Maternal and fetal insulin levels at birth in women with polycystic ovary syndrome: data from a randomized controlled study on metformin

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Abstract

Context: Metformin is suggested to reduce pregnancy complications in women with polycystic ovary syndrome (PCOS). Metformin crosses the placenta and therapeutic concentrations are measured in the fetal circulation. Whether metformin treatment in pregnant PCOS women affects maternal and fetal insulin concentrations at birth is not clarified.

Objectives: To investigate the possible effect of metformin on insulin concentrations in umbilical cord blood and the possible association between maternal and fetal insulin concentrations.

Design: Post-hoc analysis of a subgroup of PCOS women participating in a double-blind randomized controlled trial.

Setting: University hospital setting.

Participants: Women with PCOS ($n = 118$), aged 19–39 years.

Main outcome measures: Maternal and umbilical cord insulin concentrations immediately after birth.

Results: At delivery women randomized to metformin had lower insulin concentrations than those randomized to placebo (259 ± 209 vs 361 ± 261 pmol/l; $P = 0.020$). No difference was found in insulin concentrations in umbilical venous ($P = 0.95$) and arterial ($P = 0.39$) blood between the metformin and placebo groups. The arteriovenous difference was also equal between the groups ($P = 0.38$). Insulin concentrations were higher in the umbilical vein than in the umbilical artery independent of randomization (70 ± 51 vs 45 ± 48 pmol/l; $P < 0.0005$).

Conclusions: In PCOS, metformin treatment during pregnancy resulted in lower maternal insulin concentrations at delivery. Metformin treatment did not affect fetal insulin concentrations. Higher insulin concentrations in the umbilical vein indicate that the placenta somehow secretes insulin to the fetus. The possibility of placental insulin secretion to the fetus deserves further investigations.

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Introduction

Polycystic ovary syndrome (PCOS) is a heterogeneous syndrome affecting 6.5–15% of women in fertile age, depending on the criteria used and the population studied (1, 2). PCOS is characterized by polycystic ovaries, oligo-amenorrhea, and hyperandrogenism. It is associated with maternal obesity (3), insulin resistance (4), metabolic syndrome and type II diabetes (5, 6), dyslipidemia (4), endometrial cancer, and possibly cardiovascular diseases later in life (4). Increased prevalence of pregnancy complications and adverse pregnancy outcomes is reported in PCOS women (7). Continuing metformin therapy during pregnancy in women with PCOS has been suggested to reduce pregnancy complications such as gestational hypertension and adverse pregnancy outcomes like miscarriage and gestational diabetes (8, 9). However, a recent randomized controlled trial (RCT) failed to show an effect of metformin on gestational diabetes mellitus, preeclampsia, or preterm birth in PCOS women (10).

Metformin passes the placenta and is present in the fetal circulation at therapeutic concentrations (11). Despite no difference in birth weight, 1-year-old children exposed to metformin *in utero* weighed on average 0.5 kg more than children exposed to placebo (12). The possible intrauterine effect of metformin on the fetus, with possible long-term effects on the child and adult, remains to be established.

This *post-hoc* analysis comprises a subgroup of women with PCOS participating in a placebo-controlled RCT (10). We investigated the possible effect of metformin on insulin concentrations in the umbilical cord blood. Correlations between maternal and fetal insulin concentrations were analysed and fetal umbilical arterial and venous insulin concentrations were compared. In addition we examined the placenta immunohistochemically for insulin-positive cytotrophoblasts.

Subjects and methods

Study design

The present study is a substudy of 'The Metformin treatment in pregnant PCOS women' (The PregMet) study (10). Inclusion criteria were PCOS diagnosed according to The Rotterdam Criteria (13), age 18–45 years, gestational age between 5 and 12 weeks, and a singleton viable fetus shown on ultrasonography. Exclusion criteria were alanine aminotransferase (ALT) >90 IU/l, serum creatinine concentration >130 µmol/l,

known alcohol abuse, previously diagnosed diabetes mellitus or fasting serum glucose >7.0 mmol/l at time of inclusion, treatment with oral glucocorticoids or use of drugs known to interfere with metformin.

The diagnosis of PCOS was based on documentation in the nonpregnant state before the actual pregnancy, all diagnosed by a gynecologist. Two hundred and seventy-three pregnancies (in 257 women) were randomly assigned to either 2000 mg metformin daily or placebo from the first trimester to delivery. Detailed description of the materials and methods has been published previously (10).

The present study comprises those women in the PregMet study who gave birth at St Olavs Hospital, in Trondheim. Of the 164 PCOS women delivering at the University Hospital in Trondheim, placentas and maternal and umbilical cord blood samples were collected from 134. Due to shortage of serum, both arterial and venous umbilical and maternal insulin concentrations were unavailable in 16 women–offspring pairs. The present study population thus consists of 118 PCOS women and their offspring.

The Committee for Medical Research Ethics of Health Region IV, Norway, and The Norwegian Medicines Agency approved the study. Written informed consent was obtained from each patient before inclusion and the Declaration of Helsinki was followed throughout the study. The study was conducted according to the principles of 'Good Clinical Practice' and the trial is registered at www.clinicaltrials.gov as NCT00159536.

Measurements

The umbilical cord was clamped by two clamps, one close to the baby and the other close to the mother before the delivery of the placenta. Blood was separately collected from the umbilical artery and the umbilical vein by aspiration with an injection needle. After collection of umbilical cord blood samples, venous blood was drawn from the mother within 1 h after delivery. Blood samples were then centrifuged within 1 h and stored at 5–8 °C. Within 24 h, the samples were stored in a –80 °C freezer. The placentas were stored in 5% formaldehyde solution and processed at the Department of Pathology, St Olavs Hospital, Trondheim, Norway.

Insulin was measured in one series in stored serum samples by ELISA method using kits and reagents supplied by the manufacturer (DRG Instruments GmbH, Marburg, Germany). The intra- and inter-assay coefficient of variation (CV) was 2.7 and 7.6% respectively.

Immunohistochemical analysis of the placenta

Formalin-fixed slices were taken from 15 placentas according to a standard protocol. The slices were paraffin-embedded and cut in 4 µm sections and stained with hematoxylin-eosin. Two sections from each placenta, one central and one from the maternal side, were selected for immunohistochemistry with antibodies against insulin (anti-insulin pAb, clone A0564, DakoCytomation, Glostrup, Denmark).

Outcomes

Main outcome measures were insulin concentrations in maternal vein, umbilical artery, and umbilical vein.

Data

All data entry, data management, and data analyses were carried out at the Department of Laboratory Medicine, Children's and Women's Health and at the Unit for Applied Clinical Research, Department of Cancer Research and Molecular Medicine, Norwegian University of Science and Technology.

Statistical analysis

Statistical analyses were carried out using PASW version 20 (Predictive Analytics Soft Ware, IBM Corporation, New York, NY 10589, USA). Due to uneven distribution of many variables, independent samples Mann-Whitney *U* test for comparison between groups and related samples Wilcoxon signed-rank test for comparison between variables were used as appropriate. For correlation analyses, Pearson correlations were used. *P* values <0.05 were considered significant. No adjustments for multiple testing were carried out.

Results

Study population

Maternal and newborn characteristics of both study groups are given in Table 1. No difference was found between the groups in maternal age, height, weight, BMI, fasting glucose, or 2-h glucose after an oral glucose tolerance test (OGTT). No difference was found in birth weight, birth length, and head circumference or placenta weight.

Metformin effects on maternal and newborn insulin concentrations

At delivery, but not in the third trimester, women randomized to metformin treatment had lower insulin

Table 1 Characteristics of PCOS women according to treatment group.

	Treatment group	<i>n</i>	Mean ± s.d.	<i>P</i> value ^a
Maternal characteristics at inclusion				
Age (years)	Placebo	61	30.2 ± 4.1	0.87
	Metformin	57	30.1 ± 4.1	
Height (cm)	Placebo	61	168.1 ± 5.7	0.52
	Metformin	57	167.4 ± 6.0	
Weight (kg)	Placebo	61	82.7 ± 19.0	0.97
	Metformin	57	83.9 ± 23.0	
BMI (kg/m ²)	Placebo	61	29.3 ± 6.5	0.93
	Metformin	57	30.0 ± 8.1	
Fasting glucose (mmol/l)	Placebo	61	4.55 ± 0.45	0.20
	Metformin	57	4.46 ± 0.45	
2-h glucose (mmol/l)	Placebo	61	4.99 ± 1.35	0.63
	Metformin	57	4.89 ± 1.52	
Fasting insulin (pmol/l)	Placebo	61	113 ± 55	0.59
	Metformin	57	110 ± 62	
Newborn characteristics				
Weight (g)	Placebo	61	3511 ± 601	0.78
	Metformin	57	3506 ± 602	
Length (cm)	Placebo	60	50.1 ± 2.7	0.76
	Metformin	56	50.8 ± 6.2	
Head circumference (cm)	Placebo	61	35.1 ± 1.7	0.23
	Metformin	56	35.6 ± 1.6	
Placental weight (g)	Placebo	56	657 ± 158	0.79
	Metformin	55	652 ± 164	

^aIndependent samples Mann-Whitney *U* test.

concentrations than those randomized to placebo (259 ± 209 vs 361 ± 261 pmol/l; *P*=0.020). In a subgroup analysis of women with vaginal delivery (*n*=92), metformin-treated women had significantly (*P*=0.010) lower insulin levels. In women with operative delivery (*n*=25), a nonsignificant (*P*=0.47) difference of the same magnitude was seen (data not shown). We found no difference in umbilical venous (*P*=0.95), arterial (*P*=0.39), and arterio-venous (AV) difference (*P*=0.38) in insulin concentrations in metformin- and placebo-exposed newborns (Table 2).

AV umbilical insulin difference

Insulin concentrations were higher in the umbilical vein than in the umbilical artery (69.8 ± 51.4 vs 44.9 ± 47.8 pmol/l; *P*<0.0005). When analyzing according to randomization, this difference persisted both in the metformin group (72.0 ± 56.0 vs 42.6 ± 47.4 pmol/l; *P*<0.0005) and in the placebo group (67.7 ± 47.0 vs 47.0 ± 48.4 pmol/l; *P*<0.0005). The distribution of the umbilical AV difference in insulin concentrations is shown in Fig. 1.

Table 2 Effect of metformin on insulin levels in PCOS mothers in the third trimester and their children at delivery.

	Treatment group	n	Mean $\pm$ s.d.	P value ^a
Maternal vein week 32 (pmol/l)	Placebo	57	167 $\pm$ 90	0.12
	Metformin	56	150 $\pm$ 93	
Maternal vein week 36 (pmol/l)	Placebo	56	158 $\pm$ 74	0.32
	Metformin	54	161 $\pm$ 106	
Maternal vein at delivery (pmol/l)	Placebo	61	361 $\pm$ 261	0.020
	Metformin	56	259 $\pm$ 209	
Fetal vein (pmol/l)	Placebo	61	67.7 $\pm$ 47.0	0.95
	Metformin	57	72.0 $\pm$ 56.0	
Fetal artery (pmol/l)	Placebo	61	47.0 $\pm$ 48.4	0.39
	Metformin	57	42.6 $\pm$ 47.4	
Arteriovenous difference (pmol/l)	Placebo	61	-20.6 $\pm$ 53.4	0.38
	Metformin	57	-29.4 $\pm$ 37.3	

^aIndependent samples Mann–Whitney *U* test.

Immunohistochemical examination

The placenta sections chosen for the pilot study were from random cases with different insulin concentrations in the umbilical vein and artery. Positive staining could not be obtained and further examination was, therefore, not carried out.

Correlation analyses

Arterial and venous insulin concentrations in the umbilical cord correlated positively with birth weight ($r=0.20$; $P=0.029$ and $r=0.31$; $P=0.001$), head circumference ($r=0.21$; $P=0.022$ and $r=0.19$; $P=0.038$), and placental weight ($r=0.32$; $P=0.001$ and $r=0.38$; $P<0.0005$). Birth length did not correlate with umbilical insulin concentrations. Maternal insulin concentrations did not correlate with umbilical arterial or venous insulin concentrations.

Discussion

The main findings of the present study are that: i) metformin treatment in PCOS women from the first trimester until delivery reduces maternal insulin concentrations at delivery; ii) fetal insulin concentrations are not affected by maternal metformin treatment; iii) fetal insulin concentrations correlate positively with birth weight, head circumference, and placental weight; and iv) insulin concentrations in the umbilical vein are higher

than in the umbilical artery irrespective of metformin or placebo treatment.

In the nonpregnant state metformin regulates blood glucose by reducing insulin resistance and thereby also insulin concentrations. Accordingly, the insulin-lowering effect of metformin on maternal side is not surprising and is in accordance with the literature from nonrandomized studies (14). It is, however, not in accordance with our data from a previous pilot study on pregnant PCOS women, where no effect of metformin on glucose homeostasis was seen, and it is not in accordance with the large RCT from which the present substudy originates (10, 15). Possibly, metformin effects on glucose homeostasis at delivery differ from the effect during the rest of pregnancy. Labor is, however, a situation that may induce a number of physiological fluctuations, perhaps also affecting insulin. We did not standardize for example fasting state, duration of labor, vomiting, and venous infusion or fluids, as this would have been both nearly impossible to standardize in a clinical setting and also unethical. As the study is an RCT, there should theoretically be no systematic biases, although it cannot be excluded. It is remarkable, though, that we found differences in insulin levels at delivery while the same women had equal fasting insulin levels during the rest of the pregnancy.

Figure 1

Distribution of umbilical arteriovenous difference in insulin levels.

We have previously reported that metformin passes the placental barrier and is present in the fetal circulation in therapeutic concentrations (11). If metformin affects glucose homeostasis in the fetus, we would expect lower umbilical insulin concentrations in the metformin group. We did not find such an effect, indicating that the presence of metformin in the fetal circulation does not have a major effect on glucose homeostasis in the fetus.

Insulin is suggested to be a fetal growth factor (16), and hyperinsulinemia in the offspring of otherwise healthy mothers is suggested to be associated with being large for gestational age (LGA) (17). We found that fetal insulin concentrations correlated positively to birth weight, head circumference, and placental weight in women with PCOS. The results from the present PregMet substudy, in which no effect of metformin treatment was found either on birth weight or length at birth, support our observation of no effect of metformin on umbilical insulin concentrations.

The most striking finding in this *post-hoc* analysis was the significantly higher insulin concentrations in the umbilical vein than in the umbilical artery. This was a completely unexpected finding and the comparison of umbilical arterial and venous insulin concentrations was not a predefined question.

Only negligible amounts of maternal insulin and insulin analogs cross the placental barrier at therapeutic concentrations (18, 19, 20). This explains the lack of correlation between maternal venous insulin concentrations and umbilical venous insulin concentrations (20, 21). The source or sources of insulin in the fetoplacental unit are not completely understood, although the fetal pancreas is known to start secreting insulin in response to fetal blood glucose at a given time (22) and there seems to be a placental factor that stimulates fetal β -cells to secrete insulin (23).

Studies with a small number of participants report similar insulin concentrations in umbilical vein and umbilical artery in babies born to healthy mothers (20). One study examined large infants of untreated diabetic mothers and found that these infants had different umbilical venous and arterial insulin concentrations, although the difference was not consistent in direction (24). The present study is the largest to compare insulin concentrations separately in the umbilical artery and vein. This study is also the first on women with PCOS.

Increasing C-peptide with increasing gestational duration in women with diabetes mellitus type 1 was reported in 2009. C-peptide levels declined rapidly *postpartum* (25). Placenta-derived insulin growth factors

were suggested to stimulate maternal β -cell hyperplasia and secretion. Whether the β -cells really were located in the maternal pancreas or if they disappeared along with the placenta *postpartum* is not known. Furthermore, placental stem cells have recently been reported to be capable of reprogramming into β -cell progenitors (26). Considerably higher placental than maternal arterial and venous concentrations of the human insulin analog insulin lispro was reported in an *in vitro* perfusion study, suggesting that the placenta either traps and stores insulin or that the placenta produces insulin or an insulin-like substance (27). Already back in the 1960s, insulin was injected in 28 term-pregnant women after initiation of labor with the result of placental insulin trapping and subsequent degradation (28). The possibility of a rapid placental insulin secretion during labor, but not earlier during pregnancy, cannot be ruled out and could explain the high umbilical venous insulin concentrations in the present study. Our observation is also compatible with another possibility, namely placental insulin production in PCOS pregnancies.

Although it is tempting to interpret our finding of higher insulin concentration in the umbilical vein as a result of placental insulin production, there might be other explanations. First, and previously mentioned, insulin could be taken up from the maternal circulation, stored in the placenta, and then released into the fetal circulation as an acute event during labor. Second, local degradation of insulin in the umbilical cord as a consequence of clamping is a possibility. A local degradation could theoretically differ between the artery and the vein due to different endothelium in the two vessel types and thus lead to different insulin concentrations. However, the midwives who sampled the umbilical blood tended to draw blood from the artery before the vein. If local degradation took place, this order of sampling would tend to decrease the difference in insulin concentrations. Third, blood samples from the umbilical cord were stored for up to an hour before centrifugation and freezing, possibly leading to different insulin degradation. Based on these theories, we tried to detect insulin-positive cytotrophoblasts in the placenta by immunohistochemistry, but failed for unknown reasons.

The strengths of the study are the RCT design and the large number of participants with concomitant measurements of umbilical venous and arterial insulin concentrations.

Conclusion

Metformin treatment, from first trimester to delivery in women with PCOS, reduced maternal insulin

concentrations at delivery, while fetal insulin concentrations were unaffected. Fetal insulin concentrations correlated positively with birth weight, head circumference, and placental weight. In offspring of PCOS women, insulin concentrations were higher in the umbilical vein than in the umbilical artery, suggesting that the placenta somehow delivers insulin to the fetus in PCOS pregnancies.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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