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Condensation (23 words): Children of obese mothers who were exposed to metformin *in-utero* had improved cardiovascular profile including central hemodynamics and diastolic left ventricular functional parameters.

Short title: Metformin use in obese pregnant women follow-up trial

AJOG at a Glance

A. Why was this study conducted?

Maternal obesity is associated with adverse cardiometabolic outcome in the offspring. The purpose of this study was to assess whether *in utero* exposure to metformin can impact on cardiometabolic profile and body fat distribution in the offspring of obese mothers who participated in the Metformin in Obese Pregnant women randomized controlled trial.

B. Key findings

Children of obese mothers who were exposed to metformin *in-utero* had improved central hemodynamics and left ventricular diastolic functional indices. No harmful effect on body composition was noted

C. What does this add to what is known?

The results of the study suggest that metformin has a beneficial effect on cardiovascular system of the offspring of obese mothers. The clinical implications of this finding require further exploration.

ABSTRACT

BACKGROUND: Maternal obesity increases the risk for pregnancy complications and adverse neonatal outcome and it has also been associated with long lasting adverse effects in the offspring, including increased body fat mass, insulin resistance and increased risk for premature cardiovascular disease. Lifestyle interventions in pregnancy have produced none or modest effects in reducing adverse pregnancy outcomes in obese mothers. Metformin use in Obese Pregnant women trial was associated with reduced adverse pregnancy outcomes and had no effect on birthweight. However, the long-term implications of metformin on the health of offspring remain unknown.

OBJECTIVE: The purpose of this study was to assess whether prenatal exposure to metformin can improve the cardiovascular profile and body composition in the offspring of obese mothers.

STUDY DESIGN: In 151 children from the Metformin use in Obese-Pregnant women trial we measured body composition, peripheral blood pressure and arterial pulse wave velocity. Central hemodynamics (central blood pressure and augmentation index) were estimated using the Vicorder device. Left ventricular cardiac function and structure were assessed by echocardiography.

RESULTS: Children were 3.9±1.0 years of age and 77 were exposed to metformin prenatally. There was no significant difference in peripheral blood pressure, arterial stiffness and body composition apart from gluteal and tricep circumferences which were lower in the metformin group ($p<0.05$). The metformin, compared to the placebo group, had lower central hemodynamics (mean adjusted decrease -0.707mmHg for aortic systolic blood pressure, -1.65mmHg for aortic pulse pressure and -2.68% for augmentation index, $p<0.05$ for all) and lower left ventricular diastolic

function (adjusted difference in left atrial area -0.525cm^2, in isovolumic relaxation time -0.324msec and in pulmonary venous systolic wave 2.97cm/s, $p<0.05$ for all). There were no significant differences in metabolic profile between the groups.

CONCLUSION: Children of obese mothers who were prenatally exposed to metformin, compared to those exposed to placebo, have lower central hemodynamic and cardiac diastolic indices. These results suggest that administration of metformin in obese pregnant women may potentially have a beneficial cardiovascular effect for their offspring.

Key words: obesity, pregnancy, children, prenatal exposure, offspring outcome, placebo, follow up

INTRODUCTION

Maternal obesity increases the risk for pregnancy complications[1-3] and adverse neonatal outcomes[4] and it may also have long lasting adverse effects in the offspring, including, increased body fat mass and systolic blood pressure (SBP) in childhood, increased insulin resistance and dyslipidemia both in childhood and young adulthood, and increased risk for premature all-cause mortality and hospital admissions for cardiovascular events.[5-7]

Randomized controlled trials on overweight and obese women during pregnancy have investigated the effect of interventions in reducing adverse pregnancy outcomes, however very few have reported the influences of these interventions on the health of the offspring.[8-10] Following dietary and lifestyle interventions in obese mothers, pregnancy outcomes have been largely unaffected and changes in body fat distribution of the offspring have been none or only modest.[10] Pharmacological interventions might produce a greater response and to date only few trials examined the effect of metformin in obese non-diabetic women in reducing adverse pregnancy outcomes.[11-14] Although the primary outcome, birthweight, was similar between groups in both studies, experimental and clinical data suggest that in utero exposure to metformin use can have long term effects in the offspring by modifying processes which regulate fat accumulation and cardiovascular health.[15]

To investigate this hypothesis, we followed up children from the Metformin in Obese Pregnant women trial to assess whether in utero exposure to metformin can improve

the cardiometabolic profile and body fat distribution in the offspring of obese mothers.

METHODS

Study population and study design

Our study population consisted of the offspring from the Metformin in Obese Pregnant women trial; in this trial obese (Body Mass Index-BMI >35kg/m^2) non-diabetic pregnant women were randomised to receive metformin or placebo from 12 to 18 weeks' gestation until delivery in three NHS maternity hospitals in the United Kingdom.[11] In this study we aimed to invite the mothers of the 393 cases with livebirths to bring their child to the Harris Birthright Research Centre for detailed cardiometabolic phenotyping. The examinations were conducted by one trained clinical research fellow (OP) who was blinded to all maternal information including arm of randomisation.

Ethical approval for the study was obtained from the London-Surrey Borders Research Ethics Committee (REC no 08/H0806/80). Signed informed consent was obtained from the parents and assent from the child when possible.

Adiposity measures

The following measurements were recorded whilst children were standing with the arms hanging down to the side: first, weight and height, second, arm relaxed, arm flexed and tense, waist, gluteal, mid-thigh and calf circumferences using a flexible

tape with 0.5 cm width and 0.5 mm precision, and third, skinfold thickness at the biceps, triceps, subscapular, supra-spinal and medial calf using a calibrated Harpenden caliper according to ISAK (International Society for Advancement of Kinanthropometry). All anthropometric measurements were performed in duplicate and the mean of the measurements is provided.

Body fat distribution was determined as previously reported with the BIA-ACC device (BIOTEKNA, Inc., Venice, Italy),[16 17] with the children dressed in light clothing without wearing any shoes. Information about weight gain since birth was obtained by measurements recorded by health visitors in the Red book.

Hemodynamic measurements and vascular measurements

Peripheral SBP and diastolic blood pressure (DBP) were measured as the average of the last 2 seated readings with an automated oscillometric device (Welch Allyn spot vital signs) in the right arm using the appropriate sized-cuff after a 5-minute rest. Carotid to femoral pulse wave velocity was measured using the Vicorder device (Software Version 4.0; Skidmore Medical Limited, Bristol, United Kingdom). The method has been previously described and has excellent intra and inter- observer repeatability and ease of use in childhood.[18] The device also determines brachial oscillometric blood pressure using a cuff placed around the upper arm. Central blood pressure parameters (aortic SBP and pulse pressure and augmentation index) are then derived from brachial blood pressure waveforms by applying a previously described brachial to aortic transfer function.[19]

Measures of cardiovascular function and structure

Conventional and tissue Doppler echocardiography was performed using a Philips CX50 system according to American Society of Echocardiography guidelines.[20] Measures which were assessed included left ventricular mass (LVM) and relative wall thickness, measures of systolic and diastolic function: peak systolic mitral annular tissue velocity and midwall fractional shortening and peak mitral annular velocities in early diastole (e') a measure of diastolic relaxation. The ratio of early diastolic transmitral flow velocity E/E' was calculated. Left atrial (LA) area was measured in the apical 4 chamber view at the ventricular end systole. LVM measurements were normalized to height 2.7 as indexed LVM (LVMI). LVM Z-scores were calculated for all children.[21] Global strain analysis included the average of all 16 segments and the peak systolic strain values were reported by using 2D speckle-tracking software version 3.0. RV systolic function was also assessed by tricuspid annular plane systolic excursion. All measurements were performed by the same clinical fellow who was trained in paediatric echocardiography.

Biomarker analysis

A 5 mL non-fasting venous blood sample was taken using standard procedures for children whose parents have agreed to venipuncture. A numbing cream was applied 30 min prior to venipuncture to minimize discomfort. Serum lipids [total cholesterol, triglycerides, and high-density lipoprotein (HDL) cholesterol and low-density cholesterol (LDL)] were measured by modification of the standard Lipid Research Clinics Protocol using enzymatic reagents for lipid determination. All assay

coefficients of variation were <5%. High sensitivity C-reactive protein and leptin and adiponectin were measured using ELISA methods. All samples were separated and frozen at -80^{o}C within 1 hour of collection.

Statistical analysis

Continuous variables are expressed as mean ± standard deviation (SD) or median and inter-quartile range (IQR) if not following the normal distribution. Numerical variables are presented as n (%). Normality of distribution was graphically evaluated by histograms and Q-Q plots. Inverse rank normalization was employed to allow for unbiased estimates of effect sizes in regression analysis of dependent cardiometabolic parameters that deviated from Gaussian distribution.

Comparison of anthropometric, hemodynamic and cardiometabolic parameters in offspring of mothers who were exposed to metformin or placebo in pregnancy was performed using independent samples Student's T-Test or the non-parametric Mann-Whitney test as well as chi-squared test. Subsequently, we used multivariable linear regression analysis to identify independent determinants of the cardiometabolic profile of the offspring. Adiposity measures and cardiovascular measures with a signal of difference ($p<0.1$) in unadjusted comparisons between groups were used as outcome variables. Independent variables in regression models were pre-specified on the basis of biological plausibility and observed differences between the total randomized population of the Metformin in Obese Pregnant women trial and the current sample and no selection procedure was followed. The use of metformin in pregnancy was inserted in all models as a factor variable and its effect size was adjusted for available exposure variables. In detail, multivariable regression models

for heamodynamic and cardiac outcomes included offspring age, mother's age at conception, sex, weight, height, race, blood pressure indices and heart rate. For adiposity outcomes, we used the same set of confounders. Possible collinearity among covariates in regression analysis was explored through assessment of the Variance Inflation Factor. We compared nested multivariable models with and without appropriate terms (likelihood ratio tests) to assess potential effect modification of metformin by sex. In certain analyses of dichotomous outcomes (i.e. highest versus lower tertiles of trans-mitral flow ratio), adjusted logistic regression analysis was employed.

Multi-level linear mixed model analysis was used to examine the impact of metformin exposure during pregnancy on longitudinal measurements of weight gain of the offspring across a range of approximately 7 years. The linear mixed model analysis included two random effects (random slope and random intercept) with unstructured variance-covariance and was adjusted for time intervals between sequential measurements, exposure to placebo or metformin, gender, race, changes in height and mother's conception age as fixed effects.

Statistical analysis was performed by Stata package, version 13.1 (StataCorp, College Station, Texas USA). All tests were two-sided and statistical significance was deemed at $p<0.05$.

Our sample size of 151 subjects, allocated in two unequal groups of 77 and 74 participants, provided power of 80% to detect a clinically significant difference of 0.5*IQR in weight and/or 0.5*SD in SBP between children who were exposed to metformin and placebo, respectively. The dispersion parameters of SD and IQR for power calculations were retrieved from previous published data. Power analysis for

the non-parametric Mann-Whitney test was based on 2,000 simulations with resampling.

RESULTS

Study population

In the Metformin in Obese Pregnant cohort there were 393 live births; 86 (11.8%) were not contactable, and of the 307 (78.2%) that were invited to participate in this study 156 (50.8%) refused. In total, 151 (38.5%) children were assessed, including 77 from the metformin and 74 from the placebo groups. Compared with the total Metformin in Obese Pregnant cohort, mothers in the current study were older (32.8 ±5.2 years vs. 31.4 ±5.8, $p<0.01$) but no other differences in the risk factor profile and in the incidence of pregnancy complications were noted (Supplementary Table 1). The characteristics of the current study population are described in Table 1. As previously shown, obese mothers treated with metformin during pregnancy had reduced gestational weight gain and incidence of preeclampsia as compared to women who received placebo.[11]

Adiposity phenotype

Children in the metformin, compared to the placebo group, had no significant difference in weight, height, body mass index, skinfold and body fat distribution measurements, but had lower gluteal circumference (56.5 vs 58.3 cm, $p<0.05$) and tricep circumference (30.2 vs 31.4 cm $p<0.05$) (Table 2). After adjustment for age, sex, race, weight and height, metformin exposure was independently associated with

decreased gluteal circumference (mean difference after inverse rank normalization = -0.183, 95% confidence intervals -0.344 to -0.022, p<0.03) and tricep circumference (mean difference = -0.189, 95% confidence intervals (CI) -0.376 to -0.001, p<0.05). The rate of weight gain from birth to early childhood was also comparable between the two groups (p=0.579 for interaction of time*group classification, i.e. metformin or placebo) (Supplementary Figure 1).

Cardiac and metabolic measurements

Children in the metformin, compared to the placebo group, had shorter isovolumic relaxation time and smaller left atrial area and higher pulmonary vein peak systolic Doppler velocity value (Table 3). These associations remained after multivariable adjustment (mean adjusted difference -0.324 msec, -0.525 cm^2 and 2.97cm/s for Isovolumic relaxation time, left atrial area and pulmonary venous systolic wave, respectively, p<0.05 for all) (Table 4, Figure 1). In addition, exposure to metformin was associated with decreased odds ratio (OR) for increased early to late trans-mitral flow ratio (OR=0.48 for increased tertile of peak doppler blood flow mitral valve velocity in early diastole (E wave) to peak velocity flow during atrial contraction (A wave)metformin vs placebo, p<0.05) after adjustment for the age of the mother and child, sex, weight, height, race and SBP. However, this association was further attenuated when heart rate was also included in the analysis. No difference in tissue Doppler measurements was noted between groups. Measures of cardiac systolic function, including strain measurements, and measurement of LVM were similar in the two groups. No association was found between maternal pregnancy body mass

index and offspring cardiovascular parameters in the placebo and metformin exposed group.

The metabolic profile was assessed in 39 infants from each group and there were no significant differences between the metformin and placebo groups (Table 2).

Hemodynamic parameters and vascular phenotype

In the metformin, compared to the placebo group, there was no significant difference in peripheral SBP and DBP; pulse pressure was lower (36.1±4.2vs. 37.5±5.1 mmHg, $p<0.05$) but this difference was attenuated following adjustment for current adiposity. After multivariable adjustment, children who were exposed to metformin during pregnancy had lower aortic pulse pressure (mean adjusted difference: -1.65mmHg, 95% CI -2.98 to -0.314, $p<0.02$), aortic SBP (mean adjusted difference: -0.707mmHg, 95% CI -1.21 to -0.201, $p=0.007$) and augmentation index (-2.68%, 95% CI -5.35 to -0.01, $p=0.049$) (Table 4, Figure 1). Carotid to femoral pulse wave velocity was similar in the two groups.

COMMENT

Main findings of the study

This study has demonstrated that *in-utero* exposure to metformin, compared to placebo, is associated with reduced central blood pressure and augmentation index and lower cardiac diastolic indices at the age of 4 years, and has no harmful effect

on body composition and metabolic profile. These results suggest a putative beneficial effect of metformin to the cardiovascular system of the offspring and if replicated and ideally supported by longer term data can have important clinical implications including a rationale for the clinical use of metformin in obese mothers during pregnancy to protect the cardiovascular health of the offspring.

Interpretation of results and comparison with existing literature

A number of different studies have suggested that maternal obesity can have adverse cardiometabolic implications for the offspring.[5 8 9] However it remains unknown whether interventions during pregnancy can modify this link. In the current study, offspring of obese mothers were exposed in utero to metformin or placebo as part of the Metformin in Obese Pregnant women trial. To assess cardiovascular profile in offspring, we used a variety of methods to define early arterial and cardiac changes. We used an oscillometric device, Vicorder, to estimate central hemodynamics and assess arterial stiffness between carotid to femoral segment. This technique has been widely used in childhood, is reproducible, operator independent and its measurements have been validated to invasive measurements in both children and adults.[19 22] We showed that children of obese mothers who were exposed to metformin *in-utero* had improved central hemodynamics as assessed by central SBP, central pulse pressure and augmentation index whereas no difference in peripheral blood pressure or arterial stiffness could be detected when current adiposity was accounted for in the analysis. These results are novel and are in keeping with data which suggest that changes in aorta and central blood pressure may precede any alterations in the peripheral circulation.[23 24] They are also in agreement with a previous study where *in-utero* exposure to metformin did not

354 modify peripheral blood pressure in 2 year old children compared to those exposed
355 to insulin treatment in the context of gestational diabetes.[25]

356

357 Considering that central aortic blood pressure and augmentation index reflect the
358 pressure that the heart and the brain are directly exposed to these results may have
359 important clinical implications.[26] In adults, central SBP has been shown to be better
360 at predicting future cardiovascular events, however, such a link remains to be
361 established in children as central hemodynamics are not routinely measured in
362 clinical practice.[27] The results from the current study, address a conclusion of the
363 2016 European Society of Hypertension guidelines, which highlighted the need to
364 increase knowledge in pediatrics and assess central blood pressure especially in
365 children who are at increased risk for hypertension as a means to better stratify their
366 cardiovascular risk.[27]

367

368 Functional alterations in the heart, usually precede structural changes and, in this
369 study, we used a variety of echocardiographic parameters to characterize systolic
370 and diastolic left ventricular functional changes. We found that in the metformin,
371 compared to the placebo group, isovolumic relaxation time was shorter, left atrial
372 area was smaller and pulmonary venous systolic waveform was increased and these
373 associations remained following adjustment for a number of hemodynamic and
374 anthropometric parameters. Although values remained within normal range for age,
375 these findings suggest that *in-utero* exposure to metformin is associated with better
376 early diastolic myocardial relaxation, but it remains uncertain whether these changes
377 will translate in long term cardiovascular benefit. We found no significant differences
378 between the metformin and placebo groups in left ventricular systolic function or left

ventricular mass. Data derived from animal studies suggest that diastolic dysfunction leading to increased diastolic filling pressures are present early in the development of obesity and contribute to the reduction of cardiac reserve and exercise intolerance in obese population.[28] The mechanism by which *in-utero* exposure to metformin may affect diastolic function in postnatal life remains speculative. It is possible that changes in myocardial architecture or myocyte metabolism occur *in-utero* as a consequence of exposure to a hyperglycemic environment and metformin may modify these processes.[29] It is also possible that metformin may be involved in epigenetic alterations, which potentially affect arterial and cardiac growth later in life.[30]

Prenatal metformin crosses the placenta and exposes the fetus to a dose that would not be used in a newborn or child and concerns were raised that this may also have long term programming effects on fetal metabolism and body weight and composition. In relation to metabolism, in a small subgroup of children we measured a series of parameters, including lipid profile, inflammatory markers and leptin and adiponectin and found similar blood levels in the placebo and metformin groups. Although the lack of association between prenatal metformin exposure and metabolic abnormalities is consistent with data from the PregMed and MiG trial,[31 32] the limited sample size of our study population does not allow firm conclusions to be drawn about late metabolic consequences of in utero exposure to metformin. In relation to body weight, we used a variety of anthropometric measurements to assess fat content and its distribution and found no significant difference between the metformin and placebo groups in fat content, distribution or weight gain since birth. These results contradict data from the PregMet trial and from two recent meta-

404 analyses which indicated that prenatal exposure to metformin is associated with
405 increased offspring weight.[32-35] There are two possible explanations for such
406 inconsistency in results: first, in our study we intentionally aimed to recruit children
407 before the adiposity rebound period to minimize the confounding effect of additional
408 risk factors which accumulate with increasing age,[36] whereas previous studies
409 revealed associations between prenatal exposure to metformin and increased weight
410 around the age of 9 years,[32 37] and second, our study was confined to obese women,
411 whereas previous studies examined women with either polycystic ovary syndrome or
412 gestational diabetes that may be associated with different in utero environments.
413
414 **Strengths and limitations**
415
416 The main strengths of our study are first, recruitment from a randomized controlled
417 trial with high compliance rate where metformin or placebo was given from the early
418 second trimester until the end of pregnancy and second, recording of measurements
419 by a research fellow who was blinded to randomization. The main limitation is that
420 only 38.5% of children from the original cohort had body composition assessment
421 and 19.8% had blood sample for metabolic measurements. Although there were no
422 statistically significant differences in maternal or fetal characteristics between our
423 study population and the original cohort, we cannot exclude that this is due to type II
424 error as there was a trend towards a higher frequency of risk factors such as Afro-
425 Caribbean race and gestational diabetes, in the studied population compared to the
426 original cohort.
427
428 **Clinical perspective**

Metformin crosses the placental and although the safety of the medication has been demonstrated during pregnancy, a number of studies have provided conflicting results regarding its long term effects on the health of the offspring. Our study is the first to report on the impact of in utero exposure to metformin in the offspring of obese non-diabetic mothers. By performing a detailed cardiometabolic phenotype we were able to show improved cardiac and vascular indices in children who were prenatally exposed to metformin compared to those exposed to placebo. In addition, no difference in body composition and metabolic profile were noted between groups. Although the differences between groups are small and their long term clinical significance remains unknown, the current findings are important as they suggest that metformin use in obese pregnant mothers is not only associated with reduced weight gain during pregnancy and incidence of preeclampsia for the mother[11] but with potential cardiovascular benefit for their offspring. Further studies, however, will be needed to confirm the long term absence of any harmful effects of in utero exposure to metformin in the different systems of the offspring before clinical use of this medication in obese mothers is advocated.

Conclusion

Our study suggests that *in-utero* metformin exposure in the context of maternal obesity, is associated with hemodynamic and echocardiographic changes which might have a direct cardioprotective effect on the offspring. Differences between groups were small and their clinical significance remains questionable, therefore further long term studies are needed to determine whether these changes translate

in long-term cardiovascular benefit and provide a rationale for use of metformin in obese mothers to protect the cardiovascular system of the offspring.

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582 **FIGURE LEGEND**

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584 **Figure 1.** Comparison of measurements of infant cardiovascular function in the
585 metformin and placebo groups. Open circles show the observed values and the
586 black circles with vertical lines represent adjusted mean estimates along with 95%
587 confidence intervals after controlling for baby's and mother's age, race, weight,
588 height, systolic blood pressure and heart rate. The adjusted p values were 0.049 for
589 Augmentation Index, 0.001 for left atrial area, 0.004 for Pulmonary Veins Systolic
590 wave and 0.013 for isovolumic relaxation time.

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Table 1. Characteristics and history of the population as allocated in the two groups.

Characteristic	Placebo N=74	Metformin N=77	P-value
Gender of offspring (%male)	50	49.4	0.94
Age in years at follow up	37 (5.2)	37.5 (5.8)	0.60
Race			0.86
White	44 (59.5)	49 (63.6)	
Black	25 (33.8)	23 (29.9)	
South Asian	5 (6.8)	5 (5.5)	
Weight at 12 weeks gestation (Kg)	106 (97-121)	104 (93 -114)	0.17
Body mass index at 12 weeks gestation (kg/m^2)	40 (4.8)	39.6 (5.1)	0.67
Gestational weight gain (Kg)	7.1 (4.2-9.6)	3.7 (1.2-7)	<0.001
Smoking	4 (5.4)	5 (6.5)	0.78
Preeclampsia	6 (8.1)	1 (1.3)	<0.05
Gestational diabetes mellitus	12 (16.2)	13 (16.9)	0.91

BMI = Body mass index

P-values are derived from Independent's samples Student's T test or Mann-Whitney test and chi-squared test. Nominal variables are presented as n and percentages and continuous variables as mean (standard deviation).

Table 2. Comparison of metabolic and body composition parameters of offspring of obese mothers.

	Placebo	Metformin	P value
Metabolic profile	**N=39**	**N=39**	
Cholesterol (mmol/L)	3.7 (0.64)	3.8 (0.66)	0.32
Low density lipoprotein (mmol/L)	1.5 (0.33)	1.5 (0.33)	0.43
High density lipoprotein (mmol/L)	2.3 (0.54)	2.4 (0.60)	0.41
Triglycerides (mmol/L)	0.91 (0.69-1.38)	0.94 (0.74-1.73)	0.74
Non high density lipoprotein (mmol/L)	2.47 (2.13-2.93)	2.70 (2.25-2.96)	0.31
C-reactive protein (mg/L)	0.39 (0.22-1.25)	0.62 (0.27-2.22)	0.50
Adiponectin (mg/L)	13.30 (9.81-15.48)	13.10 (11.60-14.82)	0.60
Leptin (µg/L)	2.50 (1.70 -5.78)	2.07 (1.53-2.93)	0.18
Body composition	**N=74**	**N=77**	
Birth weight (kg)	3.5 (3.1-3.7)	3.32 (3.0-3.68)	0.28
Weight (kg)	18.7 (15.6-21.2)	17.3 (15.7-20.1)	0.15
Height (m)	1.04 (0.99-1.09)	1.02 (0.97-1.07)	0.25
Body mass index (kg/m^2)	17.4 (2.1)	17.0 (2.0)	0.27
Waist Circumference (cm)	52.3 (50.0-55.2)	51.3 (49.4-53.8)	0.12
Gluteal Circumference (cm)	58.3 (54.5-62.0)	56.5 (53.5-59.5)	0.02
Triceps circumference (cm)	31.4 (29.0-34.7)	30.2 (27.8-32.7)	0.04
Calf circumference (cm)	22.5 (21.1-24.2)	21.9 (20.4-23.3)	0.18
Triceps skinfold (mm)	11.3 (9.2-13)	10.8 (9.0-12.6)	0.44
Biceps skinfold (mm)	5.6 (4.9-6.8)	5.2 (4.4-6.2)	0.10
Subscapularis skinfold (mm)	6.7 (5.8-8.7)	6.7 (5.7-8.7)	0.77
Supraspinal skinfold (mm)	5.8 (4.8-7.3)	5.8 (4.4-7.0)	0.24
Medial calf skinfold (mm)	11.1 (9.1-13.7)	11.1 (9.5-12.7)	0.80
Free fat mass (kg)	16.2 (14.1-18.2)	15.6 (14.0-17.2)	0.17
Fat mass (kg)	2 (1.1-3.3)	2.2 (1.2-2.8)	0.92
Maximum oxygen uptake	48.1 (44.2-53.4)	48.6 (45.4-51.6)	0.75
Total body water (L)	14.1 (12.2-16.1)	14.0 (12.0-15.7)	0.27
Extracellular water (L)	7.6 (7.2- 7.9)	7.4 (7.1-7.8)	0.11
Intracellular water (L)	6.4 (4.8-8.2)	7.0 (4.7-8.1)	0.47

Data are presented as mean (standard deviation) or median (interquartile range).

P-values are derived from Independent's samples Student's T test or Mann-Whitney test and chi-squared test.

636 **Table 3.** Comparison of cardiovascular indices between offspring of obese mothers
637 according to randomization status.
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	Placebo	Metformin	P-value
	N=74	N=77	
Systolic blood pressure (mmHg)	98.3 (7.0)	96.8 (5.7)	0.16
Diastolic blood pressure (mmHg)	60.7 (5.0)	60.8 (5.2)	0.93
Pulse pressure (mmHg)	37.5 (5.13)	36.0 (4.2)	<0.05
Aortic systolic blood pressure (mmHg)	96.2 (6.4)	94.2 (5.8)	<0.05
Aortic pulse pressure (mmHg)	35.5 (4.8)	33.4 (4.7)	<0.01
Augmentation index (%)	22 (18-30)	18 (15-29)	0.09
Pulse wave velocity (m/sec)	5.1 (4.7-5.5)	5.0 (4.7-5.5)	0.93
Heart rate (bpm)	97 (91-105)	99 (92-106)	0.21
Cardiac measurements			
Left ventricular mass indexed (grams)	29.8 (25.0-34.0)	30.1 (25.3-33.5)	0.98
FS (%)	31.8 (29.5-34.1)	31.8 (29.3-34.4)	0.95
IVSD (mm)	0.45 (0.4-0.51)	0.44 (0.40-0.49)	0.44
IVSD z-score	-0.77(-1.20--0.23)	-0.76 (-1.19--0.39)	0.83
TAPSE (cm)	1.80(1.7-1.9)	1.8 (1.7-1.9)	0.75
TAPSE z-score	0.46 (-0.31-1.23)	0.46 (-0.31-1.23)	0.64
Isovolumic relaxation time (mm)	50 (40-60)	50 (40-50)	0.03
Left atrium (cm^2)	6.1 (5.3-6.9)	5.5 (4.9-6.2)	0.001
E (cm/sec)	89.4 (83.3-95.0)	88.2 (80.7-95.5)	0.59
A (cm/sec)	53.4 (47.5-61.0)	57.1 (50.5-61.8)	0.22
Ratio of E to A	1.64 (1.45-1.89)	1.55 (1.43-1.71)	0.17
A duration (msec)	80 (80-90)	80 (80-90)	0.81
Deceleration time (msec)	90 (80-100)	90 (80-100)	0.84
Isovolumic contraction time (msec)	50 (40-60)	50 (40-50)	0.92
e' lateral (cm/sec)	18.0 (16.4-20.3)	17.9 (16.4-19.4)	0.56
e' septal (cm/sec)	12.4 (11.8-13)	12.5 (11.6-13.3)	0.86
e'(avg) (cm/sec)	15.4 (13.9-16.2)	15.2 (14.1-15.9)	0.57
a' lateral (cm/sec)	7.22 (6.40-8.37)	7.07 (6.19-8.14)	0.53
a' septal (cm/sec)	5.6 (5.0-6.5)	5.5 (4.9-6.2)	0.32
a' (avg) (cm/sec)	6.48 (5.78-7.44)	6.45 (5.58-7.10)	0.44
E/e' (avg)	5.86 (5.36-6.44)	5.88 (5.30-6.59)	0.82
E/e'	4.96 (4.35-5.58)	4.93 (4.46-5.76)	0.77
Pulmonary vein systolic wave (cm/sec)	53 (51-56)	56 (52-61)	<0.01
Pulmonary vein diastolic wave (cm/sec)	56 (51-61)	57 (53-60)	0.50
Global longitudinal strain overall (%)	-19 (-21--18)	-19.6 (-21--17.75)	0.86

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640 Data are presented as mean (standard deviation) or median (interquartile range).

P-values are derived from Independent's samples Student's T test or Mann-Whitney test and chi-squared test.

FS = fraction shortening; IVSD = Interventricular septum thickness at end of diastole; TAPSE = Tricuspid annular plane systolic excursion; E = Peak velocity of early diastolic transmitral flow; A = Peak velocity of late transmitral flow; e' = Peak velocity of early diastolic mitral annular motion as determined by pulsed wave Doppler; a' = peak velocity of diastolic mitral annular motion as determined by pulsed wave Doppler.

675 **Table 4.** Multivariable regression analysis for main cardiometabolic outcomes of the
676 study.
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	Untransformed	P-value	Inverse rank Transformation	P-Value
	Adjusted mean decrease (95% confidence interval)		Adjusted mean decrease (95% confidence interval)	
Central hemodynamics				
Aortic systolic blood pressure	-0.71 (-1.21 to -0.20)	<0.01	-	
Aortic pulse pressure	-1.65 (-2.98 to -0.31)	0.02	-	
Augmentation Index	-2.68 (-5.35 to -0.01)	<0.05	-0.32 (-0.633 to -0.007)	<0.05
Diastolic indices				
Isovolumic relaxation time	-3.2 (-5.78 to -0.70)	0.01	-0.34 (-0.61 to -0.07)	0.02
Left atrial area	-0.53 (-0.84 to -0.201)	<0.01	-0.43 (-0.68 to -0.17)	<0.01
Pulmonary veins: systolic wave	2.97 (0.94 to 5.0)	<0.01	0.41 (0.08 to 0.73)	0.01

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679 All models are adjusted for age and mother's age, sex, race, weight, height, systolic
680 blood pressure and heart rate.
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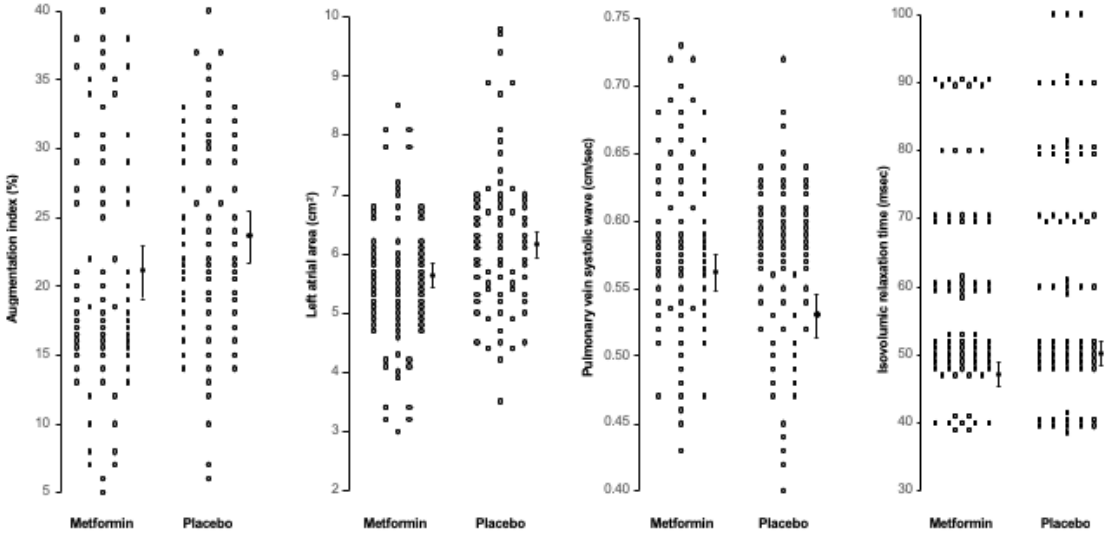

Figure 1