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RESEARCH ARTICLE

Gestational weight gain, appetite regulating hormones, and metformin treatment in polycystic ovary syndrome: A longitudinal, placebo-controlled study

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Abstract

Objective: To explore mechanisms that modulate gestational weight gain (GWG) in women with polycystic ovary syndrome (PCOS) and healthy controls.

Design: Sub-sample of randomised controlled trials (PCOS) combined with a prospective cohort (controls).

Setting: Eleven Norwegian, Swedish, and Icelandic hospitals.

Population: Pregnant women with PCOS treated with metformin (PCOS-M, $n = 36$) or placebo (PCOS-P, $n = 37$), and healthy pregnant women (HC, $n = 15$).

Methods: Serum levels of the appetite regulating hormones leptin, ghrelin, allopregnanolone, and soluble leptin receptor (sOB-R) were determined in the first and third trimesters.

Main Outcome Measures: Excessive GWG (eGWG) relative to body mass index according to Institute of Medicine (IOM) guideline. Serum leptin/sOB-R ratio, or free-leptin-index (FLI), as biomarker of leptin sensitivity. Serum ghrelin and allopregnanolone levels.

Results: The overall prevalence of eGWG was 44% (38/86). Women with eGWG had higher first and third trimester FLI ($P < 0.001$), and lower third trimester allopregnanolone levels ($P = 0.003$) versus women with non-eGWG. The prevalence of eGWG was lower in PCOS-M versus PCOS-P (28% versus 62%, odds ratio = 0.4, 95% CI 0.2–0.8, $P = 0.005$). FLI decreased during pregnancy in PCOS-M ($P = 0.01$), but remained unaltered in PCOS-P and HC. Ghrelin and allopregnanolone levels were comparable in PCOS-M, PCOS-P and HC throughout pregnancy.

Conclusion: Excessive GWG is associated with enhanced leptin resistance, and attenuated physiological increase in serum allopregnanolone levels during pregnancy. Metformin reduces the risk for eGWG and improves leptin sensitivity in pregnant women with PCOS.

KEYWORDS
allopregnanolone, gestational weight gain, ghrelin, leptin resistance, metformin, PCOS

Tweetable Abstract: Metformin counteracts excessive weight gain and leptin resistance in pregnant women with polycystic ovary syndrome.

1 | INTRODUCTION

Polycystic ovary syndrome (PCOS)[1] is the most common endocrine disorder in women of reproductive age, affecting 5–20%.[2] Observational studies indicate that women with PCOS have greater gestational weight gain (GWG) compared with weight-matched controls[3] and, in particular, overweight women[4] tend to exceed GWG recommendations issued by the Institute of Medicine (IOM).[5] Excessive GWG is associated with sub-optimal pregnancy outcome and long-term obesity development[6] and is therefore essential to prevent. Metformin use during pregnancy is increasing, and randomised trials report less GWG compared with placebo treatment.[7–10] Metformin has been proposed to induce weight loss by interfering with appetite stimulation and leptin sensitivity[11] (Figure S1). However, these mechanisms have not been studied in pregnancy.

The adipocyte hormone leptin promotes satiety when binding to hypothalamic leptin receptors (OB-R).[12] A compensatory physiological leptin resistance counteracts this response in normal pregnancy and ensures a positive energy balance and adequate nutrition to the fetus[13] when serum leptin levels rise due to placental production and accumulation of fat stores.[14,15] Serum leptin binds to the soluble iso-form of the leptin receptor (sOB-R) and only unbound leptin is biologically active.[12] The leptin/sOB-R-ratio or free leptin index (FLI) is a biomarker of leptin sensitivity that increases when hypothalamic leptin sensitivity attenuates.[12,13] Serum leptin levels correlate positively with GWG in non-obese women,[16,17] but whether sOB-R levels or FLI correlates with GWG is unexplored. sOB-R is formed by proteolytic cleavage of membrane-bound OB-R-ectodomains and reflects expression of OB-R in peripheral tissues.[18] Lower sOB-R levels[19] and placental OB-R mRNA levels[20] have been reported in women with PCOS than in controls.

Placental production raises serum levels of ghrelin[21] and the progesterone metabolite allopregnanolone during pregnancy.[22] Ghrelin counteracts hypothalamic leptin action and promotes hunger,[11] and rodent studies have shown that allopregnanolone enhances ghrelin effects by inducing hyperphagia.[23,24] Serum allopregnanolone levels correlate positively with uncontrolled eating in non-pregnant obese women with PCOS.[25] Third trimester serum allopregnanolone levels correlate positively with GWG in healthy women[22] but allopregnanolone or ghrelin have not been studied in pregnant women with PCOS.

We hypothesised that appetite-regulating hormones produced by the placenta modulate GWG, and that metformin decelerates GWG by mechanisms involving these hormones. The purpose of this study was to gain more insight into these mechanisms by exploring serum levels of leptin, sOB-R, allopregnanolone and ghrelin longitudinally in pregnant women with PCOS randomised to metformin or placebo, and in healthy controls.

2 | METHODS

2.1 | Patient involvement and funding

No patients or patient organisations were involved in the planning of this study. Grants from the Research Council of Norway, Västerbotten County Council, and Umeå University Foundations supported the study. The funders had no role in designing the study, collecting, analysing, and interpreting the data, or writing the article.

2.2 | Subjects

The 73 women with PCOS included in the present study were a sub-sample of subjects enrolled between 2005 and 2017 in one of two randomised, double-blind, placebo-controlled multi-centre trials studying the effect of metformin on pregnancy complications in PCOS (PregMet,[7] PregMet2[8]). The Rotterdam criteria[1] were used for PCOS diagnosis, which was confirmed and documented prior to the pregnancy. The sub-sample was restricted to nulliparous women with body mass index (BMI) <30 at inclusion, from whom serum samples from inclusion and gestational week 36 were available. All eligible participants in PregMet2 were included. In addition, all 16 eligible participants in PregMet from whom snap-frozen placenta samples were available were included. We further included 15 healthy women, referred to as healthy controls, for comparison. They were recruited in early pregnancy among nulliparous women with BMI <30 and no history of physical or psychological illnesses, attending antenatal clinics affiliated with Stockholm South General Hospital, Sweden, between 2015 and 2016. PCOS was ruled out if they, prior to the pregnancy, had regular menstrual cycles of <35 days, no clinical manifestations of hyperandrogenism and no fertility problems. A single viable fetus was confirmed by ultrasound. Two healthy controls enrolled in the study were excluded due to pre-eclampsia and gestational diabetes development to confine the control group to healthy women with uncomplicated pregnancies.

All participants gave written informed consent prior to inclusion. Ethical approval was obtained from the Committee for Medical Research Ethics of Health Region IV, Norway (145–05), Regional Committee for Health Research Ethics of Central Norway (2011/1434), National Bioethics Committee of Iceland (VSNb2012100011/03.10), Regional Ethical Review Board, Stockholm, Sweden (2012/1200-31/2), and Regional Ethical Review Board, Umeå, Sweden (2014-257-31M; 2016-190-32M). The study was conducted according to the Declaration of Helsinki.

2.3 | Study design

All participants followed essentially the same protocol.[7,8] Thirty-six of the women with PCOS were randomised

to treatment with a daily dose of 2000 mg metformin (PCOS-M) and 37 women to placebo (PCOS-P), from inclusion between gestational week 5 and 13 until delivery. Compliance was assessed by structured questions at study visits and categorised as good, acceptable or poor. Category definitions are displayed in Table S1. Adverse effects were recorded, and dosage was adjusted if necessary. Study visits were scheduled between 0700 and 1100 hours, and venous blood samples were collected after an overnight fast. The blood samples were allowed to clot for 30 minutes before centrifugation to collect serum. The supernatants were aliquoted into plastic vials, immediately placed in the freezer and kept stored at −80°C until further analyses. Body weight was measured with the participants wearing light clothes and no shoes, and blood pressure was measured in the sitting position after minimum rest of 10 minutes. In parallel with the study, the participants followed the regular pregnancy surveillance programme at their local maternal healthcare clinic.

2.4 | Gestational weight gain

Total GWG was the recorded weight at gestational week 36, minus the recorded weight at inclusion. Average GWG per week was calculated by dividing total GWG by the number of days between the two weight recordings, multiplied by seven, and categorised as low, recommended or excessive in accordance with the 2009 US Institute of Medicine (IOM) guideline.[5] Recommended second and third trimester GWG is 0.35–0.50 kg per week when pre-pregnancy BMI is 18.5–24.9, and 0.23–0.33 kg when BMI is 25–29.9.[5]

2.5 | Hormonal assays

Fasting serum concentrations were determined from samples obtained at inclusion, and at gestational week 36 (±1 week). Leptin and ghrelin concentrations were quantified by multiplex Bio-Plex Assay (#171B7009M, Leptin; #171B7004M, Ghrelin, Bio-Rad) with Bio-Plex MANAGER software version 6.1.1.794 using Bio-Plex 200 System (Bio-Rad). sOB-R was quantified by Human Quantikine Leptin R ELISA kit (R&D System). All assays were performed according to the manufacturers' instructions and samples were run in duplicate. Inter-assay coefficient of variation (CV) was 8%, 8% and 4%, and intra-assay CV was 2%, 3% and 4% for leptin, ghrelin and sOB-R, respectively. Allopregnanolone was quantified by Admescope Oy (Oulu, Finland) in duplicate samples using UPLC/MS/MS Waters Acquity with Waters-TQ-S triple quadrupole mass spectrometer and Phenomenex Kinetex XB C18 columns. Oxime derivatisation with hydroxylamine was used in accordance with Keski-Rahkonen et al.[26] to increase sensitivity. The detection limit was 0.002 ng/ml.

2.6 | Outcome of pregnancy

Data regarding the pregnancy, delivery and perinatal period were extracted from the PregMet and PregMet2 databases for the participants with PCOS and from the medical records for the healthy controls. No core outcome set was used. The Harmonising Research Outcomes in PCOS core outcome set (HARP)[27] was published after collection of data for this study. However, all outcomes included in the HARP pregnancy domain[27] were reported in the PregMet[7]/PregMet2[8] trials, from which our subsample originates.

2.7 | Statistics

The sample size calculation is based on data from a study by Lundquist et al.[22] who reported equivalent serum allopregnanolone levels and body weight in early pregnancy in women with subsequent low (<11 kg) versus high (>11 kg) GWG (median 6 versus 14 kg), and 15 nmol/litre difference in allopregnanolone levels between these groups in late pregnancy. We considered 8 kg a clinically relevant difference in GWG. To detect a difference of 15 nmol/l (4.8 ng/ml) between groups, with a power of 80%, alpha = 0.05, and a two-sided test, 34 women in each group was deemed sufficient. For the other hormones, this sample size was estimated to be sufficient based on the results reported by Kos et al.[27] who included 12 pregnant women per group and found a difference between groups of 40 pmol/l (0.08 ng/ml) in serum leptin, 0.08 ng/ml in serum ghrelin and 51 pmol/l in serum sOB-R to be statistically significant ($P < 0.05$) and corresponding to a statistically significant difference in GWG ($P < 0.01$).

Mann–Whitney *U*-test and Fisher's exact test were used for analysis of baseline characteristics and pregnancy outcome. Within-group analysis of appetite-regulating hormone levels was by repeated measures analysis of variance (ANOVA), and between-group analysis was by one-way ANOVA or analysis of covariance (ANCOVA). At baseline, analyses were controlled for BMI and gestational age in days. At gestational week 36, between-group analyses were controlled for baseline levels. Variables with non-normal distribution were log-transformed, and robust 95% confidence intervals were determined using 1000 bootstrapped samples. Sidak's correction was used for multiple comparisons adjustments, and the significance level was set at $P < 0.05$. All statistical analyses were performed using SPSS, software version 26.

2.8 | Procedures

The modulating effect of metformin on GWG was first evaluated against total GWG, and secondly against categories of compliance to the IOM GWG guideline[5] based on BMI at inclusion for assessment of the effect on an established

measure of clinical importance for pregnancy outcome. Prior to further analyses, free leptin index (FLI) was calculated as the ratio of leptin (ng/ml) to sOB-R (ng/ml) for assessment of leptin sensitivity,[13] and data from women with low and normal GWG were pooled and referred to as women with non-excessive GWG (non-eGWG). Thereafter, FLI and serum levels of leptin, sOB-R, ghrelin and allopregnanolone were evaluated in women with excessive GWG (eGWG) versus non-excessive GWG to assess associations between GWG and leptin sensitivity and appetite-regulating hormones. Finally, metformin treatment was evaluated against FLI and serum levels of leptin, sOB-R, ghrelin and allopregnanolone to assess leptin sensitivity and appetite-regulating hormones as possible mediators of the mechanism of action that underlies metformin's modulating effect on GWG.

3 | RESULTS

3.1 | Baseline characteristics and pregnancy outcome

There was no difference in baseline parameters between the metformin, placebo and control groups at inclusion (Table 1). At delivery, median birthweight was lower in the metformin group than in the placebo group ($P = 0.02$) (Table 2). Characteristics of women with eGWG and non-eGWG are presented in Table S2.

3.2 | Gestational weight gain and metformin

We found no correlation between BMI and total GWG ($r_s = 0.14$, 95% CI −0.13 to 0.38) but normal weight women with PCOS were less likely to have eGWG compared with overweight women (13/45 versus 20/28, odds ratio [OR] 0.4, 95% CI 0.2–0.6, $P < 0.001$).

In women with PCOS, median total GWG was lower in the metformin group than in the placebo group (9.0 versus 11.6 kg, $P = 0.01$) (Table 2). We further found that women in the metformin group were less likely to exceed recommended average weekly GWG (28% versus 62%, OR 0.4, 95% CI 0.2–0.8, $P = 0.005$) but also more likely to gain weight below recommendations (33% versus 8%, OR 4.1, 95% CI 1.5–15.5, $P = 0.01$) compared with the placebo group (Table 2).

3.3 | Gestational weight gain and appetite-regulating hormones

We found that women with eGWG had higher first trimester leptin levels ($P < 0.001$) and lower sOB-R levels ($P = 0.035$), and consequently FLI was higher in this group of women ($P = 0.001$) than in women with non-eGWG (Figure 1). By the third trimester, sOB-R levels had increased in both groups (Table S3) but less in women with eGWG ($P < 0.001$) (Figure 1). Leptin levels, on the other hand, had only increased in women with eGWG (Table S3). FLI was thereby further increased compared with first trimester levels in women with eGWG but had decreased in women with non-eGWG (Figure 1, Table S3). By contrast, ghrelin levels were lower in women with eGWG ($P = 0.01$) and decreased at a similar rate as in women with non-eGWG (Figure 1). There was no difference in allopregnanolone levels in the first trimester, and although allopregnanolone levels increased considerably in both groups (Table S3) there was less increase in women with eGWG ($P = 0.008$) (Figure 1).

TABLE 1 Baseline characteristics of women with polycystic ovary syndrome, randomised to metformin (PCOS-M) or placebo (PCOS-P) from first trimester to delivery, and healthy controls (HC)

				P-value	
Characteristic	PCOS-M *n* = 36	PCOS-P *n* = 37	HC *n* = 13	PCOS-M versus PCOS-P	PCOS[a] versus HC
Age (years)	29 (19–38)	29 (23–35)	29 (24–38)	0.97	0.65
Height (cm)	166 (160–176)	167 (153–176)	168 (160–176)	0.97	0.54
Weight (kg)	64 (52–85)	67 (50–92)	64 (55–71)	0.48	0.53
BMI (kg/m^2)	23.4 (19.3–29.8)	24.2 (19.6–29.7)	23.3 (18.9–25.3)	0.30	0.29
sBP (mmHg)	112 (95–131)	113 (94–143)	110 (105–120)	0.68	0.59
dBP (mmHg)	70 (60–85)	72 (60–88)	70 (50–80)	0.17	0.29
Smoking (*n*)	1 (3)	1 (3)	0	>0.999	>0.999
Gestational age at inclusion (days)	79 (49–90)	71 (52–90)	79 (60–91)	0.37	0.22

Note: Values displayed are median (min-max) or numbers (%).

Comparisons between groups by Mann-Whitney *U*-test for continuous variables, and by Fisher's exact test for categorical variables. Significant group difference was set at $P < 0.05$.

Abbreviations: BMI, body mass index; dBP, diastolic blood pressure; PCOS, polycystic ovary syndrome; sBP, systolic blood pressure.

[a]PCOS = pooled baseline data from PCOS-M and PCOS-P.

TABLE 2 Pregnancy outcome in women with polycystic ovary syndrome, randomised to treatment with either metformin (PCOS-M) or placebo (PCOS-P) from the first trimester to delivery, and in healthy controls (HC)

				P-value		
Outcome	**PCOS-M** ***n*** **= 36**	**PCOS-P** ***n*** **= 37**	**HC** ***n*** **= 13**	**PCOS-M versus PCOS-P**	**PCOS-M versus HC**	**PCOS-P versus HC**
Adherence to study medication						
Good (*n*)	24 (67)	28 (75)	N/A	0.45	N/A	N/A
Acceptable (*n*)	11 (30)	8 (22)	N/A	0.43	N/A	N/A
Poor (*n*)	1 (3)	1 (3)	N/A	>0.999	N/A	N/A
Total GWG (kg)	9.0 (5.2–21.0)	11.6 (3.8–18.1)	11.2 (7.1–16.4)	0.01	0.11	0.80
GWG per week (kg)	0.35 (0.20–0.84)	0.49 (0.14–0.68)	0.46 (0.31–0.72)	0.04	0.11	0.99
GWG by IOM category relative to BMI at inclusion						
Low (*n*)	12 (33)	3 (8)	0	0.01	0.02	0.56
Normal (*n*)	14 (39)	11 (30)	8 (62)	0.47	0.20	0.05
Excessive (*n*)	10 (28)	23 (62)	5 (38)	0.005	0.50	0.20
Gestational age at delivery (days)	282 (261–295)	282 (264–296)	285 (271–295)	0.35	0.07	0.20
GDM (*n*)	6 (17)	4 (11)	N/A	0.51	N/A	N/A
PE (*n*)	0	1 (3)	N/A	>0.999	N/A	N/A
Birthweight (g)	3360 (2590–4170)	3590 (2280–4840)	3650 (2860–4170)	0.02	0.06	0.74
Apgar score						
1 min (*n*)	9 (6–10)	9 (1–10)	9 (5–10)	0.59	0.65	0.89
5 min (*n*)	10 (7–10)	10 (2–10)	10 (8–10)	0.86	0.11	0.10
10 min (*n*)	10 (9–10)	10 (6–10)	10 (9–10)	0.16	0.94	0.31
Apgar <7 at 5 min (*n*)	0	1 (3)	0	>0.999	N/A	>0.999

Note: Values displayed are median (min-max) or numbers (%).

Comparisons between groups by Mann-Whitney *U*-test for continuous variables, and by Fisher's exact test for categorical variables. Significant group difference was set at $P < 0.05$.

Abbreviations: GDM, gestational diabetes mellitus; GWG, gestational weight gain; IOM, Institute of Medicine; N/A, not applicable; PCOS, polycystic ovary syndrome; PE, pre-eclampsia.

3.4 | Appetite-regulating hormones and metformin

There was no difference in baseline levels between women with PCOS randomised to metformin or placebo regarding any of the investigated hormones (Table S4). Healthy control women had higher baseline sOB-R levels compared with women with PCOS ($P < 0.001$) when controlling for gestational age (Figure 2). At gestational week 36, both the placebo group and the control group had increased their serum leptin levels ($P < 0.001$ and $P = 0.006$, respectively) in parallel with increasing sOB-R levels ($P < 0.001$ and $P < 0.001$, respectively). FLI was thereby kept constant over time (Table S4). By contrast, FLI had decreased from baseline to gestational week 36 in the metformin group ($P = 0.01$) (Table S4). To investigate whether this was an effect of metformin, we analysed the change over time by comparing hormone levels and FLI between study groups at gestational week 36 while controlling for baseline levels. We found that metformin treatment in PCOS was associated with a greater increase in sOB-R levels from baseline compared with placebo and healthy controls ($P = 0.002$ and $P = 0.009$, respectively) and a smaller increase in leptin levels ($P = 0.005$ and $P = 0.01$, respectively) (Figure 2). Metformin treatment was thereby associated with a significantly different change in FLI over time ($P = 0.004$ versus placebo, $P = 0.006$ versus controls) (Figure 2). Ghrelin decreased and allopregnanolone increased equally in the metformin, placebo and control groups (Figure 2, Table S4).

Given the apparent effect of metformin on leptin sensitivity, we repeated analyses of hormone levels and FLI relative to eGWG and non-eGWG after excluding women in the metformin group. This did not change the overall effect on leptin sensitivity or third trimester allopregnanolone levels, but first trimester ghrelin levels were no longer significantly lower in women with eGWG (Table S3). Table S5 presents sub-group analyses in women with eGWG and non-eGWG for the metformin, placebo and control groups, respectively.

4 | DISCUSSION

4.1 | Main findings

Metformin treatment resulted in less GWG and reduced the risk for excessive GWG in women with PCOS. The underlying mechanism appears to involve improved leptin sensitivity, evidenced by decreasing FLI from the first to the third

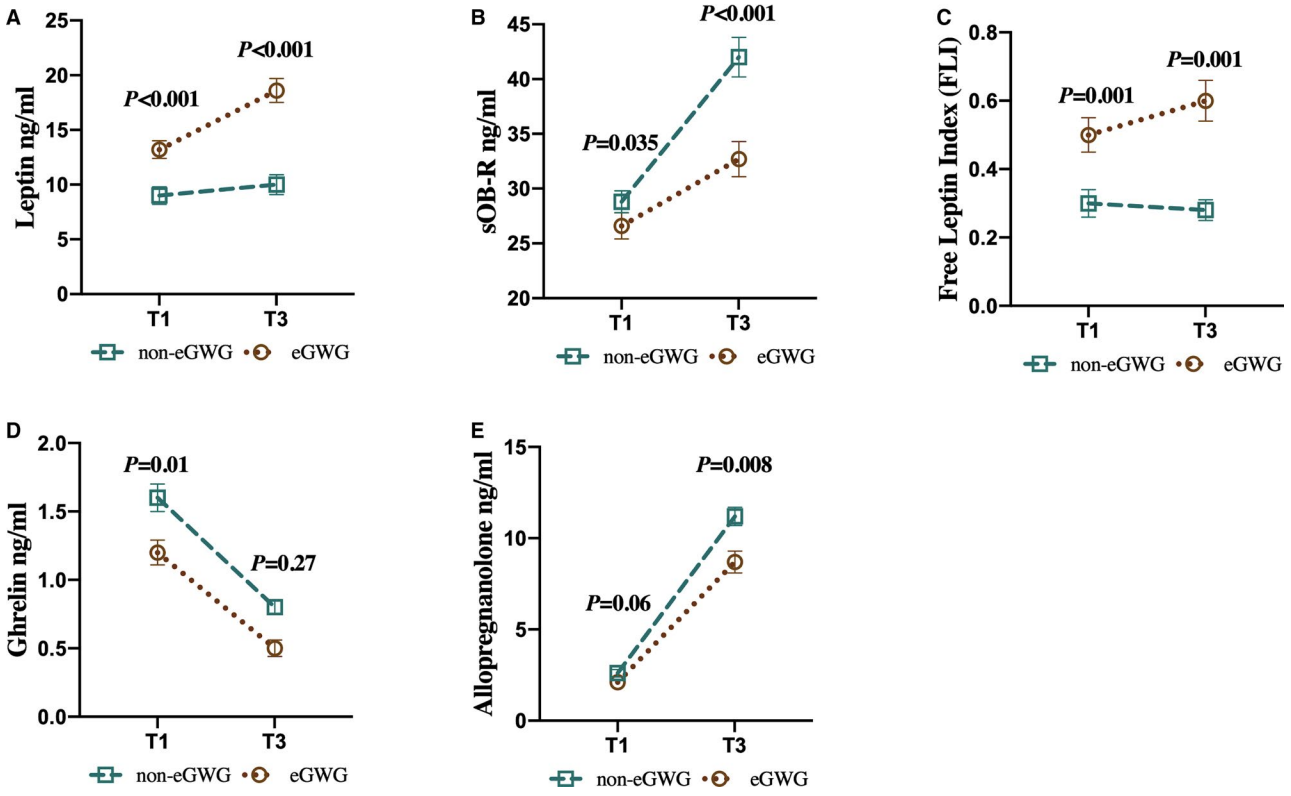

FIGURE 1 Unadjusted serum levels (mean ± SEM) are displayed for (A) leptin, (B) soluble leptin receptor (sOB-R), (C) free leptin index (FLI), (D) ghrelin, (E) allopregnanolone in women with non-excessive gestational weight gain (non-eGWG) and excessive GWG (eGWG) relative to first trimester BMI in accordance with the 2009 Institute of Medicine (IOM) guideline for gestational weight gain.[5] Pooled data from women with polycystic ovary syndrome randomised to metformin or placebo treatment from inclusion to delivery and healthy control women of samples obtained at inclusion in the first trimester (T1) and at gestational week 36 (T3). ANCOVA was used to compare T1 levels between groups while controlling for gestational age at inclusion. ANCOVA was used to assess difference in change from T1 to T3 (Δ) between groups by comparing T3 levels while controlling for T1 levels

trimester. Women with excessive GWG were more leptin-resistant and showed less of an increase in serum allopregnanolone levels during pregnancy.

4.2 | Strengths and limitations

Our study is the first to investigate leptin sensitivity and serum levels of ghrelin and allopregnanolone in pregnant women with PCOS, and the first to explore the effects of metformin on appetite-regulating hormones in pregnancy. The randomised placebo-controlled design and the repeated measurements are methodological strengths of the study. Another strength is that GWG was calculated from measurements obtained at documented study visits, eliminating recall bias. We evaluated metformin and appetite-regulating hormones against different categories of compliance with the IOM GWG guideline[5] which facilitates interpretation of the clinical relevance of our results. A possible weakness in the study is that only primiparous women were included. Although we studied women with all four PCOS phenotypes, we included only non-obese individuals from an ethnically homogeneous group of Nordic women, which may limit the generalisability of our results. Another limitation is that relatively few participants were included, which undermines sub-group analyses due to reduced power. Our results should therefore be interpreted with some caution and need to be confirmed by others.

4.3 | Interpretation

4.3.1 | Excessive GWG

Based on observational data, the IOM GWG guideline recommends a more restricted GWG with increasing pre-pregnancy BMI for optimal pregnancy outcome.[5] A statistically significant treatment effect on total GWG must therefore be related to BMI and cannot be expected to improve pregnancy outcome unless the likelihood of keeping GWG within recommendations increases. The randomised OPTIMISE trial has shown that diet and lifestyle advice is not superior to standard care in preventing eGWG in normal weight women,[28] and the GRoW trial concluded that metformin is not superior to placebo in preventing eGWG in overweight or obese women.[10] By contrast, we found a lower risk for eGWG in metformin-treated normal weight and overweight women with PCOS compared with placebo, although the rate of eGWG in the metformin group was similar to the GRoW trial.[10]

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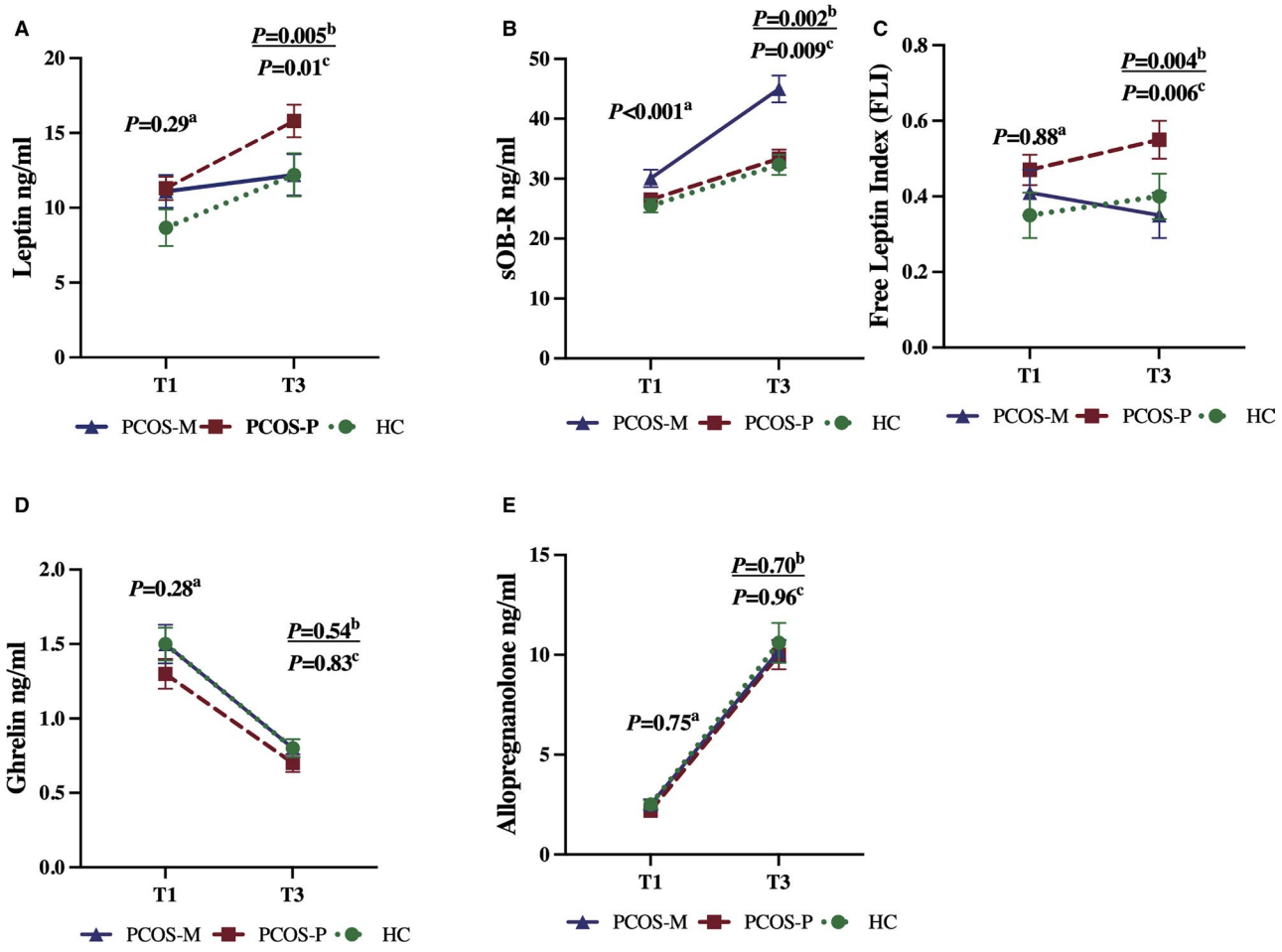

FIGURE 2 Unadjusted serum levels (mean ± SEM) are displayed for (A) leptin, (B) soluble leptin receptor (sOB-R), (C) free leptin index (FLI), (D) ghrelin, (E) allopregnanolone in women with polycystic ovary syndrome (PCOS) randomised to metformin (PCOS-M) or placebo (PCOS-P), and in healthy control women (HC). Analysis of covariance (ANCOVA) was used to compare women with PCOS (PCOS-M + PCOS-P) to HC at baseline in the first trimester (T1), while controlling for gestational age and body mass index at inclusion. ANCOVA was used to assess difference between groups in change of serum levels from the first to the third trimester (Δ) by comparing serum levels at gestational week 36 (T3) while controlling for baseline levels. There was no difference between PCOS-P and HC at T3 in (A–E) (results not displayed)

4.3.2 | Excessive GWG and leptin resistance

Walsh et al.[29] found higher leptin levels during pregnancy in healthy women with eGWG compared with non-eGWG regardless of BMI. Our study confirms this also in women with PCOS and provides additional information, as we found attenuated increase in sOB-R levels and elevated FLI in women with eGWG. This indicates that leptin resistance is associated with eGWG. Leptin resistance is usually defined as diminished leptin effects in combination with hyperleptinaemia, and is associated with reduced sOB-R levels.[30] Increased free leptin levels are believed to compensate for decreased hypothalamic leptin sensitivity.[30] Leptin resistance is a normal physiological response in pregnancy. However, serum leptin levels usually increase in parallel with sOB-R levels during pregnancy, leaving FLI unaltered.[13,31,32] Our study demonstrates this in women with PCOS, as FLI remained unchanged in our placebo group, as well as in the control group. The mechanisms underlying leptin resistance in pregnancy have not been established, but impaired leptin transport over the blood–brain barrier (BBB) has been proposed to underlie central leptin resistance.[30] Membrane-bound short isoforms of the OB-R are believed to mediate leptin transport over the BBB, and sOB-R regulates transport by preventing clearance of leptin from the circulation.[12,30] There are also indications of altered BBB leptin transport in pregnancy, as the leptin ratio between cerebrospinal fluid and serum is reduced compared with the non-pregnant state.[32–34] Gustafson et al.[35] further demonstrated in an animal study that leptin transport from the peripheral circulation to hypothalamus was completely suppressed in late pregnancy. Impaired BBB leptin transport is thereby relevant to consider as a mechanism to target in prevention of eGWG.

4.3.3 | Metformin and leptin resistance in pregnancy

No previous studies have investigated how metformin affects leptin resistance in pregnancy. However, the

decreasing FLI that we found in the metformin group indicates that metformin counteracts pregnancy-related physiological leptin resistance. In non-pregnant leptin-resistant animals, metformin decreases serum leptin levels and increases hypothalamic leptin levels, thus appearing to restore impaired leptin transport from the peripheral circulation to hypothalamus.[36,37] A meta-analysis has previously concluded that metformin decreases serum leptin levels in non-pregnant women with PCOS.[38] Our study extends this conclusion and demonstrates that metformin suppresses leptin levels in pregnant women with PCOS as well. Romualdi et al.[39] found no effect of metformin on sOB-R levels in non-pregnant women with PCOS, which contradicts the enhanced sOB-R levels that we found in the metformin group. However, a larger study by Liu et al.[19] found increased sOB-R levels in women with PCOS after 6 months of metformin treatment, which supports our results. Increased sOB-R levels parallels increased expression of membrane-bound OB-R-isoforms in hypothalamus and the liver, and is associated with weight loss in metformin-treated animals.[36,40] Shedding of hepatic membrane-bound OB-R ectodomains into the circulation is the most abundant source of sOB-R.[40] Upregulated expression of hepatic OB-R is therefore a possible explanation for the enhanced increase in sOB-R levels that we found in the metformin group in the third trimester, although we cannot confirm this with our study design.

4.3.4 | GWG and appetite-stimulating hormones

We found no overall effect of metformin on allopregnanolone or ghrelin concentrations compared with placebo. The attenuated increase in allopregnanolone levels from baseline to gestational week 36 in women with eGWG was unexpected, as Lundqvist et al.[22] in contrast found enhanced increase during pregnancy among women with high total GWG compared with women with low total GWG. However, they did not relate their results to BMI, whereas we categorised GWG relative to first trimester BMI in accordance with the IOM guideline.[5] We noticed that overweight PCOS women in our study had lower first and third trimester allopregnanolone levels compared with normal weight women (data not shown), and negative correlations between first trimester BMI and second trimester allopregnanolone levels have previously been reported in women with depression and unselected controls.[41] These aspects likely explain our disparate results.

5 | CONCLUSION

Excessive GWG is associated with enhanced leptin resistance in both early and late pregnancy, and with attenuated increase in serum allopregnanolone levels in late pregnancy. Appetite-regulating pathways thereby appear to be involved in GWG modulation. Metformin reduces the risk for excessive GWG and improves leptin sensitivity in pregnant women with PCOS but does not affect allopregnanolone or ghrelin serum levels. Thus, metformin appears to modulate GWG by mechanisms that counteract physiological leptin resistance in pregnancy.

5.1 | Practical and research implications

Excessive GWG is a major clinical challenge. Many women find it difficult to adhere to GWG recommendations, and existing prevention programmes have limited effects. Leptin sensitivity appears to be associated with adherence to GWG recommendations. Future research should aim for deeper insight into this mechanism as a potential target in the prevention of eGWG. Metformin appears effectively to counteract leptin resistance and prevent eGWG in PCOS. However, nearly one-third over-responded in our study and gained below recommendations, all of whom had BMI <25. Awareness of this possible side-effect is warranted, as inadequate GWG may have negative implications for pregnancy outcome.

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DISCLOSURE OF INTERESTS

JM, EV, TL and ED declare no conflicts of interest. MB is a member of the advisory board for Asarina Pharma and declares no other conflicts of interest. Completed disclosure of interest forms are available to view online as supporting information.

CONTRIBUTIONS TO AUTHORSHIP

MB was principal investigator and designed the study. JM, EV and ED participated in the design of the study. JM, EV, TL and MB collected the data. JM analysed the data with support from statistician JE. JM and MB interpreted the results. JM wrote the draft with support from MB. The draft was revised by EV, TL, ED and MB, all of whom approved the final version.

DETAILS OF ETHICS APPROVAL

All PCOS participants gave their oral and written informed consent, which included future research regarding effects of metformin treatment during pregnancy prior to inclusion in the PregMet and PregMet2 trials. The healthy participants gave their oral and written informed consent prior to inclusion in this study. The study was approved by the Committee for Medical Research Ethics of Health Region IV, Norway (145–05, PregMet), the Regional Committee for Health Research Ethics of Central Norway (2011/1434. PregMet2), the National Bioethics Committee of Iceland

(VSNb2012100011/03.10, PregMet2), the Regional Ethical Review Board in Stockholm (2012/1200-31/2, PregMet2) and the Regional Ethical Review Board in Umeå, Sweden (2014-257-31M; 2016-190-32M, Healthy controls).

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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SUPPORTING INFORMATION

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