**The association of maternal gestational hyperglycemia with breastfeeding duration and markers of milk production**

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Short running head:Maternal hyperglycemia and breastfeeding success

Abbreviations used: 2hPG, 2 hour-post glucose challenge; AGA, appropriate-for-gestational age; EPDS, Edinburgh Postnatal Depression Scale; FPG, fasting plasma glucose; GDM, gestational diabetes mellitus; GLM , general linear regression ; GUSTO, Growing Up in Singapore Towards healthy Outcomes; IADPSG, International Association of Diabetes and Pregnancy Study Groups; ICP-OES , inductively coupled plasma optical emission spectrometry; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; LGA, large-for-gestational age; NICU, neonatal intensive care unit; OGTT, oral glucose tolerance test; SGA, small-for-gestational age; STAI, State-Trait Anxiety Inventory.

**ABSTRACT**

**Background:** Previous studies focusing on the association between gestational diabetes and breastfeeding duration have been inconclusive.

**Objectives:** We aimedto determine whether maternal gestational hyperglycemia is associated with the duration of breastfeeding and the concentrations of markers linked to breastmilk production.

**Methods:** Data from the prospective, multi-ethnic Growing Up in Singapore Towards healthy Outcomes (GUSTO) study was used to assess the association of fasting plasma glucose (FPG) and 2-hour post challenge glucose (2hPG) measured at 26-28 weeks’ gestation with duration of breastfeeding and concentrations of protein, lactose, citrate, sodium, potassium and zinc in breastmilk 3 weeks postpartum.

**Results:** Of the 1035 participants, 5.2% and 9.5% had elevated FPG and 2hPG, respectively, consistent with a diagnosis of gestational diabetes mellitus (GDM) based on International Association of Diabetes and Pregnancy Study Groups criteria. FPG ≥ 5.1 mmol/L was associated with a crude reduction in median breastfeeding duration of 2.3 months. In a model adjusted for maternal pre-pregnancy BMI and intention to breastfeed, FPG ≥ 5.1 mmol/L predicted earlier termination of any breastfeeding (Adjusted hazard ratio [95% CI]: 1.47 [1.04, 2.08]) but not full breastfeeding (1.08 [0.76, 1.55]). 2hPG ≥ 8.5 mmol/L was not significantly associated with the durations of any or of full breastfeeding (0.86 [0.62, 1.19] and 0.85 [0.62, 1.18], respectively). Maternal FPG was significantly and positively associated with breastmilk sodium and sodium-to-potassium (Na:K) ratio (Adjusted coefficient [95% CI]: 1.28 [1.08, 1.51] and 1.29 [1.08, 1.54], respectively), but not with other measured breastmilk components.

**Conclusions:** Women with FPG ≥ 5.1 mmol/L during pregnancy breastfeed for a shorter duration. Future work involving measurement of milk production is needed to determine whether low milk production predicts breastfeeding duration among women with elevated FPG.

**Keywords:** Asian, breastfeeding, breastmilk components, gestational hyperglycemia

**Introduction**

Optimal infant feeding is important for a child’s growth and development. As such, WHO and UNICEF recommend exclusive breastfeeding for the first six months of a child’s life with continued breastfeeding for the first two years (1). Many benefits of breastfeeding have been documented, including reduced risk of child infections, better child cognition and protection against breast cancer among women (2). Despite this, recent statistics indicated only 41% of infants under six months of age are exclusively breastfed (3). It is widely believed and accepted that nearly all women are biologically capable of breastfeeding successfully (4, 5), and if a mother is not successful, it is attributed either to social, psychological or behavioural factors (2, 6-8). However, as crucial morphological and functional development of the breast occur during early pregnancy and continue throughout pregnancy (9), pathophysiological events occurring during pregnancy, such as gestational diabetes mellitus (GDM)/gestational hyperglycemia, may also be a risk factor for early weaning.

Maternal metabolism undergoes profound alterations during pregnancy. During this period, glucose use is favored by the fetus, forcing maternal tissues to increase their use of alternative energy sources; this leads to decreased insulin sensitivity. Several hormones and placental cytokines are believed to be responsible for this altered milieu (10). GDM occurs when decreased insulin sensitivity or increased insulin resistance is not matched by a sufficient increase in insulin secretion, resulting in hyperglycemia of variable severity (11). Maternal hyperglycemia thus reflects an environment of altered concentrations of a network of substances (12), which has implications on the development of the breast during pregnancy. The branching and elongation of milk ducts during early pregnancy followed by the proliferation and differentiation of the breast alveolar epithelium into lactocytes, are orchestrated by the neuro- and endocrine systems involving placental hormones (e.g. estrogen, progesterone, human placental lactogen), metabolic hormones (e.g. insulin, growth hormone, cortisol) and prolactin (13). Altered pregnancy environment as a result of maternal hyperglycemia will likely disrupt the normal development of the breast during pregnancy, negatively affecting breastmilk production; decreased milk production will mean shorter duration of breastfeeding.

Only a few studies have examined the association of maternal hyperglycemia with breastfeeding duration and the findings have been inconsistent (14). Most studies were conducted in the US (14) and methods for diagnosing GDM have varied, with many using self-reports of GDM and many others do not provide details on how GDM was diagnosed (15-17). The majority of studies to date have not examined fasting and post challenge glucose concentrations separately in relation to breastfeeding duration even though risks for maternal and perinatal outcomes appear to differ depending on which oral glucose tolerance test (OGTT) thresholds are used (*i.e.,* fasting, 1h, 2h, or their combinations) (18), and this may be due to differences in pathophysiological mechanisms underlying elevated fasting glucose and elevated postprandial glycemia (18, 19). Further, no study thus far has examined the impact of maternal plasma glucose levels as a continuum on breastfeeding duration despite known continuous associations of maternal glucose concentrations with adverse maternal and child outcomes (20, 21).

Here, we used data from the Growing Up in Singapore Towards healthy Outcomes study (GUSTO) to examine whether maternal gestational hyperglycemia is associated with a shorter duration of breastfeeding and with lower milk production defined by elevated breastmilk sodium, protein and sodium-to-potassium (Na:K) ratio, and lower citrate, lactose and zinc concentrations (22-26).

**Methods**

**Participants and study design**

Recruitment for the “Growing Up in Singapore Towards healthy Outcomes” (GUSTO) study took place between 2009 and 2010 among women in their first trimester of pregnancy attending the maternity units of two major Singaporean public hospitals, KK Women’s and Children’s Hospital and the National University Hospital. Women were eligible to join the study if they were between 18-46 years old, not diagnosed with type 1 diabetes and not on any psychotropic medications or undergoing chemotherapy. Only women of Chinese, Malay or Indian ethnicity (the three main ethnic groups in Singapore) with homogenous parental ethnic background were eligible for the study; this was in line with the GUSTO study’s primary objective of evaluating early life factors that contribute to metabolic compromise and altered body composition among the three ethnic groups suggested to differ in their developmental patterns of metabolic functions (27). Details of the cohort have been published previously (28). Written informed consent was obtained from all participants, and the study (NCT01174875; <https://clinicaltrials.gov/>) was granted ethical approval by both the National Healthcare Group Domain Specific Review Board and SingHealth Centralized Institutional Review Board.

Only women who gave birth to singleton children born at term (≥ 37 weeks gestation) were considered as eligible participants for this study (n = 1086). Women were included based on the availability of information on breastfeeding duration and/or breastmilk composition. The final sample sizes considered for data analyses are shown in Supplementary Figure 1.

**Collection of data and biosamples**

Participant educational attainment, maternal age and ethnicity were collected during the first trimester of pregnancy through interviewer-administered questionnaires. Maternal pre-pregnancy BMI (kg/m2) was derived from pre-pregnancy weight reported by the women at 11-14 weeks gestation and maternal height measured at 26-28 weeks gestation (using SECA 213 Stadiometer). At 26-28 weeks’ gestation, an oral glucose tolerance test (OGTT) was performed (detailed in the section below), the women reported whether they had any previous breastfeeding experience, their intended breastfeeding duration after delivery, and they also completed two self-administered questionnaires: Edinburgh Postnatal Depression Scale (EPDS) and the State-Trait Anxiety Inventory (STAI). The EPDS is a screening tool that has been used during and after pregnancy to rate the intensity of depressive symptoms in the past 7 days via 10-items, scored between 0 and 3. Women with antenatal EPDS scores ≥ 15 were considered as having probable depression during pregnancy (29). The STAI is used to assess anxiety levels using two subscales (State and Trait anxiety), each with 20 items scored from 0 to 4 (30). Total scores of trait and state subscales were used as a continuum in this study, with higher scores indicating greater anxiety. Information on parity, mode of delivery, any admission to neonatal intensive care unit (NICU), child sex and birth weight was extracted from medical records. Infants were classified into birth-weight-for-gestational-age percentiles using local population references according to the method described by Mikolajczyk et al. (31), and infants who were <10th percentile were considered small-for-gestational-age (SGA), 10-90th percentile were appropriate-for-gestational-age (AGA), and >90th percentile were large-for-gestational-age (LGA). At the 3-week postnatal visit conducted at home by trained research coordinators, mothers provided information on their in-hospital breastfeeding experiences, including any skin-to-skin soon after delivery and whether baby was breastfed within hours of birth. Mothers also reported their current breastfeeding practices, given the option to provide breastmilk samples if they were still breastfeeding (more details on milk collection in the sections below), indicated whether they experienced any pain while breastfeeding, including pain from mastitis or a breast abscess, and whether they took any antibiotics for mastitis or a breast abscess.

**Maternal glycemia**

At 26-28 weeks’ gestation, pregnant women underwent a 2h 75g OGTT. Fasting and 2h post OGTT venous blood samples were collected in fluoride-containing tubes. Plasma glucose concentrations were measured by colorimetry via a glucose oxidase enzymatic method [Advia 2400 Chemistry system (Siemens Medical Solutions Diagnostics) using Beckman LX20 Pro analyzer (Beckman Coulter)]. As recruitment for GUSTO started in 2009 (prior to the publication of International Association of Diabetes and Pregnancy Study Groups [IADPSG] criteria in 2010), women were diagnosed for GDM using 1999 WHO criteria: ≥ 7.0 mmol/L for fasting plasma glucose (FPG) and/or ≥ 7.8 mmol/L for 2h post-glucose (2hPG) (32). Women diagnosed with GDM (n=183; 17.8%) were subsequently treated according to clinical protocols at KK Women’s and Children’s Hospital and National University Hospital through diet modification (n=160; 87.4%) or the use of insulin (n=14; 7.7%); 9 (4.9%) women were untreated. For this current study, FPG and 2hPG were used as continuous and categorical exposures; for the categorical exposure, cut-offs used were based on the IADPSG criteria of ≥ 5.1 mmol/L for FPG, and ≥ 8.5 mmol/L for 2hPG (33).

**Breastfeeding practices**

Breastfeeding practices were captured using interviewer-administered questionnaires. Participants were asked whether the child was still breastfed and the extent of breastfeeding were captured as ‘exclusive’, ‘predominant’, ‘partial’ breastfeeding or ‘formula feeding’, based on WHO breastfeeding definitions (34, 35). The questionnaire was conducted with each mother at every postnatal visit starting from week 3, then at 3-monthly intervals from month 3 to month 18 (when mothers were asked to record responses for every month within the 3-month interval between visits), and then yearly intervals from year 2 to year 4. At each time point, children who were exclusively or predominantly breastfed were combined into one group – the ‘full breastfeeding’ group (36). The durations of any and of full breastfeeding were calculated based on when participants indicated they stopped exclusive/predominant breastfeeding (full breastfeeding duration) or when breastfeeding ceased completely (duration of any breastfeeding). The duration of breastfeeding, in number of days, was subsequently converted to ‘number of months’ for ease of interpretation.

**Breastmilk composition**

Breastmilk samples (16 mL) were collected at 3 weeks postpartum. On the day of the home visit, mothers were asked not to empty one breast for at least 2 hours, and to express all breastmilk from that breast between 6am to 10am into a milk bottle either by hand/manual expression or by using mother’s own breast pump. Expressed milk samples were kept in the fridge. During the home visit, breastmilk was warmed to 38°C, gently mixed and divided into 2mL aliquots. Samples were then transported on ice back to the laboratory and stored at -80°C. Prior to shipping, samples were thawed, mixed thoroughly then aliquoted and shipped on dry ice to The University of Western Australia, Perth and to Liggins Institute, Auckland. Samples were kept frozen at -80°C until further analysis.

For analysis of total protein, lactose and citrate, milk samples were thawed and defatted by centrifugation (10000 x *g*, 10 minutes). Total protein concentration of the defatted milk sample was determined by the modified Bradford method using Bio-Rad protein assay dye reagent concentrate (500-006; Bio-Rad Laboratories) (37). The human milk protein standard for the assay was determined by the Kjeldahl method (38), with detection limit of 0.016 g/L and interassay coefficient of variation (CV) of 4.7%. The recovery (SD) of a known amount of protein added to the milk samples was 103.6% (3.7%). Lactose concentration of the defatted milk sample was determined by a validated enzymatic spectrophotometric assay (22, 39), with detection limit of 1.3 g/L and interassay CV of 5.3%. The recovery (SD) of a known amount of lactose added to the milk samples was 102.0% (2.3%). Citrate concentration of the defatted milk sample was determined by enzymatic spectrophotometric assay (22) with detection limit of 0.28 g/L and interassay CV of 8.7%. The recovery (SD) of a known amount of citrate added to the milk samples was 101.1% (3.3%).

For sodium (Na), potassium (K) and zinc (Zn) measurements, whole human milk samples were thawed and acid digested. The digested samples were then analyzed for concentrations of Na, K and Zn by inductively coupled plasma optical emission spectrometry (ICP-OES) (5100, Agilent technologies, Santa Clara, CA, USA) in triplicate (40). Standard solutions of Na and K (50-500 µg/ml, High Purity Standard, USA) and Zn (2- 8 µg/mL, Sigma Aldrich, Australia) were diluted with 18.2 MΩ water. Na was measured at 568.263nm and 568.821nm; K was measured at 766.491 nm and 769.897 nm; Zn was measured at 202.548 nm and 213.857 nm. The results of Na, K and Zn obtained from those specific wavelengths were averaged and converted from g/ml to mmol/L using the molecular weights of Na, K and Zn (23, 39 and 65 g/mol, respectively). The interassay CVs for Na, K and Zn were 9%, 5% and 3% respectively. The recovery (SD) of a known amount of Na added to the milk samples was 98% (9%), for K it was 99% (5%) and for Zn it was 102% (3%). Blank preparations using the same batch of tubes as those used to analyse breastmilk samples for Na, K and Zn were found to be negative for mineral contamination.

For the analyses of milk cortisol and cortisone concentrations, milk samples (100 µL) were thawed and vortexed for 20 s before being transferred to glass tubes containing 100 µL internal standards (12 ng/mL cortisol d4, 60 mg/mL corticosterone d8). Milk steroids were measured by liquid chromatography mass spectrometry (LC-MS) (Ion Max APCI source on a Finnigan TSQ Quantum Ultra AM triple quadrupole mass spectrometer) as published previously (41).

**Data analyses**

Overall participant characteristics and characteristics categorized according to FPG and 2hPG are described as mean (SD) for continuous variables, or as frequencies and proportions for categorical variables; differences in characteristics between the categories were subsequently tested using t-tests and chi-squared tests for continuous and categorical data, respectively. We examined the influence of maternal glycemia on any and on full breastfeeding durations using Cox regression models. As unfavorable early breastfeeding practices are known to impact breastfeeding duration, we examined the relationship between maternal hyperglycemia and early breastfeeding practices using Poisson regression models. Characteristics with P < 0.1 between the categories of FPG and 2hPG were included in the adjusted regression models.

To examine the associations of maternal glycemia and concentrations of breastmilk components 3 weeks postpartum, we fit general linear regression (GLM) models. For these analyses, maternal glycemia was modeled as a continuous exposure and not as cut-offs due to small sample sizes (*n*=5 for FPG ≥ 5.1mmol/L and *n*=18 for 2hPG ≥ 8.5 mmol/L). Sodium and cortisol concentrations, and Na:K ratios were heavily right-skewed thus were log-transformed for models while all other components were retained in their natural units. Consequently, coefficients and standard errors for sodium, cortisol and Na:K models were exponentiated and expressed in %-difference, while all others were expressed in respective concentration unit-difference, in response to maternal glycemia. Models were adjusted for maternal age (years), ethnicity (Chinese, Malay, Indian), maternal education (secondary and below, technical school, tertiary), parity (primiparous, multiparous) and maternal pre-pregnancy BMI (kg/m2).

Several sensitivity analyses were performed. Firstly, as obesity and high FPG are often comorbid, we examined whether maternal pre-pregnancy BMI modified the relationship of FPG on breastfeeding durations; interaction terms were not significant (P > 0.10). Second, as many women with high FPG were clinically managed through diet modification only or with insulin treatment, we additionally adjusted models for GDM treatment when examining the association of FPG and breastmilk components; the trends of associations remained similar (Supplementary Table 1). This sensitivity analysis was not done for the 2hPG analyses because 99% women with high 2hPG received treatment. Third, as insulin treatment could independently result in shortened breastfeeding duration (42), we excluded all women who received insulin treatment from the analysis and examined the association of FPG with breastfeeding duration; the associations remained the same (Supplementary Table 2). Fourth, as concentrations of breastmilk components may be affected by mastitis due to increased ‘leakiness’ of tight junctions between lactocytes, we excluded mothers with probable mastitis (*n*=5); the association of maternal glycemia during pregnancy and concentrations of breastmilk components remained the same (results not shown). Fifth, even though mothers were asked to collect milk in the morning, 13% of the milk samples were collected in the afternoon/evening; results were similar when we further adjusted for time of collection (AM vs PM) (results not shown). Finally, we performed multiple imputations by chained equations on missing covariates (0-16.2%) by imputing the missing data with all other variables in the model (43). Estimates from pooled analysis of ten imputations are similar to estimates from the unimputed dataset and hence are not presented. We considered a P-value < 0.05 as the threshold for significant difference assuming model-dependent null hypotheses of no differences. All statistical analyses were performed using IBM SPSS software (Version 25).

**Results**

Details of participants included in the study are shown in Table 1. When stratified by gestational FPG levels, those with elevated FPG (≥ 5.1 mmol/L) were more likely to be of Malay and Indian ethnicity, had higher pre-pregnancy BMI, had higher FPG and 2hPG concentrations, more often received treatment for GDM, delivered large-for-gestational-age infants, delivered via cesarean section, less likely to have had skin-to-skin or breastfed baby soon after birth and were less likely to have breastfed for at least 6 months compared to women with FPG < 5.1 mmol/L (Table 1). Women with 2hPG ≥ 8.5 mmol/L had significantly higher FPG and 2hPG concentrations, and were more likely to be treated for GDM when compared to women with 2hPG < 8.5 mmol/L. Characteristics of participants with glycemia levels above both thresholds, *i.e.* FPG ≥ 5.1 mmol/L and 2hPG ≥ 8.5, are presented in Supplementary Table 3.

The median breastfeeding durations estimated from crude survival analysis were 3.5 months among women with FPG < 5.1 mmol/L and 1.2 months among women with FPG ≥ 5.1 mmol/L, a difference of 2.3 months. When we examined the association of FPG on breastfeeding duration, women with FPG ≥ 5.1 mmol/L were 1.47 times more likely to stop breastfeeding at any point in time as compared to those with FPG < 5.1 mmol/L (P=0.029); however, the estimates for full breastfeeding were much more variable (P = 0.67) (Table 2). No significant relationships with breastfeeding duration were observed when FPG was modeled as a continuous exposure. 2hPG was not significantly associated with earlier termination of any or of full breastfeeding (Table 2). We also examined the association of FPG and 2hPG with early breastfeeding practices; no significant associations were noted (Table 2).

Breastmilk samples at 3 weeks postpartum were collected and analysed from a subset of women (*n*=189). Compared to women who did not have breastmilk collected, a significantly higher proportion of women included in the breastmilk analyses were Chinese, of higher education, intended to breastfeed for ≥ 6M, had skin-to-skin contact soon after birth, breastfed baby within hours of birth and breastfed their child for a longer duration, while a lower proportion had probable depression during pregnancy (Supplementary Table 4). We examined the associations of FPG and 2hPG with the concentrations of breastmilk components. There were significant positive associations between gestational FPG and breastmilk sodium concentration, and Na:K ratio in the adjusted models, where 1 mmol/L increase in FPG was associated with 28% increase in sodium concentration and with 29% increase in Na:K ratio; associations were not significant for the other breastmilk components (Table 3). There were no significant associations of 2hPG with any of the breastmilk components studied (Table 3).

**Discussion**

Breastmilk is the gold standard in infant nutrition but global breastfeeding rates are low. Here, we examined whether maternal gestational hyperglycemia may be a factor contributing to the low breastfeeding rates. Findings from our multi-ethnic Asian cohort suggest that elevated FPG, but not 2hPG, reduces the median breastfeeding duration by 2.3 months, and is associated with elevated breastmilk sodium concentration and Na:K ratio, possibly indicative of low milk production.

We hypothesized that mothers with hyperglycemia during pregnancy would have lower milk production and shorter breastfeeding duration compared to normoglycemic mothers. Maternal gestational hyperglycemia, as opposed to diagnosed GDM, was used as the exposure in this study for two main reasons. First, blood glucose levels 1h post-OGTT (1hPG), a part of the current criteria for GDM diagnosis, were not collected in this cohort at a time when the 1999 WHO criteria for GDM diagnosis was still in use, and as such, women with elevated 1hPG would not have been represented if GDM was used as the exposure in this study. It is clinically important to include glucose levels at the 1h time-point as over a third of GDM diagnoses were made based on an elevated 1hPG alone and/or in combination with elevated 2hPG (44). Second, it has previously been shown by others (18), and supported by data from this cohort (21), that risks for maternal and perinatal outcomes differ depending on which OGTT thresholds are used (*i.e.,* fasting, 1h, 2h, or their combinations) and thus it seems clinically more relevant to investigate the association of gestational hyperglycemia, as opposed to diagnosed GDM, with breastfeeding success. There is also ongoing uncertainty regarding the “ideal” testing approach for GDM and accompanying international variability in diagnostic criteria for GDM (see Hillier TA *et al.* 2021) (45), further strengthening the case for using hyperglycemia. Using plasma glucose levels as continuums as well as adopting IADPSG cut-offs, we showed that mothers with FPG ≥ 5.1 mmol/L during pregnancy were at a higher risk of early weaning than those with FPG < 5.1 mmol/L. Pre-pregnancy obesity and insulin treatment were previously shown to be independent risk factors of delayed lactogenesis (42). As expected, women with FPG ≥ 5.1 mmol/L in our study tended to have higher pre-pregnancy BMI and a larger proportion received insulin treatment; nevertheless, the association between FPG ≥ 5.1 mmol/L and shorter breastfeeding duration remained even after accounting for these two risk factors. Women with FPG ≥ 5.1 mmol/L in our study were also more likely to deliver by cesarean section and have poorer early breastfeeding practices; these are factors often associated with shorter breastfeeding duration (46, 47). In an earlier study, we showed that delivery mode was not an independent risk factor for breastfeeding success in our cohort (36), however, poorer early breastfeeding practices were. One possible reason why a higher proportion of women with FPG ≥ 5.1 mmol/L exhibit unfavorable early breastfeeding practices may be related to their stressful labor or delivery (48) as these women are more likely to deliver a LGA baby and to undergo intrapartum cesarean section. This implies that the shorter breastfeeding duration in response to elevated FPG could have resulted from initial breastfeeding practices which were unfavorable. However, results from further analyses did not support this as elevated FPG was not a significant a risk factor for unfavourable early breastfeeding practices.

It is not immediately clear why elevated FPG, but not 2hPG, is associated with early cessation of breastfeeding. A possible reason lies in the different pathophysiological mechanisms underlying FPG vs 2hPG which result in different metabolic abnormalities (18, 19); this is supported by data from our cohort as FPG appears to exert a greater and more sustained influence on child growth and adiposity than 2hPG (21, 49). We are unaware of other studies that have examined the association of FPG and breastfeeding duration. Results from previous studies, comparing duration of breastfeeding among mothers with or without diagnosed GDM have been inconsistent, with some showing shorter breastfeeding duration in relation to GDM, while others found no association (14). Two studies have previously examined the association of plasma glucose levels post-glucose challenge in relation to breastfeeding duration. In the Spanish study, there was a higher risk of early exclusive breastfeeding cessation among those with elevated (≥ 7.8mmol/L but < 10.6 mmol/L), compared to those with normal, 1h glucose levels (50). In the second study involving 2030 Vietnamese women, women with IADPSG-defined GDM, or those with elevated 2hPG, had shorter durations of any breastfeeding, but there was no mention of findings in relation to elevated FPG (51). In contrast, women with elevated 2hPG levels in our cohort were not at a higher risk of breastfeeding for a shorter duration. Differences in clinical treatments may explain the discrepancy; almost all women with 2hPG ≥ 8.5 mmol/L in our cohort were treated either with dietary intervention or insulin, while no treatment information was available for the Vietnamese cohort (51). More studies using plasma glucose levels, as opposed to using GDM diagnosis cut-offs, are needed to confirm our findings.

The concentrations of some breastmilk components were also examined in this study in relation to maternal gestational hyperglycemia. Previous studies using breastmilk from mothers who gave birth to preterm infants (23, 52) and mothers with milk supply concerns (24) suggest that perturbation of breastmilk sodium, Na:K ratio, citrate, protein, lactose and zinc may predict breast dysfunction, resulting in low milk production. In our study, only the concentration of sodium, and the Na:K ratio showed significant positive association with increasing levels of FPG. Nevertheless, all other breastmilk components showed trends consistent with breast dysfunction despite not reaching statistical significance, and this may in part be due to the smaller sample sizes of women included in the breastmilk analyses. Breastmilk cortisol concentrations, which is in a dynamic equilibrium with plasma cortisol concentrations, also appeared to be higher among those with elevated FPG. Several studies have examined the link between breastmilk Na:K ratio or sodium concentrations with breastfeeding success. In one study, elevated Na:K ratio was reported to be more prevalent in mothers with a milk supply concern, and it was predictive of early weaning (24). In another study, an elevation of sodium concentration appear to indicate impaired lactogenesis with a high risk of breastfeeding failure (53). The combined use of multiple breastmilk components as biomarkers of milk production have been studied, with results suggesting a dose-response relationship between the number of breastmilk biomarkers within the normal range (as determined from published references) and volume of breastmilk produced (23, 26). Taken together, our findings suggest that elevated FPG impairs breastmilk production to the extent that women wean early. Future studies on a larger number of women with milk samples and measurement of milk productions will be crucial to confirm or refute our findings.

Strengths of our study include its prospective study design, with breastfeeding practices recorded at close intervals thus reducing recall bias. Information on many relevant covariates were included to reduce confounding. We examined maternal hyperglycemia comprehensively using both FPG and 2hPG as continuums as well as using clinically recommended cut-offs, and excluded women with pre-existing type 2 diabetes. We also measured levels of multiple breastmilk components using well-established laboratory methods.

There are some limitations to this study. First, as breastmilk collection was voluntary, those who had lower intention to breastfeed were under-represented as indicated by relatively longer breastfeeding duration in our sample (Supplementary Table 4). However, our sample is the clinically relevant population, as those with lower intention are likely to cease breastfeeding early for reasons other than differences in milk production. Moreover, hyperglycemia status did not strongly differ between sampled and un-sampled groups, suggesting selection bias is limited. Second, glycemia during breastfeeding, instead of pregnancy, may have contributed to our findings because information on adherence to GDM treatment and subsequent glycemia levels during breastfeeding were not collected. However, blood glucose levels for the majority of women with gestational hyperglycemia return to normoglycemic levels during the early weeks postpartum (≤ 6 weeks) (54); this early period corresponds to a time when breastmilk samples were collected in our study and also when breastmilk production is thought to have been established (55), suggesting that glycemia during breastfeeding is unlikely to explain, to a large extent, the results of our study. Third, there may have been Zn contaminants in the milk collection equipment as they were not acid-washed. Nevertheless, the mean ±SD (2.02 ± 0.64 mg/L) and range (0.3 to 3.8 mg/L) of breastmilk Zn in our study were comparable to, or lower than, Zn concentrations of breastmilk collected at similar lactation periods in other studies (56, 57), thus suggesting minimal, if any, contamination from other sources of Zn. Fourth, because we did not collect data on mother’s milk production, we are unable to associate concentrations of breastmilk components to milk production directly. Finally, our ability to draw firmer conclusions from the results on breastmilk composition were limited because of the small number of breastmilk samples analyzed; however, trends from the different breastmilk components collectively support the hypothesis that maternal gestational glycemia may affect breast development.

In summary, this study shows that independent of a woman’s pre-pregnancy BMI, those with FPG ≥ 5.1 mmol/L during pregnancy are at a higher risk of having a 2.3 month shorter breastfeeding duration. Future work involving the measurement of milk production will be needed to confirm the role of low milk production in influencing breastfeeding duration and the impact on child health and disease risk.

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**Contribution statement** Y-S.C., P.D.G., K.M.G., F.Y., K.H.T., S-Y.C., L.P.S., J.G.E. designed and led the GUSTO study. M.E.W., S-Y.C. and W.W.P. conceptualized and designed the current study; D.F., W.W.P., C.T.L, M.H.V., S.P., M.C.C., S.B.L. and C.Y.C. conducted research and/or collected the data. W.W.P. performed statistical analysis, with guidance from Y.H.C. and J.H. W.W.P. wrote the draft of manuscript; J.G.E., D.T.G., J.H., S-Y.C. and M.E.W. critically reviewed the manuscript. All authors read and approved the final manuscript. W.W.P. is the guarantor 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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Table 1 Study participant characteristics1

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | Fasting plasma glucose (FPG) | | | 2hour-post challenge glucose (2hPG) | | |
|  | All participants | < 5.1 mmol/L | ≥ 5.1 mmol/L |  | < 8.5 mmol/L | ≥ 8.5 mmol/L |  |
| Characteristics | (n=1035) | (n=981) | (n=54) | *P* value2 | (n=937) | (n=98) | *P* value2 |
| *Maternal characteristics* |  |  |  |  |  |  |  |
| Maternal age (yr)3 | 30.6 ± 5.1 | 30.6 ± 5.1 | 31.0 ± 5.3 | 0.55 | 30.4 ± 5.1 | 32.7 ± 4.8 | 0.38 |
| Ethnicity4 |  |  |  | 0.004 |  |  | 0.37 |
| Chinese | 587 (56.8) | 568 (58.0) | 19 (35.2) |  | 527 (56.3) | 60 (61.2) |  |
| Malay | 261 (25.2) | 241 (24.6) | 20 (37.0) |  | 242 (25.9) | 19 (19.4) |  |
| Indian | 186 (18.0) | 171 (17.4) | 15 (27.8) |  | 167 (17.8) | 19 (19.4) |  |
| Maternal Education4 |  |  |  | 0.09 |  |  | 0.31 |
| Secondary and below | 303 (29.6) | 290 (29.9) | 13 (24.5) |  | 280 (30.3) | 23 (23.5) |  |
| Technical school | 358 (35.0) | 332 (34.3) | 26 (49.1) |  | 323 (35.0) | 35 (35.7) |  |
| Tertiary | 361 (35.3) | 347 (35.8) | 14 (26.4) |  | 321 (34.7) | 40 (40.8) |  |
| Maternal pre-pregnancy BMI (kg/m2)3,4 | 22.7 ± 4.5 | 22.5 ± 4.3 | 27.2 ± 5.0 | <0.001 | 22.5 ± 4.4 | 24.5 ± 4.7 | 0.17 |
| Parity |  |  |  | 0.44 |  |  | 0.54 |
| Primiparous | 474 (45.8) | 452 (46.1) | 22 (40.7) |  | 432 (46.1) | 42 (42.9) |  |
| Antenatal EPDS4 |  |  |  | 0.35 |  |  | 0.36 |
| Score ≥ 15 | 69 (7.4) | 64 (7.2) | 5 (10.9) |  | 65 (7.6) | 4 (4.9) |  |
| Antenatal STAI score3,4 | 70.8 ± 17.8 | 70.8 ± 17.8 | 70.8 ± 17.5 | 0.99 | 71.1 ± 17.9 | 68.4 ± 17.2 | 0.63 |
| FPG (mmol/L)3 | 4.3 ± 0.5 | 4.3 ± 0.3 | 5.6 ± 0.7 | <0.001 | 4.3 ± 0.4 | 4.7 ± 0.8 | <0.001 |
| 2hPG (mmol/L)3 | 6.5 ± 1.5 | 6.4 ± 1.3 | 8.0 ± 2.3 | <0.001 | 6.2 ± 1.1 | 9.4 ± 1.1 | <0.001 |
| Treated for GDM | 174 (16.9) | 147 (15.1) | 27 (50.0) | <0.001 | 77 (8.3) | 97 (99.0) | <0.001 |
| Diet modification only | 160 (15.5) | 140 (14.3) | 20 (37.0) |  | 73 (7.8) | 87 (88.8) |  |
| Insulin treatment | 14 (1.4) | 7 (0.7) | 7 (13.0) |  | 4 (0.4) | 10 (10.2) |  |
| *Child characteristics* |  |  |  |  |  |  |  |
| Child sex |  |  |  | 0.89 |  |  | 0.96 |
| Female | 488 (47.2) | 463 (47.2) | 25 (46.3) |  | 442 (47.2) | 46 (46.9) |  |
| Child birthweight category4 |  |  |  | 0.005 |  |  | 0.22 |
| SGA | 132 (12.9) | 130 (13.4) | 2 (3.8) |  | 125 (13.5) | 7 (7.3) |  |
| AGA | 740 (72.2) | 705 (72.5) | 35 (67.3) |  | 667 (71.8) | 73 (76.0) |  |
| LGA | 153 (14.9) | 138 (14.2) | 15 (28.8) |  | 137 (14.7) | 16 (16.7) |  |
| Delivery mode4 |  |  |  | 0.022 |  |  | 0.82 |
| Vaginal | 686 (71.2) | 659 (72.1) | 27 (54.0) |  | 627 (71.3) | 59 (70.2) |  |
| Intrapartum Cesarean Section | 149 (15.5) | 137 (14.0) | 12 (24.0) |  | 137 (15.6) | 12 (14.3) |  |
| Non-labor Cesarean Section | 129 (13.4) | 118 (12.9) | 11 (22.0) |  | 116 (13.2) | 13 (15.5) |  |
| Admission to NICU4 | 15 (1.6) | 14 (1.5) | 1 (2.0) | 0.79 | 13 (1.5) | 2 (2.4) | 0.52 |
| *Breastfeeding Practices* |  |  |  |  |  |  |  |
| Planned breastfeeding duration4 |  |  |  | 0.30 |  |  | 0.89 |
| < 6 Months | 271 (26.8) | 259 (27.1) | 12 (22.6) |  | 246 (26.9) | 25 (26.3) |  |
| ≥ 6 Months | 630 (62.4) | 598 (62.5) | 32 (60.4) |  | 569 (62.2) | 61 (64.2) |  |
| Undecided | 109 (10.8) | 100 (10.4) | 9 (17.0) |  | 100 (10.9) | 9 (9.5) |  |
| Prior breastfeeding experience4 | 484 (47.2) | 457 (47.0) | 27 (50.9) | 0.57 | 435 (46.8) | 49 (51.0) | 0.43 |
| Skin-to-skin immediately/soon after birth4 | 534 (61.6) | 512 (62.4) | 22 (47.8) | 0.049 | 490 (62.3) | 44 (55.0) | 0.20 |
| Baby breastfed within hours of birth4 | 502 (57.9) | 481 (58.6) | 21 (45.7) | 0.08 | 461 (58.6) | 41 (51.3) | 0.21 |
| Duration of Any breastfeeding ≥ 6 months4 | 377 (40.4) | 367 (41.4) | 10 (21.3) | 0.006 | 337 (39.9) | 40 (44.9) | 0.36 |
| Duration of Full breastfeeding ≥ 3 months4 | 175 (18.4) | 169 (18.7) | 6 (12.2) | 0.26 | 151 (17.6) | 24 (25.8) | 0.052 |

1Values presented are n (%) unless indicated. 2hPG, 2 hour-post glucose challenge; AGA, appropriate-for-gestational age; EPDS, Edinburgh Postnatal Depression Scale; FPG, fasting plasma glucose; GDM, gestational diabetes mellitus; LGA, large-for-gestational age; NICU, neonatal intensive care unit; SGA, small-for-gestational age; STAI, State-Trait Anxiety Inventory.

2t-tests and chi-squared tests were performed for continuous and categorical data, respectively.

3Values are Mean ± SD.

4Variables with missingness: Antenatal EPDS (n=101; 9.8%), antenatal STAI (n=69; 6.7%), planned BF duration (n=25; 2.4%), maternal pre-pregnancy BMI (n=88; 8.5%), ethnicity (n=1; 0.1%), maternal education (n=13, 1.3%), child birthweight category (n=10; 1.0%), treated for GDM (n=5, 0.5%), delivery mode (n=71, 6.9%), admission to NICU (n=71, 6.9%), prior breastfeeding experience (n=9, 0.9%), baby breastfed within hours of birth (n=168, 16.2%), skin-to-skin immediately/soon after birth (n=168, 16.2%), duration of any breastfeeding ≥ 6 months (n=101, 9.8%), duration of full breastfeeding ≥ 3 months (n=83, 8.0%).

Table 2 The associations1 of pregnancy fasting plasma glucose (FPG) and 2 hour-post challenge glucose (2hPG) with early cessation of any2 and of full3 breastfeeding, and with early breastfeeding practices.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Duration of any breastfeeding** | **Duration of full breastfeeding** | **Skin-to-skin after birth** | **Breastfeeding soon after birth** |
|  | (*n*= 976) | (*n*= 963) | (*n*= 867) | (*n*= 867) |
|  | HR (95%CI) | HR (95%CI) | RR (95%CI) | RR (95%CI) |
| **FPG4** |  |  |  |  |
| FPG (continuous) | 1.05 (0.87, 1.28) | 1.04 (0.86, 1.25) | 0.97 (0.84, 1.11) | 0.91 (0.78, 1.07) |
| FPG < 5.1 mmol/L | Reference | Reference | Reference | Reference |
| FPG > 5.1 mmol/L | 1.47 (1.04, 2.08)5 | 1.08 (0.76, 1.55) | 1.05 (0.79, 1.39) | 0.93 (0.65, 1.33) |
| **2hPG6** |  |  |  |  |
| 2hPG (continuous) | 0.94 (0.87, 1.00) | 0.94 (0.88, 1.01) | 0.99 (0.94, 1.05) | 0.97 (0.91, 1.03) |
| 2hPG < 8.5 mmol/L | Reference | Reference | Reference | Reference |
| 2hPG > 8.5 mmol/L | 0.86 (0.62, 1.19) | 0.85 (0.62, 1.18) | 1.11 (0.82, 1.50) | 0.96 (0.71, 1.30) |

1Cox regression models were fitted to examine the associations of pregnancy glycemia and early cessation of any and of full breastfeeding, and Poisson regression models were fitted to examine the associations of pregnancy glycemia and early breastfeeding practices.

2Includes infants who were fed any amount of breastmilk, regardless of what other food or drink they received.

3Includes infants who were exclusively or predominantly fed breastmilk.

4When examining durations of breastfeeding as outcomes, models were adjusted for maternal education, ethnicity, pre-pregnancy BMI, 2hPG, GDM treatment, birthweight percentile, mode of delivery, any skin-to-skin soon after birth and any breastfeeding soon after birth. When examining early breastfeeding practices as outcomes, models were adjusted for maternal education, ethnicity, pre-pregnancy BMI, 2hPG, GDM treatment, birthweight percentile and mode of delivery.

5Significant at *P* <0.05.

6Models were adjusted for FPG, GDM treatment. 2hPG, 2 hour-post glucose challenge; FPG, fasting plasma glucose; GDM, gestational diabetes mellitus.

Table 3 The association1 of pregnancy FPG and 2hPG with markers of low breastmilk production, cortisol and cortisone measured at 3 weeks postpartum (*n*=189)

|  |  |  |  |
| --- | --- | --- | --- |
|  | Unadjusted | Model 1 | Model 2 |
| **FPG (continuous)** | β (95%CI) | β (95%CI) | β (95%CI) |
| Protein (g/L) | 1.26 (0.37, 2.15)2 | 1.09 (0.17, 2.02)2 | 0.94 (-0.02, 1.91) |
| Sodium (% change in mg/L)3 | 1.38 (1.17, 1.63)2 | 1.28 (1.09, 1.51)2 | 1.28 (1.08, 1.51)2 |
| Potassium (mg/L) | -4.07 (-20.07, 11.94) | -5.20 (-22.24, 11.84) | -5.97 (-23.66, 11.72) |
| Lactose (g/L) | -4.01 (-8.22, 0.20) | -2.94 (-7.34, 1.47) | -2.93 (-7.57, 1.71) |
| Citrate (mmol/L) | -0.18 (-0.49, 0.13) | -0.13 (-0.45, 0.19) | -0.19 (-0.52, 0.14) |
| Zinc (mg/L) | -0.08 (-0.27, 0.11) | -0.06 (-0.26, 0.14) | -0.06 (-0.26, 0.15) |
| Cortisol (% change in ng/mL)3 | 1.50 (0.94, 2.41) | 1.62 (0.98, 2.67) | 1.55 (0.92, 2.60) |
| Cortisone (ng/mL) | 0.05 (-1.15, 1.24) | 0.56 (-0.69, 1.80) | 0.47 (-0.80, 1.75) |
| Na:K (% change in ratio)3 | 1.39 (1.17, 1.64)2 | 1.29 (1.09, 1.53)2 | 1.29 (1.08, 1.54)2 |
| **2hPG (continuous)** |  |  |  |
| Protein (g/L) | 0.16 (-0.13, 0.46) | 0.11 (-0.19, 0.41) | 0.04 (-0.28, 0.35) |
| Sodium (% change in mg/L)3 | 1.00 (0.95, 1.06) | 1.01 (0.95, 1.06) | 1.00 (0.95, 1.06) |
| Potassium (mg/L) | -2.35 (-7.56, 2.86) | -2.60 (-8.08, 2.89) | -3.05 (-8.79, 2.70) |
| Lactose (g/L) | 0.09 (-1.30, 1.48) | -0.04 (-1.47, 1.39) | 0.05 (-1.47, 1.57) |
| Citrate (mmol/L) | 0.03 (-0.06, 0.12) | 0.02 (-0.07, 0.11) | 0.02 (-0.07, 0.11) |
| Zinc (mg/L) | 0.03 (-0.03, 0.09) | 0.02 (-0.04, 0.08) | 0.03 (-0.04, 0.09) |
| Cortisol (% change in ng/mL)3 | 0.99 (0.85, 1.16) | 1.01 (0.86, 1.19) | 0.99 (0.83, 1.17) |
| Cortisone (ng/mL) | -0.07 (-0.46, 0.32) | -0.01 (-0.42, 0.39) | -0.07 (-0.49, 0.34) |
| Na:K (% change in ratio)3 | 1.01 (0.95, 1.07) | 1.01 (0.96, 1.07) | 1.01 (0.95, 1.07) |

1General linear regression models were fitted to examine these associations.

Model 1 included adjustments for maternal age, parity, maternal education and ethnicity

Model 2 included adjustments for confounders in Model 1 + pre-pregnancy BMI

2Significant at *P*<0.05.

3Results for sodium, cortisol and Na:K have been exponentiated. 2hPG, 2 hour-post glucose challenge; FPG, fasting plasma glucose.