Insulin action in women with polycystic ovary syndrome and its relation to gestational diabetes

Marlieke A. de Wilde¹,*, Angelique J. Goverde¹, Susanne M. Veltman-Verhulst¹, Marinus J. C. Eijkemans¹,2, Arie Franx³, Bart C. J. M. Fauser¹, and Maria P. H. Koster¹,3

¹Department of Reproductive Medicine and Gynaecology, University Medical Centre Utrecht, 3584 CX Utrecht, The Netherlands
²Julius Centre for Health Sciences and Primary Care, University Medical Centre Utrecht, 3584 CX Utrecht, The Netherlands
³Department of Obstetrics, University Medical Centre Utrecht, 3584 EA Utrecht, The Netherlands

*Correspondence address. Heidelberglaan 100, 3584 CX Utrecht, The Netherlands. Tel: +31-887556978; Fax: +31-887555507; E-mail: m.dewilde-2@umcutrecht.nl

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STUDY QUESTION: How does insulin action change during pregnancy in women with polycystic ovary syndrome (PCOS) who develop gestational diabetes (GDM) compared with women with PCOS who do not?

SUMMARY ANSWER: Women with PCOS who develop GDM already show disturbed insulin action early in pregnancy.

WHAT IS KNOWN ALREADY: Pregnant women with PCOS are at increased risk of developing GDM compared with women without PCOS.

STUDY DESIGN, SIZE, DURATION: This study represents a post hoc analysis of a subgroup of pregnant women with PCOS participating in a multicentre prospective cohort study. A total of 72 women were included.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Women with PCOS and a wish to conceive were included before conception and followed during pregnancy. Insulin, glucose, homeostasis model assessment of insulin resistance (HOMA-IR), sex hormone-binding globulin (SHBG) and testosterone were analysed at three different time points in women who developed GDM and women who did not.

MAIN RESULTS AND THE ROLE OF CHANCE: Seventy-two pregnant women with PCOS were included of which 22 (31%) women developed GDM. Both insulin levels and HOMA-IR were significantly higher at each sampling point in women with PCOS who developed GDM. SHBG levels were significantly lower before conception and in the second trimester compared with women who did not develop GDM. Testosterone concentrations were significantly lower before conception in women who developed GDM. After adjusting for BMI, waist circumference and waist/hip ratio, the differences in insulin, HOMA-IR, SHBG and testosterone levels remained largely the same.

LIMITATIONS, REASONS FOR CAUTION: Selection bias cannot be excluded since only women from one centre with a complete blood sampling set were included in this study.

WIDER IMPLICATIONS OF THE FINDINGS: The knowledge that women with PCOS who develop GDM already have a disturbed insulin action early in pregnancy is likely to be useful in considering the pathophysiology processes underlying this disorder in this specific group of women.

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Key words: PCOS / pregnancy / gestational diabetes
**Introduction**

Gestational diabetes (GDM), one of the most common complications of pregnancy, has become a serious health problem worldwide, due to its increasing incidence with reported values as high as 0.4–7.3% of all pregnancies (Reece et al., 2009). GDM is associated with distinct short-term and long-term effects on both mother (e.g. metabolic syndrome, type 2 diabetes) and child (e.g. large for gestational age, impaired post-natal growth, childhood obesity, type 2 diabetes) (Catalano et al., 2003; Reece et al., 2009; Nolan, 2011).

During normal pregnancy, a state of insulin resistance develops in order to provide sufficient nutrition for the fetus. Additionally, glucose levels and free fatty acids rise and insulin production in the mother increases. Sensitivity for insulin is highest around 13 weeks of gestation and decreases during the rest of the pregnancy, resulting in the lowest sensitivity for insulin in the third trimester (Ryan, 2003). Women develop GDM when there is a lack of insulin action in body tissues (Yamashita et al., 2000; Metzger et al., 2007; Landon and Gabbe, 2011). This condition resolves directly after delivery (Ryan, 2003). Women, who are obese, have an evidently increased risk of developing GDM (Chu et al., 2007).

Women with polycystic ovary syndrome (PCOS) also have an increased risk of developing GDM, likely due to higher insulin resistance before pregnancy (Boomsma et al., 2006; Kjerulf et al., 2011). PCOS, characterized by oligo- or anovulation, clinical (acne, hirsutism) or biochemical hyperandrogenism and polycystic ovaries, is present in 6–15% of women of reproductive age (Rotterdam ESHRE/ASRM-Sponsored PCOS consensus workshop group, 2004; Norman et al., 2007). PCOS is often accompanied by infertility, obesity, insulin resistance and dyslipidaemia (Fauzer et al., 2012). Our group has previously proposed that women with PCOS who are at risk of developing GDM can be identified even before conception (de Wilde et al., 2014). Another study demonstrated that sex hormone-binding globulin (SHBG) in the first trimester is a predictor for GDM and that women who develop GDM have significantly higher testosterone concentrations compared with controls (Thadani et al., 2003). An in vitro study showed that hyperinsulinaemia reduces the synthesis of SHBG in the liver (Plymate et al., 1988). During normal pregnancy, testosterone levels increase; however, the physiological mechanism remains unclear. In PCOS pregnancy, hyperandrogenism could be explained by high insulin levels, which inhibit the placental aromatase activity, which converts androgens to estrogens. Moreover, it is suggested that increased insulin levels during pregnancy could stimulate androgen production directly (Sir-Petermann et al., 2002).

Data of mechanisms of pregnancy complications in women with PCOS are scant, and usually address comparisons between women with PCOS versus women with normal cycles. We compared levels of insulin, glucose, homeostasis model assessment insulin resistance (HOMA-IR), SHBG and testosterone in women with PCOS throughout pregnancy to gain more understanding in the course of development of GDM. We hypothesize that in women with PCOS who develop GDM, insulin sensitivity is already disturbed early in pregnancy with consecutively higher glucose levels, lower SHBG levels and higher testosterone concentrations compared with women with PCOS who do not develop GDM.

**Materials and Methods**

**Design**

This study was part of the CoPPer study (Complications of PCOS Pregnancy: evaluating risk), a large prospective multicentre study in the Netherlands. Women diagnosed with PCOS and a wish to conceive were included between April 2008 and April 2012 in four hospitals in the Netherlands (de Wilde et al., 2014). PCOS was diagnosed according to the Rotterdam 2003 consensus criteria, when two of the three following criteria were present: Oligo- or anovulation, clinical or biochemical hyperandrogenism and polycystic ovaries (Rotterdam ESHRE/ASRM-Sponsored PCOS consensus workshop group, 2004). After PCOS was diagnosed, standardized phenotyping followed (Goverde et al., 2009). Women were excluded in case of missing data, age <18 years or >45 years, a language barrier and pre-existing type 2 or type 1 diabetes mellitus. For this post hoc analysis, women who were included at the University Medical Centre Utrecht (UMCU) and of whom blood assessments at three different sampling moments were available.

**Ethical approval**

The CoPPer study was approved by the institutional review board of the UMCU. All women who participated in this study gave written informed consent. The study was registered with Clinicaltrials.gov, number NCT00821379.

**Clinical assessments**

All women underwent standardized preconception screening upon inclusion, as described previously (Goverde et al., 2009). This screening included a questionnaire, a physical examination, a transvaginal ultrasound scan of the uterus and ovaries and metabolic and endocrine measurements. Based on the Adult Treatment Panel III (ATP III) criteria, metabolic syndrome was diagnosed when three or more of the following features were present: waist circumference >88 cm, blood pressure ≥130 mmHg/≥85 mmHg, fasting glucose ≥5.6 mmol/l, triglycerides ≥1.7 mmol/l, high-density lipoprotein (HDL) cholesterol ≤1.3 mmol/l (Grundy et al., 2005). The free androgen index (FAI) was calculated before conception: ([testosterone (nmol/l) × 100]/SHBG (nmol/l)). Women with an FAI of >4.5 are considered to be hyperandrogenic (van Santbrink et al., 1997). All women underwent an oral glucose tolerance test prior to conception (OGTT; 75-g glucose load, 2 h follow-up) in order to rule out pre-existing type 2 diabetes (American Diabetes Association, 2010). Subsequently, most women started fertility treatment by ovulation induction because of oligo- or anovulation. Metabolic and endocrine measurements were repeated 1 year after preconception screening, only when a pregnancy was not yet achieved.

When pregnancy was achieved, subjects received obstetric care according to national guidelines. According to these guidelines, no metformin was used in these patients, neither was it given before conception. At 10–12 weeks of gestational age, a venous blood sample was obtained and sparse serum was stored. GDM was diagnosed or ruled out in the second trimester, at a gestational age of 24–26 weeks, when all women underwent an oral glucose tolerance test (100-g glucose load, 3 h follow-up). When two or more plasma glucose levels exceeded the given threshold after a 100-g glucose load, GDM was diagnosed: fasting glucose ≥5.3 mmol/l, 1 h glucose ≥10.0 mmol/l, 2 h glucose ≥8.6 mmol/l, 3 h glucose ≥7.8 mmol/l (American Diabetes Association, 2003). Neonatal hypoglycaemia was defined as a glucose level <2.6 mmol/l. Definitions of other adverse outcomes have been described previously (de Wilde et al., 2014).

Blood samples were all drawn after overnight fasting. Glucose, insulin, SHBG and total testosterone levels were determined at three, or in some cases four, moments: (i) at standardized screening before conception, (ii) 1 year after screening (i.e. if not yet pregnant), (iii) in the first trimester,
at 10–12 weeks of gestational age, and (iv) in the second trimester, at 24–26 weeks of gestational age (when GDM was diagnosed or ruled out). Subsequently, the HOMA-IR score was calculated for each sampling time: \([\text{glucose (mmol/l)} \times \text{insulin (mU/l)}] / 22.5\). Because blood sampling cannot be performed at the beginning of pregnancy, the most recent blood assessment before conception was used as a proxy for baseline marker levels. This blood sample was not taken into account if the time between blood withdrawal and pregnancy was >1.5 years (547 days).

Glucose levels were already measured as part of the CoPPer study, the other parameters were measured for this post hoc analysis. Blood samples were analysed at the laboratory of the UMCU. Glucose concentrations were determined, using the Beckman DxC clinical chemistry analyser. Insulin and SHBG concentrations were measured, using an electrochemiluminescence immunoassay on the Modular E170 (Roche Diagnostics GmbH, D-68298 Mannheim, Germany). Testosterone was measured after diethyl ether extraction, using an in-house competitive radio-immunoassay, employing a polyclonal anti-T-antibody (Dr. Pratt AZG 3290). \([1,2-3\text{H}(\text{N})]\) T (NET-387, DuPont NEN Nederland B.V.) was used as a tracer following chromatographic verification of its purity.

Statistical analysis
Baseline characteristics were compared between women who developed GDM and women who did not. Chi-square tests were used to calculate P-values for categorical variables and Mann–Whitney U-tests were performed on continuous variables.

Differences between GDM and non-GDM pregnancies in the longitudinal course of fasting insulin, fasting glucose, HOMA-IR, SHBG and testosterone at three different sampling moments were assessed by performing a linear mixed model analysis, using the three sampling moments as a categorical variable. A logarithmic transformation was applied to the values, and differences were tested both in level (main effect) and in pattern over time (interaction of GDM group with time). All analyses, except for the baseline characteristics, were adjusted for BMI, waist circumference and waist/hip ratio. Only the P-values of the models adjusted for BMI are shown in the results section. After these univariate analyses, we performed a multivariate logistic regression analysis with backward elimination to find out which parameters have a direct association with GDM.

All data were analysed, using the SPSS Statistics (IBM SPSS, Inc., Chicago, IL, USA version 20.0) and R version 2.9.0. (http://www.r-project.org/).

Results
In the CoPPer study, 326 women with PCOS and a wish to conceive were included prior to conception. An ongoing pregnancy was achieved in 189 (58%) of these women. A total of 106 (56%) women were included at the UMC Utrecht. The blood assessment set was complete in 72 (68%) women who were therefore included in this study (Supplementary Figure S1). Twenty-two (31%) of these women were diagnosed with GDM at 24–26 weeks of gestation. Twelve (55%) women were treated with a diet after diagnosis, nine (41%) with a diet and insulin and the treatment of one (5%) woman was unknown.

Baseline characteristics at standardized screening prior to conception for women who did and did not develop GDM are given in Table I. Women who developed GDM had a significantly higher waist circumference \((P = 0.012)\), waist/hip ratio \((P = 0.004)\), fasting insulin level \((P < 0.001)\), HOMA-IR \((P < 0.001)\), FAI \((P = 0.006)\) and a higher incidence of metabolic syndrome \((P < 0.001)\) compared with women who did not develop GDM. SHBG levels were significantly lower \((P < 0.001)\) in women who developed GDM. The number of women of non-European descent was significantly higher in the GDM group than in the non-GDM group \((P = 0.013)\). The weight gain (Table II) compared with before conception in the GDM group was not statistically different from the non-GDM group in the first and second trimesters, with P-values of 0.626 and 0.766, respectively.

Changes in glucose, insulin, HOMA-IR, SHBG and testosterone from before conception, during pregnancy until the second trimester in women who developed GDM versus women who did not, are given in Table II and Fig. 1A–E. After adjustment for BMI, glucose levels were significantly different \((P < 0.001)\) between the groups in the second trimester (Fig. 1A). The glucose model remained unaffected after adjustment for waist circumference and waist/hip ratio. Glucose concentrations changed significantly over time \((P = 0.003)\). This pattern was significantly different between women with GDM and women without GDM, that is, there was a stronger increase of glucose levels in women who develop GDM.

At all sampling moments, and after adjustment for BMI, insulin concentrations were significantly higher in women who developed GDM compared with women who did not (Fig. 1B). The insulin model remained unaffected after adjustment for waist circumference and waist/hip ratio. Insulin levels changed significantly over time in both groups \((P < 0.001)\). This pattern was similar for women who developed GDM and women who did not develop GDM.

After adjustment for BMI, HOMA-IR was significantly increased at all sampling moments in women who developed GDM compared with women who did not (Fig. 1C). The HOMA-IR model also remained unaffected after adjustment for waist circumference and waist/hip ratio. HOMA-IR did not change significantly over time.

SHBG concentrations turned out to be significantly lower before conception \((P < 0.001)\) and during the second trimester \((P = 0.004)\) in the GDM group compared with the non-GDM group (Fig. 1D). The SHBG model remained unaffected after adjustment for waist circumference and waist/hip ratio. In both groups, the SHBG concentrations also increased significantly over time \((P = 0.015)\). This pattern was significantly different between the groups, that is, the increase in SHBG was stronger in the non-GDM group \((P < 0.001)\).

Women who developed GDM had a significantly lower testosterone concentration before conception \((P = 0.03)\) compared with women who did not (Fig. 1E). The testosterone model remained unaffected after adjustment for waist circumference and waist/hip ratio and testosterone concentrations did not change significantly over time.

After multivariate analysis with backward elimination, only HOMA-IR turned out to have a direct effect on the development of GDM \((P < 0.001)\), whereas the other parameters (insulin, SHBG and testosterone) did not. Glucose was not included in the multivariate analysis, since GDM was diagnosed based on glucose levels.

Discussion
The current study was a post hoc analysis of a subgroup of women with PCOS, participating in a previously published prospective cohort study (de Wilde et al., 2014). Levels of glucose, insulin, HOMA-IR, SHBG and testosterone were assessed before conception as well as in the first and second trimesters of pregnancy and were compared between women who did or did not develop GDM. We have demonstrated that women with PCOS who developed GDM already have significantly higher insulin concentrations and HOMA-IR scores before conception.
compared with women who did not, a pattern that persists during the first and second trimesters of pregnancy. SHBG was significantly decreased at these moments in the GDM group and testosterone was only decreased prior to conception.

A remarkably high incidence (31%) of GDM was observed in our population of women with PCOS, even while the women participating in this study had a relatively low BMI. This increased incidence could be explained by the higher BMI and high incidence of metabolic syndrome in women with PCOS. However, after adjusting for BMI, there was still a significant difference in the levels of the assessed parameters between the two groups.

As can be explained by the fact that GDM diagnosis is based on glucose levels, these levels were significantly higher in the second trimester of pregnancy in women who developed GDM compared with women who did not. During the course of normal pregnancy in healthy women, physiological insulin resistance develops with the highest level of resistance in the third trimester and the highest level of insulin sensitivity in the late first trimester (Catalano et al., 1999; Yamashita et al., 2000; Ryan, 2003; Landon and Gabbe, 2011). The mechanism of insulin resistance is not yet fully understood, but it is suggested to be caused by the elevation of free fatty acids during pregnancy (Catalano, 2010). One study compared healthy women who developed GDM with healthy women who were normal glucose tolerant during pregnancy (Kirwan et al., 2001). In that study, women who developed GDM had increased insulin levels compared with the normal glucose tolerant group, but with the same low glucose levels in the first trimester of pregnancy.

### Table 1 Baseline characteristics before conception of women with polycystic ovary syndrome (PCOS) who developed gestational diabetes (GDM) and women who did not. Data are median [IQR] or number (%).

<table>
<thead>
<tr>
<th>Demographic characteristics</th>
<th>Total study group (n = 72)</th>
<th>Women who developed GDM (n = 22)</th>
<th>Women who did not develop GDM (n = 50)</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (years)</td>
<td>29.6 [26.8–31.8]</td>
<td>30.5 [27.9–34.5]</td>
<td>29.0 [26.8–31.1]</td>
<td>0.085</td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
<td></td>
<td></td>
<td>0.013</td>
</tr>
<tr>
<td>European descent</td>
<td>67 (93)</td>
<td>18 (82)</td>
<td>49 (98)</td>
<td></td>
</tr>
<tr>
<td>Non-European descent</td>
<td>5 (7)</td>
<td>4 (18)</td>
<td>1 (2)</td>
<td></td>
</tr>
<tr>
<td>Smoking</td>
<td>6 (8)</td>
<td>2 (9)</td>
<td>4 (8)</td>
<td>0.747</td>
</tr>
<tr>
<td>Cycle disorder</td>
<td></td>
<td></td>
<td></td>
<td>0.627</td>
</tr>
<tr>
<td>Oligomenorrhoea</td>
<td>55 (67)</td>
<td>16 (73)</td>
<td>39 (78)</td>
<td></td>
</tr>
<tr>
<td>Amenorrhoea</td>
<td>17 (24)</td>
<td>6 (27)</td>
<td>11 (22)</td>
<td></td>
</tr>
<tr>
<td>Pregnancy after treatment (n = 71)</td>
<td></td>
<td></td>
<td></td>
<td>0.065</td>
</tr>
<tr>
<td>No treatment</td>
<td>9 (13)</td>
<td>3 (14)</td>
<td>6 (12)</td>
<td></td>
</tr>
<tr>
<td>Ovulation induction (clomiphene citrate, follicle-stimulating hormone)</td>
<td>49 (69)</td>
<td>13 (59)</td>
<td>36 (72)</td>
<td></td>
</tr>
<tr>
<td>IVF/ICSI</td>
<td>13 (18)</td>
<td>6 (27)</td>
<td>7 (14)</td>
<td></td>
</tr>
<tr>
<td>Clinical measures (preconception)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.4 [21.6–28.9]</td>
<td>27.4 [22.7–33.8]</td>
<td>23.8 [21.2–28.1]</td>
<td>0.055</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>84 [75.0–95.0]</td>
<td>94.5 [77.0–103.3]</td>
<td>81.0 [73.0–89.0]</td>
<td>0.012</td>
</tr>
<tr>
<td>Waist/hip ratio</td>
<td>0.8 [0.7–0.9]</td>
<td>0.9 [0.8–0.9]</td>
<td>0.8 [0.7–0.8]</td>
<td>0.004</td>
</tr>
<tr>
<td>Metabolic syndrome</td>
<td>10 (14)</td>
<td>8 [36]</td>
<td>2 (4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Polycystic ovaries</td>
<td>71 (99)</td>
<td>22 (100)</td>
<td>49 (98)</td>
<td>0.504</td>
</tr>
<tr>
<td>Acne</td>
<td>16 (22)</td>
<td>6 (27)</td>
<td>10 (20)</td>
<td>0.494</td>
</tr>
<tr>
<td>Hirsutism*</td>
<td>5 (7)</td>
<td>1 (5)</td>
<td>4 (8)</td>
<td>0.595</td>
</tr>
<tr>
<td>Fasting glucose (mmol/l)</td>
<td>5.0 [4.8–5.3]</td>
<td>5.1 [4.9–5.3]</td>
<td>5.0 [4.7–5.1]</td>
<td>0.075</td>
</tr>
<tr>
<td>Fasting insulin (mU/l)</td>
<td>6.0 [4.0–9.8]</td>
<td>9.0 [6.0–13.3]</td>
<td>4.0 [3.0–8.0]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>1.3 [0.8–2.1]</td>
<td>2.1 [1.4–2.9]</td>
<td>0.9 [0.7–1.8]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Testosterone (nmol/l)</td>
<td>2.3 [1.7–2.8]</td>
<td>1.9 [1.5–2.6]</td>
<td>2.3 [1.8–2.9]</td>
<td>0.190</td>
</tr>
<tr>
<td>Steroid hormone-binding globulin (nmol/l)</td>
<td>57.5 [40.0–76.5]</td>
<td>39.0 [25.8–49.8]</td>
<td>63.5 [49.3–85.0]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Delivery</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gestational age at delivery</td>
<td>39.3 [38.3–40.4]</td>
<td>39.1 [38.3–39.9]</td>
<td>39.6 [38.7–40.7]</td>
<td>0.114</td>
</tr>
<tr>
<td>Birthweight (g)</td>
<td>3405 [2923–3710]</td>
<td>3363 [2860–3738]</td>
<td>3405 [2975–3721]</td>
<td>0.995</td>
</tr>
</tbody>
</table>

HOMA-IR, homeostasis model assessment insulin resistance.

*Ferriman–Gallwey score > 8.

*P-values were calculated based on the comparison of women who developed GDM with women who did not develop GDM.
Table II  The course of some variables in women who developed gestational diabetes (GDM) and women who did not from before conception until the second trimester during pregnancy. Data are median [IQR].

<table>
<thead>
<tr>
<th>Outcome variables</th>
<th>Preconception</th>
<th>First trimester</th>
<th>Second trimester</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Women with</td>
<td>Women without</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GDM (n = 22)</td>
<td>GDM (n = 50)</td>
<td></td>
</tr>
<tr>
<td>Gestational age (weeks)</td>
<td>-22.4 [-7.1 - 41.4]</td>
<td>-23.0 [-9.7 - 32.7]</td>
<td>11.8 [11.2 - 12.3]</td>
</tr>
<tr>
<td>Weight gain compared with preconception (kg)</td>
<td>-</td>
<td>-</td>
<td>2.6 [-2.0 - 6.0]</td>
</tr>
<tr>
<td>Fasting glucose (mmol/l)</td>
<td>5.1 [4.9–5.4]</td>
<td>5.0 [4.8–5.1]</td>
<td>4.9 [4.6–5.1]</td>
</tr>
<tr>
<td>Fasting insulin (mU/l)</td>
<td>9.0 [6.0–13.3]</td>
<td>4.0 [3.0–8.0]</td>
<td>10.9 [8.0–14.0]</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>2.1 [1.4–2.9]</td>
<td>0.9 [0.7–1.7]</td>
<td>2.5 [1.7–3.1]</td>
</tr>
<tr>
<td>Sex hormone-binding globulin (nmol/l)</td>
<td>40.5 [26.0–50.3]</td>
<td>63.5 [50.8–85.0]</td>
<td>197.5 [135.0–317.5]</td>
</tr>
<tr>
<td>Testosterone (nmol/l)</td>
<td>1.9 [1.5–2.6]</td>
<td>2.3 [1.8–2.9]</td>
<td>4.3 [2.6–6.0]</td>
</tr>
</tbody>
</table>

HOMA-IR, homeostasis model assessment insulin resistance.

Figure 1 Fasting glucose (A), fasting insulin (B), homeostasis model assessment of insulin resistance (HOMA-IR), (C) sex hormone-binding globulin (SHBG) (D) and testosterone (E) before conception and during the first and second trimesters of pregnancy in women with polycystic ovary syndrome (PCOS) who developed gestational diabetes (GDM) (red) versus women with PCOS who did not (green). The results are presented as median and IQR with P-values above the boxplots. The whiskers extend to the most extreme value, which is not more than 1.5 times the IQR away from the median. The small circles above the boxplots represent outliers, which is more than 1.5 times the IQR away from the median.
Based on these results, we expected that insulin sensitivity in the GDM group might be not as high compared with physiological pregnancies in the first trimester, with compensatory higher insulin concentrations and higher HOMA-IR scores as a result. Glucose concentrations were expected to be low or normal due to the expected higher insulin levels in the GDM group. In our study, we indeed found increased insulin and HOMA-IR levels in the GDM group with normal glucose levels in the first trimester. Possibly, insulin sensitivity in women who develop GDM is not as high as in normal glucose-tolerant women in the first trimester, with increased insulin concentrations as a result in order to keep glucose concentrations within normal ranges. In addition, insulin levels and HOMA-IR levels were already significantly increased prior to conception in the GDM group compared with the non-GDM group. These findings fit the hypothesis that the risk of developing GDM is already present early in pregnancy.

In women with PCOS, pregnancy complications are associated with higher androgen levels and increased insulin resistance compared with women with PCOS who have uncomplicated pregnancies (Falbo et al., 2010). It has been reported that during normal pregnancy, SHBG levels increase around 5–10 fold due to the placental estrogen production (Anderson, 1974). On the other hand, SHBG levels also decrease due to inhibition of SHBG synthesis by hyperinsulinaemia (Plymate et al., 1988). In our study, SHBG strongly increased during pregnancy in both study groups. However, SHBG levels were significantly lower before conception and during the second trimester in the GDM group compared with the non-GDM group. A possible explanation for this observation is that the hyperinsulinaemia associated with GDM may have an attenuating effect on SHBG secretion caused by placental estrogens.

Testosterone concentrations were slightly increased during pregnancy in women who developed GDM compared with women who did not, although these differences were not statistically significant. In contrast, testosterone was significantly lower before conception in women who developed GDM since testosterone is significantly correlated to SHBG (Anderson, 1974; Jayagopal et al., 2003; Tsai et al., 2004). The rise in testosterone concentrations during pregnancy could be explained by the observed increase of SHBG. Since the increase of SHBG was higher in women who developed GDM compared with women who did not, testosterone might also increase more in women who develop GDM. However, despite the low total testosterone concentrations before conception, women who developed GDM were considerably more hyperandrogenic (FAI 5.65) than women who did not (FAI 3.07). This is supported by the finding that the hyperandrogenic PCOS phenotype seems to be more insulin resistant than the non-hyperandrogenic phenotype (Daan et al., 2014).

A limitation of this study was that potential selection bias could have occurred, since only women with complete blood sampling in the UMCU were selected for analysis. Women who were potentially at risk for a complicated pregnancy before conception could be more motivated for blood sampling than women who were not. However, after comparing demographic characteristics, BMI and the occurrence of GDM during pregnancy, no significant differences were found between women who were included versus women who were not (data not shown). Further, shortly after our study ended, a new guideline was published by the American Diabetes Association posing the 75-g OGTT as test of choice, using the exact same cut-off points as the 100-g OGTT (American Diabetes Association, 2012). This means that the number of women who developed GDM in our study might be overestimated. However, we expect that the differences in all parameters between women who developed GDM and women who did not, would have been even larger when the new guidelines had been followed. Another limitation is the fact that all women in our study population only underwent an OGTT at 24–26 weeks of gestation according to the study protocol and Dutch guidelines. Women can develop GDM later on in pregnancy and therefore there might be a few women in the non-GDM group who eventually developed GDM. However, based on maternal and neonatal outcomes in our study, we have no reason to assume that this might have occurred. Finally, we did not make a distinction between different PCOS phenotypes. Yet, these phenotypes have different metabolic features and associations might therefore be different within phenotypes. Because of the sample size of the GDM group, we decided not to perform subgroup analysis, because effect sizes would become too small.

In conclusion, this study showed a significantly disturbed insulin action in women with PCOS who develop GDM compared with women who do not. However, there is still overlap between groups, so there are probably more mechanisms and pathways contributing to the development of GDM. With this study, more understanding in the course of insulin action was achieved in women with PCOS during pregnancy and the development of GDM. This can be useful, considering the pathophysiology of this disorder in this specific group of women. By performing high-powered studies, future research could focus on the prediction of GDM, with the aim to reduce the prevalence of GDM and adverse consequences for both mother and baby.

**Supplementary data**

Supplementary data are available at http://humrep.oxfordjournals.org.

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**Authors’ roles**

A.J.G., M.J.C.E., B.C.J.M.F. and M.P.H.K. designed the study. M.A.d.W. and S.M.V.-V. collected the data. M.A.d.W., A.J.G., S.M.V.-V., A.F., M.J.C.E., B.C.J.M.F. and M.P.H.K. analysed and interpreted the data and wrote the manuscript. B.C.J.M.F. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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**Conflict of interest**

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