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Original article

Decreased beta-cell function in breastfeeding obese and non-obese women: A prospective observational study

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SUMMARY

Background & aims: Obesity is associated with lower breastfeeding rates. The underlying pathophysiological mechanisms are not well-understood, but there is increasing evidence on an association between parameters of maternal glucose metabolism and prolactin concentrations. In this cross-sectional observational study we investigate the relationship between breastfeeding, maternal obesity, and maternal glucose metabolism postpartum with beta cell function as a primary outcome measure.
Methods: We investigated 106 women (44% obese) prospectively recruited during the pregnancy, who underwent a 75 g – 2 h oral glucose tolerance test (OGTT) between the 3rd and 5th months postpartum. At this time point, we tested the relationship between breastfeeding status, maternal prolactin concentrations, maternal obesity, and fasting and dynamic indices of glucose metabolism using multivariate logistic regression in a post hoc analysis of prospective observational data.
Results: During the study visit at a mean of 122 (SE 9.3) days after delivery, 47% of obese women and 68% of non-obese women were breastfeeding ($p < 0.05$). Lactation and higher prolactin concentrations were associated with lower prepregnancy weight and lower postpartum insulin concentrations. Prehepatic beta-cell function was decreased in both obese (mean (SD); 0.16 (0.04) vs. 0.19 (0.05), $p < 0.05$) and non-obese (0.12 (0.05) vs. 0.16 (0.06), $p < 0.01$), lactating women. Obese lactating women have significantly lower first (1135.1 (306.7) pmol/L vs. 1517.3 (475.8) pmol/L, $p < 0.01$) and second phase insulin secretion (mean (SD), 300.2 (70.7) pmol/L vs. 393.1 (115.5) pmol/L, $p < 0.01$) as shown by Stumvoll indices when comparing to obese non-lactating women. Prehepatic beta-cell function and Stumvoll 1st phase insulin secretion index, but not BMI, were independently and negatively associated with breastfeeding and circulating prolactin concentrations.
Conclusions: Beta-cell function during lactation relates to breastfeeding and circulating prolactin concentrations independently of obesity. The well-known positive effects of lactation on maternal and offspring outcomes might reflect a causative relationship of higher breastfeeding rates in metabolically healthier women.

1. Introduction

Prolactin increases physiologically during pregnancy and breastfeeding, playing an indispensable role in lactation [1–3]. Maternal obesity is associated with lower breastfeeding rates and with a lower prolactin response to suckling [4–6]. The pathophysiological link between obesity and impaired breastfeeding is not well-understood, but there is increasing evidence that disturbances in glucose tolerance impact lactation [7,8]. Although lactation is associated with positive metabolic outcomes for mothers and offspring, large randomized trials do not show any positive effects of active interventions for extending the breastfeeding period on long-term outcomes [9–12].

Prolactin receptors are expressed not only in the mammary gland, but also in the pancreas, liver, adipose tissue and hypothalamus, and prolactin exerts also metabolic properties [1]. In animal

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models, moderately increased prolactin concentrations stimulate beta-cell proliferation and insulin secretion [13–15]. In line with these data, higher prolactin levels are associated with a lower incidence of diabetes in humans [16]. In contrast, very high prolactin concentrations do not have insulinotropic effects, but impair glucose-induced insulin secretion and exacerbate insulin resistance [13]. Hyperprolactinemia in patients with prolactinoma is associated with increased insulin resistance and adverse lipid profile, which improve after dopamine agonist therapy [17–19]. The pregnancy-induced increase in prolactin is thought to play a role in the adaptation of carbohydrate metabolism, ensuring the availability of glucose for the fetal/placental compartment [1,2].

Higher prolactin levels were also related to obesity, and normalization of prolactin levels leads to weight loss [20]. Mice lacking the prolactin receptor are protected against high-fat-diet-induced obesity [21]. Prolactin secretion in obese patients decreases following bariatric surgery [22]. The link between hyperprolactinemia and obesity is also in line with the insulinotropic effects of prolactin, as obesity is associated with increased insulin resistance and a compensatory increase in beta-cell function [23]. This positive association between adiposity and high prolactin levels does not seem to exist during breastfeeding, where the opposite is observed: impaired prolactin secretion in response to suckling in obese women [4]. Breastfeeding is associated with increased mobilization of fat from maternal depots, ensuring adequate milk production, so the pathophysiological role of prolactin might be different in the context of the changed hormonal milieu accompanying lactation [3].

To date, there are no data on the relationship between higher prolactin concentrations during breastfeeding, obesity and beta-cell function in lactating women. Here we address this question, investigating fasting and OGTT-based indices of insulin secretion and insulin sensitivity in relation to breastfeeding and circulating prolactin concentrations in 106 (44% obese) women three to five months postpartum in a post hoc analysis of prospective observational data.

2. Material and methods

2.1. Study participants and design

The study population includes 106 pregnant women of all BMI categories recruited during the pregnancy in a prospective observational study and investigated during a study visit performed between the 3rd and 5th months after delivery. The protocol was approved by the institutional ethics committee of the Medical University of Vienna (EK Nr. 2022/2012 & 771/2008) and was performed in accordance with the principles of the Declaration of Helsinki. All participants were recruited after signing informed consent. Inclusion criteria were: a singleton pregnancy and age $\geq$18 years. Exclusion criteria were: pre-existing diabetes, chronic and/or infectious diseases, significant psychiatric disorders or inability to follow instructions related to the studies due to language difficulties. All study subjects were monitored during their pregnancy following the national guidelines [24]. As we are a tertiary health care centre taking care of higher risk pregnancies, a high number of cases with GDM is represented in our cohort. All participants received information about metabolic advantages of breastfeeding and recommended breastfeeding durations according to WHO as routinely performed in all pregnancies. During the study visit performed 3–5 months postpartum, all women underwent a 75 g − 2 h oral glucose tolerance test (OGTT) with blood samples taken at baseline, 30, 60, 90 and 120 min for the measurement of glucose, insulin and c-peptide. All OGTTs were performed after at least 8 h overnight fasting and were scheduled between 7 and 9 am in the morning. In addition, a baseline blood sample was performed for the determination of serum prolactin, lipid parameters and blood chemistry parameters. We performed blood examinations in the early to mid-follicular phase in all non-lactating women. Information regarding pre-pregnancy weight and breastfeeding status was obtained by interviewing the patient. The current weight was measured to the nearest 0.1 kg on calibrated electronic scales (SECA 877/888) wearing no shoes and light clothes; the average value of two measurements was used. Maternal height was measured with a stadiometer (SECA 206, SECA, Birmingham, UK) using the average of two measurements. Waist circumference was measured twice at the midpoint between the lower border of the rib cage and the iliac crest. The average of two measurements was calculated. Systolic and diastolic blood pressure and heart rate were measured on the left arm with an appropriate-sized cuff with an OMRON 705 device (Kyoto, Japan). Patients were in resting position for at least 2–3 min before testing. An average of two measurements taken 1 min apart was recorded. Information on gestational diabetes mellitus (GDM) was obtained from the medical records.

2.2. Assays

All samples were analysed in our ISO 9001 certified central laboratory at the General Hospital in Vienna (AKH Wien). Methods are available under the homepage of the institute of laboratory medicine, www.kimcl.at. Shortly, Prolactin was measured using ECLIA method with a sensitivity of 0.05 ng/ml and a coefficient of variation (CV) of 4%. Serum prolactin samples >25 ng/ml were pre-treated with the polyethylene glycol precipitation (PEG) method. Insulin and c-peptide were measured using CLIA with a sensitivity of 2μIU/ml and a CV of 4–7% and 0.08 ng/ml and a CV of 3–4%, respectively. Glucose was measured colorimetrically.

2.3. Calculation of baseline and dynamic parameters of insulin secretion and sensitivity

Insulin resistance, insulin sensitivity and insulin secretion indices were calculated (HOMA, OGIS, Disposition Index, Insulinogenic Index, Stumvoll first and second phase insulin secretion). The HOMA Index (homeostasis model assessment) is a surrogate estimate of hepatic insulin resistance calculated as the product of fasting plasma insulin (FPI) and fasting plasma glucose (FPG), divided by the constant 22.5 (FPIxFPG/22.5) [25]. The oral glucose insulin sensitivity index (OGIS) is an estimate of peripheral insulin sensitivity (liver, muscle and adipose tissue) as described previously (due to complexity formula not shown, online calculation tool available from http://webmet.pd.cnr.it/ogis/) [26]. The insulinogenic index (IGI) is an estimate of initial insulin secretion, thus showing potential secretory deficiencies, defined as Δinsulin (0–30′)/Δglucose (0–30') and the disposition index is an estimate of beta-cell capacity for insulin production calculated as the product of insulin sensitivity (1/fasting insulin) and insulin secretion (IGI). Stumvoll indices, indicating first and second phase insulin secretion, were calculated as $728 + 3.537 \times$ Insulin 0min $- \ 120.3 \times$ Glucose 60min $+ 1.341 \times$ Insulin 60min $+ 21.27 \times$ BMI for estimated Stumvoll 1st phase beta cell function and $208 + 0.335 \times$ Insulin 60min $- 26.33 \times$ Glucose 60min $+ 0.887 \times$ Insulin 0min $+ 3.933 \times$ BMI for estimated Stumvoll 2nd phase beta cell function [27]. Prehepatic beta-cell function was calculated as fasting c-peptide divided by fasting glucose (C-peptide 0min/Glucose 0min) and posthepatic beta-cell function was calculated as fasting insulin divided by fasting glucose (Insulin 0min/Glucose 0min). Areas under the curves (AUC) were obtained using trapezoidal rule. The ratio of the AUCs of glucose and insulin (AUC Glucose 0–120min/AUC Insulin 0–120min) were used to calculate a further proxy of beta-cell function.

2.4. Statistical methods

Descriptive data analysis was performed for all parameters. Continuous variables were summarized by mean ± SD and categorical variables by counts and percentages. Assumption of Gaussian distribution of parameters was decided by visual assessment of histograms and calculation of skewness using Kolmogorov–Smirnov test. Categorical parameters were analyzed using Chi2 test. Non-parametrically distributed parameters were log transformed for further statistical analysis. Two-way ANOVA was performed with different glycemic and lipid parameters as independent variable and with fixed factors BMI (<30 and >30 kg/m^2) and lactation (yes/no). In the post hoc analysis, multiple testing was adjusted with Tuckey correction. Correlation analysis was performed using Spearman's correlation. Parameters correlating significantly with prolactin concentrations in univariate analysis were entered in a stepwise multiple regression analysis for detecting independent associations with prolactin concentrations. Multivariate logistic regression was performed to detect independent predictors of breastfeeding. Multicollinearity was tested in all linear and logistic regression models. Only parameters with a variance inflation factor below 3.5 were included in the multivariate and logistic regression analysis. In the final linear and logistic regression models Stumvoll's first phase, fasting prehepatic and posthepatic beta-cell function, HOMA Index, BMI before pregnancy, weight gain, age, total cholesterol and triglycerides were included. Several regression models were calculated with stepwise exclusion of the major significant parameter. Glejser test was used to test for heteroscedasticity in the primary linear regression model. Pairwise deletion was performed for cases with missing records. The differences between slopes and intercepts of lactating and non-lactating groups were tested using general linear models. As this is a post hoc analysis we did not perform any power analysis. Statistical analysis was performed using SPSS 24.0 (SPSS Inc, Chicago, USA). A two-sided p-value <0.05 was considered statistically significant. GraphPad Prism 7 (GraphPad Software, Inc., La Jolla, USA) was used to visualize data.

3. Results

Characteristics of the study population according to BMI and lactation status are presented in Table 1 and show significant differences between the groups in anthropometric, glycemic and metabolic parameters. Glucose and insulin parameters over time (0–120 min) are shown in Fig. 1 (Fig. 1A and B). Weight, waist and hip circumferences were significantly higher in women with BMI>30 kg/m^2 (lactating and non-lactating) compared with women with BMI < 30 kg/m^2. Fasting insulin and hepatic insulin resistance, shown as HOMA index, were significantly lower in lactating mothers with BMI < 30 kg/m^2 compared with lactating and non-lactating mothers with BMI > 30 kg/m^2. Glucose over time was significantly higher at 30min in lactating women with BMI < 30 kg/m^2 compared with non-lactating women with BMI > 30 kg/m^2 (159.0 ± 23.1 vs. 128.5 ± 40.3 mg/dL; p = 0.025) after Tuckey correction for multiple testing in a post hoc test (Fig. 1A). Insulin over time was significantly increased at baseline and 60 min after glucose ingestion in non-lactating women with BMI > 30 kg/m^2 compared with lactating women with BMI < 30 kg/m^2 (11.7 ± 7.2 vs 5.1 ± 5.4μIU/mL; p = 0.001 at baseline; 85.7 ± 9.7 vs. 50.1 ± 6.0μIU/mL; p = 0.011 at 60min) and BMI>30 kg/m^2 (85.7 ± 9.7 vs. 52.2 ± 6.1 μIU/mL; p = 0.021 at 60min) after correction for multiple testing (Fig. 1B). First phase insulin secretion, shown as insulinogenic index (IGI), was significantly lower in lactating mothers with BMI < 30 kg/m^2 when compared to non-lactating mothers with BMI > 30 kg/m^2. No differences between groups were found in insulin sensitivity (OGIS) and disposition index. Prolactin levels were significantly higher in lactating women (obese and non-obese). Rates of GDM and impaired glucose tolerance diagnosed postpartum, as well as history of GDM were comparable in obese and non-obese women independently of breastfeeding. Lactating mothers were less likely to be obese than non-lactating mothers (36% (22/62) vs. 57% (25/44), p < 0.047). In women with BMI > 30 kg/m^2, the rate of breastfeeding was significantly lower at the time of blood examination (47% (22/47) vs 68% (40/59), p = 0.047). Among lactating women the majority were exclusively breastfeeding (n = 57/62 exclusive breastfeeding, n = 5/62 breastfeeding and supplementary food). No significant differences in glucometabolic parameters were found between these groups (data not shown), but due to low numbers no further analysis was performed.

Differences in fasting, dynamic and global indices of insulin secretion between lactating and non-lactating mothers stratified to BMI groups (non-obese, obese) are shown in Fig. 1 and Table 1. Prolactin levels were significantly higher in obese and non-obese mothers who breastfeed (Fig. 1C). Lactating women (obese and non-obese) had lower first and second phase insulin secretion, as shown by Stumvoll indices (Fig. 1D and E), and lower pre- and posthepatic beta-cell function (Supplement Fig. 1A and B). Total insulin secretion, presented as AUC ratio of insulin and glucose, was lower in breastfeeding women (Fig. 1F).

We also found differences in lipid parameters between groups according to lactation status and BMI (Fig. 2). Triglyceride levels were significantly lower in lactating obese mothers when compared with non-lactating obese mothers. Small differences between groups were also observed for total cholesterol, HDL cholesterol and LDL cholesterol with lactating women with BMI < 30 kg/m^2 featuring mostly higher levels.

The relationships between prolactin concentrations, baseline characteristics and indices of insulin sensitivity and beta-cell function are presented in Table 2. As expected, a strong correlation between prolactin and breastfeeding exists. Prolactin correlated negatively with weight, BMI before pregnancy, the time span between birth and blood sampling, and positively with total cholesterol and LDL cholesterol. Strong negative correlations were found between prolactin and fasting glycemic parameters, as well as several postload glucose parameters including markers of insulin resistance and secretion.

Breastfeeding negatively correlated with weight, BMI before pregnancy, postpartum hip circumference, the time span from birth until blood sampling and triglycerides, whereas positive correlations were found for total cholesterol and HDL cholesterol. Similar to prolactin concentrations, also breastfeeding correlates negatively with fasting and postload glycemic parameters including insulin resistance and secretion.

Stepwise multivariate regression analysis (Table 3) revealed Stumvoll's first phase insulin secretion (B = −0.410, p = 0.001) as the main variable associated with prolactin concentrations, whereas other variables did not contribute significantly to the model. Glesjer test in this model ruled out heteroscedasticity issues.

In a multivariate logistic regression analysis, we tested for parameters independently associated with breastfeeding. In our model only Stumvoll 1st phase insulin secretion was independently associated with breastfeeding (OR 0.996 (95% CI 0.993–0.999, p = 0.004). A 100 unit increase in Stumvoll 1st phase index decreases the odds for breastfeeding by factor 4 and therefore breastfeeding is less likely with increasing insulin secretion.

The associations of prolactin, BMI before pregnancy and insulin secretory parameters (prehepatic insulin secretion, a fasting parameter and Stumvoll first phase, a dynamic parameter) according to lactation status are shown in Fig. 3. Fig. 3A and B shows a significant negative correlation between prolactin and insulin secretion in lactating women, which is not visible in non-lactating

Table 1
Characteristics of the study population divided according to BMI groups (<30, >30 kg/m^2) based on postpartum BMI and breastfeeding status 3–5 months after delivery.

Parameter	BMI<30 kg/m^2		BMI>30 kg/m^2		p#
	Non-lactating	Lactating	Non-lactating	Lactating	
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	
N	19	40	25	22	
Anthropometrics					
Age (y)	33.2 (5.6)	33.3 (4.4)	32.0 (4.0)	33.6 (5.0)	0.65
Weight before pregnancy (kg)	66.8 (8.5) [g, j]	64.1 (9.2) [g, j]	94.5 (11.3) [c, e]	94.1 (17.8) [c, e]	<0.001
BMI before pregnancy (kg/m^2)	24.4 (3.4) [g, j]	23.8 (3.1) [g, j]	34.7 (3.9) [c, e]	34.7 (5.8) [c, e]	<0.001
BMI postpartum (kg/m^2)	25.2 (3.1) [g, j]	24.7 (3.3) [g, j]	35.8 (4.7) [c, e]	35.8 (5.2) [c, e]	<0.001
Waist circumference postpartum (cm)	92.3 (7.8) [g, j]	91.6 (9.3) [g, j]	112.1 (10.7) [c, e]	111.3 (11.9) [c, e]	<0.001
Hip circumference postpartum (cm)	104.2 (8.7) [g, j]	102.6 (8.4) [g, j]	122.5 (8.4) [c, e]	123.4 (12.0) [c, e]	<0.001
Days between birth and postpartum visit	203 (169) [c, f]	89 (35) [e, h]	121 (66) [a]	120 (89) [d]	<0.01
MAP (mmHg)	95 (15)	96 (9)	99 (10)	97 (13)	0.60
Glycemic parameters					
Fasting glucose (mg/dL)	85.8 (5.7)	85.3 (9.3)	87.8 (11.0)	84.0 (7.1)	0.53
Fasting insulin (μIU/mL)	8.9 (7.9) [a]	5.1 (5.4) [d, j, g]	11.8 (8.2) [a]	8.6 (4.1) [a]	<0.001
HOMA IR	1.9 (1.7) [a]	1.1 (1.3) [d, j, g]	2.6 (2.2) [c]	1.8 (1.0) [c]	<0.001
OGIS	495.5 (49.5)	450.1 (78.6)	439.4 (68.6)	470.3 (28.1)	0.19
Insulinogenic index	1.1 (0.9)	0.5 (0.3) [i]	1.6 (1.4) [b]	0.9 (0.9)	<0.05
Disposition index	0.26 (0.38)	0.14 (0.11)	0.20 (0.19)	0.13 (0.12)	0.39
Stumvoll 1st phase	1163.6 (494.3) [h]	852.8 (367.7) [f,j]	1517.3 (475.8) [c, d f]	1135.1 (306.7) [a, i]	<0.001
Stumvoll 2nd phase	305.8 (116.9) [h]	236.4 (88.3) [j]	393.1 (115.5) [c, d, f]	300.2 (70.7) [i]	<0.001
Prehepatic fasting betacellfunction	0.16 (0.06) [b]	0.12 (0.05) [d, f, j]	0.19 (0.05) [c, f]	0.16 (0.04) [b, h]	<0.001
Posthepatic fasting betacellfuction	13.0 (11.4) [a]	7.2 (7.0) [d, f, j]	16.0 (10.1) [c]	12.6 (5.5) [a]	<0.001
	N (%)	N (%)	N (%)	N (%)	
Categorical parameters					
GDM	7/106 (6.6)	19/106 (17.9)	16/106 (15.1)	9/106 (8.5)	0.40
History of GDM	6/94 (6.4)	18/94 (19.1)	8/94 (8.5)	8/94 (8.5)	0.61
Impaired Glucose Tolerance	1/104 (1.0)	5/104 (4.8)	4/104 (3.8)	1/104 (1.0)	0.56

#global test (ANOVA),
[a] For p<0.05 vs Lactating, BMI <30 kg/m^2,
[b] For p<0.01 vs Lactating, BMI <30 kg/m^2,
[c] For P<0.001 vs Lactating, BMI <30 kg/m^2,
[d] For p<0.05 vs Non-lactating, BMI <30 kg/m^2,
[e] For P<0.001 vs Non-lactating, BMI <30 kg/m^2,
[f] For p<0.05 vs Lactating, BMI >30 kg/m^2,
[g] For P<0.001 vs Lactating, BMI >30 kg/m^2,
[h] For p<0.05 vs Non-lactating, BMI >30 kg/m^2,
[i] For p<0.01 vs Non-lactating, BMI >30 kg/m^2,
[j] For P<0.001 vs Non-lactating, BMI >30 kg/m^2 after correction for multiple comparison (LSD), BMI = body mass index, MAP = mean arterial pressure, AUC = area under the curve, HOMA = homeostasis model assessment, OGIS = oral glucose insulin sensitivity, PEG = polyethylene glycol precipitation, GDM = gestational diabetes mellitus.

women; with significant differences between slopes and intercepts in Fig. 1A ($p = 0.001$) and Fig. 1B ($p = 0.006$). Insulin secretion is positively associated with BMI levels in lactating and non-lactating women (Figs. 3C and D). Nevertheless, the difference between regression lines in these two groups was not significant (Fig. 3C ($p = 0.976$) and Fig. 3D ($p = 0.717$)).

We did not find any differences in insulin resistance, sensitivity and beta-cell function between lactating women with and without GDM (Supplement Table 1).

4. Discussion

Here we investigate the relationship between breastfeeding, prolactin concentrations and maternal indices of fasting and postprandial insulin secretion and sensitivity 3–5 months postpartum, showing that lactation is associated with decreased prehepatic beta-cell function and decreased glucose-induced insulin secretion in obese women. We confirm previous data on the negative relationship between breastfeeding and prepregnancy body weight, thereby finding out that beta-cell function, but not BMI, is independently associated with breastfeeding.

The hormonal milieu of lactation if dominated by increased baseline and suckling-induced prolactin concentrations [3]. Insufficient prolactin secretion (e.g. in patients with hypopituitarism) leads to agalactia [28]. In obese females, there is a decreased prolactin secretion in response to suckling, and this finding was thought to be the main reason for impaired lactation in maternal obesity [4]. Nevertheless, a relationship between glucose metabolism and the ability to breastfeed was observed, with even small impairments of glucose metabolism linked to significant differences in lactation [7]. In the present study, we included obese and non-obese women without diabetes, but with partial impairment of glucose metabolism as 51% of obese women and 46% of non-obese women had GDM.

Obesity is associated with increased insulin resistance and a compensatory augmentation in insulin secretion. So increased beta-cell function is responsible for maintaining a normal glucose tolerance, and over time, beta-cell failure leads to increased postprandial plasma glucose levels (disturbed glucose tolerance) and later on to diabetes [23]. Genetic predisposition leads over time to a deterioration of glucose homeostasis even in relatively young and healthy people, also independently from BMI [29]. In the present study, we show a significantly lower beta-cell function in breastfeeding women when compared to non-breastfeeding women, and this finding is consistent in obese and non-obese women. Beta-cell function of non-obese women, who do not breastfeed, is comparable to that of obese women who breastfeed. Different indices of beta-cell function (fasting prehepatic beta-cell function, Stumvoll 1st phase insulin secretion index) are independently associated with breastfeeding and maternal prolactin concentrations. Furthermore, both lean and obese lactating mothers have lower insulin resistance as seen by HOMA-IR or fasting plasma insulin

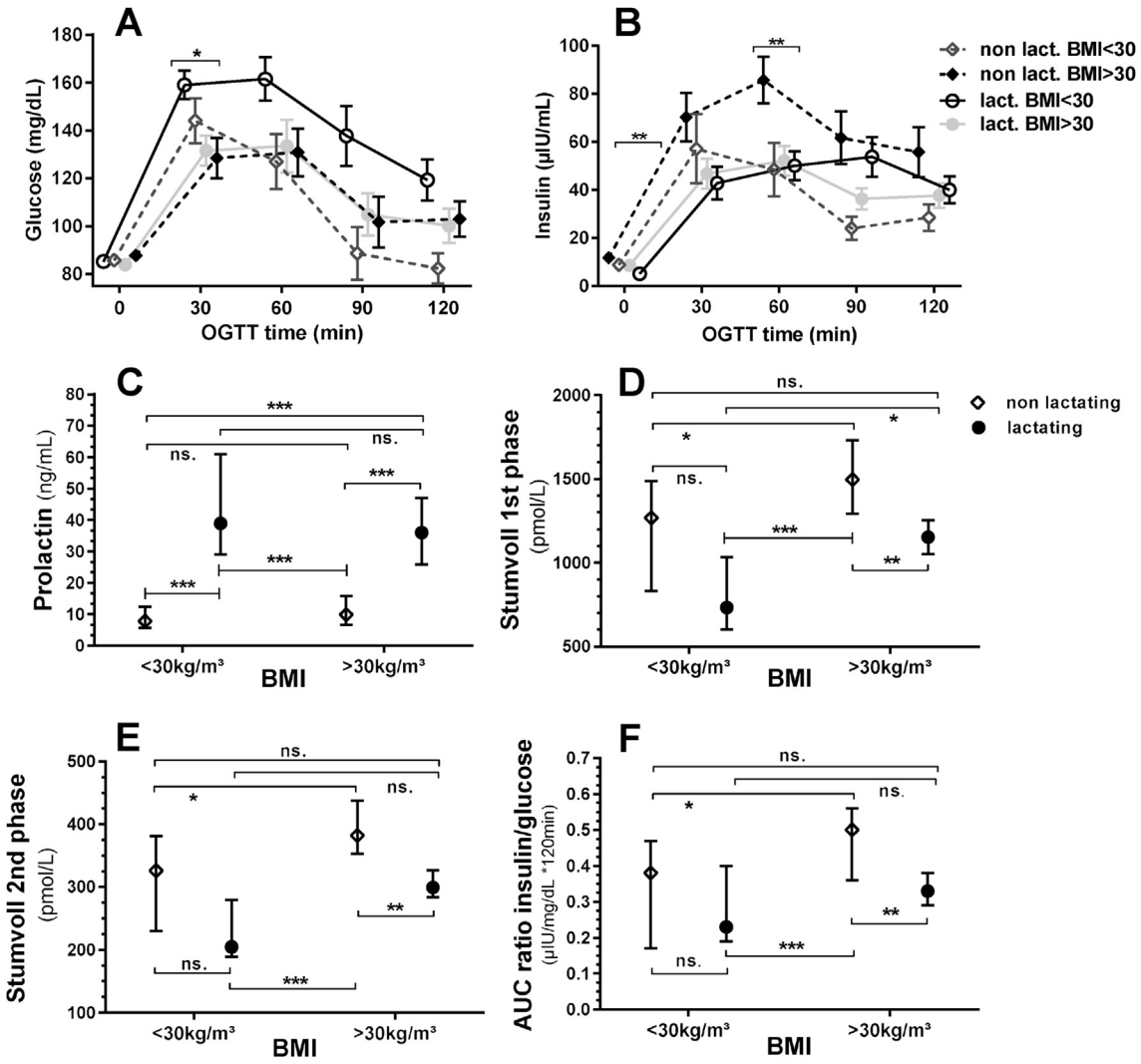

Fig. 1. Glycemic parameters in lactating or non-lactating women according to BMI three to five months postpartum (*p < 0.05, **p < 0.01, ***p < 0.001, ns. non significant).

levels and lower insulin secretion. However, we did not find differences in peripheral insulin sensitivity as measured by OGIS or the disposition index.

Pregnancy and puerperium lead to significant changes in glucose metabolism, increasing peripheral insulin resistance and directing the nutrient flow to the placenta/fetus [30]. Increased prolactin concentrations during pregnancy enhance beta-cell proliferation and function [31]. Postpartum, the nutrient flow in women who breastfeed needs to be redirected towards milk production from the mammary gland, and insufficient glucose supply impairs lactation [32]. Increased glucose concentrations post glucose-load in the presence of low circulating insulin levels in healthy lean lactating women ensure the availability of glucose for lactose synthesis, which is not an insulin-dependent process. Indeed, the beginning of lactation is supported by significant changes in glucose metabolism, starting with beta-cell mass contraction, possibly even apoptosis, and decreased insulin secretion, which accompany the reduction in peripheral insulin resistance [30]. These changes are not only governed by the immediate fall in placental lactogen levels, but also by the increase in progesterone accompanying the whole lactation period [30,33]. In addition, maternal metabolism during lactation is dominated also by increased lipolysis and maternal fat mobilisation, which ensures the production of long-chain polyunsaturated fatty acid (PUFA), arachidonic acid and, especially, docosahexaenoic acid (DHA), which are limited in the environment, but apparently indispensable for the rapidly growing infant brain [34]. Lipolysis is also known to contribute to decreasing beta-cell function [23]. Taken together, the pathophysiology of pregnancy and lactation requires a certain beta-cell plasticity, enabling adequate increases during pregnancy and immediate decreases in beta-cell function postpartum. This plasticity may not be easy to reach in obese insulin resistant mothers, but also in lean mothers with impaired beta-cell function because of genetic or aging reasons.

In the present study, we confirm the data from animal studies, finding decreased beta-cell function in lactating women, and in addition show that beta-cell function is an independent, but negative predictor of prolactin concentrations. To our knowledge, this is the only pathophysiological situation where increased prolactin concentrations are linked to decreased beta-cell function. In non-lactating states, prolactin stimulates insulin secretion [31,35]. On the other side, also insulin stimulates rPRL promoter activity and prolactin secretion from GH3 mammosomatotroph cells [36]. The main function of prolactin during breastfeeding is played within the mammary gland, so it appears that a good beta-cell plasticity resulting in a postpartum decrease in beta-cell function

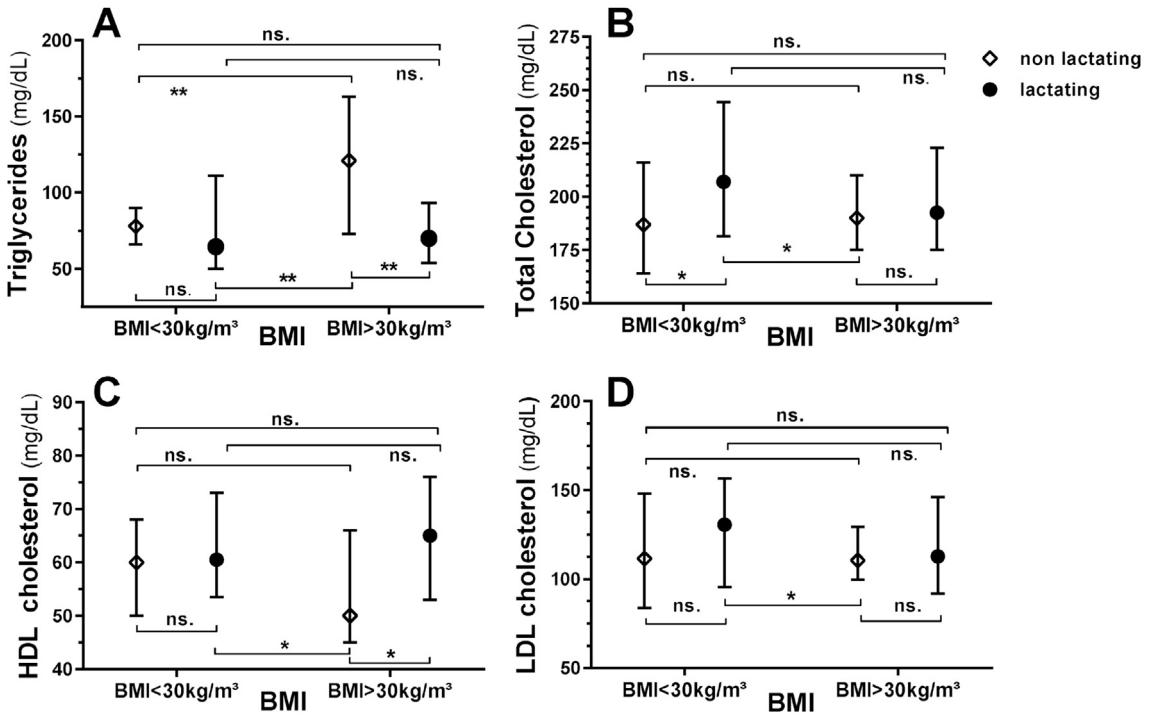

Fig. 2. Lipid parameters in lactating or non-lactating women according to BMI three to five months postpartum (*p < 0.05, **p < 0.01, ***p < 0.001, ns. non significant).

Table 2
Univariate Spearman's correlations of Prolactin and Breastfeeding with baseline characteristics, fasting and OGTT-based indices of glucose metabolism in all subjects.

	Univariate Prolactin		Univariate Breastfeeding	
	Spearman's ρ	p	Spearman's ρ	p
	N = 106		**N = 106**	
Baseline				
Age	0.073	0.457	0.098	0.318
Weight before pregnancy	**−0.259**	**0.01**	**−0.266**	**0.006**
BMI before pregnancy	**−0.205**	**0.036**	**−0.249**	**0.010**
Waist circumference postpartum	−0.155	0.121	−0.170	0.088
Hip circumference postpartum	−0.138	0.167	**−0.196**	**0.048**
Days between birth and postpartum visit	**−0.392**	**<0.001**	**−0.236**	**0.015**
Triglyceride	−0.140	0.155	**−0.301**	**0.002**
Total cholesterol	**0.227**	**0.020**	**0.200**	**0.04**
HDL cholesterol	0.096	0.329	**0.250**	**0.010**
LDL cholesterol	**0.196**	**0.045**	0.146	0.135
Prolactin	–	–	**0.774**	**<0.001**
Fasting				
Fasting glucose	−0.097	0.332	−0.113	0.252
Fasting insulin	**−0.324**	**0.001**	**−0.264**	**0.009**
Fasting C-peptide	**−0.381**	**<0.001**	**−0.411**	**<0.001**
HOMA-IR	**−0.307**	**0.002**	**−0.297**	**0.003**
Prehepatic beta-cell function	**−0.315**	**0.002**	**−0.429**	**<0.001**
Posthepatic beta-cell function	**−0.395**	**<0.001**	**−0.278**	**0.006**
Post glucose load				
OGIS	−0.121	0.388	0.034	0.804
Insulinogenic Index	**−0.376**	**0.003**	**−0.296**	**0.020**
Stumvoll 1st phase	**−0.443**	**<0.001**	**−0.477**	**<0.001**
Stumvoll 2nd phase	**−0.436**	**<0.001**	**−0.477**	**<0.001**
Disposition index	**−0.292**	**0.025**	−0.150	0.251
AUC insulin/glucose	**−0.415**	**0.001**	**−0.447**	**<0.001**
AUC glucose	**0.381**	**0.003**	**0.302**	**0.019**
AUC insulin	−0.212	0.113	**−0.292**	**0.026**
Impaired glucose tolerance	0.059	0.552	−0.029	0.772
GDM	−0.099	0.316	−0.070	0.475
Lactation	**0.774**	**<0.001**	–	–

BMI = body mass index, AUC = area under the curve, HOMA = homeostasis model assessment, OGIS = oral glucose insulin sensitivity, PEG = polyethylene glycol precipitation, GDM = gestational diabetes mellitus.
Boldface indicates significant differences.

Table 3
Multivariate regression with prolactin as dependent variable.

	Standardized β	p	Standardized β	p
	All parameters		Without Stumvoll 1st phase	
Stumvoll 1st phase	**−0.410**	**0.001**	–	–
Fasting prehepatic beta-cell function	−0.028	ns	**−0.275**	**0.007**
Total cholesterol	0.222	ns	**0.265**	**0.009**
Fasting posthepatic beta-cell function	−0.050	ns	−0.064	ns
HOMA-IR	0.009	ns	0.016	ns
BMI before pregnancy	0.006	ns	−0.015	ns
Age	0.140	ns	0.030	ns
Weight gain*	0.197	ns	0.126	ns
Triglyceride	0.138	ns	0.182	ns

HOMA-IR = homeostasis model assessment of insulin resistance, * weight gain in pregnancy.
Boldface indicates significant differences.

might exert a permissive effect on lactation, allowing prolactin to exert its primary evolutionary role. Nevertheless, we cannot exclude that the hormonal milieu of breastfeeding might per se contribute to the decreased insulin resistance, and therefore to the increased beta-cell function in breastfeeding women.

Here we also describe an independent correlation between prolactin and cholesterol concentrations. Similarly, cohort studies have demonstrated high cholesterol and LDL-cholesterol concentrations in patients with prolactinoma, and a significant decrease in both these lipids following normalisation of circulating prolactin concentrations [18,19,37]. A different relationship was observed in the Framingham cohort, where only female patients had higher odds of low HDL cholesterol for each 5-mg/dL increment in baseline prolactin [38]. Therefore, we conclude that the positive association

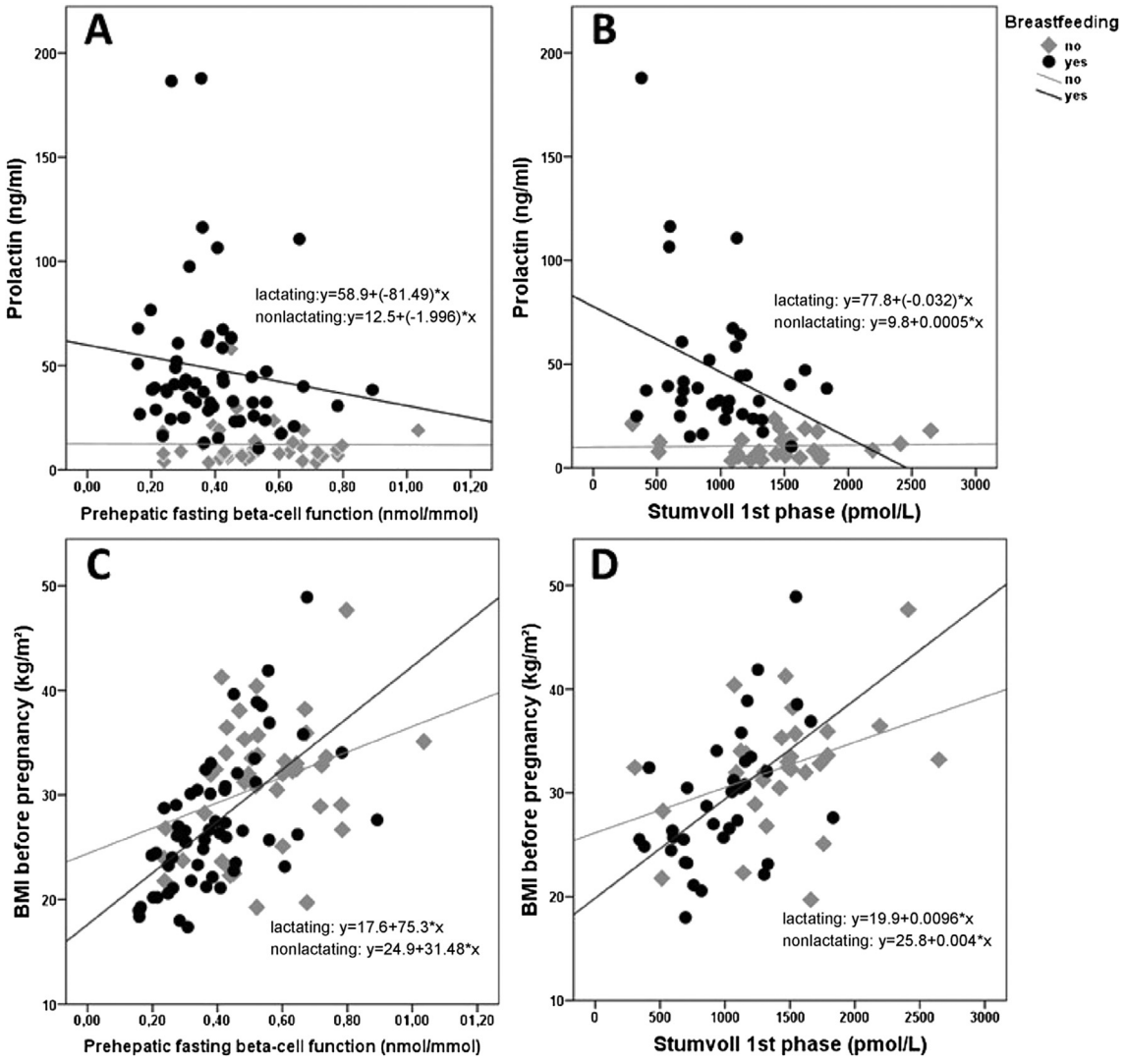

Fig. 3. First phase and fasting insulin secretory parameters according to prolactin levels, BMI and breastfeeding status (yes/no) in women 3–5 months postpartum.

between circulating prolactin and cholesterol concentrations is not restricted to the breastfeeding period but is apparently found only in situations with supraphysiological prolactin levels. The underlying mechanisms are not known. Prolactin receptor deficient mice display lower body weight and fat depots, and this phenotype is more distinct in females [39]. Nevertheless, hyperprolactinemia inhibits pituitary gonadotropin secretion, breastfeeding is also associated with an inhibition of gonadotropin-releasing-hormone leading to lactational amenorrhoea, and the accompanying hypogonadism might mediate the effects on lipid metabolism [1,33,37]. In addition, we confirm previous studies finding lower triglyceride concentrations in breastfeeding women, supporting the fact that maternal nutrients are utilised for ensuring milk production.

Lactation is associated with positive metabolic outcomes for both mother and offspring [9,10]. Nevertheless, large randomized trials using active interventions for extending the breastfeeding period have failed to show any positive effect on the later development of overweight/obesity in the offspring [11,12]. Our data find a direct relationship between beta-cell function and breastfeeding, independently of obesity. As breastfeeding seems to be associated with a better ability of the beta-cell to adapt its function to the hormonal changes accompanying pregnancy and puerperium, the positive effects of breastfeeding on the mother might be a simple consequence of the better metabolic status of mothers who are able to breastfeed. Similarly, offspring outcomes may reflect genetic and environmental influences that determine the maternal metabolic status. Taken together, the positive effects of lactation on maternal and child outcomes might not necessarily be a consequence of lactation, but a causative relationship reflecting the higher breastfeeding rate in metabolically healthy women.

A limitation of this study is the cross sectional design which does not allow to make stronger conclusions or report about causalities instead of crude associations. As breastfeeding cannot be randomized, we tried to overcome this limitation through the prospective inclusion of all women already during the pregnancy. In addition, we only have a small sample size of women with supplementary feeding, which does not allow further analysis or reliable interpretation. Supplementary feeding in comparison to exclusive breastfeeding could affect the maternal metabolism and thus further investigation with larger sample size is necessary A further limitation is that the General hospital in Vienna is a tertiary center with referrals of high risk pregnancies, so our study cohort includes obese and non-obese women with a high rate of metabolic disturbances (as seen in the high number of women with GDM). Strength of the present study is the prospective design, as all obese and non-obese women recruited in early pregnancy were studied during the postpartum phase, showing real-life data on the rate of breastfeeding and its relationship to different fasting and OGTT-related indices of glucose metabolism.

Taken together, here we show that maternal beta-cell function is independently and negatively associated with breastfeeding, with lower beta-cell function found in both lean and obese women who breastfeed. The ability to switch from the higher beta-cell function during pregnancy to a decreased beta-cell function at puerperium, might reflect the grade of maternal beta-cell plasticity. Further studies are needed for investigating the effect of interventions in improving beta-cell-plasticity on breastfeeding rates, maternal and fetal outcomes.

Statement of authorship

J.H. and G.V. contributed to the conception of the study, wrote the initial manuscript draft, performed the analyses, read and corrected draft versions. A.K.-W. contributed to the conception and design of the trial, acquisited funding, read and corrected the initial manuscript and corrected draft versions. K.L., L.W., M.L., C.W., D.B.-T, read the paper and contributed significantly to editing and preparation of the final manuscript. All authors approved the final manuscript. J.H., G.V. and A.K-W. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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Conflicts of interest

The authors do not have any conflict of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.clnu.2018.11.035.

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