A nutritional supplement taken during preconception and pregnancy influences human milk macronutrients in women with overweight/obesity and gestational diabetes mellitus: A Randomized Controlled Trial

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Keywords: human milk, macronutrients, gestational diabetes mellitus, maternal BMI, maternal nutrition

Abstract

**Rational** Maternal overweight/obesity and gestational diabetes mellitus (GDM) are associated with an increased risk of their offspring developing overweight/obesity or type 2 diabetes later in life. However, the impact of maternal overweight/obesity and dysglycemia on human milk (HM) macronutrient composition is not well understood.

**Objective** Through a double-blind randomized controlled trial, we investigated the effects of maternal supplementation from preconception throughout pregnancy until birth on HM macronutrient concentrations, in association with maternal and infant factors including maternal pre-pregnancy body mass index (BMI) and GDM status. In addition, we aimed to characterize longitudinal changes in HM macronutrients.

**Methods** The control supplement contained calcium, iodine, iron, β-carotene, and folic acid. The intervention supplement additionally contained zinc, vitamins B2, B6, B12, D3, probiotics and myo-inositol. HM samples were collected across 7 time points from 1 week to 12 months from Singapore and New Zealand. HM macronutrient concentrations were measured using a MIRIS Human Milk Analyser. Potential differences in HM macronutrient concentrations were assessed using linear mixed models with a repeated measures design.

**Results** Overall, HM macronutrient concentrations were similar between control and intervention groups. Among the control group, overweight/obesity BMI and GDM were associated with higher HM fat and energy concentrations over the first 3 months. Such associations were not observed among the intervention group. Of note, mothers with GDM in the intervention group had lower HM fat by 10% (p=0.049) and energy by 6% (p=0.029) than mothers with GDM in the control group. Longitudinal changes in HM macronutrient concentrations over 12 months of lactation in New Zealand showed that HM fat and energy decreased in the first 6 months then increased until 12 months. HM lactose and crude protein gradually decreased from 1 week to 6 months then remained relatively constant until 12 months.

**Conclusions** Maternal overweight/obesity or GDM were associated with increased HM fat and energy levels. We speculate the intervention taken during preconception and pregnancy altered the impact of maternal BMI or GDM status on HM macronutrient composition. Further studies are required to identify the mechanisms underlying altered HM macronutrient concentration and to determine any long-term effects on offspring health.

Trial registration: ClinicalTrials.gov, identifier: NCT02509988, Universal Trial Number U1111‑1171-8056. Registered on 16 July 2015. This is an academic-led study by the EpiGen Global Research Consortium.

# Introduction

Human milk (HM) provides the essential nutrients and bioactive factors infants need for growth and development (1,2). The World Health Organization recommends infants to be exclusively breastfed for at least 6 months (3), which has been associated with long-term infant outcomes including lower risks of obesity (4,5) type 2 diabetes (6), infections (7), allergies, and asthma (8,9), and positive neurodevelopmental outcomes (10). Some HM components have been associated with specific outcomes. For example, HM oligosaccharides have been associated with altered risks of allergies or infections (11,12) and cognitive developmental scores in infants (13). Further, a recent pre-clinical study demonstrated that myo-inositol promotes neuronal connectivity, providing insights into the role of myo-inositol in infant brain development (14). HM macronutrient composition has been associated with infant body composition up to 12 months of age (15,16). However, there is limited and inconsistent evidence in this area thus requires further research.

In addition to bioactive compounds such as immunological components and growth and metabolic hormones, HM provides nutrients and energy for infant growth and development. HM contains approximately 3.8% fat, 7% lactose, and 1% protein, each contributing about 50%, 40-45%, and 5‑6% to total energy (17,18). Infant formula contains similar proportions of macronutrients as in HM with fat providing about 45-50% of total energy, carbohydrate 40-45%, and protein about 8-12% (18). While infant formula is a standardized solution over specific age ranges, HM is a dynamic compound, changing during a feed (19), throughout the day (20), and over the course of lactation (21). Moreover, HM macronutrient composition may vary according to a range of maternal and infant factors, but these associations are not well understood. With the exception of fatty acids, maternal diet is reported to have no association with HM macronutrients (22). Positive associations have been reported between maternal body mass index (BMI) and HM fat and energy (23–26), and between maternal age and HM fat and carbohydrate (27–30). In addition, negative associations have been reported between infant gestational age and HM protein (27,31), fat and lactose (21,30,32). There have been inconsistent observations on the influence of maternal GDM status (27,33,34), infant sex (26,35–38), parity (28,39), and mode of delivery (24,32,36,40) on HM macronutrient composition.

The Nutritional Intervention Preconception and During Pregnancy to Maintain Healthy Glucose Metabolism and Offspring Health (NiPPeR) study was a double-blind, randomized controlled trial investigating the effects of a micronutrient supplement during preconception and pregnancy on maternal pregnancy outcomes and infant growth (41). Adequate maternal micronutrient status during pregnancy and lactation is essential for both mothers and infants (42). Micronutrient supplementation during pregnancy has been associated with lower risks of adverse pregnancy outcomes (43). For example, folic acid supplementation decreased occurrence of neural tube defect in the offspring (44); a combined supplementation of folic acid and iron reduced the risk of post-partum hemorrhage (45); calcium supplementation lowered the risk of pre-eclampsia (46); and vitamin D supplementation was associated with reduced risks of pre-eclampsia and preterm birth (47). In our recent publications, we showed that micronutrient supplementation during pre-conception and pregnancy increased HM concentrations of zinc (48) and vitamin D (49) in the first 3 months of lactation. However, there remains a paucity of data on the potential impact of micronutrient supplementation during preconception and pregnancy on HM macronutrient composition during lactation. Therefore, the aim of this study was to describe HM macronutrient composition following micronutrient supplementation during preconception and pregnancy in association with maternal and infant factors including maternal pre-pregnancy BMI and GDM status.

# Materials and Methods

## Study design

A detailed protocol for the NiPPeR study (ClinicalTrials.gov, identifier: NCT02509988, Universal Trial Number U1111-1171-8056; registered on 16 July 2015) has been published previously (41). Briefly, the NiPPeR study was a double-blind, randomized, controlled trial investigating the effects of a nutritional supplement taken from preconception and during pregnancy on maternal pregnancy and infant outcomes. The control supplement comprised of micronutrients that are present in commonly used pregnancy supplements: calcium, iron, iodine, folic acid, and vitamin A. The NiPPeR intervention supplement additionally contained zinc, vitamin B2, vitamin B6, vitamin B12, vitamin D3, myo-inositol and probiotics (Table 1). The study supplements were packaged as a powder form in sachets and were taken twice daily, as a drink reconstituted with water. Adherence to the study protocol was assessed by sachet counting, with good adherence defined as at least 60% of the sachets consumed (50). The primary outcome of gestational glycaemia and the secondary outcome of GDM did not differ between control and intervention groups (50). The study was conducted in Southampton (UK), Singapore, and Auckland (New Zealand) and ethics approval was obtained at each site [Southampton – Health Research Authority National Research Ethics Service Committee South Central Research Ethics Committee (15/SC/0142); Singapore – the National Healthcare Group Domain Specific Review Board (2015/00205); and New Zealand – Northern A Health and Disability Ethics Committee (15/NTA/21)]. All participants provided written informed consent.

## Study participants

Participants were recruited by self-referral after study information was distributed through local and social media advertisements. The key inclusion criteria were women aged 18-38 years who were planning to conceive within 6 months. The full inclusion, exclusion and withdrawal criteria have been reported previously (41) and are provided in Supplementary Table 1. Eligible participants were randomised in a 1:1 ratio to either the control or the intervention group through the electronic study database (41), and stratified by site and ethnicity to ensure balanced allocation of participants.

## Human milk sample collection

HM samples were collected in Singapore until 3 months (July 2016 to March 2019) and in New Zealand until 12 months of lactation (May 2017 to November 2019); practical constraints precluded collection in the UK (Figure 1). Samples were collected at four time points common to both sites: 1 week (±3 days), 3 weeks (±5 days), 6 weeks (±5 days), and 3 months (±10 days). In New Zealand, there were additional HM collections at 6 months (±14) days, 9 months (±14 days), and 12 months (±14 days) (seven time points in total). In Singapore, samples were only collected until 3 months due to logistical constraints. Mothers were asked to refrain from breastfeeding for 2 hours prior to sample collection from the breast where samples would be collected. Under the supervision of trained staff, whole HM samples were collected in the morning from a single breast pumped for 15 minutes using an Ameda Lactaline breast pump (Ameda, Inc, Murarrie, Australia) until fully emptied. Following collection, HM samples were homogenised, then stored at –80ºC until analysis. HM samples were not collected if the mother had ceased breastfeeding, her milk supply was low, or there were complications with milk expression. Figure 1 shows the number of samples analysed at each time point.

## Human milk macronutrient analysis

HM fat, energy, lactose, and crude protein were quantified via infrared transmission spectroscopy using a Miris Human Milk Analyser (HMA) (Miris, Uppsala, Sweden) following the manufacturer’s protocol. 1-3 mL of frozen HM samples were slowly thawed overnight at 4ºC. Prior to analysis, they were warmed to 40ºC in a water bath and homogenized using a Miris Ultrasonic Processor (Miris, Uppsala, Sweden) at a processing time of 1.5 s/mL. Quality control was performed using a HM sample with known macronutrient composition after every 20 samples and clean and check performances were done every 10 samples.

## Statistical analysis

Descriptive statistics were calculated for maternal, infant, and birth-related characteristics. For intergroup comparisons, the independent samples *t-*test was used for continuous variables and Chi-square tests were used to compare categorical variables between randomization groups and between sites. For HM macronutrient measurements, samples with one or more macronutrient measurement value of ‘0’ were excluded from analysis due to a possible risk of sample dilution. In addition, we adopted a conservative approach to removing outliers from analysis, excluding values outside the mean ± 5 $×$ standard deviations (SD) (Supplementary Table 3).

Potential intervention effects on HM macronutrient concentrations were examined on the samples collected in the first 3 months of lactation only, collected in both Singapore and New Zealand. Key parameters included in linear multilevel models were randomization group, visit, their interaction term (group\*visit), study site, maternal pre-pregnancy BMI, infant gestational age at birth, and adherence to the study supplements as a continuous variable. Participant study ID number was also included as a random factor to account for the repeated measurements. Subgroup analyses were performed to examine potential intervention effects over the first 3 months of lactation separately for Singapore and New Zealand. Temporal changes in HM macronutrients from 1 week to 12 months of lactation are described for the New Zealand site only as samples from the later time points were not available in Singapore.

As a secondary analysis, potential interaction between the intervention and maternal metabolic status (pre-pregnancy BMI or GDM) on HM macronutrients were examined for the first 3 months of lactation in fully adjusted models. Pre-pregnancy BMI was defined using the WHO classification (51): underweight <18.5 kg/m2, normal weight 18.5–24.9 kg/m2, overweight 25.0–29.9 kg/m2, and obesity ≥30 kg/m2.

As an exploratory analysis, potential impacts of other maternal and infant factors on HM macronutrients were assessed. The mean HM macronutrient concentrations in the first 3 months were compared between binary categories of maternal ethnicity (Non-Asian vs Asian), maternal age (< 35 vs ≥ 35 years old), delivery mode (Vaginal vs Cesarean-section), parity (Primiparous vs Multiparous), infant sex (Male vs Female) and infant gestational age (Term/Post-term ≥ 37 weeks vs Preterm < 37 weeks). Interactions between a given maternal/infant factor and the intervention group were tested but none were significant.

Study outcomes are reported as the back-transformed least-square means (i.e., adjusted means) for each group or the adjusted mean difference (aMD) between groups and their respective 95% confidence intervals (CI). The aMD for back-transformed values represent proportional differences between the comparison groups. Statistical analysis was carried out using IBM SPSS Statistics for Windows, Version 26 (IBM Corp., Armonk, NY, USA) and SAS Version 9.4 (SAS Institute Inc., Cary, NC, USA). Graphs were created with GraphPad Prism version 9.3.1 (GraphPad Software, San Diego, California, USA). All statistical tests were two-sided with significance maintained at p<0.05, without adjustments for multiple comparisons or imputation of missing values.

# Results

## Study population

In total, 336 of 387 participants (86.8%) from Singapore and New Zealand sites who continued to the postpartum stage of the study provided at least one HM sample in the first 12 months of lactation (Figure 1). Maternal pre-pregnancy BMI (p=0.047) and passive smoking rates (p=0.035) were lower in the intervention group compared with control. Other baseline and perinatal characteristics, including GDM rates, were similar between control and intervention groups (Table 2). In Singapore, most participants were of Chinese ethnicity (78.5%), while in New Zealand, most were White Caucasian (75.8%) (p<0.001). Compared to the New Zealand cohort, the Singapore participants had lower pre-pregnancy BMI (p<0.001), a higher GDM rate (p<0.001), more vaginal deliveries (p=0.023), lower infant birth weight (p<0.001), and shorter gestation (p<0.001), but the incidence of preterm delivery was not different between the two sites (Supplementary Table 4).

## Impact of intervention on HM macronutrients

During the first 3 months of lactation, the mean HM macronutrient concentrations did not differ between the intervention and control groups with Singapore and New Zealand sites combined (Figure 2), and when each site was analysed separately (Supplementary Table 5).

## Maternal pre-pregnancy BMI and GDM status on HM macronutrients

Among the control group, HM from mothers with overweight/obesity BMI had higher fat [aMD (95% CI) = 1.11 (1.01, 1.21), p=0.023] and energy [aMD (95% CI) = 1.05 (1.01, 1.10), p=0.017] levels than mothers with under/normal weight BMI (Figure 3). Such differences were not observed in the intervention group. Similarly, among the control group, HM fat [aMD (95% CI) = 1.11 (1.01, 1.22), p=0.030] and energy [aMD (95% CI) = 1.06 (1.01, 1.11), p=0.011] levels were higher in mothers with GDM compared to those without GDM (Figure 4). Of note, mothers with GDM in the intervention group had lower HM fat [aMD (95% CI) = 0.90 (0.81, 1.00), p=0.049] and energy [aMD (95% CI) = 0.94 (0.90, 0.99), p=0.029] than mothers with GDM in the control group.

## Changes in HM macronutrients over time in New Zealand (0-12 months)

Among the New Zealand cohort, longitudinal changes in HM macronutrient concentrations were observed in the first 12 months of lactation (Figure 5; Supplementary Table 6). HM fat and energy followed a similar decreasing pattern in the first 6 months, after which they all increased until 12 months of lactation (Figure 5A–B). HM lactose gradually decreased from 1 week to 12 months of lactation but there was little intra-individual variation in absolute concentrations during this time (Figure 5C). HM crude protein continuously decreased from 1 week to 6 months, after which it remained relatively constant until 12 months of lactation (Figure 5D).

## Other maternal and infant factors and HM macronutrients

HM macronutrient concentrations were affected by some maternal factors (Supplementary Table 7). HM lactose was lower in milk from younger mothers (< 35 vs ≥ 35 years old, p=0.020) and energy content was greater following vaginal delivery (p=0.034). Also, energy (p=0.023) and crude protein (p=0.022) concentrations were higher following first childbirth compared to higher order of births (primiparous vs multiparous). HM macronutrient composition did not differ between maternal ethnicity (Non-Asian vs Asian), infant sex (Male vs Female), and infant gestational age (Term/Post-term vs Preterm).

1. **Discussion**

In our study, HM macronutrients were overall not influenced by the NiPPeR intervention supplement taken preconception and during pregnancy. However, in the subpopulation of control group mothers with overweight/obesity or GDM, fat and energy levels were higher compared to the under/normal weight or non-GDM mothers, respectively, over the first 3 months of lactation. Such differences were not observed among the intervention group. Furthermore, among mothers with GDM, the intervention group had lower HM fat and energy levels than those mothers in the control group. This suggests the impact of GDM status on HM macronutrients was altered by the NiPPeR intervention supplement.

Previous studies have reported that HM macronutrient composition is tightly regulated and is not strongly affected by maternal diet nutritional supplementation (2,25,52), with the exception of fatty acids (22). As such, the NiPPeR intervention supplement consumed in the pre-lactation period was not expected to strongly impact HM total fat, energy, lactose and protein concentrations. Some studies have reported a positive association between maternal BMI and HM fat and energy (23–26), as observed in the present study. The impact of maternal GDM on HM macronutrients is less well understood. Among women with GDM, some have observed higher carbohydrate in colostrum (33), energy in colostrum, transitional and mature milk (27), while others have reported lower fat and energy content in mature milk (34). Differences between study findings may be due to pre-analytical variations in HM collection protocols and processing. Fat is known to be the most dynamic component of HM but regulatory mechanisms underlying fat synthesis or transport in HM are not well understood. It has been speculated that metabolic dysregulation commonly reported in individuals with GDM, such as hyperglycemia, dyslipidemia and insulin resistance, may contribute to increased HM fat in these mothers (53,54). In the current study, using standardized collection methods, GDM status was associated with higher HM fat and energy levels in the control group but not in the intervention group. This suggests that the associations between GDM and HM macronutrients could have been modified by some components in the NiPPeR intervention supplement. Previous studies have observed that supplementation of myoinositol (55–57), probiotics (58–61), and zinc (62,63) for 6-8 weeks in women already diagnosed with GDM at 24-28 weeks of gestation, improved glycemic control in these women, as reflected in lower maternal circulating insulin, glucose, triglycerides, total cholesterol and LDL-cholesterol and increased insulin sensitivity. In the current study, we speculate that myo-inositol, probiotics, and zinc components in the NiPPeR intervention supplement, postulated to act as insulin sensitizers, modified glucose or lipid metabolism in these mothers, leading to altered HM macronutrient composition. Further studies are required to assess the potential benefits of lower HM fat and energy and infant outcomes, particularly as relates to growth during infancy and adiposity later in life.

The average HM fat, energy, lactose and protein concentrations over 12 months observed in the current study are comparable to those reported previously: fat 3.0–4.0 g/100mL, energy 61–65 kcal/100mL, lactose 6.6–7.1 g/100mL, and protein 0.9–1.4 g/100mL (64–66). Further observed in the current study, HM macronutrients displayed various patterns of change over 12 months of lactation. As observed in previous studies, HM lactose remained relatively constant (19,64,67), and protein decreased (19,32,64,68) until 6 months. Conversely, HM fat initially increased until 3 months, decreased from 3 to 6 months, then increased again from 6 to 12 months of lactation with HM energy content following a similar trajectory. Others have also observed a decrease in HM fat in early lactation followed by an increase in later stages of lactation (67,69), reporting a positive correlation between HM fat and lactation stage (70). It has been suggested that such changes in HM fat are related to adaptation to changes in infant feeding and energy requirements during development. From about 6 months of age, infants start eating solid foods and breastfeeding becomes complementary. As a result, a reduction in milk volume could be counterbalanced by an increase in fat content to provide sufficient energy for the infant.

Previous studies have investigated the potential influences of maternal ethnicity (17), maternal age (27–30), infant gestational age (21,27,30–32), infant sex (26,35–38), parity (28,39), and mode of delivery (24,32,36,40) on HM macronutrients. However, the results are conflicting, and the underlying mechanisms are not well understood. It has been suggested that anatomical changes of the mammary gland with maternal age (30,71), and successive pregnancies (28), different hormonal releases associated with mode of delivery (72) and gestational period (73), and different energy demands according to infant sex (35) could contribute to altered HM macronutrient composition. In the current study, we did not observe differences in HM macronutrients according to maternal ethnicity, infant sex, or gestational age. Although we observed a relationship between maternal age and lactose, delivery mode and energy, and parity and energy and crude protein, the magnitude of differences ranged from 2% to 5% in the first 3 months of lactation. While these were statistically significant observations, further research is required to understand the physiological significance of such small absolute changes in HM macronutrient levels in relation to infant outcomes.

# Strengths and limitations

This study has a few strengths to note: i) HM macronutrient composition was examined from a large cohort of diverse ethnic groups, ii) standardized HM sample collection, processing and analytic methods were used, and iii) the visit windows were tightly controlled, each time point being a distinctive stage of lactation. As longitudinal samples could not be collected from all participants, a repeated measures design was used for statistical analysis. There are some limitations to be acknowledged in the present study. The IADPSG criteria was used for determining GDM status and site-specific diagnostic criteria were not considered. Also, treatment for GDM was an independent decision by the local clinicians and specific for each site. While diet treatment was more common in Singapore, medication treatment with insulin or metformin was more common in New Zealand. Due to the imbalances in sample sizes for treatment types between control and intervention groups, and between sites, any potential impact of GDM treatment modality on HM macronutrients could not be assessed. Finally, infants of this cohort were born generally healthy, none under 28 weeks gestation (extremely preterm) and only 24 infants (7.1%) had low birth weight (< 2500 g). This precluded investigation of potential influences of extreme infant characteristics on HM macronutrients.

# Conclusions

In this study, we observed that maternal overweight/obesity and GDM were associated with increased HM fat and energy levels among controls but not in the intervention group. This suggests that the intervention supplement during preconception and pregnancy altered the impact of a high maternal BMI and GDM status on HM macronutrient composition. Further studies are required to identify the components in the intervention supplement responsible for altering HM macronutrient composition, characterize the underlying mechanisms, and determine any long-term effects on offspring health.

# Conflict of Interest

KMG, S-YC, and WSC are part of an academic consortium that has received grants from Société des Produits Nestlé SA relating to the submitted work, and from Abbott Nutrition, Danone, and BenevolentAI Bio Ltd. outside the submitted work. SMH, JGBD, MHV, SD, FH, KMG, S-YC, SKT, and WSC are co-inventors on patent filings by Société des Produits Nestlé SA relating to the NiPPeR intervention or its components. FH, SD, and SKT are employees of Société des Produits Nestlé SA.

# Author Contributions

KMG, S-YC, and WSC led the design of the original study. The present sub-study was developed and undertaken by SMH, JGBD, MHV, SD, FH, SKT, and WSC. SMH performed the laboratory analyses. SMH and JGBD performed the statistical analyses. SMH led the manuscript wring. SKT and WSC supervised all aspects of the present study. All authors contributed to interpretation, manuscript revision, read and approved the final version.

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# Funding

Public good funding for this investigator-led study is through the Medical Research Council (U.K.) (MRC) as part of an MRC award to the MRC Lifecourse Epidemiology Unit (MC\_UU\_12011/4), the Singapore National Research Foundation, the National Medical Research Council (SG) (NMRC) (NMRC/TCR/012-NUHS/2014), the National University of Singapore (NUS), the Agency for Science, Technology and Research (SG) as part of the Growth, Development and Metabolism Programme of the Singapore Institute for Clinical Sciences (H17/01/a0/005), and as part of Gravida, a New Zealand Government Centre of Research Excellence. KMG is supported by the National Institute for Health Research (NIHR Senior Investigator (NF-SI-0515-10042) and NIHR Southampton Biomedical Research Centre (NIHR203319)), and the European Union (Erasmus+ Programme ImpENSA 598488-EPP-1-2018-1-DE-EPPKA2-CBHE-JP). S-YC is supported by a Singapore NMRC Clinician Scientist Award (NMRC/CSA-INV/0010/2016; MOH-CSAINV19nov-0002). Funding for provision of the intervention and control drinks and to cover aspects of the fieldwork for the study was provided by Société des Produits Nestlé SA under a research agreement with the University of Southampton, Auckland UniServices Ltd., Singapore Institute for Clinical Sciences, National University Hospital Singapore PTE Ltd., and NUS. For the purpose of Open Access, the authors have applied a Creative Commons Attribution (CC BY) license to any Author Accepted Manuscript version arising from this submission.

# Acknowledgments

SMH is currently receiving University of Auckland Doctoral Scholarship. The authors thank the participants and their families for their enthusiastic involvement in the study, the study research staff and hospital clinical staff at participating centres and operational support staff for contributions to the trial, and the members of the independent data monitoring and safety committee for invaluable contributions and for overseeing the conduct of the trial.

# Data Availability Statement

The datasets presented in this article are not publicly available because public sharing of the data was not part of the original participant informed consent. Requests to access the datasets should be directed to the corresponding author.

# References

1. Andreas NJ, Kampmann B, Mehring Le-Doare K. Human breast milk: A review on its composition and bioactivity. Early Hum Dev. 2015;91(11):629–35.

2. Ballard O, Morrow AL. Human Milk Composition: Nutrients and Bioactive Factors. Pediatr Clin North Am. 2013;60(1):49–74.

3. World Health Organization. Global strategy for infant and young child feeding. World Health Organization; 2003.

4. Yan J, Liu L, Zhu Y, Huang G, Wang PP. The association between breastfeeding and childhood obesity: a meta-analysis. BMC Public Health. 2014;14(1):1267.

5. Owen CG, Martin RM, Whincup PH, Smith GD, Cook DG. Effect of infant feeding on the risk of obesity across the life course: A quantitative review of published evidence. Pediatrics. 2005;115(5):1367–77.

6. Horta BL, Loret De Mola C, Victora CG. Long-term consequences of breastfeeding on cholesterol, obesity, systolic blood pressure and type 2 diabetes: A systematic review and meta-analysis. Acta Paediatr Int J Paediatr. 2015;104:30–7.

7. Ladomenou F, Moschandreas J, Kafatos A, Tselentis Y, Galanakis E. Protective effect of exclusive breastfeeding against infections during infancy: A prospective study. Arch Dis Child. 2010;95(12):1004–8.

8. Lodge C, Tan D, Lau M, Dai X, Tham R, Lowe A, et al. Breastfeeding and asthma and allergies: A systematic review and meta-analysis. Acta Paediatr Int J Paediatr. 2015;104:38–53.

9. Oddy WH. Breastfeeding, Childhood Asthma, and Allergic Disease. Ann Nutr Metab. 2017;70(2):26–36.

10. Horta BL, Loret De Mola C, Victora CG. Breastfeeding and intelligence: A systematic review and meta-analysis. Acta Paediatr Int J Paediatr. 2015;104:14–9.

11. Doherty AM, Lodge CJ, Dharmage SC, Dai X, Bode L, Lowe AJ. Human milk oligosaccharides and associations with immune-mediated disease and infection in childhood: A systematic review. Front Pediatr. 2018;20(6):91.

12. Masi AC, Embleton ND, Lamb CA, Young G, Granger CL, Najera J, et al. Human milk oligosaccharide DSLNT and gut microbiome in preterm infants predicts necrotising enterocolitis. Gut. 2021;70(12):2273–82.

13. Berger PK, Plows JF, Jones RB, Alderete TL, Yonemitsu C, Poulsen M, et al. Human milk oligosaccharide 2’-fucosyllactose links feedings at 1 month to cognitive development at 24 months in infants of normal and overweight mothers. PLoS One. 2020;15(2):1–12.

14. Paquette AF, Carbone BE, Vogel S, Israel E, Maria SD, Patil NP, et al. The human milk component myo-inositol promotes neuronal connectivity. Proc Natl Acad Sci. 2017;120(30):e2221413120.

15. de Fluiter KS, Kerkhof GF, van Beijsterveldt IALP, Breij LM, van de Heijning BJM, Abrahamse-Berkeveld M, et al. Longitudinal human milk macronutrients, body composition and infant appetite during early life. Clin Nutr. 2021;40(5):3401–8.

16. Vieira Queiroz De Paula M, Grant M, Lanigan J, Singhal A. Does human milk composition predict later risk of obesity? A systematic review. BMC Nutr. 2023;9(1):1–10.

17. Jenness R. The composition of human milk. Semin Perinatol. 1979;3(3):225—239.

18. Joeckel RJ, Phillips SK. Overview of infant and pediatric formulas. Nutr Clin Pract. 2009;24(3):356–62.

19. Saarela T, Kokkonen J, Koivisto M. Macronutrient and energy contents of human milk fractions during the first six months of lactation. Acta Paediatr. 2005;94(9):1176–81.

20. Kent JC, Mitoulas LR, Cregan MD, Ramsay DT, Doherty DA, Hartmann PE. Volume and frequency of breastfeedings and fat content of breast milk throughout the day. Pediatrics. 2006;117(3):e387-95.

21. Bauer J, Gerss J. Longitudinal analysis of macronutrients and minerals in human milk produced by mothers of preterm infants. Clin Nutr. 2011;30(2):215–20.

22. Aumeistere L, Ciproviča I, Zavadska D, Andersons J, Volkovs V, Ceļmalniece K. Impact of maternal diet on human milk composition among lactating women in Latvia. Med. 2019;55(5):1–12.

23. Leghi GE, Netting MJ, Middleton PF, Wlodek ME, Geddes DT, Muhlhausler BS. The impact of maternal obesity on human milk macronutrient composition: A systematic review and meta-analysis. Nutrients. 2020;12(4):934.

24. Burianova I, Bronsky J, Pavlikova M, Janota J, Maly J. Maternal body mass index, parity and smoking are associated with human milk macronutrient content after preterm delivery. Early Hum Dev. 2019;137:104832.

25. Bzikowska-Jura A, Czerwonogrodzka-Senczyna A, Olędzka G, Szostak-Węgierek D, Weker H, Wesołowska A. Maternal nutrition and body composition during breastfeeding: Association with human milk composition. Nutrients. 2018;10(10):1379.

26. Bzikowska-Jura A, Sobieraj P, Szostak-Węgierek D, Wesołowska A. Impact of infant and maternal factors on energy and macronutrient composition of human milk. Nutrients. 2020;12(9):1–14.

27. Dritsakou K, Liosis G, Valsami G, Polychronopoulos E, Skouroliakou M. The impact of maternal- and neonatal-associated factors on human milk’s macronutrients and energy. J Matern Neonatal Med. 2017;30(11):1302–8.

28. Bachour P, Yafawi R, Jaber F, Choueiri E, Abdel-Razzak Z. Effects of smoking, mother’s age, body mass index, and parity number on lipid, protein, and secretory immunoglobulin a concentrations of human milk. Breastfeed Med. 2012;7(3):179–88.

29. Lubetzky R, Sever O, Mimouni FB, Mandel D. Human Milk Macronutrients Content: Effect of Advanced Maternal Age. Breastfeed Med. 2015;10(9):433–6.

30. Hausman Kedem M, Mandel D, Domani KA, Mimouni FB, Shay V, Marom R, et al. The effect of advanced maternal age upon human milk fat content. Breastfeed Med. 2013;8(1):116–9.

31. Gidrewicz DA, Fenton TR. A systematic review and meta-analysis of the nutrient content of preterm and term breast milk. BMC Pediatr. 2014;14(1):1–14.

32. Fumeaux CJF, Garcia-Rodenas CL, De Castro CA, Courtet-Compondu MC, Thakkar SK, Beauport L, et al. Longitudinal analysis of macronutrient composition in preterm and term human milk: A prospective cohort study. Nutrients. 2019;11(7):1525.

33. Korkut S, Köse Çetinkaya A, Işlk S, Özel S, Gökay N, Sahin A, et al. Macronutrient Composition of Colostrum in Mothers with Gestational Diabetes Mellitus. Breastfeed Med. 2022;17(4):322–5.

34. Shapira D, Mandel D, Mimouni FB, Moran-Lev H, Marom R, Mangel L, et al. The effect of gestational diabetes mellitus on human milk macronutrients content. J Perinatol. 2019;39(6):820–3.

35. Powe CE, Knott CD, Conklin-Brittain N. Infant sex predicts breast milk energy content. Am J Hum Biol. 2010;22(1):50–4.

36. Hahn WH, Song JH, Song S, Kang N mi. Do gender and birth height of infant affect calorie of human milk? An association study between human milk macronutrient and various birth factors. J Matern Neonatal Med. 2017;30(13):1608–12.

37. Quinn EA. No evidence for Sex Biases in Milk Macronutrients, Energy, or Breastfeeding Frequency in a Sample of Filipino Mothers. Am J Phys Anthropol. 2013;152(2):209–16.

38. Hosseini M, Valizadeh E, Hosseini N, Khatibshahidi S, Raeisi S. The Role of Infant Sex on Human Milk Composition. Breastfeed Med. 2020;15(5):341–6.

39. Nommsen LA, Lovelady CA, Heinig MJ, Lönnerdal B, Dewey KG. Determinants of energy, protein, lipid, and lactose concentrations in human milk during the first 12 mo of lactation: the DARLING Study. Am J Clin Nutr. 1991 Feb 1;53(2):457–65.

40. Dizdar EA, Sari FN, Degirmencioglu H, Canpolat FE, Oguz SS, Uras N, et al. Effect of mode of delivery on macronutrient content of breast milk. J Matern Neonatal Med. 2014;27(11):1099–102.

41. Godfrey KM, Cutfield W, Chan SY, Baker PN, Chong YS, Aris IBM, et al. Nutritional Intervention Preconception and During Pregnancy to Maintain Healthy Glucose Metabolism and Offspring Health (“NiPPeR”): Study protocol for a randomised controlled trial. Trials. 2017;18(1):1–12.

42. Allen LH. Multiple micronutrients in pregnancy and lactation: An overview. Am J Clin Nutr. 2005;81(5):1206–12.

43. Parisi F, di Bartolo I, Savasi VM, Cetin I. Micronutrient supplementation in pregnancy: Who, what and how much? Obstet Med. 2019;12(1):5–13.

44. De-Regil LM, Peña-Rosas JP, Fernández-Gaxiola AC, Rayco-Solon P. Effects and safety of periconceptional oral folate supplementation for preventing birth defects. Cochrane Database Syst Rev. 2015;2015(12).

45. Christian P, Khatry SK, LeClerq SC, Dali SM. Effects of prenatal micronutrient supplementation on complications of labor and delivery and puerperal morbidity in rural Nepal. Int J Gynecol Obstet. 2009;106(1):3–7.

46. Khaing W, Vallibhakara SAO, Tantrakul V, Vallibhakara O, Rattanasiri S, McEvoy M, et al. Calcium and vitamin D supplementation for prevention of preeclampsia: A systematic review and network meta-analysis. Nutrients. 2017;9(10):1–23.

47. De-Regil L, Palacios C, Lombardo L, Peña-Rosas J. Vitamin D supplementation for women during pregnancy: Summary of a Cochrane review. Cochrane Database Syst Rev. 2016;1.

48. Han SM, Devaraj S, Derraik JGB, Vickers MH, Huang F, Dubascoux S, et al. A nutritional supplement containing zinc during preconception and pregnancy increases human milk zinc concentrations. Front Nutr. 2023;10(9):1034828.

49. Han SM, Huang F, Derraik JGB, Vickers MH, Redeuil K, Campos-Giménez E, et al. A micronutrient supplement during preconception and pregnancy increases human milk vitamin D but not B vitamin concentrations. Clin Nutr. Forthcoming 2023.

50. Godfrey KM, Barton SJ, El-Heis S, Kenealy T, Nield H, Baker PN, et al. Myo-Inositol, Probiotics, and Micronutrient Supplementation From Preconception for Glycemia in Pregnancy: NiPPeR International Multicenter Double-Blind Randomized Controlled Trial. Diabetes Care. 2021;44(5):1091–9.

51. Balasubramanian S, Ganesh R. Vitamin D deficiency in exclusively breast-fed infants. Indian J Med Res. 2008;127(3):250–5.

52. Bravi F, Wiens F, Decarli A, Pont AD, Agostoni C, Ferraroni M. Impact of maternal nutrition on breast-milk composition: a systematic review. Am J Clin Nutr. 2016;104(3):646–62.

53. Mohammad MA, Sunehag AL, Haymond MW. Effect of dietary macronutrient composition under moderate hypocaloric intake on maternal adaptation during lactation. Am J Clin Nutr. 2009;89(6):1821–7.

54. Fujimori M, França EL, Fiorin V, Morais TC, Honorio-França AC, de Abreu LC. Changes in the biochemical and immunological components of serum and colostrum of overweight and obese mothers. BMC Pregnancy Childbirth. 2015;15(1):1–8.

55. Corrado F, D’Anna R, di Vieste G, Giordano D, Pintaudi B, Santamaria A, et al. The effect of myoinositol supplementation on insulin resistance in patients with gestational diabetes. Diabet Med. 2011;28(8):972–5.

56. Fraticelli F, Celentano C, Zecca I Al, Di Vieste G, Pintaudi B, Liberati M, et al. Effect of inositol stereoisomers at different dosages in gestational diabetes: an open-label, parallel, randomized controlled trial. Acta Diabetol. 2018;55(8):805–12.

57. D’Anna R, Corrado F, Loddo S, Gullo G, Giunta L, Di Benedetto A. Myoinositol plus α-lactalbumin supplementation, insulin resistance and birth outcomes in women with gestational diabetes mellitus: a randomized, controlled study. Sci Rep. 2021;11(1):1–5.

58. Dolatkhah N, Hajifaraji M, Abbasalizadeh F, Aghamohammadzadeh N, Mehrabi Y, Abbasi MM. Is there a value for probiotic supplements in gestational diabetes mellitus? A randomized clinical trial. J Heal Popul Nutr. 2015;33(1):1–8.

59. Karamali M, Dadkhah F, Sadrkhanlou M, Jamilian M, Ahmadi S, Tajabadi-Ebrahimi M, et al. Effects of probiotic supplementation on glycaemic control and lipid profiles in gestational diabetes: A randomized, double-blind, placebo-controlled trial. Diabetes Metab. 2016;42(4):234–41.

60. Babadi M, Khorshidi A, Aghadavood E, Samimi M, Kavossian E, Bahmani F, et al. The Effects of Probiotic Supplementation on Genetic and Metabolic Profiles in Patients with Gestational Diabetes Mellitus: a Randomized, Double-Blind, Placebo-Controlled Trial. Probiotics Antimicrob Proteins. 2019;11(4):1227–35.

61. Ebrahimi FS, Rad AH, Mosen M, Abbasalizadeh F, Tabrizi A, Khalili L. Effect of L. acidophilus and B. lactis on blood glucose in women with gestational diabetes mellitus: A randomized placebo-controlled trial. Diabetol Metab Syndr. 2019;11(1):1–7.

62. Karamali M, Heidarzadeh Z, Seifati SM, Samimi M, Tabassi Z, Hajijafari M, et al. Zinc supplementation and the effects on metabolic status in gestational diabetes: A randomized, double-blind, placebo-controlled trial. J Diabetes Complications. 2015;29(8):1314–9.

63. Ostadmohammadi V, Samimi M, Mobini M, Zarezade Mehrizi M, Aghadavod E, Chamani M, et al. The effect of zinc and vitamin E cosupplementation on metabolic status and its related gene expression in patients with gestational diabetes. J Matern Neonatal Med. 2019;32(24):4120–7.

64. Chang N, Jung JA, Kim H, Jo A, Kang S, Lee SW, et al. Macronutrient composition of human milk from Korean mothers of full term infants born at 37-42 gestational weeks. Nutr Res Pract. 2015;9(4):433–8.

65. Wojcik KY, Rechtman DJ, Lee ML, Montoya A, Medo ET. Macronutrient Analysis of a Nationwide Sample of Donor Breast Milk. J Am Diet Assoc. 2009;109(1):137–40.

66. Cooper AR, Barnett D, Gentles E, Cairns L, Simpson JH. Macronutrient content of donor human breast milk. Arch Dis Child Fetal Neonatal Ed. 2013;98(6):2012–4.

67. Thakkar SK, Giuffrida F, Cristina CH, De Castro CA, Mukherjee R, Tran LA, et al. Dynamics of human milk nutrient composition of women from singapore with a special focus on lipids. Am J Hum Biol. 2013;25(6):770–9.

68. Yang T, Zhang Y, Ning Y, You L, Ma D, Zheng Y, et al. Breast milk macronutrient composition and the associated factors in urban Chinese mothers. Chin Med J (Engl). 2014;127(9):1721–5.

69. Mitoulas LR, Kent JC, Cox DB, Owens RA, Sherriff JL, Hartmann PE. Variation in fat, lactose and protein in human milk over 24h and throughout the first year of lactation. Br J Nutr. 2002;88(1):29–37.

70. Czosnykowska-łukacka M, Królak-Olejnik B, Orczyk-Pawiłowicz M. Breast milk macronutrient components in prolonged lactation. Nutrients. 2018;10(12):1–15.

71. Stone J, Warren RML, Pinney E, Warwick J, Cuzick J. Determinants of percentage and area measures of mammographic density. Am J Epidemiol. 2009;170(12):1571–8.

72. Nissen E, Uvnäs-Moberg K, Svensson K, Stock S, Widström AM, Winberg J. Different patterns of oxytocin, prolactin but not cortisol release during breastfeeding in women delivered by Caesarean section or by the vaginal route. Early Hum Dev. 1996;45(1–2):103–18.

73. Léké A, Grognet S, Deforceville M, Goudjil S, Chazal C, Kongolo G, et al. Macronutrient composition in human milk from mothers of preterm and term neonates is highly variable during the lactation period. Clin Nutr Exp. 2019;26:59–72.

74. Department of Health. Dietary reference values : a guide. London: HMSO Publications Centre; 1991.

75. Dietetics Department NUH. Vitamins & Minerals Chart. National University Hospital. Singapore; 2006.

76. National Health and Medical Research Council, Australian Government Department of Health and Ageing, New Zealand Ministry of Health. Nutrient Reference Values for Australia and New Zealand Including Recommended Dietary Intakes. Canberra: National Health and Medical Research Council; 2006.

77. Metzger BE. International Association of Diabetes and Pregnancy Study Groups recommendations on the diagnosis and classification of hyperglycemia in pregnancy. Diabetes Care. 2010;33(3):676–82.

78. Cole TJ, Williams AF, Wright CM. Revised birth centiles for weight, length and head circumference in the UK-WHO growth charts. Ann Hum Biol. 2011;38(1):7–11.

79. Pike KC, Crozier SR, Lucas JSA, Inskip HM, Robinson S, Roberts G, et al. Patterns of fetal and infant growth are related to atopy and wheezing disorders at age 3 years. Thorax. 2010;65(12):1099–106.

**Figure 1.** CONSORT diagram for the number of human milk (HM) samples analysed for macronutrients in the NiPPeR study.

Reasons for exclusion during the preconception phase have been published previously (50), while reasons for exclusion during pregnancy and birth in Singapore (SGP) and New Zealand (NZL) are provided in Supplementary Table 2. ‡There were no HM samples collected in the United Kingdom (UK), so all participants from that site were excluded from this diagram. \* Number of participants who provided at least one HM sample during the first 12 months of lactation. † Number of participants who provided at least one HM sample during the first 3 months of lactation.

**Figure 2.** Macronutrient concentrations in human milk (HM) in control and intervention groups in the NiPPeR study, during the first 3 months of lactation: (A) fat, (B) energy, (C) lactose, and (D) crude protein. Data are the least-square means (i.e., adjusted means) for each group, adjusted for randomisation group, visit, an interaction term (group\*visit), study site, maternal pre-pregnancy BMI, gestational age at birth, and adherence. Error bars represent the respective 95% confidence intervals.

**Figure 3.** Average macronutrient concentrations in human milk (HM) in control and intervention groups by maternal BMI status in the NiPPeR study over first 3 months of lactation: (A) fat, (B) energy, (C) lactose, and (D) crude protein. Data are the least-square means (i.e., adjusted means) for each group, adjusted for randomisation group, BMI status, group\*BMI interaction term, visit, study site, gestational age at birth, and adherence. Error bars represent the respective 95% confidence intervals. \*p<0.05.

**Figure 4.** Average macronutrient concentrations in human milk (HM) in control and intervention groups by maternal GDM status in the NiPPeR study over first 3 months of lactation: (A) fat, (B) energy, (C) lactose, and (D) crude protein. Data are the least-square means (i.e., adjusted means) for each group, adjusted for randomisation group, GDM status, group\*GDM interaction term, visit, study site, gestational age at birth, and adherence. Error bars represent the respective 95% confidence intervals. \*p<0.05.

**Figure 5.** Macronutrient concentrations in human milk (HM) in control and intervention groups in New Zealand in the NiPPeR study during 12 months of lactation: (A) fat, (B) energy, (C) lactose, and (D) crude protein. Data are the least-square means (i.e., adjusted means) for each group, adjusted for randomisation group, visit, an interaction term (group\*visit), maternal pre-pregnancy BMI, gestational age at birth, and adherence. Error bars represent the respective 95% confidence intervals. \*p<0.05 for a difference between intervention and control groups at a given time point.

**Table 1.** Detailed nutrient composition of the intervention and control drinks in the NiPPeR study.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Group** | **Nutrient** | **Intervention** | **Control** | **Daily dose** | **Recommended range#** |
| Minerals | Calcium | ✓ | ✓ | 150 mg | 700 – 1300 mg |
| Iodine | ✓ | ✓ | 150 µg | 140 – 220 μg |
| Iron | ✓ | ✓ | 12 mg | 14.8 – 27 mg |
| Zinc | ✓ | 🗶 | 10 mg | 7 – 15 mg |
| Vitamins | A (β-carotene) | ✓ | ✓ | 720 µg | 700 – 750 μg |
| B2 (Riboflavin) | ✓ | 🗶 | 1.8 mg | 1.38 – 1.46 mg |
| B6 (Pyridoxine) | ✓ | 🗶 | 2.6 mg | 1.2 – 1.9 mg |
| B9 (Folic acid) | ✓ | ✓ | 400 µg | 300 – 600 μg |
| B12 (Cobalamin) | ✓ | 🗶 | 5.2 µg | 1.5 – 2.6 μg |
| D3(Cholecalciferol) | ✓ | 🗶 | 400 IU (10 μg) | 5 – 10 μg |
| Other | Myo-inositol | ✓ | 🗶 | 4 g | n/a |
| *Lactobacillus rhamnosus* \* | ✓ | 🗶 | >1 × 109 CFU | n/a |
| *Bifidobacterium animalis* ssp. *lactis* † | ✓ | 🗶 | >1 × 109 CFU | n/a |

# Recommended ranges for daily intake during pregnancy according to the reference nutrient intake for the UK (74), recommended dietary allowance for Singapore (75), and recommended daily intake for New Zealand (76).

**\*** NCC 4007 (CGMCC 1.3724)

† NCC 2818 (CNCM I-3446)

Abbreviations: CFU, colony-forming units; n/a, not applicable. **Table 2.** Baseline and perinatal characteristics of control and intervention groups in the NiPPeR study who provided at least one human milk sample in the first 12 months of lactation.

|  |  |  |
| --- | --- | --- |
|  | **Overall (n = 336)** | **Pearson Chi-Square** **or T-test p-value** |
| **Control** | **Intervention** |
| n | 169 (50.3%) | 167 (49.7%) |  |
| Adherence (%) | 89.2 (82.9 – 95.9) | 90.4 (82.9 – 96.0) | n.s. |
| Duration of supplementation (days) | 405.2 ± 105.0 | 393.4 ± 98.2 | n.s. |
| Ethnicity | n.s |
|  Caucasian | 69 (40.8%) | 66 (39.5%) |
|  Chinese | 70 (41.4%) | 69 (41.3%) |
|  South Asian | 10 (5.9%) | 10 (6.0%) |
|  Malay | 10 (5.9%) | 10 (6.0%) |
|  Other | 10 (5.9%) | 12 (7.2%) |
| Age at delivery (years) | 31.9 ± 2.9 | 32.3 ± 3.2 | n.s. |
|  < 35 | 145 (85.8%) | 130 (77.8%) | n.s |
|  ≥ 35 | 24 (14.2%) | 37 (22.2%) |
| BMI (kg/m2) | 24.4 ± 5.2 | 23.3 ± 4.4 | 0.047 |
|  Under/normal weight | 115 (68.0%) | 128 (76.6%) | n.s. |
|  Overweight | 31 (18.3%) | 25 (15.0%) |
|  Obesity | 23 (13.6%) | 13 (7.8%) |
|  Missing | – | 1 (0.6%) |
| Highest level of education | n.s. |
|  Bachelor’s degree or higher | 136 (80.5%) | 135 (80.8%) |
|  Lesser qualification\* | 33 (19.5%) | 32 (19.2%) |
| Household income quintile | n.s. |
|  5 (lowest) | 4 (2.4%) | 1 (0.6%) |
|  4 | 12 (7.1%) | 16 (9.6%) |
|  3 | 43 (25.4%) | 43 (25.7%) |
|  2 | 60 (35.5%) | 55 (32.9%) |
|  1 (highest) | 44 (26.0%) | 42 (25.1%) |
|  Missing | 6 (3.6%) | 10 (6.0%) |
| Smoking during pregnancy | 0.035 |
|  None | 133 (78.7%) | 148 (88.6%) |
|  Passive | 33 (19.5%) | 16 (9.6%) |
|  Active | 3 (1.8%) | 3 (1.8%) |
| GDM  | n.s. |
|  No GDM | 123 (72.8%) | 119 (71.3%) |
|  GDM | 41 (23.4%) | 42 (25.1%) |
|  Excluded | 5 (3.0%) | 6 (3.6%) |
| Mode of delivery | n.s. |
|  Vaginal delivery | 124 (73.4%) | 119 (71.3%) |
|  Caesarean section | 45 (26.6%) | 48 (28.7%) |
| Birth weight (kg) | 3.24 ± 0.54 | 3.23 ± 0.53 | n.s. |
|  Appropriate for gestational age | 143 (84.6%) | 142 (85.0%) | n.s. |
|  Large for gestational age | 10 (5.9%) | 6 (3.6%) |
|  Small for gestational age | 16 (9.5%) | 19 (11.4%) |
| Gestational age (weeks) | 39.2 ± 1.6 | 39.2 ± 1.5 | n.s. |
|  Preterm  | 14 (8.3%) | 11 (6.6%) | n.s. |
|  Term or post-term | 155 (91.7%) | 156 (93.4%) |
| Parity | n.s |
|  Primiparous | 113 (66.9%) | 95 (56.9%) |
|  Multiparous | 56 (33.1%) | 72 (43.1%) |
| Infant sex | n.s. |
|  Male | 75 (44.4%) | 79 (47.3%) |
|  Female | 94 (55.6%) | 88 (52.7%) |

Data are n (%), mean ± standard deviation (SD), or median (Q1 – Q3). Adherence to the study protocol was determined by sachet counting. Duration of supplementation calculated by counting the number of days from randomization date to delivery date. Body mass index (BMI) status was defined as per WHO: under/normal weight <25.0 kg/m2, overweight 25.0–29.99 kg/m2, obesity ≥30.0 kg/m2. Gestational diabetes mellitus (GDM) was defined by International Association of Diabetes and Pregnancy Study Groups criteria (77). Birth weight categories determined by Royal College of Paediatrics and Child Health 2009 U.K.-World Health Organization growth charts.(78). Gestational age was determined using a pre-specified algorithm as previously described (79) with preterm defined as birth <37 weeks of gestation, and term or post-term as birth at ≥37 weeks of gestation.

**\*** Including incomplete and complete high school qualifications, and other tertiary level qualifications below bachelors (e.g., diploma or certificate).

n.s., not statistically significant.