of the prespecified outcomes reported.

2.5 | Study characteristics

We extracted bibliographic details (e.g. author, year of publication), animal model maternal characteristics (e.g. species, feeding pattern, chosen comorbidity and how this was induced—e.g. diet-induced in the case of diabetes or obesity, or streptozotocin-induced in the case of diabetes), intervention characteristics (e.g. route, dose and timing of metformin administration, exposure duration) and outcome measures (e.g. type of outcomes, number of dams and numbers of offspring, sex of offspring, duration of follow-up and timing of outcome assessment). A table of characteristics of included articles is shown in Table 1.

2.6 | Data extraction

Two authors (D.R. and E.v.H.) conducted the data extraction from eligible studies with the use of a piloted data extraction form in duplo. Means, standard deviations (SDs) or standard errors (SEs), and number of animals (number of litters and number of offspring in total) were extracted for both control and experimental groups for all outcomes. In case an outcome was measured at multiple time points in the offspring, the last time point was extracted for the overall meta-analyses. In addition, if available, the outcome was extracted at 4 and 8 weeks of age for the subgroup analysis. ImageJ was used to extract results from figures.[29] In case determinants, or outcomes of interest were presented in the manuscript in a way that was insufficient for inclusion in the meta-analysis, authors were contacted for more information.

2.7 | Risk of bias assessment

The methodological quality of all selected studies was independently evaluated by two reviewers (E.v.H and A.v.d.W.) using the SYRCLE risk of bias tool for animal studies.[30] A 'yes' score indicates low risk of bias; a 'no' score indicates high risk of bias; and a '?' score indicates unclear risk of bias. Reporting of all essential methodological details is generally low in animal experiments.[31] To overcome the resulting problem of judging many risk of bias domains as 'unclear risk of bias', we added two items regarding reporting quality: (1) did the authors report any measure of randomization, and (2) did the authors report any measure to ensure blinding. For these items, a 'yes' score indicates reported and 'no' indicated unreported. We did not exclude studies based on poor quality. No aggregated quality was determined.

2.8 | Data synthesis

The statistical analyses were conducted using Comprehensive Meta-Analysis (CMA) software (version 3.0). Meta-analyses were performed for each outcome with more than three studies available. Per outcome mean, SD and *n* were extracted. To avoid data loss, separate analyses were conducted for outcomes presented per litter and per total offspring, as some studies reported outcomes in mean per total offspring and some reported in mean per litter. If a control group (as a whole) was used in more than one comparison, correction for multiple testing (*n*/times control group was used) was performed. If mean, SD or *N* could not be obtained by contacting the authors, or extracted using ImageJ,[32] the study was excluded. In case *N* was displayed in a range, the lowest number was used. The standardized mean difference (SMD) (hedges g) and 95% confidence interval (95% CI) was calculated for individual comparisons. SMD was used as a statistical measure to standardize and compare the treatment effects across studies with different species and different units of measurements for an outcome. We used the random effects model, which takes into account the precision of individual studies and the variation between studies and

weights each study accordingly. I^2 was used to determine the level of between-study heterogeneity. We displayed summary statistics for all meta-analyses, regardless of the degree of heterogeneity. Predefined subgroup analyses were planned for species, strain, sex, timing of outcome assessment (4 weeks of age, 8 weeks of age, last time point in individual experiments), dose and type of model used and were only conducted in case ≥10 independent comparisons from ≥5 individual studies were available per subgroup. In case of 15 or more independent studies, we also assessed the risk of publication bias in Stata (StataCorp 2019 Stata Statistical Software: Release 16; College Station, TX). by assessing funnel plots and conducting Eggers regression and trim-and-fill analyses We plotted the standardized mean differences against a sample size-based precision estimate ($1/\sqrt{n}$)[33] because SMDs may cause funnelplot distortion.[33] We considered *p*-values of lower than 0.05 as statistically significant.

3 | RESULTS

3.1 | Study selection

The systematic literature search in PubMed and EMBASE yielded 666 unique references (Figure 1, PRISMA flowchart). Out of these, 77 were included after screening on title and abstract. Thirty-seven articles met our inclusion criteria. Three articles did not provide numbers of animals or SD and could therefore not be included in our meta-analysis. Therefore, 34 articles were included with 51 comparisons ($n = 3133$ offspring of three species, $n = 716$ litters of three species). The references of the included articles can be found in Data S1.

3.2 | Study characteristics

In Table 1, the characteristics of the studies included in this meta-analysis are provided. Most studies used mice and rats ($n = 20$ used 378 litters, 1685 offspring of mice, $n = 15$ used $n = 307$ litters, 1380 offspring of rats). Two studies used pigs ($n = 157$ offspring). Metformin was administered orally in most studies ($n = 36$). In only one study, metformin was administered via injection. Most animal models employed normal (chow) diet fed ad libitum during pregnancy in both experimental and control animals ($n = 21$, $n = 311$ litters, 1554 offspring). A number of comparisons used a high-fat diet in both the metformin group and control group ($n = 14$, $n = 282$ litters, $n = 1340$ animals). A variety of methods for disease induction were used including streptozotocin-induced diabetes ($n = 3$), a fructose-rich diet ($n = 1$), obesity otherwise induced ($n = 2$) and a diet-controlled model of intrauterine growth restriction ($n = 2$). The dose of metformin administration differed between species (from 50 mg/kg/day to 850 mg/day). The frequency of administration was once daily in 35 studies and once weekly in two studies. Most studies included both offspring sexes in the analyses ($n = 27$ both sexes, $n = 8$ female, $n = 8$ male).

3.3 | Risk of bias assessment

An overall summary of the risk of bias assessment of the included studies is shown Figure 2. In general, the majority of items assessed in the risk of bias analysis showed an unclear risk of bias, due to insufficient reporting of essential methodological details, which is also

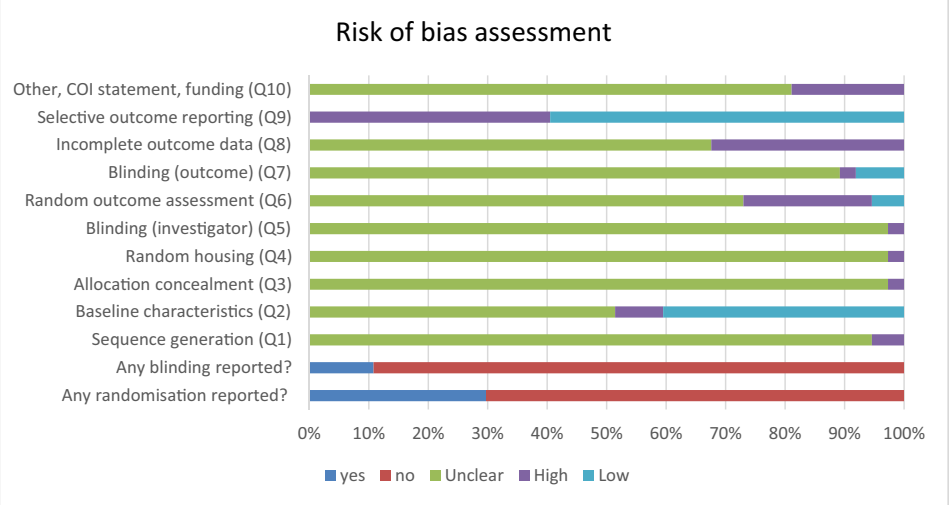

FIGURE 2 Summary of risk of bias assessment.

made transparent by the two question we have added regarding reporting quality. The answers to all questions of the assessment per individual study are displayed separately in the Table S1.

3.4 | Meta-analysis

Table 2 shows a summary of the meta-analysis that we were able to perform including the effects of the main analyses per outcome. Overall, we found no significant effects on any of our predefined outcomes in the primary analyses.

TABLE 2 Overall effect per outcome.

	Per litter		Total offspring	
	Effect	***N* (*n*)**	**Effect**	***N* (*n*)**
Anthropometric outcomes				
Birthweight	⊷	33 (25)	⊷	26 (20)
Placental weight	⊷	12 (8)	⊷	11 (8)
Placental size	N/a	0 (0)	N/a	2 (1)
Body Length	⊷	3 (3)	⊷	5 (4)
Offspring weight	⊷	19 (9)	⊷	21 (12)
Cardiovascular and metabolic outcomes				
Body fat	⊷	4 (2)	⊷	6 (3)
Glucose value	⊷	12 (5)	⊷	10 (7)
Insulin value	⊷	8 (5)	⊷	6 (3)
Glucose tolerance 60 min	⊷	5 (3)	⊷	11 (6)
Glucose tolerance largest effect	⊷	5 (3)	⊷	9 (5)
Insulin tolerance	N/a	3 (2)	N/a	2 (1)
Mean arterial pressure	N/a	4 (1)	N/a	0 (0)
Systolic blood pressure	N/a	0 (0)	N/a	0 (0)
Diastolic blood pressure	N/a	0 (0)	N/a	0 (0)
Triglycerides	⊷	8 (4)	N/a	4 (2)
Total cholesterol	⊷	7 (4)	N/a	2 (1)
LDL	N/a	2 (2)	N/a	3 (2)
HDL	N/a	2 (2)	N/a	3 (2)
Cortisol	N/a	0 (0)	N/a	0 (0)

Note: If no significant effect could be determined, it is presented as ⊷.↑ shows a significantly positive effect for metformin (SMD >0 and $p < 0.05$) and ↓ a significantly negative effect for metformin (SMD <0 and $p < 0.05$). N/a shows there was insufficient data to perform meta-analyses. *N* shows the number of comparisons per outcome in the overall meta-analyses, (*n*) is the number of studies reporting the specific outcome.

3.4.1 | Perinatal anthropometric outcomes

Birth weight could be extracted from 27 studies ($n = 557$ litter, $n = 2979$ offspring), seven studies did not report birth weight.[34–39] There was no significant difference in birth weight between offspring of mothers with metformin use and offspring of mothers without metformin use (Figure 3. and b), SMD −0.11 [95% CI −0.29; 0.06], $I^2 = 78\%$; SMD for birth weight per litter 0.08 [95% CI −0.24; 0.41] $I^2 = 73\%$. Placental weight was measured in 11 studies ($n = 187$ litter, $n = 1084$ offspring).[35,40–47] No significant effect of metformin administration on placental weight was identified (SMD 0.13 [95% CI −0.14; 0.40], $I^2 = 41\%$ for all individual offspring, SMD 0.09 [95% CI −0.18; 0.36] $I^2 = 0\%$ for placental weight per litter. Body length at birth was obtained from five studies ($n = 60$ litter, $n = 283$ offspring) and showed no differences,[43,47–50] SMD 0.12 [95% CI −0.15; 0.38], $I^2 = 5\%$ for all offspring, SMD 0.14 [95% CI −0.35; 0.62] $I^2 = 1\%$ for body length per litter (Figure 4).

3.4.2 | Post-natal anthropometric outcomes

We found no significant effect on weight at last time point (ranging from 6 weeks to 6 months) identified in the individual experiments ($n = 358$ offspring, $n = 1540$ litters, SMD −0.13 [95% CI −0.26; 0.00], $I^2 = 27\%$ for all offspring, SMD −0.13 [95% CI −0.35; 0.09] $I^2 = 16\%$ per litter (Figure 5a,b).

3.4.3 | Body composition, glucose homeostasis and lipids

Five studies ($n = 157$ offspring, $n = 69$ dams) reported body fat percentage (SMD −0.16 [95% CI −1.44; 1.11] $I^2 = 92\%$ for all offspring and SMD −0.31 [95% CI −0.94–0.32] $I^2 = 46\%$ per litter).[37–39,51,52] None of the outcomes of glucose homeostasis differed between the two groups, Figure 6. Eleven studies presented glucose concentration ($n = 556$ offspring, $n = 172$ dams, SMD 0.07 [95% CI −0.61; 0.75] $I^2 = 92\%$ for all offspring and SMD −0.14 [95% CI −0.48; 0.20] $I^2 = 31\%$ per litter).[36,37,39,43,49,51,53–57] Eight presented glucose concentration 60 min after a glucose load injection ($n = 520$ offspring, $n = 92$ dams, SMD −0.10 [95% CI −0.54; 0.34] $I^2 = 80\%$ for all offspring, SMD −0.93 [95% CI −1.99; 0.13] $I^2 = 83\%$ per litter).[36,37,49,51–53,58,59] The same eight studies that presented glucose concentration at 60 min were used to obtain the largest difference between groups in glucose concentration during a glucose tolerance test (SMD −0.22 [95% CI −1.09; 0.65] $I^2 = 94\%$ for all offspring, SMD −1.36 [95% CI −2.83; 0.12] $I^2 = 90\%$ per litter). Seven studies ($n = 404$ offspring, $n = 180$ litters)

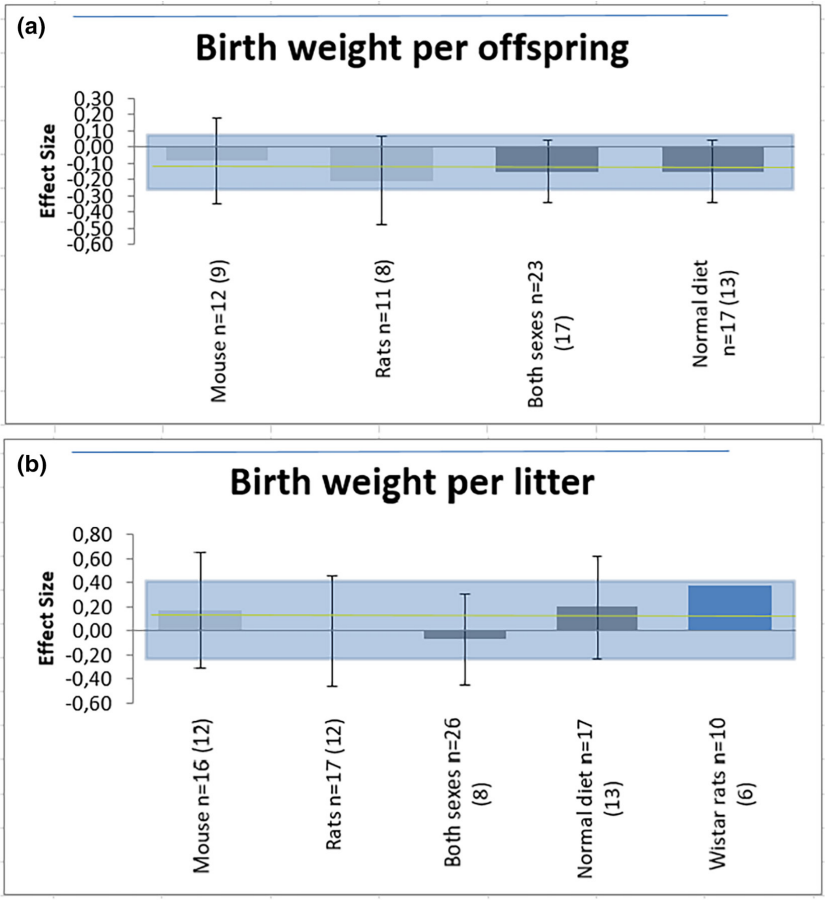

FIGURE 3 Results of the overall analyses and subgroup effects regarding the effect of metformin on birthweight (a) Birthweight in all offspring, (b) average birthweight per litter. *N*: number of comparisons (number of studies). The yellow line and blue area indicate the overall summary effect and the 95% confidence interval. The effect of subgroup analyses are depicted in the individual bars. The height represents the pooled effect size (Hedges g). The black lines represent the ±95% confidence interval.

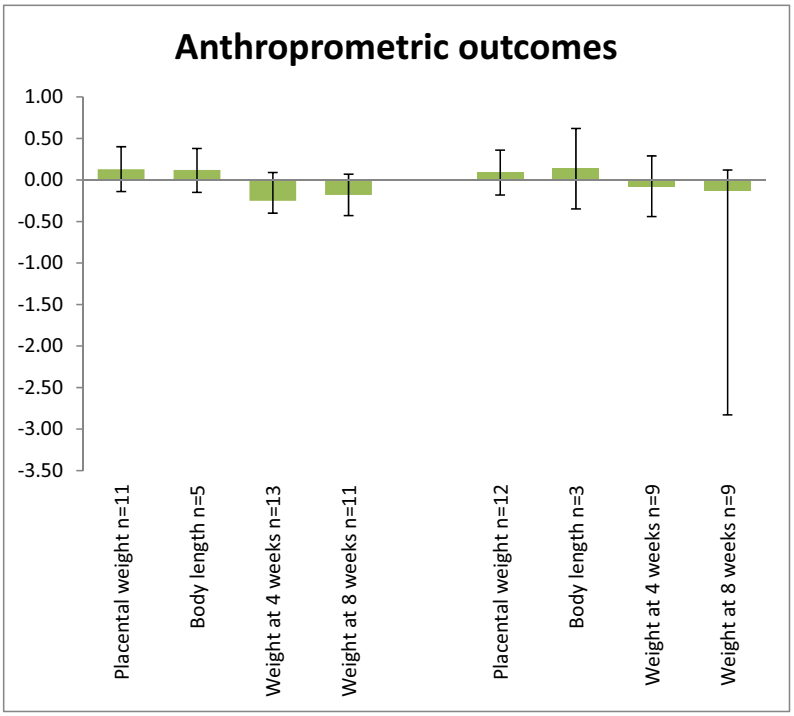

FIGURE 4 Results of the overall analyses regarding the effect of metformin compared with placebo on anthroprometric outcomes. *N*: number of comparisons. Effect size in standardized mean difference (all animals, all ages, all offspring sexes).

reported fasting insulin values (SMD −0.32 [95% CI −0.98; 0.35] $I^2 = 76\%$ for all offspring, SMD 0.33 [95% CI −0.29; 0.95] $I^2 = 75\%$ per litter).

In addition, five studies reported fasting triglycerides ($n = 193$ litters) and total cholesterol ($n = 146$ dams). These outcomes could only be extracted per litter and showed

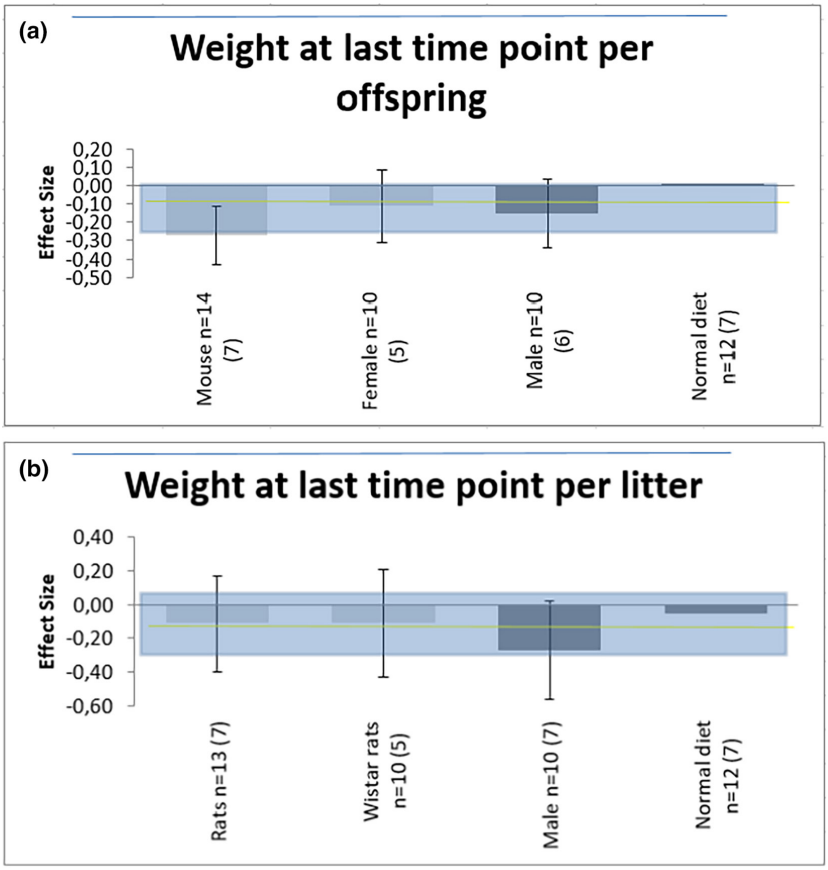

FIGURE 5 Subgroup comparisons of the effect of metformin on weight at the last measured timepoint. (a) Birthweight in all offspring, (b) average birthweight per litter. *N*: number of comparisons (number of studies). The yellow line and blue area indicate the overall summary effect and the 95% confidence interval. The effect of subgroup analyses are depicted in the individual bars. The height represents the pooled effect size (Hedges g). The black lines represent the ±95% confidence interval.

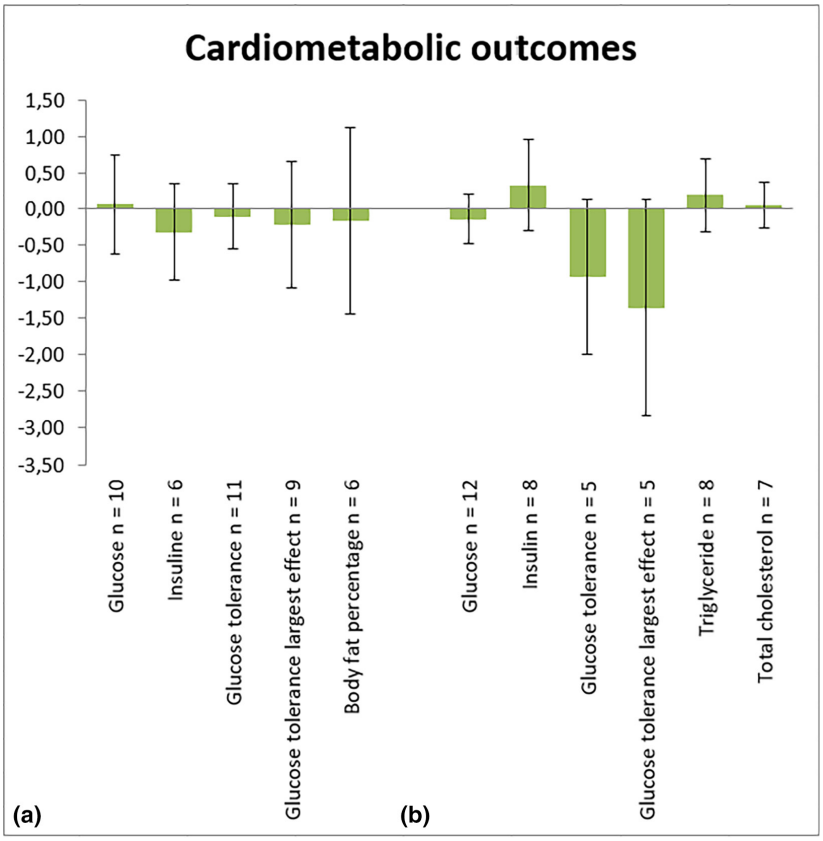

FIGURE 6 (a) Cardiometabolic outcome in all offspring, (b) cardiometabolic outcome per litter. Results of the measures of glucose and lipid homeostasis among offspring of metformin during gestation (all animals, all ages, all offspring sexes), effect size in standardized mean difference. *N*: number of comparisons.

no effects (triglyceride: SMD 0.19 [95% CI −0.32; 0.69] $I^2 = 65\%$ and total cholesterol: SMD 0.05 [95% CI −0.26; 0.36] $I^2 = 0\%$).

3.5 | Outcomes for which meta-analyses were not performed

Since only one study reported on placental size, mean arterial pressure, HDL and LDL cholesterol, and none reported on systolic or diastolic blood pressure or cortisol concentrations, we did not perform meta-analyses on these outcomes.

3.6 | Subgroup analyses, sensitivity analyses and publication bias

Predefined subgroup analyses were carried out for offspring sex (male only, female only, both sexes), species (mouse, rat, pig), strain (C57B16, CD-1, FAAH, Female friendly, RCS-10, Sprague–Dawley, Wild type and Wistar), timing of outcome assessment (4 weeks, 8 weeks) and type of model (normal diet, high-fat diet, GDM, LPS alone, obese, diet-controlled IUGR model).

For birthweight, we performed subgroup analyses for mice, rats, both sexes and normal diet and in addition for outcomes per litter for Wistar rats and high-fat diet. Results of the subgroup analyses are displayed in Figure 3. and b, mice: SMD −0.08 [95% CI −0.35; 0.18] $I^2 = 73\%$ for all offspring; SMD 0.17 [95% −0.31; 0.65] $I^2 = 75\%$ per litter, rats: SMD −0.21 [95% CI −0.48; 0.07] $I^2 = 86\%$ for all offspring and SMD 0.00 [95% −0.46; 0.46] $I^2 = 71\%$ per litter, both sexes: SMD −0.15 [95% −0.34; 0.04] $I^2 = 80\%$ for all offspring and SMD −0.07 [95% −0.45; 0.320] $I^2 = 76\%$ per litter, Wistar: SMD 0.38 [95% −0.24; 1.00] $I^2 = 0\%$ per litter, normal diet: SMD 0.00 [95% −0.22; 0.23] $I^2 = 0\%$ for all offspring, SMD 0.20 [95% −0.23; 0.62] $I^2 = 0\%$ per litter and high-fat diet: SMD −0.05 [95% −0.59; 0.50] $I^2 = 68\%$.

Regarding placental weight we were able to perform subgroup analysis for the mixed sex group (SMD 0.13 [−0.14; 0.40] $I^2 = 40\%$ for all offspring, SMD 0.16 [−0.16; 0.48] $I^2 = 0\%$ per litter).

We also performed subgroup analyses for the outcome weight at last time point identified in the individual experiments for mice, female, male, normal diet, weight at 4 weeks and 8 weeks of age in all offspring (Figure 5a, mice: SMD −0.27 [95% CI −0.43; −0.11] $I^2 = 16\%$, $p = 0.00$, male: SMD −0.15 [95% −0.34; 0.04] $I^2 = 58\%$, female: SMD −0.11 [95% −0.31; 0.09] $I^2 = 0\%$, normal diet: SMD 0.01 [95% −0.11; 0.14] $I^2 = 0\%$, weight at 4 weeks of age (SMD −0.25 [95% CI −0.40; 0.09], $I^2 = 10\%$), weight at 8 weeks of age (SMD −0.18 [95% CI −0.43; 0.07], $I^2 = 18\%$). In addition, subgroup analysis for weight at last time point identified in the individual experiments measured per litter were performed for rats, Wistar, male sex, normal diet, weight at 4 weeks and 8 weeks of age (Figure 5b, rats: SMD −0.11 [95% −0.40; 0.17] $I^2 = 0\%$, Wistar: SMD −0.11 [95% −0.43; 0.21] $I^2 = 0\%$, male: SMD −0.27 [95% −0.56; 0.02] $I^2 = 39\%$, normal diet: SMD −0.05 [95% −0.32; 0.22] $I^2 = 0\%$, 4 weeks of age: SMD −0.08 [95% CI −0.44; 0.29] $I^2 = 13\%$, 8 weeks of age SMD −0.13 [95% CI −0.59; 0.33] $I^2 = 20\%$). Effect estimates were similar for subgroups indicating no differences in the effect of metformin exposure on outcomes based on sex, species, strain or type of model. The only outcome we found to be affected by in utero exposure to metformin was among mice in the total offspring group, which demonstrated a significantly lower weight at the last measured time point after metformin exposure in utero (SMD −0.27 [95% CI −0.43; −0.11] $I^2 = 16\%$. We were not able to perform and thereby confirm this finding when this outcome was measured per litter due to insufficient numbers of studies.

Publication bias was assessed for birthweight, both per litter and in all offspring. Trim-and-fill analysis per litter indicated no missing studies for birthweight. For all offspring, the trim-and-fill analysis showed nine missing studies for birthweight, Egger's regression analysis indicated no significant effect ($p = 0.20$) (Figures S2 and S3).

4 | COMMENT

In this systematic review and meta-analysis in animal studies, we investigated the effects of metformin on fetal and neonatal outcomes, as well as offspring outcomes in later life. With the data available, we found no evidence of metformin affecting offspring growth or cardiometabolic parameters, including measures of adiposity. However, heterogeneity was high and the reporting of methodology often limited making it difficult to make any firm conclusion.

We included 37 studies ($n = 3133$ offspring of three species, $n = 716$ litters of three species) and found no significant effects of intrauterine metformin exposure on birth weight, weight of the offspring at different time points, markers of glucose homeostasis, measures of adiposity as well as other (bio)markers of cardiometabolic health. Our subgroup analyses found no evidence for specific effects based on offspring sex, species, strain, model or timing of outcome assessment. We were however not able to eliminate all heterogeneity with our subgroup analyses. This could be explained because for some planned subgroup analyses, there were insufficient studies to perform these analyses. Most importantly, most comparisons

used lean healthy dams fed normal diets (41%), although in humans metformin is generally used in women with pronounced insulin resistance, such as obesity, PCOS, GDM or T2DM.[3,9–11,60–62] We were not able to perform subgroup analyses for high-fat-diet models and/or diabetes models due to insufficient number of studies. Besides, we were not able to run all analyses for male and female offspring separately, although effects may show sexual dimorphism.[63] In addition, the high heterogeneity could be due the difference in doses applied—a factor that we would have liked to explore in post hoc analyses to assess possible dose–response relationships, but which was not possible due to insufficient number of studies. Moreover, duration of exposure could be a factor influencing potential effects. The only significant effect we found was that exposure to metformin compared with controls resulted in a lower offspring weight at the last time point identified in the individual experiments in mice, which was measured between 4 and 20 weeks comparable to human ranging from childhood to adulthood. However, this finding should be interpreted with caution since the effect size was small and the confidence interval close to zero, the effect is not confirmed in other species, and could be the result of co-linearity.

Following trial evidence of its non-inferiority in terms of perinatal outcomes compared with insulin alone, metformin has now become one of the first choice treatment options for gestational and type 2 diabetes in pregnancy.[1,7,61,64] In addition, insulin has several disadvantages. It is associated with maternal hypoglycaemia, maternal weight gain and people are burdened with storage, self-monitoring and frequent subcutaneous injections. However, since metformin is known to cross the placental barrier, the hypothesis that intrauterine exposure to metformin may have long-term implications on offspring's health, remains topic of debate[14,15] and affects clinician's willingness to prescribe metformin. Human in vitro and in vivo studies have established that metformin crosses the placenta, with concentrations at delivery in the umbilical artery and vein ranging from non-detectable up to maternal therapeutic concentrations.[14–16,65,66] Theories why metformin, despite substantial placental passage, does not exert lasting effects in the fetus include that the early human embryo may be unresponsive to metformin due to low mitochondrial content and negligible metformin transporter expression.[67] In addition, negligible distribution and metabolism by the fetus and elimination by the placenta has been suggested.[15] Although evidence is conflicting,[14] some studies showed that placental tissue retained only small amounts of metformin and that this concentration is unlikely to affect its function.[16,66] Furthermore, metformin provides important effects through the gastro-intestinal system. Therefore, subsequent to portal vein drug delivery, concentration of metformin in the maternal intestine and liver could be even higher than in the systemic circulation, and hence, fetal toxicity is limited.[68]

4.1 | Clinical relevance and human studies

When comparing our offspring outcomes with evidence from humans studies some differences stand out. In humans, intrauterine metformin exposure has been investigated in different conditions of pregnancy including obesity, GDM, PCOS, preeclampsia and metabolic syndrome.[1–5,9–11,69] Two meta-analysis in humans followed up offspring born in RCTs comparing either metformin to insulin in women with GDM[70] or metformin to insulin or placebo in mother with GDM or PCOS.[23] Both examined infant and childhood growth with a maximum follow-up of 9 years. Both studies suggested that maternal metformin treatment was associated with offspring adiposity in mid-childhood based on higher BMI scores[70] or higher weight (but not length or BMI scores).[23] One RCT included in both meta-analyses suggested higher abdominal and visceral fat volumes in children exposed to metformin compared with insulin.[5] However, a recent follow-up study of an RCT on metformin in T2DM during pregnancy found no differences in anthropometrics.[21] In addition, an observational study examining the cardiovascular effects on the offspring at 4 years of age after metformin exposure in pregnant women with obesity provided beneficial data concerning haemodynamic and cardiac diastolic indices.[71] Maternal metformin use was associated with a similar glucose metabolism and more favourable lipid profile compared with insulin in offspring of 9 years of age.[22]

The current standardized use of metformin in human pregnancy adds to the importance and urgency of why we need to know if there are any potential long-term effects to allow risk versus benefit to be assessed. Since in our analysis most animal studies used healthy lean dams fed a normal diet, we cannot rule out that metformin may exert different effects on offspring when in a maternal environment with increased insulin resistance. The fact that we included studies with maternal adiposity, high-fat diet, or hyperglycaemia, none of which demonstrated an increase in adverse outcomes, lends too little support to this limitation. This is a research priority for future studies. Most human pregnancies are exposed to metformin in the third trimester. Animal models, in particular rodent models, do not include a fetal model of third trimester human pregnancy, as they are born in relative immaturity compared with humans.[72] Adipose tissue is laid down in the human fetus in the third trimester, which may also offer

an explanation for the fact that findings are at odds with findings in human studies.

4.2 | Strengths and limitations

To our knowledge, this is the first systematic review of intrauterine metformin exposure in animal models that describes offspring's anthropometry, cardiovascular and metabolic outcomes. A major strength of the present study, is the large number of studies included in this review, and the possibility to explore underlying heterogeneity in comparison to existing evidence in human studies. In addition, in general, animals mature faster than humans and are thus commonly used as models for ageing.[73] With anthropometric data up to 5 months of age, we were able to examine longer term outcomes in offspring after intrauterine metformin exposure.

This study has several limitations which might impact the generalizability and validity of our findings. The majority of the studies used rodent models (mice or rats). The difference between rodents and humans may be more evident than with larger animal models.[74] Because only two studies used pigs, it was not possible to explore the effect of various species using subgroup analysis which hampers the translation to the clinical situation. Most studies used diets to induce comorbidity and/or used healthy animals in their models. This is in discrepancy with the use of metformin in pregnancy, where it is usually prescribed in women with insulin resistance and/or hyperglycaemia. Moreover, our study was not able to study dose-effect associations. Another limitation is the poor repowrting of methodological details (randomization, blinding, power calculation, and unclear reporting of either offspring or dams), an issue commonly reported in meta-analyses of animal studies. The poor reporting of outcome assessment per litter or per offspring and the big discrepancy between numbers and litters is notable and adds to the concerns of quality. This reduces the reliability of our conclusions, as we cannot rule out some of the studies included were of inferior quality. It is highly recommended to use guidelines such as the ARRIVE guidelines and GSPC for reporting to improve the reporting quality of animal studies.[75,76] In addition, some of our predefined outcomes were only reported in a small number of studies or were not reported at all, preventing us from conducting meta-analyses for some of the outcomes. Furthermore, the included studies were heterogeneous by a diversity in study designs. We addressed this issue by using a random effects model rather than a fixed effect model for our meta-analysis and conducted subgroup analyses and sensitivity analyses. Despite these efforts, heterogeneity remained high for some outcome measures, impacting the certainty of the evidence. Lastly, it could be beneficial to investigate other antidiabetic agents, including glibenclamide and Sodium-Glucose Co-Transporter 2 inhibitors, as they might be alternatives or adjuncts to metformin.

5 | CONCLUSIONS

In this first systematic review and meta-analysis of animal studies, we found no effects of intrauterine metformin exposure on offspring's anthropometry, cardiovascular or metabolic outcomes. Nevertheless, heterogeneity was high and reporting of methodology often limited making it difficult to make any definite conclusions. Based on the results of this systematic review future research should focus on the effects of metformin in older offspring age groups, and on outcomes which have gone uninvestigated to date. In addition, it is highly recommended to use guidelines such as the ARRIVE guidelines and GSPC for reporting to improve the reporting quality of animal studies.

CONFLICT OF INTEREST STATEMENT

None.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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