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The impact of lactation on glucose and insulin response and CRP concentration in women with prior GDM diagnosed according to WHO criteria — a prospective 18-month observation

ABSTRACT

Introduction. Gestational diabetes mellitus (GDM) is defined as glucose intolerance with an onset or first recognition during pregnancy. Previous GDM predisposes the woman to prediabetes or overt diabetes later in life. Lactation seems to have a protective impact on metabolic profile of the women with previous GDM but the results of available studies are conflicting. The aim of our study was to prospectively investigate in a 18-month observation whether lactation duration and intensity influences glucose and insulin response among women with prior GDM, diagnosed according to WHO criteria.

Material and methods. The study population consisted of 144 white caucasian women that were initially included in the study. During enrollment visit, between 26th and 30th week of gestation, maternal medical history, the result of 75 g oral glucose tolerance test (75 g OGTT), and anthropometric parameters were collected. Blood samples were collected for additional tests. Final analysis comprised 68 subjects (47.2%) that participated in the follow-up visit 18 months after delivery. Data on delivery and lactation as well as anthropometric data were gathered and 75 g OGTT was performed. The participants were then compared according to lactation duration [longer (> 12 weeks) or shorter (≤ 12 weeks)] or lactation intensity [more intensively (> 70% of the total infant milk consumption coming from breastfeeding) or less intensively (< 70% of the total infant milk consumption coming from breastfeeding)].

Results. 53 (78%) women breastfed more than 12 weeks, and 52 (76%) had intensive lactation. The women lactating longer than 12 weeks had significantly higher body weight (p = 0.038) and BMI (p = 0.001) than the women lactating for a shorter period of time. There was a 3-fold higher number of women treated with insulin in the group lactating for a longer period of time (p = 0.038). The women lactating more intensively had significantly lower HOMA 2 IR (p = 0.019) compared to the women breastfeeding less intensively. They had also lower HOMA 2 %B (p = 0.05). The number of subjects with isolated impaired glucose tolerance was significantly higher in the women lactating less intensively (18.7% *vs.* 1.9%, p = 0.037). A significant negative correlation between lactation duration and fasting glucose concentration (r = –0.282, p < 0.05) as well as fasting insulin concentration (r = –0.251, p < 0.05) was detected. Similar correlation was noticed as concerns 2-h post-load insulin concentration (p = 0.05). Moreover, a significant negative correlation between lactation intensity and fasting

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insulin concentration ($r = -0.251$, $p < 0.05$) was found. In multiple regression model analysis, prepregnancy BMI and gestational weight gain appeared to be the strongest factors influencing the obtained results. Neither lactation duration nor intensity appeared as the significant factors in the model.

Conclusions. Our data provide evidence that lactation may have favorable effects on insulin and glucose response after delivery among women with prior GDM who are at high future cardiometabolic risk. The effects seems to be more evident with longer lactation duration as well as higher lactation intensity. (Clin Diabetol 2019; 8, 2: 99–109)

Key words: gestational diabetes mellitus, lactation

Introduction

Gestational diabetes mellitus (GDM) is defined as glucose intolerance with an onset or first recognition during pregnancy. It results from increasing insulin resistance during pregnancy which may exceed the compensatory rise in insulin secretion and consequently lead to the development of glucose intolerance [1]. GDM tends to remit completely short after delivery but the disturbances concerning insulin secretion and/ /or action often remain in the post partum period [2]. Previous GDM predisposes the woman to prediabetes or overt diabetes later in life. Available data indicate that the risk of diabetes is even 7-fold higher than in the women with normoglycaemic pregnancy [3] and the rate of any glucose intolerance after GDM ranges from 2.6% to 70% [4]. These differences result mainly from the time of follow-up, studied population, and the screening criteria used for GDM diagnosis. Previous GDM is also associated with an increased risk of all other features of metabolic syndrome such as obesity, hypertension and dyslipidemia [5–7]. Recent studies have also shown that the women with prior GDM have a higher risk of coronary heart disease and myocardial infarction [7].

It is widely known that intensive lifestyle modification is effective in delaying or even preventing type 2 diabetes mellitus (T2DM) in women after GDM but adopting a healthy lifestyle is usually quite difficult [8]. Lactation seems to have a protective impact on metabolic profile of the women with previous GDM. Some results of available studies suggest that it increases insulin sensitivity and/or insulin secretion and finally improves glucose tolerance, thus lowering the risk of diabetes [9–12]. Moreover, it seems to prolong the period of time to development of diabetes [13]. On the other hand, there are also studies that did not confirm the health benefits of lactation in this particular group [14]. Recently published meta-analysis has shown that longer lactation in the women with a history of GDM had reduced the risk of (T2DM) compared with shorter lactation. Additionally, exclusive lactation compared with exclusive formula feeding had also lowered the risk of (T2DM). These findings support the evidence that longer and exclusive lactation may be beneficial for (T2DM) prevention in women with previous GDM. However, the evidence relies only on observational, and in majority retrospective, studies. What is more, analyses with stratification by the participants' characteristics such as ethnicity, BMI and other affecting factors were not possible because of inadequate information. These limitations deterred the authors from drawing tailored and general conclusion for each woman in real practice [15]. It was documented that breastfeeding rate among subjects with a history of GDM is lower compared with women after normoglycaemic pregnancies [16].

Because the majority of studies was carried out short after delivery, and the longer-term studies were in vast majority retrospective, the aim of our study was to prospectively investigate in a 18-month observation whether lactation duration and intensity influences glucose and insulin response among women with prior GDM, which was diagnosed according to WHO diagnostic criteria. We hypothesized that longer and more intense lactation ameliorates insulin secretion and sensitivity as well as glucose tolerance in this group of women.

Materials and methods

Study population

This is a prospective, cohort study curried out in Outpatient Department of Diabetology in Lodz, Poland between 2013 and 2016. All the women with a singleton pregnancy, attending the first visit between 26th and 30th gestational week with confirmed GDM diagnosis, that provided written informed consent to participate in the study, were enrolled to the study. The exclusion criteria were as follows: multiple pregnancy, prior glucose intolerance/diabetes (apart from previous GDM), already initiated insulin treatment or any concomitant cardiovascular diseases. The study population consisted of 144 white caucasian women that were initially included in the study. Gestational diabetes mellitus was diagnosed according to the World Health Organization (WHO 2013) criteria. GDM was diagnosed if at least one of the following criteria were met: fasting plasma glucose between 92 and 125 mg/dL (5.1–6.9 mmol/l); 1-h — post-load glucose concentration higher than 180 mg/dL (10.0 mmol/l); 2-h — post-load glucose level between 153 and 199 mg/dL (8.5–11.0 mmol/l).

The study protocol has been approved by the Ethics Committee of the Medical University of Lodz.

Data collection

Baseline data were collected during enrollment visit between 26th and 30th week of gestation. Information on age, education, parity, smoking, family history of diabetes, history of GDM in previous pregnancies, maternal birth weight as well as data concerning the day of GDM diagnosis and the result of 75 g oral glucose tolerance test (75 g OGTT) were gathered. Body weight and height were measured and body mass index (BMI) was calculated. Prepregnancy weight was self-reported and was used to calculate prepregnancy BMI. Blood sample was collected (see the description below). The women were informed about the potential benefits of lactation and were asked for making notes concerning lactation after delivery: 1) lactation (yes/ /no), 2) lactation intensity (exclusive/partial; if partial then percent of breastfeeding in relation to the infant total daily milk consumption), and 3) lactation duration in weeks. All the women were then invited for the follow-up visit 18 months (± 1 month) after delivery. They were given a written information concerning the visit, and then they were called before the estimated follow-up date in order to increase the attendance rate. At the follow-up visit data on gestational weight gain, actual weight and insulin use during pregnancy (yes/no), perinatal outcomes as well as lactation (see above) were collected.

The women attending the follow-up visit were then compared according to lactation duration [longer (> 12 weeks) or shorter (≤ 12 weeks)] or lactation intensity [more intensively (> 70% of the total infant milk consumption coming from breastfeeding) or less intensively (< 70% of the total infant milk consumption coming from breastfeeding)].

Laboratory measurements

The first blood sample was taken during pregnancy at baseline visit. Plasma glucose concentration was measured enzymatically while plasma insulin concentration was measured by RIA (Roche Diagnostics). Glycated haemoglobin (HbA_{1c}) was measured using HPLC method, and high sensitive C-reactive protein concentration was evaluated using immunoassay. 18 months after delivery a 75 g 2-h OGTT was performed in the morning after an overnight fast. Blood samples were collected at 0 and 120 minutes for the measurement of plasma glucose and insulin concentrations. All the participants were given written recommendations before delivery, as well as a telephone call just before the follow-up visit, concerning the standards of OGTT performance. They were also asked to breastfeed the infants just before the glucose load, and not during OGTT, as lactation may potentially affect both glucose and insulin concentrations. The test was performed after the meeting with the investigator to ensure that all the above conditions are met. All the tests were carried out in the central laboratory. Impaired fasting glycemia (IFG), impaired glucose tolerance (IGT), and diabetes mellitus (DM) were diagnosed according to WHO criteria.

The homeostasis model assessment for insulin sensitivity index (HOMA 2 %S), homeostasis model assessment for β-cell function index (HOMA 2 %B), and homeostasis model assessment for insulin resistance index (HOMA 2 IR) were calculated using the mathematical model developed by Jonathan Levy et al [17]. Calculations were done by the "HOMA Calculator" developed by Diabetes Trial Unit of The Oxford Centre for Diabetes, Endocrinology and Metabolism available for download and use on DTU's site. The QUICKI index was estimated by the following formula: 1/[log (fasting insulin μU/mL) + log (fasting glucose mg/dL)], https:// //www.dtu.ox.ac.uk/homocalculator/ [18].

Statistical analysis

Statistical analyses were performed using the PQStat statistical package, license no. 01500256 (PQStat Software, Poznań, Poland). Continuous data were expressed as mean ± standard deviation (SD) and categorical variables as percentages. The Kolmogorov--Smirnov test was first used to confirm whether the variables had a normal distribution. Normally-distributed dependent and independent variables were compared using the Student's t-test. For variables with a non-normal distribution, independent variables were compared with the Mann-Whitney test, and dependent variables with the Wilcoxon test. Fisher's exact test was used for comparison of proportions. Correlations were identified with the Pearson's correlation coefficient for parametric variables, and the Spearman's rank correlation coefficient for non-parametric variables. Multiple regression linear analysis was done to assess the relationship between lactation duration/intensity and both glucose and insulin response 18 months after delivery, with covariates such as: pregestational BMI, gestational weight gain and family history of DM. Statistical significance was defined as $p < 0.05$.

Results

Of all 144 women included in the study 68 participated in the follow-up visit and finally this group was taken into consideration in the analysis. 53 (78%) women breastfed more than 12 weeks, and 52 (76%)

Table 1. The characteristics of the study group at baseline visit in pregnancy week 26–30 according to the lactation duration

Parameters	Whole group (n = 68)	Lactation < 12 weeks (n = 15)	Lactation > 12 weeks (n = 53)	p
Age (years)	34.1 ± 4.3	35.3 ± 4.5	33.8 ± 4.8	0.297
Multiparity, n (%)	35 (51.5)	4 (26.6)	31 (58.5)	0.041
Smoking, yes, n (%)	15 (22.1)	3 (20.0)	11 (20.6)	0.761
Education				
Secondary, n (%)	29 (42.6)	8 (53.3)	21 (39.6)	0.386
Post secondary, n (%)	39 (57.4)	7 (46.7)	32 (60.4)	0.248
Family history of DM, yes, n (%)	37 (54.4)	6 (40.0)	31 (58.4)	
History of GDM, yes, n (%)	3 (4.4)	1 (6.7)	2 (3.7)	0.532
Maternal birth weight [g]	3212 ± 500	3212 ± 373	3245 ± 489	0.826
Prepregnancy weight [kg]	68.8 ± 15.5	68.0 ± 11.5	69.35 ± 15.56	0.771
Prepregnancy BMI [kg/m^2]	25.1 (CI 24.0–26.1)	25.3 (CI 22.4–28.6)	23.9 (CI 22.8–25.8)	0.604
Actual body weight [kg]	79.2 ± 16.2	77.0 ± 10.2	80.3 ± 16.9	0.502
Actual BMI [kg/m^2]	27.1 (CI 26.3–27.8)	27.1 (CI 24–28.4)	27.2 (CI 25.4–28.8)	0.915
OGTT result during pregnancy				
Fasting glucose [mg/dL], n = 68	86 ± 12	86 ± 15	85 ± 12	0.788
1-h post-OGTT glucose [mg/dL], n = 56	175 ± 27	176 ± 33	173 ± 28	0.725
2-h post-OGTT glucose [mg/dL], n = 68	155 ± 21	154 ± 26	152 ± 21	0.758
Time of GDM diagnosis (gestational week)	28.1 ± 2.4	27.46 ± 1.8	27.96 ± 2.78	0.746
Measurements between 26th and 30th gestational week				
Fasting glucose [mg/dL]	81 ± 11.8	80.36 ± 8.73	80.65 ± 13.7	0.947
Fasting insulin [μIU/ml]	16.6 ± 9.07	14.77 ± 5.86	13.57 ± 9.25	0.887
HbA_{1c} (%)	5.18 ± 0.34	5.25 ± 0.44	5.227 ± 0.34	0.807
hsCRP [mg/dL]	4.11 ± 3.04	3.67 ± 1.8	4.6 ± 3.4	0.241

CI — confidence interval; GDM — gestational diabetes mellitus; BMI — body mass index; OGTT — oral glucose tolerance test; hsCRP — high-sensitivity C-reactive protein

had intensive lactation. Only three women that attended the follow-up visit were not lactating at all. They were included in the group of shorter or less intensive lactation, respectively. The characteristics of the study groups according to lactation duration and intensity are presented in Table 1 and 2.

Majority of women breastfed longer than 12 weeks. In a group of subjects that were breastfeeding longer than 12 weeks there was a higher proportion of multipara (p = 0.041), and higher incidence of positive family history of DM (p = 0.021). However, both groups did not differ significantly in relation tobaseline variables such as age, history of GDM, anthropometric parameters, OGTT result and additional measurements performed at enrollment. Similarly, according to lactation intensity the women from both groups did not differ significantly in relation to baseline parameters during pregnancy. Women lactating more intensively tented to have lower prevalence of diabetes in the family and their educational level was frequently higher than secondary school, but these differences were insignificant.

The characteristics of women at the 18 months visit are presented in Tables 3 and 4.

18 months after delivery, the women lactating longer than 12 weeks had significantly higher body weight (p = 0.038) and BMI (p = 0.001) than the women lactating for a shorter period of time. Additionally, 2-h postload insulin concentration was one third lower in this group of women but this difference was not statistically significant. There was 3-fold higher number of women treated with insulin in the group lactating for a longer period of time (p = 0.038). The remaining parameters did not differ significantly according to lactation duration. The women lactating more intensively had significantly lower HOMA 2 IR (p = 0.019) compared to the women breastfeeding less intensively. They had also lower HOMA 2 %B, and this difference was approaching statistical significance (p = 0.05). The number of subjects with isolated impaired glucose tolerance was significantly higher in the women lactating less intensively (18.7% *vs.* 1.9%, p = 0.037). The remaining parameters did not differ significantly according to lactation intensity.

Table 2. The characteristics of the study group at baseline visit in pregnancy week 26–30 according to the lactation intensity

Parameters	Whole group (n = 68)	Lactation > 70% (n = 52)	Lactation < 70% (n = 16)	p
Age (years)	34.1 ± 4.3	34.1 ± 4.4	33.18 ± 3.7	0.439
Multiparity, n (%)	35 (51.5)	28 (53.8)	7 (43.7)	0.527
Smoking, yes, n (%)	15 (22.1)	11 (21.1)	4 (25.0)	0.739
Education				
Secondary, n (%)	29 (42.6)	20 (38.4)	9 (56.2)	0.257
Post secondary, n (%)	39 (57.4)	32 (61.6)	7 (43.8)	
Family history of DM, yes, n (%)	37 (54.4)	25 (48.0)	12 (75.0)	0.08
History of GDM, yes	3 (4.4)	2 (3.8)	1 (6.25)	0.552
Maternal birth weight [g]	3212 ± 500	3225 ± 519	3123 ± 484	0.473
Prepregnacy weight [kg]	68.8 ± 15.5	67.9 ± 14.4	67.0 ± 14.0	0.828
Prepregnancy BMI [kg/m^2]	25.1 (CI 24.0–26.1)	23.8 (CI 22.6–25.3)	25.3 (CI 21.8–26.3)	0.948
Actual body weight [kg]	79.2 ± 16.2	77.8 ± 14.8	78.1 ± 14.0	0.931
Actual BMI [kg/m^2]	27.1 (CI 26.3–27.8)	26.3 (CI 25.6–27.4)	27.9 (CI 24.7–29.5)	0.426
OGTT result during pregnancy				
Fasting glucose [mg/dL], n = 52	86 ± 12	86 ± 12	85 ± 14	0.780
1-h post-OGTT glucose [mg/dL], n = 49	175 ± 27	176 ± 28	174 ± 27	0.802
2-h post-OGTT glucose [mg/dL], n = 52	155 ± 21	154 ± 21	156 ± 19	0.735
Time of GDM diagnosis (gestational week)	28.1 ± 2.4	28 ± 2.4	28.5 ± 2.3	0.735
Measurements between 26th and 30th gestational week				
Fasting glucose [mg/dL]	81 ± 11.8	80 ± 9.5	80.5 ± 16	0.969
Fasting insulin [μIU/ml]	16.6 ± 9.07	12.5 ± 7.2	16.8 ± 12.2	0.260
HbA_{1c} (%)	5.18 ± 0.34	5.17 ± 0.31	5.18 ± 0.39	0.978
hsCRP [mg/dL]	4.11 ± 3.04	3.88 ± 2.97	5.19 ± 3.4	0.193

CI — confidence interval; GDM — gestational diabetes mellitus; BMI — body mass index; OGTT — oral glucose tolerance test; hsCRP — high-sensitivity C-reactive protein

A significant negative correlation between lactation duration and fasting glucose concentration ($r = -0.282$, $p < 0.05$) (Figure 1) as well as fasting insulin concentration ($r = -0.251$, $p < 0.05$) (Figure 2) was detected. Similar correlation was noticed as concerns 2-h post-load insulin concentration but this relationship had borderline significance ($p = 0.05$) (Figure 3). Moreover, a significant negative correlation between lactation intensity and fasting insulin concentration ($r = -0.251$, $p < 0.05$) was found (Figure 4). No other significant correlations were found according to lactation duration nor intensity.

In multiple regression model analysis, according to data suggested in literature, we used the following factors that might have impact on fasting and post-OGTT glucose and insulin concentrations as well as CRP concentration, lactation duration, lactation intensity, prepregnancy BMI, family history of DM, and gestational weight gain (Table 5). Covariates that were used in the analysis were the most frequently chosen in the analyses of other authors, as it was described in a recent meta-analysis [15].

Our model was correct for the influence on concentrations of CRP, fasting glucose, fasting insulin and 2-h post-load insulin and for the indices such as HOMA 2 %B and HOMA 2 IR. Prepregnancy BMI and gestational weight gain appeared to be the strongest factors influencing the obtained results. Neither lactation duration nor intensity appeared as the significant factors in the model.

Discussion

The aim of our study was to investigate the influence of lactation on insulin and glucose response 18 months after delivery in the group of women with previous GDM. The impact of lactation duration and intensity was analyzed. Results from our observation reveal that a vast majority of women (93.8%) with prior GDM breastfed their infant. In our trial this number was relatively higher that reported in other studies [9, 19], which may result from greater health awareness as our participants were educated regarding the advantages of breastfeeding. In our study the inci-

Table 3. The characteristics of the study group at the 18 month visit according to lactation duration

Parameters	Whole group (n = 68)	Lactation < 12 weeks (n = 15)	Lactation > 12 weeks (n = 53)	p
Actual body weight [kg]	67.8 ± 16.9	65.9 ± 14.5	71.2 ± 16.7	0.038
Actual BMI [kg/m^2]	24.4 (CI 23.0–25.8)	22.2 (CI 21.0–23.9)	25.5 (CI 24.3–31.1)	0.001
Gestational weight gain [kg]	9.5 ± 9.15	9.0 ± 5.9	11.0 ± 5.7	0.276
Insulin treatment in pregnancy, yes, n (%)	27 (39.7)	2 (26.6)	23 (43.4)	0.371
OGTT result				
Fasting glucose [mg/dL]	92 (CI 89–95)	93 (CI 88–98)	89.5 (CI 86–94)	0.38
2-h post-OGTT glucose [mg/dL]	107 (CI 101–113)	107 (CI 96–129)	104 (93–109)	0.40
Fasting insulin [μIU/ml]	9.63 ± 6.5	9.37 ± 4.32	9.66 ± 2.06	0.88
2-h post-OGTT insulin [uIU/ml]	50.1 ± 30.0	66.7 ± 41	46.4 ± 26.1	0.14
HOMA 2 IR	1.19 (CI 1.02–1.43)	0.92 (CI 0.38–1.54)	1.1 (CI 0.80–1.39)	0.430
HOMA 2 %S	117.6 ± 84.6	138.6 ± 97.5	103.5 ± 55.0	0.427
HOMA 2 %B	101.7 ± 41.5	95.4 ± 29.5	107.3 ± 41.5	0.506
QUICKI	0.66 ± 0.50	0.63 ± 0.32	0.67 ± 0.54	0.844
Isolated IFG, yes, n (%)	15 (22.1)	2(13.0)	13 (24.5)	0.492
Isolated IGT, yes, n (%)	5 (7.3)	1 (6.5)	4 (7.5)	1.0
IFG + IGT, yes, n (%)	1 (1.4)	0	1	–
HbA_{1c} (%)	5.16 ± 0.30	5.26 ± 0.41	5.11 ± 0.28	0.123
hsCRP [mg/dL]	1.97 (CI 1.03–2.35)	1.47 (CI 1.0–2.48)	1.02 (CI 0.83–1.7)	0.31

CI — confidence interval; BMI — body mass index; OGTT — oral glucose tolerance test; hsCRP — high-sensitivity C-reactive protein; HOMA 2 %S — homeostasis model assessment for insulin sensitivity index; HOMA 2 %B — homeostasis model assessment for β-cell function index; HOMA 2 IR — homeostasis model assessment for insulin resistance index; IFG — impaired fasting glucose; IGT — impaired glucose tolerance

dence of impaired glucose tolerance was significantly higher in the group of women lactating less intensively (p = 0.037). The results of other studies concerning this issue are still inconclusive. In the study of Kim et al., conducted in a group of Asian women 6–12 weeks after delivery, no protective effect of breastfeeding on the risk of developing diabetes was reported [8]. Although a retrospective observation of Stuebe et al. showed a beneficial effect of breastfeeding on the risk of developing diabetes, this observation was not confirmed in the subgroup of women after GDM [14]. On the other hand however, in a long-term observation of Ziegler et al. lactation after GDM pregnancy has been shown to delay diabetes by an average of 10 years (12.3 years *vs.* 2.3 years), with the lowest observed risk in women who have breastfed more than 3 months [13]. Similar conclusions come from another study, comprising 300 women with prior GDM, the prevalence of persistent hyperglycemia at 12 weeks after delivery was lower in subjects who lactated comparing to not lactating women [9]. Also, in the SWIFT study (Study of Women, Infant Feeding, and Type 2 Diabetes), performed 6–9 weeks after delivery, it was shown that the prevalence of diabetes and pre-diabetes were lower in the group of women who were breastfeeding exclusively or mainly [10]. However, in the Nurses' Health Study, longer lactation decreased the risk of type 2 diabetes in the group without GDM, while it did not in the women with previous GDM [14]. Like in Stuebe et al., in the Coronary Artery Risk Development in Young Adults Study longer lactation duration was associated with a lower incidence of the metabolic syndrome in the women with prior GDM during a 20-year observation [20]. Finally, in the recent systematic review, lactation of any intensity for more than 4 weeks to more than 12 weeks postpartum in the women with previous GDM was associated with significantly lower risk of type 2 diabetes mellitus in the long term. It is worth stressing that the impact of longer lactation was not obvious when diabetes was evaluated in early postpartum, but became more evident with longer follow-up. The possible explanation is that glucose disturbances prevalence after GDM pregnancy increases with time so it seems that at least several years of follow-up are required to objectively judge this effect.

We observed that women who breastfed longer had significantly higher body weight (p < 0.05) and BMI (p < 0.01) comparing to the subjects lactating for a shorter period of time. We found a significant negative correlation between lactation duration and both fasting glucose and insulin concentrations. Additionally, a negative correlation between lactation duration

Table 4. The characteristics of the study group at the follow-up visit according to lactation intensity

Parameters	Whole group (n = 68)	Lactation > 70% (n = 52)	Lactation < 70% (n = 16)	p
Actual body weight [kg]	67.8 ± 16.9	67.2 ± 15.2	68.5 ± 15.9	0.742
Actual BMI [kg/m^2]	24.4 (CI 23.0–25.8)	23.7 (CI 22.3–25.0)	25.1 (CI 21.3–29.0)	0.604
Gestational weight gain [kg]	9.5 ± 9.15	8.8 ± 9.3	11.1 ± 6.2	0.352
Insulin treatment in pregnancy, yes, n (%)	27 (39.7)	22 (42.3)	5 (32.2)	0.562
OGTT result				
Fasting glucose [mg/dL]	92 (CI 89–95)	92 (CI 86–97)	90 (CI 85–94)	0.769
2-h post-OGTT glucose [mg/dL]	107 (CI 101–113)	105 (CI 94–111)	102.5 (CI 92–121)	0.823
Fasting insulin [μIU/ml]	9.63 ± 6.5	9.68 ± 7.1	9.82 ± 4.42	0.987
2-h post-OGTT insulin [μIU/ml]	50.1 ± 30.0	50.3 ± 30.1	54.4 ± 29.4	0.677
HOMA 2 IR	1.19 (CI 1.02–1.43)	0.77 (CI 0.68–0.88)	1.12 (CI 0.90–1.66)	0.019
HOMA 2 %S	117.6 ± 84.6	139.7 ± 76.8	103.61 ± 74.81	0.09
HOMA 2 %B	101.7 ± 41.5	102.86 ± 31.89	133.45 ± 56.09	0.05
QUICKI	0.66 ± 0.50	0.70 ± 0.28	0.59 ± 0.24	0.144
Isolated IFG, yes, n (%)	15 (22.1)	12 (23.1)	3 (18.7)	1.0
Isolated IGT, yes, n (%)	4 (5.8)	1 (1.9)	3 (18.7)	0.037
IFG + IGT, yes, n (%)	1 (1.4)	1 (1.9)	0	–
HbA_{1c} (%)	5.16 ± 0.30	5.22 ± 0.32	5.27 ± 0.35	0.582
hsCRP [mg/dL]	1.97 (CI 1.03–2.35)	1.36 (CI 1.02–1.58)	1.94 (CI 1.32–2.45)	0.209

CI — confidence interval; BMI — body mass index; OGTT — oral glucose tolerance test; hsCRP — high-sensitivity C-reactive protein; HOMA 2 %S — homeostasis model assessment for insulin sensitivity index; HOMA 2 %B — homeostasis model assessment for β-cell function index; HOMA 2 IR — homeostasis model assessment for insulin resistance index; IFG — impaired fasting glucose; IGT — impaired glucose tolerance

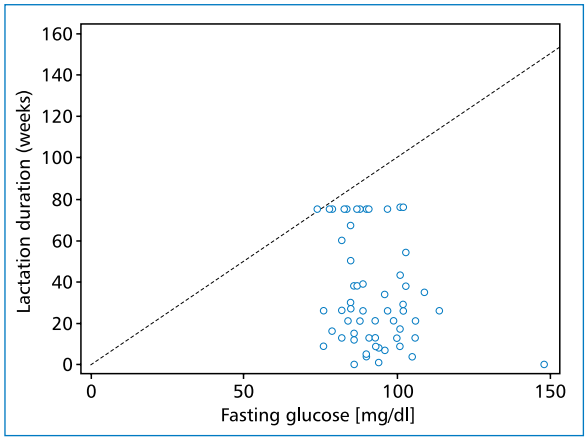

Figure 1. Correlation between lactation duration and fasting plasma glucose concentration at 18 month period

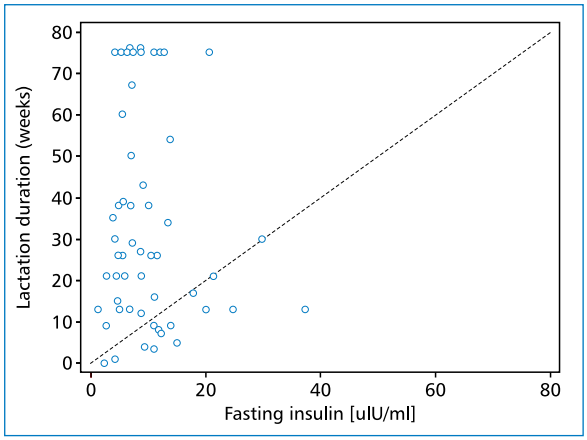

Figure 2. Correlation between lactation duration and fasting plasma insulin concentration at 18 month period

and 2-h post-load insulin concentration was noticed, approaching a level of significance (p = 0.05). Also, a significant negative correlation between lactation intensity and fasting insulin concentrations was recorded. In multiple regression model analysis however, neither lactation duration nor intensity were independent predictors of fasting and post-load glucose and insulin concentrations, which stays in line with some other observations [19]. Lactation duration appeared to affect 2-h post-OGTT insulin concentration, approaching but not reaching significance (p = 0.06). Similarly to our results, Ram et al. observed that lactation duration was inversely correlated with fasting insulin concentrations in a retrospective study comprising high number (2516) of women [12]. Additionally, other authors also showed that breastfeeding was inversely correlated

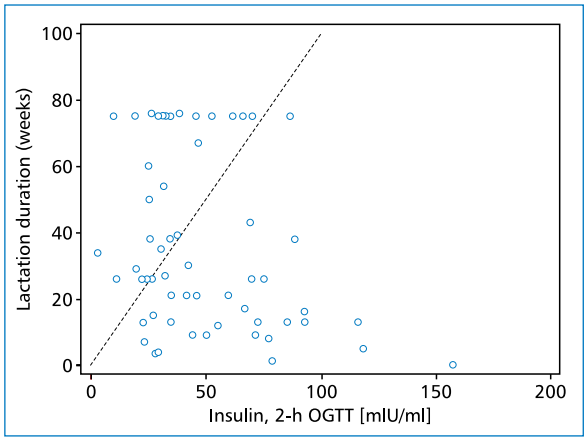

Figure 3. Correlation between lactation duration and 2-h post-OGTT insulin concentration at 18 month period

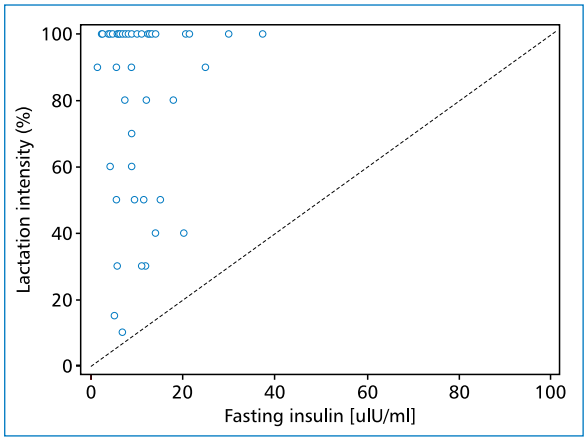

Figure 4. Correlation between lactation intensity and fasting plasma insulin concentration at 18 month period

with insulin response during OGTT [19, 21]. Also, in the SWIFT cohort, women who were breastfeeding exclusively or mostly had lower fasting insulin levels as well as reduced insulin response following a glucose challenge compared with formula feeding exclusive or mostly [10]. These results stay in line with our observations and suggest that lactation is associated with lower both fasting and post-load insulin concentrations. In our study longer lactation correlated with lower fasting glucose levels but not with 2-h post-load glucose values. Similarly, some studies have also shown improved insulin homeostasis but no changes in glucose tolerance among women who lactate, suggesting that lactation may be associated with post-load hyperinsulinemia thus maintaining normal glycemia [10, 21, 22].

Participants lactating more intensively in our study had significantly lower index of insulin resistance ($p = 0.019$) as well as insulin secretion ($p = 0.05$) compared to the women breastfeeding less intensively. The most probable explanation of these results is higher BMI and body weight in the women lactating more intensively, as these parameters were the strongest predictors of glucose and insulin response in a multivariate model. Tigas S et al., reported similar glucose results in OGTT, but in breastfeeding women post-load insulin levels were significantly lower, indicating higher insulin sensitivity [22]. In another study, higher insulin sensitivity in women who have been breastfeeding for more than 10 months have been demonstrated [19]. However, similarly to our results McManus et al. observed that insulin sensitivity was not significantly different in relation to lactation 3 months after delivery [11]. On the longer perspective, this relationship is also not so evident, as the results from the study performed 3 years after delivery did not show any association between lactation and insulin resistance [23]. Similarly, in a larger study of women with prior GDM (SWIFT), 522 subjects with prior GDM were tested shortly post partum (6–9 weeks after delivery). Intensive lactation (exclusive or almost exclusive) was associated with significantly lower HOMA-IR compared to no lactation, while no differences were found regarding the mixed or inconsistent lactation [10]. Similarly to our results, McManus has showed that in breastfeeding women, improvement in pancreatic β-cell function was observed. Also, a higher β-cell function was observed in 3 months after delivery, while there was no difference in insulin sensitivity [11]. In a study of Chouinard-Castonguay S et al., improved insulin secretion among women who lactated longer than 10 months was observed [19]. In a multiple regression model, however, lactation duration was no longer significant when other variables were included in the model.

Several hypotheses concerning potential mechanisms underlying a possible beneficial effect of lactation on glucose metabolism have been proposed. First, direct prolactin action which has been shown to stimulate insulin secretion through stimulation of β-cell proliferation by downregulating the expression of menin [24, 25]. Prolactin levels remain high during lactation, suggesting that this hormone may play a role in regulating insulin secretion and glucose homeostasis in the post partum period [26]. The similar role of oxytocin was also suggested [27]. Another suggestion is that glucose could be preferentially utilized in lactogenesis process (which is estimated for about 50 g of glucose per day) in an insulin-independent pathway, which in turn may unload the pancreatic β-cells and finally preserve long-term insulin production in lactating women [28]. Also, breastfeeding may affect

Table 5. Multiple regression linear analyses of the relationships between lactation duration and the glucose and insulin response 18 months after delivery (n = 68). Covariates included in the models were: pregestational BMI, family history of DM, parity and pregnancy weight gain

	β	p	Model r^2
Fasting glucose [mg/dL]			0.2219
Lactation duration	–0.08	0.209	
Lactation intensity	–0.036	0.526	
Prepregnancy BMI	0.610	0.108	
Family history of DM	–3.425	0.322	
Gestational weight gain	–0.251	0.0040	
2-h post-OGTT glucose [mg/dL]			0.111
Lactation duration	–0.071	0.589	
Lactation intensity	0.047	0.720	
Prepregnancy BMI	0.790	0.459	
Family history of DM	11.08	0.105	
Gestational weight gain	–0.338	0.139	
Fasting insulin [μIU/ml]			0.378
Lactation duration	0.038	0.895	
Lactation intensity	–0.04	0.179	
Prepregnancy BMI	0.978	0.0001	
Family history of DM	–2.129	0.328	
Gestational weight gain	–0.274	< 0.0001	
2-h post-OGTT insulin [μIU/ml]			0.310
Lactation duration	–0.207	0.155	
Lactation intensity	–0.057	0.687	
Prepregnancy BMI	3.618	0.0029	
Family history of DM	14.671	0.068	
Gestational weight gain	–0.94	0.0017	
HOMA 2 %S			0.100
Lactation duration	–0.723	0.123	
Lactation intensity	0.565	0.227	
Prepregnancy BMI	–5.392	0.154	
Family history of DM	4.054	0.864	
Gestational weight gain	1.343	0.092	
HOMA 2 %B			0.306
Lactation duration	0.244	0.228	
Lactation intensity	–0.237	0.242	
Prepregnancy BMI	5.426	0.0015	
Family history of DM	–11.14	0.282	
Gestational weight gain	–1.586	< 0.0001	
HOMA 2 IR			0.372
Lactation duration	0.0002	0.940	
Lactation intensity	–0.005	0.172	
Prepregnancy BMI	0.124	0.0002	
Family history of DM	–0.257	0.177	
Gestational weight gain	–0.03	< 0.0001	
QUICKI			0.055
Lactation duration	–0.003	0.232	
Lactation intensity	0.001	0.540	
Prepregnancy BMI	–0.017	0.320	
Family history of DM	-0.056	0.701	
Gestational weight gain	0.003	0.440	
CRP			0.2548
Lactation duration	–0.008	0.495	
Lactation intensity	0.0001	0.990	
Prepregnancy BMI	0.167	0.081	
Family history of DM	0.099	0.870	
Gestational weight gain	–0.060	0.0004	

BMI — body mass index; DM — diabetes mellitus; CRP — C-reactive protein; HOMA 2 %S — homeostasis model assessment for insulin sensitivity index; HOMA 2 %B — homeostasis model assessment for β-cell function index; HOMA 2 IR — homeostasis model assessment for insulin resistance index

adipocytes metabolism by mobilizing and redirecting lipids accumulated in hepatocytes and muscle cells into breast milk instead of adipocytes. Additionally, prolactin modulates the transcription factors such as STAT5 and PPARγ, and the expression of lipoprotein lipase, which are co-expressed in breast, adipose tissue, and skeletal muscle [29]. Thus, a non-lactating woman would be at higher risk of storing lipids in non-adipose tissues resulting in further imbalance between insulin secretion and sensitivity. In another study, it was observed that lactation affected both ghrelin and YY peptide concentrations, the hormones involved in hypothalamic appetite regulation, suggesting the impact of feeding on neuroendocrine pathways within the hypothalamus. A correlation between adiponectin concentration and breastfeeding duration was also noted in this study, while no relationship was found as concerns leptin concentrations. However, it should be stressed that women with prior GDM were excluded from this cohort [23].

There are some limitations of our current study that have to be addressed. First, only 68 of all 144 initially enrolled subjects attended the follow-up visit, which is relatively high proportion (in relation to the reported general follow-up rate, but still unsatisfactory in the light of statistical power. Secondly, the controlgroup of not lactating women was unavailable, which resulted from both insufficient follow-up attendance rate and the fact that the great majority of women lactated, leaving finally only 3 not lactating women of 68 subjects. However, all efforts were made to encourage the women to attend the visits, as every single participant was called by the investigator before the estimated date of the follow-up meeting. Furthermore, no control group with a negative history for GDM was available for comparison, but such methodology can be found in the literature concerning this issue [19]. There is a possibility that some confounding factors were not taken into consideration. The effect of unknown confoundings or reverse causation cannot be ruled out even in well designed and adequately analyzed studies. However, we were able to control for multiple potential confounders including age, education level, parity, family history of DM, BMI, OGTT result during pregnancy, time of GDM diagnosis, weight gain during pregnancy, maternal birth weight, and finally insulin use during pregnancy. Finally baseline data on the metabolic profile during pregnancy, as well as precise prepregnancy weight, were available in our study and these parameters did not differ between the analyzed subgroups thus allowing further objective comparison.

The strenghts of our study are that the subjects were enrolled during pregnancy, which enabled to collect all the relevant baseline data so that any information biases could be rather excluded. Secondly, initial metabolic profile could be then assessed and compared as the first blood sample was taken during the initial visit. The baseline metabolic profiles of the subjects seem to be comparable as the enrollment took place within a specified 4-week period between 26th and 30th gestational week. It is also worth mentioning that the study comprised the women with GDM diagnosed according to the current WHO diagnostic criteria, so it seems that the conclusions are rather universal and the results can be transmitted to all the countries where the new recommendations have been implemented. Additionally, the prospective design of the study gave us a certainty of obtaining detailed and accurate data. Moreover, every participant was informed before the follow-up visit on the conditions of the glucose challenge test to ensure that all the procedures will be standardized. Each OGTT was performed at one site and all the measurements were performed in the same laboratory, so there were no between-site or between-laboratory differences.

In conclusion, our data provide evidence that lactation may have favorable effects on insulin and glucose response after delivery among women with prior GDM who are at high future cardiometabolic risk. The effects seems to be more evident with longer lactation duration as well as higher lactation intensity. Therefore, our study supports a growing evidence on the health benefits of breastfeeding on maternal insulin and glucose metabolism later in life. As lifestyle modification is often difficult in real life settings, encouraging women to breastfeed may provide an important strategy for reducing the metabolic risk in the women with a history of GDM. It seems however, that further investigations, especially large scale, long-term and prospective are needed to clarify the mechanisms underlying this association.

Conflict of interest

The authors declare no conflict of interest.

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