Prolactin and breast increase during pregnancy in PCOS – linked to long-term metabolic health?

Maria Othelie Underdal MD[1,2], Øyvind Salvesen PhD[3], Anne Schmedes MD[4], Marianne Skovsager Andersen MD PhD DMSci[5✪] and Eszter Vanky MD PhD[1,2✪]

[1]Department of Clinical and Molecular Medicine, Faculty of Medicine and Health Sciences, Norwegian University of Science and Technology, Trondheim, Norway and [2]Department of Obstetrics and Gynecology, St. Olavs Hospital, University Hospital of Trondheim, Trondheim, Norway, [3]Department of Public Health and Nursing, Faculty of Medicine and Health Sciences, Norwegian University of Science and Technology, Trondheim, Norway, [4]Department of Biochemistry and Immunology, Lillebaelt Hospital, Vejle, Denmark, [5]Department of Internal Medicine, Odense University Hospital, Odense, Denmark

★Shared last authorship

Correspondence: Maria Othelie Underdal.

Postal address: Department of Obstetrics and Gynecology, St. Olavs Hospital. University Hospital of Trondheim, Olav Kyrres gt.16, 7006 Trondheim

Email: maria.o.underdal@ntnu.no

ORCID ID: https://orcid.org/0000-0002-9027-558X

Short title: Prolactin linked to metabolic health in PCOS

Keyword: Follow-up, women's health, hyperandrogenism, insulin-resistance, metformin, breastfeeding

Word count: 3060

Clinical trial registration: ClinicalTrials.gov: The PregMet study: NCT00159536.

Number of figures and tables: 5 tables, 1 figure

2. Abstract:

Objective

To explore whether gestational prolactin and breast increase are markers of metabolic health in pregnancy and on long-term, in PCOS.

Design

Follow-up study. Women with PCOS, according to the Rotterdam criteria (N=239), former participants of the randomized controlled trial (RCT) PregMet were invited, 131 participated in the current follow-up study, at mean 8 years after pregnancy.

Methods

Metformin 2000 mg/day or placebo from 1st trimester to delivery in the original RCT. No intervention in the current study.

Prolactin was analyzed in the first trimester and at gestational week 32 and metabolic characteristics which are part of the metabolic syndrome and measures of glucose homeostasis were examined. Metabolic health was also evaluated according to breast increase versus lack of breast increase during pregnancy.

Results

Prolactin increase in pregnancy was negatively correlated to body mass index (BMI) (p=0.007) and systolic blood pressure (p=<0.001) in gestational week 32. Prolactin at gestational week 32 was negatively correlated to BMI (p=0.044) and visceral fat area (p=0.028) at 8 years follow-up in an unadjusted model. Prolactin at gestational week 32 showed no associations to metabolic health at follow-up when baseline BMI was adjusted for. Women who reported lack of breast-increase during pregnancy, had higher BMI (p=0.034), waist-hip ratio (p=0.004), visceral fat area (p=0.050), total cholesterol (p=0.022), systolic (p=0.027) and diastolic blood pressure (p=0.011) at 8 years follow-up.

Conclusion

High prolactin levels and breast increase in pregnancy were associated with a more favorable long-term metabolic health in women with PCOS. Both prolactin and breast increase may be mediated by gestational BMI.

3. Introduction

Polycystic ovary syndrome (PCOS) is the most prevalent endocrine condition in women, with implications mainly for reproductive and metabolic health (1). Most women with PCOS have insulin resistance and hyperandrogenism (2, 3). These women are at increased risk of cardiovascular disease already during premenopausal years (4). In PCOS pregnancies, increased risk of gestational diabetes mellitus (GDM), preterm birth, and preeclampsia has been reported (5-7). However, the high number of women with GDM might be linked to higher BMI in women with PCOS (8).

Development and differentiation of the mammary glands and successive lactogenesis depend on prolactin. As a physiological adaptation to the emerging insulin resistance in pregnancy, prolactin stimulates β-cell proliferation in pancreatic islets (9). In non-pregnant women with PCOS, prolactin within normal range was inversely associated with metabolic risk markers such as waist circumference, total cholesterol, triglyceride and low-density lipoprotein (10). Furthermore, high prolactin within normal range was associated with lower prevalence of diabetes and impaired glucose tolerance in a large population-based cohort (11). Baseline prolactin was not associated to cardiovascular (CVD) risk profile at follow-up in women, around 40 years of age at inclusion, attending a longitudinal community based cohort study on CVD risk factors (12).

We have previously reported that lack of breast increase during pregnancy was linked to poorer metabolic health both in early and late pregnancy compared to women with breast increase among women with PCOS (13). Breastfeeding duration is shorter in obese women, than in normal-weight women in the general population (14, 15). Shorter breastfeeding duration has also been reported in women with PCOS compared to women without PCOS (16). A large cohort study from Australia showed similar results, but the association between short breastfeeding and PCOS diminished after adjustment for BMI. Obesity might thus be a major contributing factor to shorter breastfeeding in women with PCOS (17).

Metformin is an insulin-sensitizer, and is the first drug of choice in type 2 diabetes. According to the recent international evidence-based guidelines on treatment of PCOS, metformin could be

considered in addition to lifestyle changes, to improve weight, hormonal and metabolic outcomes in adult non-pregnant women with PCOS (18). In pregnant women with PCOS, use of metformin reduced the prevalence of late miscarriage and preterm delivery (19).

Both prolactin levels and breast increase in pregnancy may be useful as long-term risk markers for metabolic health in women with PCOS. At present, androgen status and obesity are suggested as tools in individual risk-assessment of metabolic health in PCOS; however metabolic disturbances are also increased in non-hyperandrogenic PCOS and we currently have no optimal predictors for detecting those at increased risk of metabolic and cardiovascular morbidity (20, 21).

In the present study, we aimed to explore if prolactin and breast increase during pregnancy are associated with metabolic health in pregnancy and on long-term in women with PCOS. We also investigated whether metformin affected serum prolactin levels in pregnancy.

4. Materials and Methods

A. Study design

1. The PregMet study and later follow-up studies

In the current study, we analyzed data from the original PregMet study (22) and 2 follow-up studies. The first follow-up focused on breast increment and participants provided self-reported data (13). At the second follow-up on metabolic health, participants met for follow-up examination at mean 8 years after the original study (23).

The PregMet study was a randomized, controlled, double blind, multicenter trial performed 2005 to 2009; exploring the efficacy of metformin to reduce pregnancy complications in women with PCOS. Criteria permitting inclusion in the PregMet study were: 1) PCOS diagnosed according to the Rotterdam criteria (24), 2) age 18–45 years, 3) gestational age between 5 and 12 weeks, and 4) a singleton, viable fetus shown on ultrasonography. Details on randomization, blinding and examination are described elsewhere (22). In the PregMet study, 274 pregnancies in 257 women

were assigned to an oral dose of metformin 2g/day or placebo from first trimester of pregnancy and until delivery. All participants received individual verbal and written diet and lifestyle advice. Blood pressure was measured after at least 10 minutes of rest; measured three times with 2 minutes apart and the mean of the second and third measurement was calculated. Weight and height were measured with light clothes on.

Participants in the PregMet study gave their consent to be contacted after the initial study, for possible follow-up studies. One-year post-partum, participants (N=240) were asked to complete a questionnaire on breast increment and breastfeeding. Participants who miscarried (N=3), dropped-out (N=12), or had lost their child (N=2) were not approached. Bra size was recorded as bra cup size (A=1; B=2; C=3; D=4; E=5) and bra chest circumference (70, 75, 80, 85, 90, 95 or 100cm) before and at the end of pregnancy. An increase in one bra chest circumference size results automatically in a bra cup increase of one. If a woman had 80B in early pregnancy and 85C at delivery, this implies an increase of two bra cup sizes (from B to C=1 and from 80 to 85=1) (13). Breast increase was dichotomized according to if bra size increased or not during pregnancy.

Metabolic health of the participants was reassessed from April 2014 to July 2016; 5-11 years after inclusion in the PregMet study. In all, 131 (55%) women agreed to participate in the follow-up. One hundred seventeen women met for physical examination and interview, while 14 were interviewed by phone and gave self-reported data. Metabolic characteristics which are part of the metabolic syndrome and measures of glucose homeostasis were examined. Metabolic characteristics included therefore blood pressure, anthropometric measurements, body composition, serum lipids, fasting glucose and fasting insulin. Blood pressure was measured three times, 2 minutes apart, with digital devices, with participant in sitting position after at least 15 minutes of chair rest. The mean of the second and third measurements was calculated. Height, waist and hip circumference were measured manually and rounded off to closest 0.5 cm. Body composition and weight were measured using bioelectrical impedance (Inbody 720, BIOSPACE, Seoul, Korea). The examination was performed with light clothes and no shoes.

InBody gives an estimate of total body fluids, proteins, minerals and fat and is validated against DEXA scan. Further details on recruitment, inclusion and procedures are described elsewhere (23). None of the women were postmenopausal when included in the follow-up. Contraceptives were not discontinued before the follow-up examination. All available data were included in the analyses. Two were pregnant at follow-up and excluded from the analyses.

2. Laboratory analyses

Venous blood samples were collected and processed after an overnight fast at each study site. At gestational weeks 12 and 32, a 75 g oral glucose tolerance test was performed. Hormonal analyses on prolactin, testosterone and androstenedione were performed at Department of Clinical Chemistry, Vejle County Hospital, Denmark. Prolactin concentrations were measured using a chemiluminescence immunoassay system (ADVIA Centaur XP; Siemens Healthineers, Siemens A/S, Copenhagen, Denmark). The method has a low cross-reaction with macroprolactin and has been standardized against the 3rd IRP WHO Reference Standard 84/500(25). Testosterone and androstenedione were analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS). In the analysis, plasma samples are extracted by supported liquid extraction and the eluate evaporated and reconstituted before analysis on LC-MS/MS. The analysis is calibrated by in-house prepared calibrators and the relative standard deviation is below 10 %. Quality is assured by monthly participation with satisfactory results in the external quality control program for steroid hormones from NEQAS, UK. Homeostatic model assessment (HOMA) IR was computed as (fasting glucose mmol/L x fasting insulin mU/L)/22.5. Free testosterone index was calculated as (total testosterone/SHBG) x10.

B. Statistical analyses

Baseline data were analyzed by Chi-Squared tests for categorical variables and two tailed independent samples t-tests for continuous variables according to initial

randomization. General linear model was used to examine the correlation of prolactin increase from first trimester to gestational week 32 and parameters of metabolic health and glucose homeostasis at week 32 of pregnancy. Additionally, median change in prolactin-levels from first trimester to gestational week 32 was calculated and participants dichotomized into two groups according to prolactin change under or above the median value. Then, measures of glucose homeostasis at week 32 were analyzed with two tailed independent samples t-tests according to dichotomization. General linear model was used to examine the correlation of prolactin in gestational week 32 and metabolic health and glucose homeostasis at follow-up. We present associations at follow-up both unadjusted and adjusted for maternal baseline BMI. Comparisons of women with or without breast increase and of prolactin levels according to original study randomization were done by two tailed independent samples t-test or chi square test as appropriate.

P-values < 0.05 were considered significant. Analyses were performed using Statistical Package for the Social Sciences (SPSS, version 25).

C. Ethical approval

Written informed consent was obtained from each participant before inclusion to the present follow-up and the declaration of Helsinki was followed throughout the study. “The Regional Committee for Health Research Ethics of Central Norway” approved the present study 04.04.2014, reference number: 2014/96.

5. Results

Baseline measurements of follow-up participants at first trimester of pregnancy are presented in table 1. There was no difference between the groups according to original study randomization (metformin vs. placebo) at first trimester of pregnancy (data not shown).

Increase of prolactin from first to third trimester of pregnancy was negatively correlated to BMI (p=0.007) and systolic blood pressure (p=<0.001) at week 32 of pregnancy (Table 2a). Gestational increase of prolactin was positively correlated to breast increase in pregnancy (p=0.013). Women with prolactin increase above median had lower f-glucose (p=0.041), f-insulin (p=0.048) and HOMA-IR (p=0.030) in gestational week 32 when examined according to prolactin increase above or below median (median=125 µg/l) gestational prolactin increase (Table 2b). Prolactin at gestational week 32 was negatively correlated to BMI (p=0.044) and visceral fat area (p=0.028) at mean 8 years follow-up in an unadjusted model (Table 3). The lower the prolactin level in 3rd trimester, the higher were BMI and VFA at follow-up. Gestational prolactin showed no associations to metabolic health at follow-up when baseline BMI was adjusted for.
Women who reported lack of breast increase during pregnancy, had higher BMI (31.4 vs 28.4, p=0.034), WHR (0.94 vs 0.89, p=0.004), visceral fat area (151.6 vs 118.9, p=0.050), total cholesterol (5.0 vs. 4.6, p=0.022), systolic (123 vs 117, p=0.027) and diastolic blood pressure (81 vs 76, p=0.011) at follow-up (Table 4). Levels of testosterone, SHBG and androstenedione in pregnancy and at follow-up was not associated to either prolactin or breast increase in pregnancy. Prolactin at follow-up tended to be higher in women with breast increase during pregnancy, but the difference did not reach level of significance. Metformin use from first trimester and onwards during pregnancy, had no effect on serum prolactin level at gestational week 32 (162 vs. 163), or prolactin increase from 1st trimester to gestational week 32 (120 vs. 125) compared to placebo (Table 5).

6. Discussion

In pregnant women with PCOS, increase of prolactin was negatively correlated to BMI and systolic blood pressure in third trimester of pregnancy. Women with prolactin increase above median had better glucose regulation in third trimester of pregnancy. Prolactin was negatively correlated to BMI and VFA at follow-up, but after adjustment for baseline BMI, none of these associations remained significant. Breast increase during pregnancy was associated with better

long-term metabolic health expressed by more favorable anthropometric measures, lower blood pressure and total cholesterol at follow-up.

Measures of glucose homeostasis in third trimester of pregnancy were analyzed according to prolactin increase above or below median prolactin increase. Participants were under strict surveillance for development of gestational diabetes mellitus (GDM) throughout pregnancy in the original RCT. If GDM was detected, additional treatment was initiated according to contemporary national guidelines. Theoretically, this could result in a plateau of f-glucose and we could therefore not assume linearity in our handling of variables of glucose homeostasis during pregnancy.

Prolactin may be a primary effector or a marker of metabolic health in pregnancy. Dopamine inhibits the active spontaneous secretion of pituitary prolactin. Circulating prolactin mainly effects peripheral tissue, but prolactin also activates prolactin-receptors (PRL-R) in dopaminergic neurons and thereby regulates its own release by a short-loop negative feedback mechanism. Less dopamine is secreted during pregnancy. The reduced dopamine results in hyperprolactinemia, probably via alterations in signaling pathways downstream of the PRL-R in neuroendocrine dopaminergic neurons (26). Insulin seems to be involved, directly or indirectly, in the regulation of prolactin secretion. Our findings of better glucose regulation and less insulin resistance in women with high increase of circulating prolactin during pregnancy might imply that the neuroendocrine adaptation allowing physiological increase of prolactin during pregnancy is influenced by glucose metabolism. Our findings that prolactin level in gestational week 32 was inversely correlated with BMI and visceral adiposity at follow-up further strengthen the suggestion that low prolactin in late pregnancy may act as a adiposity mediated marker of future impaired metabolic health. We are not aware of previous studies, which can explain our findings. Prolactin do stimulate β-cell proliferation in pancreatic islets as a physiological adaptation to the increasing insulin resistance that emerges during pregnancy and prolactin also reduces B-cell apoptosis when studied in-vitro (9, 27). There is thus a theoretical basis for considering prolactin as a protective factor against later diabetes.

It is intriguing that a crude measurement of breast increase in pregnancy can identify difference in metabolic profile also later in life. We have previously reported inferior metabolic health *in both first and third trimester of pregnancy* and less breastfeeding in women with PCOS, who did not experience breast increase while pregnant (13).

These results point to a link between metabolic health and both prolactin levels and breast increase, and contradict the understanding that breastfeeding *per se* improves future metabolic health by reducing maternal postpartum weight-retention and lowering the risk of obesity, type 2 diabetes and metabolic syndrome later in life (28, 29). We suggest that these observations are explained by “reverse causality”. Poor metabolic health prior to, and during, pregnancy had a negative effect on breastfeeding ability, demonstrated by lower gestational prolactin levels and no breast increase. These women also demonstrate poor long-term metabolic health measured as BMI, central obesity and higher blood pressure. These findings are in line with the results from a recently published large longitudinal cohort study, indicating pre-pregnancy metabolic health as main determinant of both breastfeeding and long-term maternal metabolic health (30).

Metformin had no impact on either increase of prolactin in pregnancy or prolactin levels in week 32 of pregnancy. Long-term administration of metformin in insulin-resistant non-pregnant women with PCOS, resulted in decrease of insulin and increase in prolactin (31). Prolactin secretion in the non-pregnant state is regulated by inhibition, predominantly by dopamine. The authors linked this observation to normalization of hypothalamic dopaminergic tone (31). In pregnancy however, randomized, *placebo* controlled studies reported no effect of metformin on glucose metabolism, i.e. prevention of GDM, or need of additional insulin treatment (19, 22, 32-34). These observations are in line with the finding of no effect of metformin on prolactin in pregnancy.

Strengths and limitations

The strengths of the study were the well-characterized cohort of women with PCOS diagnosed in accordance with the Rotterdam criteria. The cohort studied represents women with all four PCOS phenotypes and not only a selected group of PCOS women needing ART. Participants in the follow-up were representative of the original study population. To our knowledge, this is the first

study with a prospective design investigating the association between prolactin and breast increase in pregnancy and later metabolic health.

The main limitation of the study was the sample size. A higher participation rate at follow-up would have been desired. Breast increase was self-reported and a rather rough estimate, possibly also prone to bias.

In conclusion; High prolactin levels and breast increase in pregnancy were associated with a more favorable long-term metabolic health in women with PCOS. Low prolactin and no breast increase may serve as a marker of inferior metabolic health. Both prolactin and breast increase seem to be BMI mediated markers.

7. Declaration of interest, Funding and Acknowledgements

Declaration of interest:

I certify that neither I, nor my co-authors have a conflict of interest that is relevant to the subject matter or materials included in this work.

Funding:

This work was supported by The Research Council of Norway (registration number: 239987), Novo Nordisk Foundation Norway and the Norwegian University of Science and Technology/St. Olavs University Hospital-trust. Weifa AS, who supplied the drug free of charge for use in the PregMet study, and Novo Nordisk Foundation had no role in collection, analysis and interpretation of data, or writing the primary reports from these studies and was in no way involved in this follow-up study.

8. References

1. March WA, Moore VM, Willson KJ, Phillips DI, Norman RJ & Davies MJ. The prevalence of polycystic ovary syndrome in a community sample assessed under contrasting diagnostic criteria. *Human Reproduction* 2010 **25** 544-551.
2. Diamanti-Kandarakis E & Dunaif A. Insulin resistance and the polycystic ovary syndrome revisited: an update on mechanisms and implications. *Endocrine Reviews* 2012 **33** 981-1030.
3. Glintborg D, Henriksen JE, Andersen M, Hagen C, Hangaard J, Rasmussen PE, Schousboe K & Hermann AP. Prevalence of endocrine diseases and abnormal glucose tolerance tests in 340 Caucasian premenopausal women with hirsutism as the referral diagnosis. *Fertility and Sterility* 2004 **82** 1570-1579.
4. Glintborg D, Rubin KH, Nybo M, Abrahamsen B & Andersen M. Cardiovascular disease in a nationwide population of Danish women with polycystic ovary syndrome. *Cardiovascular Diabetology* 2018 **17** 37.
5. Roos N, Kieler H, Sahlin L, Ekman-Ordeberg G, Falconer H & Stephansson O. Risk of adverse pregnancy outcomes in women with polycystic ovary syndrome: population based cohort study. *BMJ* 2011 **343** d6309.
6. Kjerulff LE, Sanchez-Ramos L & Duffy D. Pregnancy outcomes in women with polycystic ovary syndrome: a metaanalysis. *American Journal of Obstetrics and Gynecology* 2011 **204** 558.e551-556.
7. Boomsma CM, Eijkemans MJ, Hughes EG, Visser GH, Fauser BC & Macklon NS. A meta-analysis of pregnancy outcomes in women with polycystic ovary syndrome. *Human Reproduction Update* 2006 **12** 673-683.
8. Palm CVB, Glintborg D, Kyhl HB, McIntyre HD, Jensen RC, Jensen TK, Jensen DM & Andersen M. Polycystic ovary syndrome and hyperglycaemia in pregnancy. A narrative review and results from a prospective Danish cohort study. *Diabetes Research and Clinical Practice* 2018.

9. Baeyens L, Hindi S, Sorenson RL & German MS. beta-Cell adaptation in pregnancy. *Diabetes, Obesity & Metabolism* 2016 **18 Suppl 1** 63-70.

10. Glintborg D, Altinok M, Mumm H, Buch K, Ravn P & Andersen M. Prolactin is associated with metabolic risk and cortisol in 1007 women with polycystic ovary syndrome. *Human Reproduction* 2014 **29** 1773-1779.

11. Wang T, Lu J, Xu Y, Li M, Sun J, Zhang J, Xu B, Xu M, Chen Y, Bi Y, et al. Circulating prolactin associates with diabetes and impaired glucose regulation: a population-based study. *Diabetes Care* 2013 **36** 1974-1980.

12. Therkelsen KE, Abraham TM, Pedley A, Massaro JM, Sutherland P, Hoffmann U & Fox CS. Association Between Prolactin and Incidence of Cardiovascular Risk Factors in the Framingham Heart Study. *J Am Heart Assoc* 2016 **5**.

13. Vanky E, Nordskar JJ, Leithe H, Hjorth-Hansen AK, Martinussen M & Carlsen SM. Breast size increment during pregnancy and breastfeeding in mothers with polycystic ovary syndrome: a follow-up study of a randomised controlled trial on metformin versus placebo. *BJOG: An International Journal of Obstetrics and Gynaecology* 2012 **119** 1403-1409.

14. Turcksin R, Bel S, Galjaard S & Devlieger R. Maternal obesity and breastfeeding intention, initiation, intensity and duration: a systematic review. *Maternal & Child Nutrition* 2014 **10** 166-183.

15. Winkvist A, Brantsaeter AL, Brandhagen M, Haugen M, Meltzer HM & Lissner L. Maternal Prepregnant Body Mass Index and Gestational Weight Gain Are Associated with Initiation and Duration of Breastfeeding among Norwegian Mothers. *Journal of Nutrition* 2015 **145** 1263-1270.

16. Vanky E, Isaksen H, Moen MH & Carlsen SM. Breastfeeding in polycystic ovary syndrome. *Acta Obstetricia et Gynecologica Scandinavica* 2008 **87** 531-535.

17. Joham AE, Nanayakkara N, Ranasinha S, Zoungas S, Boyle J, Harrison CL, Forder P, Loxton D, Vanky E & Teede HJ. Obesity, polycystic ovary syndrome and breastfeeding: an observational study. *Acta Obstetricia et Gynecologica Scandinavica* 2016 **95** 458-466.

18. Teede HJ, Misso ML, Costello MF, Dokras A, Laven J, Moran L, Piltonen T & Norman RJ. Recommendations from the international evidence-based guideline for the assessment and management of polycystic ovary syndrome. *Clinical Endocrinology* 2018 **89** 251-268.

19. Lovvik TS, Carlsen SM, Salvesen O, Steffensen B, Bixo M, Gomez-Real F, Lonnebotn M, Hestvold KV, Zabielska R, Hirschberg AL, et al. Use of metformin to treat pregnant women with polycystic ovary syndrome (PregMet2): a randomised, double-blind, placebo-controlled trial. *Lancet Diabetes Endocrinol* 2019.

20. Pinola P, Puukka K, Piltonen TT, Puurunen J, Vanky E, Sundstrom-Poromaa I, Stener-Victorin E, Linden Hirschberg A, Ravn P, Skovsager Andersen M, et al. Normo- and hyperandrogenic women with polycystic ovary syndrome exhibit an adverse metabolic profile through life. *Fertility and Sterility* 2017 **107** 788-795.e782.

21. Daan NM, Louwers YV, Koster MP, Eijkemans MJ, de Rijke YB, Lentjes EW, Fauser BC & Laven JS. Cardiovascular and metabolic profiles amongst different polycystic ovary syndrome phenotypes: who is really at risk? *Fertility and Sterility* 2014 **102** 1444-1451.e1443.

22. Vanky E, Stridsklev S, Heimstad R, Romundstad P, Skogoy K, Kleggetveit O, Hjelle S, von Brandis P, Eikeland T, Flo K, et al. Metformin versus placebo from first trimester to delivery in polycystic ovary syndrome: a randomized, controlled multicenter study. *Journal of Clinical Endocrinology and Metabolism* 2010 **95** E448-455.

23. Underdal MO, Stridsklev S, Oppen IH, Hogetveit K, Andersen MS & Vanky E. Does Metformin Treatment During Pregnancy Modify the Future Metabolic Profile in Women With PCOS? *Journal of Clinical Endocrinology and Metabolism* 2018 **103** 2408-2413.

24. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. *Fertility and Sterility* 2004 **81** 19-25.

25. Hattori N, Aisaka K & Shimatsu A. A possible cause of the variable detectability of macroprolactin by different immunoassay systems. *Clinical Chemistry and Laboratory Medicine* 2016 **54** 603-608.

26. Grattan DR, Steyn FJ, Kokay IC, Anderson GM & Bunn SJ. Pregnancy-induced adaptation in the neuroendocrine control of prolactin secretion. *Journal of Neuroendocrinology* 2008 **20** 497-507.

27. Terra LF, Garay-Malpartida MH, Wailemann RA, Sogayar MC & Labriola L. Recombinant human prolactin promotes human beta cell survival via inhibition of extrinsic and intrinsic apoptosis pathways. *Diabetologia* 2011 **54** 1388-1397.

28. Baker JL, Gamborg M, Heitmann BL, Lissner L, Sorensen TI & Rasmussen KM. Breastfeeding reduces postpartum weight retention. *American Journal of Clinical Nutrition* 2008 **88** 1543-1551.

29. Ram KT, Bobby P, Hailpern SM, Lo JC, Schocken M, Skurnick J & Santoro N. Duration of lactation is associated with lower prevalence of the metabolic syndrome in midlife--SWAN, the study of women's health across the nation. *American Journal of Obstetrics and Gynecology* 2008 **198** 268.e261-266.

30. Velle-Forbord V, Skrastad RB, Salvesen O, Kramer MS, Morken NH & Vanky E. Breastfeeding and long-term maternal metabolic health in the HUNT Study: a longitudinal population-based cohort study. *BJOG: An International Journal of Obstetrics and Gynaecology* 2018.

31. Ortega-Gonzalez C, Cardoza L, Coutino B, Hidalgo R, Arteaga-Troncoso G & Parra A. Insulin sensitizing drugs increase the endogenous dopaminergic tone in obese insulin-resistant women with polycystic ovary syndrome. *Journal of Endocrinology* 2005 **184** 233-239.

32. Syngelaki A, Nicolaides KH, Balani J, Hyer S, Akolekar R, Kotecha R, Pastides A & Shehata H. Metformin versus Placebo in Obese Pregnant Women without Diabetes Mellitus. *New England Journal of Medicine* 2016 **374** 434-443.

33. Chiswick C, Reynolds RM, Denison F, Drake AJ, Forbes S, Newby DE, Walker BR, Quenby S, Wray S, Weeks A, et al. Effect of metformin on maternal and fetal outcomes in obese pregnant women (EMPOWaR): a randomised, double-blind, placebo-controlled trial. *Lancet Diabetes Endocrinol* 2015 **3** 778-786.

34. Valdes E, Sepulveda-Martinez A, Candia P, Abusada N, Orellana R, Manukian B & Cuellar E. Metformin as a prophylactic treatment of gestational diabetes in pregnant patients with pregestational insulin resistance: A randomized study. *Journal of Obstetrics and Gynaecology Research* 2018 **44** 81-86.

Table 1. Baseline data from first trimester of pregnancy, (N=194)

Age at first trimester (years)	29.6 ± 4.4
BMI (kg/m^2)	28.7 ± 6.7
OGTT (75g)	
F-glucose (mmol/l)	4.6 ± 0.5
2-hour glucose (mmol/l)	5.5 ± 1.5
F-insulin (pmol/l)	108 ± 78
Triglycerides (mmol/l)	1.1 ± 0.6
HDL-cholesterol (mmol/l)	1.6 ± 0.4
Systolic blood pressure (mmHg)	118 ± 12
Diastolic blood pressure (mmHg)	73 ± 9.6
Total testosterone (nmol/l)	3.1 ± 1.7
SHBG (nmol/l)	211 ± 94
Androstenedione (nmol/l)	9.0 ± 6.0
PCOS phenotype n (%)	
Normoandrogenic	50 (26)
Hyperandrogenic	144 (74)
Smoking n (%)	16 (8)
Caucasian descent n (%)	192 (99)

Notes: Data presented as mean ± standard deviation or n % as appropriate **Abbreviations:** BMI, body mass index; OGTT, oral glucose tolerance test; HDL, high-density lipoprotein; SHBG, sex hormone-binding globulin

Table 2a. Correlation of prolactin-increase from first trimester to week 32 in pregnancy and clinical and biochemical data at week 32 in pregnancy, (N=194)

	Δ prolactin first trimester- week 32		
	B	CI	p-value
Prolactin w 32	0.860	0.780 – 0.941	<0.001
Age at first trimester (years)	-0.009	-0.020 – 0.003	0.131
BMI (kg/m^2)	-0.022	-0.038 – -0.006	0.007
Triglycerides (mmol/l)	-0.002	-0.005 – 0.002	0.323
HDL-cholesterol (mmol/l)	0.001	-0.001 – 0.002	0.335
Systolic blood pressure (mmHg)	-0.068	-0.098 – -0.038	<0.001
Diastolic blood pressure (mmHg)	-0.023	-0.051 – 0.004	0.098
Breast increase in pregnancy*	0.003	0.001 – 0.005	0.013
Total testosterone (nmol/l)	0.004	-0.002 – 0.010	0.196
SHBG (nmol/l)	0.257	-0.019 – 0.532	0.068
Androstenedione (nmol/l)	0.013	-0.002 – 0.028	0.101

Notes: Linear regression analyses, increase of prolactin as independent variable *Breast increase reported 1 year pp

Abbreviations: BMI, body mass index; HDL, high-density lipoprotein; SHBG, sex hormone-binding globulin

Table 2b. Glucose homeostasis at week 32 of pregnancy according to median change of prolactin from first trimester to week 32 of pregnancy (N=194)

Median Δ prolactin=125 µg/l (94-149)	< median change of prolactin (N=99)	>median change of prolactin (N=95)	p-value
F-glucose (mmol/l)	4.5 (4.3-4.6)	4.3 (4.2-4.5)	0.041
2-hour glucose (mmol/l)	6.3 (6.0-6.5)	6.1 (5.8-6.4)	0.300
F-insulin (pmol/l)	152 (139-165)	132 (145-150)	0.048
HOMA-IR	30.8 (27.8-33.7)	25.6 (22.3-28.8)	0.030

Notes: Data presented as mean (CI). Independent samples t-test were used to compare groups

Abbreviations: HOMA-IR, homeostasis model assessment-insulin resistance

Table 3. Association of prolactin at week 32 of pregnancy and clinical and biochemical data at 8 years follow-up (N=105)

	Prolactin week 32			Prolactin week 32, adjusted for 1.trimester BMI		
	B	CI	p-value	B	CI	p-value
BMI (kg/m^2)	-0.023	-0.046 – -0.001	0.044	-0.001	-0.014 – 0.013	0.935
Waist circumference (cm)	-0.039	-0.088 – 0.010	0.119	0.001	-0.038 – 0.039	0.975
Visceral fat area (cm^2)	-0.266	-0.502 – -0.030	0.028	-0.065	-0.247 – 0.116	0.474
F-glucose (mmol/l)	<0.001	-0.002 – 0.001	0.654	<0.001	-0.002 – 0.002	0.956
F-insulin (pmol/l)	-0.009	-0.033 – 0.016	0.482	0.001	-0.023 – 0.025	0.925
HOMA-IR	-0.002	-0.007 – 0.004	0.595	0.001	-0.005 – 0.006	0.805
Triglycerides (mmol/l)	-0.001	-0.002 – 0.001	0.401	<0.001	-0.002 – 0.001	0.640
HDL-cholesterol (mmol/l)	0.001	-0.001 – 0.002	0.368	<0.001	-0.001 – 0.002	0.715
Systolic blood pressure (mmHg)	-0.020	-0.070 – 0.031	0.439	-0.004	-0.055 – 0.046	0.866
Diastolic blood pressure (mmHg)	-0.014	-0.051 - 0.024	0.417	-0.003	-0.040 – 0.035	0.888
Total testosterone (nmol/l)	0.001	-0.001 – 0.003	0.214	0.001	-0.001 – 0.004	0.238
SHBG (nmol/l)	-0.011	-0.118 – 0.095	0.832	-0.012	-0.123 – 0.099	0.827
Androstenedione (nmol/l)	0.007	0.000 – 0.015	0.057	-0.006	-0.001 – 0.014	0.108

Notes: Linear regression analyses, prolactin week 32 as independent variable

Abbreviations: BMI, body mass index; HOMA-IR, homeostasis model assessment-insulin resistance; HDL, high-density lipoprotein; SHBG, sex hormone-binding globulin

Table 4. Metabolic health at 5-11 years follow-up according to breast increase (yes/no) during pregnancy (N=103)

	Lack of breast increase (N=33)	Breast increase (N=70)	p-value
Age at follow-up	38.8 (3.9)	37.5 (4.4)	0.157
BMI (kg/m^2)	31.4 (6.9)	28.4 (6.7)	0.034
Waist/hip ratio	0.94 (0.07)	0.89 (0.07)	0.004
Visceral fat area (cm^2)	151.6 (73.5)	118.9 (59.3)	0.050
F-glucose (mmol/l)	5.3 (0.8)	5.1 (0.5)	0.196
F-insulin (pmol/l)	91 (60)	78 (53)	0.306
HOMA-IR	2.8 (1.7)	2.7 (1.8)	0.734
Total cholesterol (mmol/l)	5.0 (0.9)	4.6 (0.7)	0.022
Triglycerides (mmol/l)	1.2 (0.7)	1.0 (0.5)	0.105
HDL-cholesterol (mmol/l)	1.5 (0.4)	1.5 (0.4)	0.612
Systolic blood pressure (mmHg)	123 (16)	117 (11)	0.027
Diastolic blood pressure (mmHg)	81 (11)	76 (8)	0.011
Total testosterone (nmol/l)	1.2 (0.8)	1.1 (0.6)	0.730
SHBG (nmol/l)	58 (38)	50 (24)	0.252
Androstendion (nmol/l)	4.9 (2.7)	4.6 (2.2)	0.591
Prolactin (µg/l)	8.3 (3.8)	12.4 (20.8)	0.289
Smoking n (%)	4 (12)	6 (9)	0.724
Current use of metformin n (%)	4 (12)	5 (7)	0.463

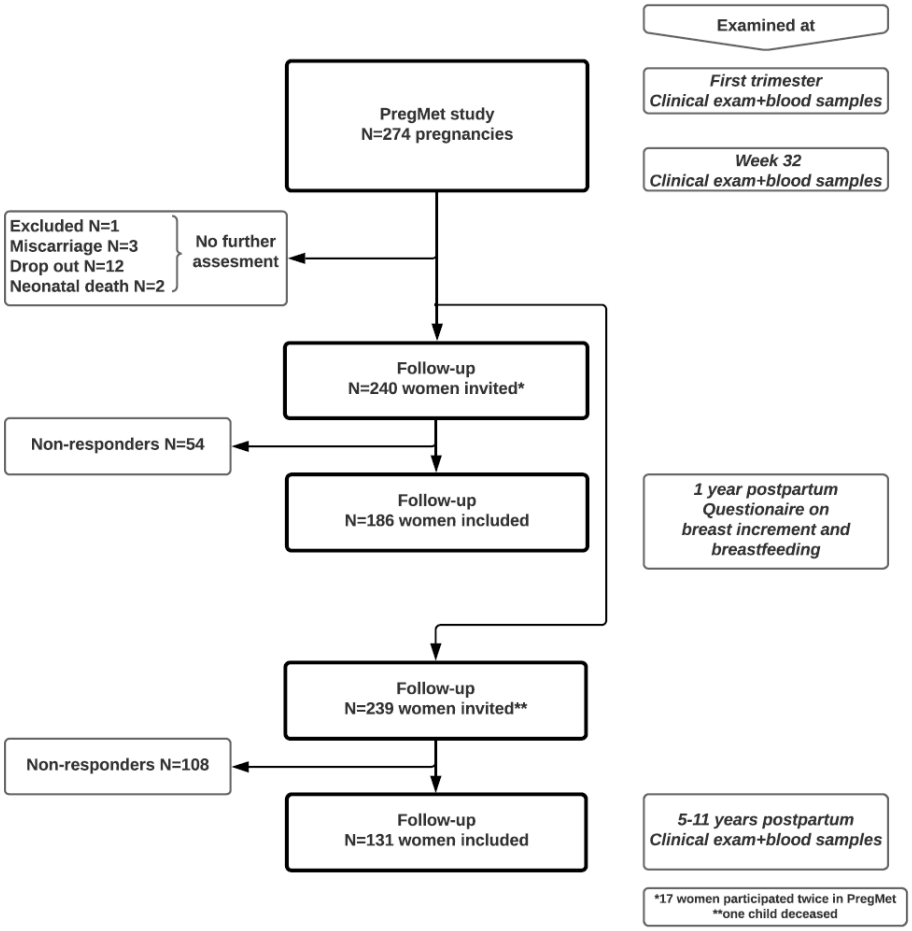
Notes: Data presented as mean ± standard deviation or n % as appropriate. Independent samples t-test and chi square test were used to compare study groups

Abbreviations: BMI, body mass index; HOMA-IR, homeostasis model assessment-insulin resistance; TG, triglyceride; HDL, high-density lipoprotein; SHBG, sex hormone-binding globulin

Table 5. Prolactin levels in women with PCOS according to original study group (metformin and placebo).

	Metformin (N=97)	Placebo (N=97)	All
	Mean (CI)	Mean (CI)	Mean (min-max)
First trimester prolactin (µg/l)	43 (36-49)	38 (32-44)	40 (7-234)
Third trimester prolactin (µg/l)	162 (150-174)	163 (152-174)	162 (10-438)
Prolactin increase from 1st to 3rd trimester (µg/l)	120 (109-130)	125 (113-136)	122 (-129-339)

Notes: Data presented as mean (CI) for groups and mean (min-max) for all. Independent samples t-test were used to compare study groups


Examined at
PregMet study
N=274 pregnancies
First trimester
Clinical exam+blood samples
Week 32
Clinical exam+blood samples
Excluded N=1
Miscarriage N=3
Drop out N=12
Neonatal death N=2
No further
assesment
Follow-up
N=240 women invited*
Non-responders N=54
Follow-up
N=186 women included
1 year postpartum
Questionaire on
breast increment and
breastfeeding
Follow-up
N=239 women invited**
Non-responders N=108
Follow-up
N=131 women included
5-11 years postpartum
Clinical exam+blood samples
*17 women participated twice in PregMet
**one child deceased