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Breast milk nutrient content and infancy growth

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Abstract

Aim: Benefits of human breast milk (HM) in avoiding rapid infancy weight-gain and later obesity could relate to its nutrient content. We tested the hypothesis that differential HM total calorie content (TCC) or macronutrient contents may be associated with infancy growth. **Methods:** HM hindmilk samples were collected at ages 4-8 weeks from 614 mothers participating in a representative birth cohort, with repeated infancy anthropometry. HM triglyceride (fat), lipid analytes and lactose (carbohydrate) were measured by ^{1}H-NMR, and protein content by the Dumas method. TCC and %macronutrients were determined. **Results:** In 614 HM samples, fat content was: [median(IQR)]:2.6 (1.7-3.6)g/100mls, carbohydrate:8.6 (8.2-8.8)g/100mls, protein:1.2 (1.1-1.2)g/100mls; TCC:61.8 (53.7-71.3)kcal/100mls. HM of mothers exclusively breast-feeding vs. mixed-feeding was more calorific with higher %fat, lower %carbohydrate and lower %protein. Higher HM TCC was associated with lower 12m body mass index (BMI)/adiposity, and lower 3-12m gains in weight/BMI. HM %fat was inversely related to 3-12m gains in weight, BMI and adiposity, whereas %carbohydrate was positively related to these measures. HM %protein was positively related to 12m BMI. **Conclusion:** HM analysis showed wide variation in %macronutrients. Although data on milk intakes were unavailable, our findings suggest functional relevance of HM milk composition to infant growth.

Keywords: breast milk, macronutrients, nutrition, weight, growth

Keynotes

- Breast feeding is associated with lower rates of infancy weight-gain and later obesity, however data on breast milk composition and relationships with growth are sparse.
- Macronutrient contents of 614 hindmilk samples were: fat: [median(IQR)]:2.6 (1.7-3.6)g/100mls, carbohydrate:8.6 (8.2-8.8)g/100mls, protein:1.2 (1.1-1.2)g/100mls.
- Whilst %carbohydrate was positively related to later infant weight and adiposity, %fat was inversely related, with % protein only positively related to body mass index, suggesting functional implications of breast milk macronutrient contents.

Introduction

It is increasingly recognised that early postnatal nutrition, as well as being critical for optimal infancy growth, may also be associated with long-term health outcomes (1). Rapid early infant weight gain predisposes to an adverse metabolic phenotype in later life, with increased risk of overweight (2), central adiposity and insulin resistance (3). The type of infant milk feeding (exclusive breast-feeding, formula-feeding or mixing feeding), as well as specific dietary compositions and volume of intake, may be important factors.

In contemporary western settings breast-feeding has been associated with slower gains in infancy weight (4) and body fat (5), and although debated has also been linked to lower risk for obesity and associated metabolic disease risk across the life-course (6). It is unclear whether the slower infancy weight gain in breast-fed babies is a result of lower total calorie intake or related to the nutrient composition of human breast milk (HM). Previous studies documenting HM energy or macronutrient contents have been recently reviewed (7) (8). Most were performed in small sample sizes and few attempted to assess the influence of HM composition on subsequent infancy growth outcomes.

The evidence on the potential effects of milk nutrient composition on infancy growth is therefore almost entirely limited to trials comparing artificial infant milk formulas. They report that higher milk protein concentrations increase infancy weight gain and predisposition to obesity (9), but there is inconsistent evidence on milk calorie contents (10, 11). In the absence of large population studies, we aimed to investigate the relationships between HM total calorie content, macronutrient contents, or individual lipid species and infancy growth, in a large UK birth cohort study. We hypothesised that specific HM composition may be associated with different patterns of weight and adiposity gain during infancy.

Patients & Methods

Study Design: The Cambridge Baby Growth Study (CBGS) is a prospective birth cohort, focussing on antenatal and early postnatal determinants of infancy growth, as previously described (5). Mothers were recruited during early pregnancy from a single antenatal centre in Cambridge (2001-2009). The whole cohort included 1585 singleton, late preterm / term (gestation ≥36 weeks) infants with measurements at birth, of whom 924 mothers were breast-feeding their infants at 8 weeks of age. A subcohort of 614 mother-infant dyads, where a breast milk sample was available, is described in the current report. The study was approved by the Cambridge local research ethics committee, and all mothers gave informed written consent.

Anthropometry: Infants were measured by trained paediatric research nurses, with weight, length, and skinfold thickness assessed in the newborn, and then at 3 and 12 months of age. Weight was measured to the nearest 1g using a Seca 757 electronic baby scale. Supine length was measured to the nearest 0.1cm using an Infantometer (SECA 416). BMI was then calculated. Skinfold thickness was measured in triplicate at four sites (triceps, subscapular, flank, quadriceps) on the left hand side of the body using a Holtain Tanner/Whitehouse Skinfold Caliper (Holtain Ltd).

Breast milk collection: To allow for comparable samples and informative macronutrient analysis, mothers who breast-fed their infants were asked to hand express hindmilk samples, after feeding their infant, between 4-8 weeks postnatally, expressing from the same breast that they last used to feed their infant. They repeated this process multiple times, keeping milk samples frozen, and a total of 100 mls of hindmilk was collected over a two week period, in order to reduce within-day and day-to-day variations. Samples were then kept frozen at -20°C, until

processed at a single time point. The pooled sample was thoroughly mixed before analysis.

Overall, infant feeding practise (exclusive breast- vs. mixed-feeding) was assessed by questionnaire at age 3 months, with detailed questions about current feeding, and age at starting supplementary formula milk feeds, as well as completely stopping breast-feeding. From this information, infants were categorised as either exclusive breast- or mixed-feeding at 8 weeks of age, contemporaneous with the breast milk collections.

Breast milk assays: Triglyceride (fat) and lactose (carbohydrate) concentrations were measured in homogenised HM samples using ^{1}H-Nuclear magnetic resonance (NMR) spectra. To determine the lipid concentrations (in mM), 400 microlitres of a homogenised HM sample was mixed with 400 microlitres $CDC1_3$ solvent for 10 minutes, and then centrifuged for 30 minutes at 10,000rpm. The non-polar fraction was then used to measure lipid concentrations, from ^{1}H-NMR spectra. Triglyceride concentration was used as a surrogate for total fat content, since this contributes 95-98% of total HM lipid content (12). A further ten lipid species were also quantified: linoleic acid, diglycerides, monoglycerides, docosahexaenoic acid, 18:1/16:1, esterified cholesterol, free cholesterol, total cholesterol, omega 3, monounsaturated fatty acid and polyunsaturated fatty acids, as described previously (13). Lactose, the major HM carbohydrate, was measured from the polar fraction of the milk sample, using ^{1}H 1D NOESY spectroscopy. Reproducibility of the NMR methods were assesssed: the coefficient of variation (CV) for NMR itself was 0.03-0.3% for lipid analysis, and 0.1-0.6% for analysis of polar metabolites, such as lactose. Analysis of different aliquots from the same sample showed CVs of 0.3-5.8% for lipids and 0.4-4.7% for the polar metabolites. NMR spectral peaks were calibrated using Topspin and analysed based on previous work (14). For protein,

total nitrogen was measured by the Dumas method, and the protein factor conversion of 6.25 used to calculate crude protein content.

Previous work has shown that storage conditions can potentially affect macronutrient content, especially fat content due to the continued activity of lipases and coalescence of fat globules (15). However, we were careful to homogenise the HM samples before analysis. There was no effect of storage time on macronutrient calories, %fat or %carbohydrate, however %protein was modestly positively associated with the storage time [% per year, B (correlation coefficient) 0.01, p=0.01]. For this reason analyses were adjusted for storage time using multiple regression.

Calculations: Age and sex-appropriate standard deviation scores (SDS) were calculated for infant weight, length and BMI measurements, adjusting for gestational age in the newborn, by comparison to the UK 1990 growth reference (16), using the LMS Pro software (17). For each of the four skinfold thicknesses an internal SDS was calculated, adjusted for age, and the mean of the four skinfolds SDS was used as estimate measure of adiposity in analyses.

The metabolisable energy content of HM was calculated using Atwater conversions, taking energy contents of 4, 4 and 9 kcal/g for protein, lactose and fat, respectively (18), and HM total calorie content (TCC) was then calculated as kcal/100mls. The nutrient density method was used to present macronutrient contents as percentages of total calorie content (i.e. %fat, %carbohydrate, and %protein) (19). In order to distinguish independent effects of the individual lipid species investigated, since the lipid species were highly inter-correlated (all Spearman's coefficients > 0.51, $p<0.0005$), we used a residual nutrient method: each lipid concentration was regressed against the triglyceride concentration, standardised residuals for each lipid species calculated and these values used in subsequent analysis.

Statistics: The demographics of the cohort subgroup with HM samples were compared to that of the entire CBGS cohort, and in particular to all mother-infant pairs who were breast-feeding (either exclusively or mixed-feeding) at 8 weeks, using t-tests, chi-squared tests or independent sample median tests.

Relationships between HM TCC, or %macronutrient contents, and infancy growth were investigated, using multivariate regression models, including the following variables: birthweight, gestational age, infant sex, nutrition type and HM storage time. Analyses were performed using SPSS version 20, and statistical significance indicated by p value <0.05.

Results

Cohort description:

The sub cohort of 614 mothers of singleton, term or later preterm infants who provided a HM sample was similar to all mother-infant pairs in CBGS who were breast-feeding (exclusively or in combination with formula-feeding, N=924). There were no differences with respect to gestational age, maternal age, maternal pre-pregnancy BMI, maternal primiparity, ethnicity, infant size at birth and subsequent growth to 12 months of age. Further details of the sub cohort are shown in Table 1.

Human milk macronutrient contents

For the 614 HM samples analysed, TCC was [median (IQR)]: 61.8 (53.7-71.3) kcal/100mls. The macronutrient composition was: fat (triglycerides) 2.6 (1.7-3.6) g/100mls; protein 1.2 (1.1-1.2) g/100mls; carbohydrate (lactose) 8.6 (8.2-8.8) g/100mls. Macronutrient contents expressed as calories per 100 mls and percentages of TCC are shown in Table 2.

HM total calorie and macronutrient contents were unrelated to mother's pre-pregnancy BMI, pregnancy weight gain, parity, gestational age at delivery or

socioeconomic status (assessed using home postcode-based index of multiple deprivation scores as reported previously (20)), and were also unrelated to infant sex (data not shown).

77% of the mothers who provided a HM sample were exclusively breast-feeding at 8 weeks; the others gave their infants both breast milk and infant formula milk (mixed feeding). HM of exclusively breast-feeding mothers contained higher TCC [medians] (62.6 vs. 58.7 kcal/100mls), higher %fat (37.6 vs. 35.0%), but lower %protein (7.3 vs. 8.3%) and %carbohydrate (54.7 vs. 57.5%), all $p<0.05$. All further analyses were adjusted for exclusive breast-feeding versus mixed feeding, using multivariate regression modeling.

Associations with infancy growth:

As shown in Table 2, HM TCC at 4-8 weeks was inversely associated with BMI ($p=0.02$) and adiposity ($p=0.008$) at age 12 months, and with 3-12 month gains in weight ($p=0.02$) and BMI ($p=0.01$). With regard to %macronutrient contents (Table 2), HM %fat was inversely associated with BMI and adiposity at 12 months, and inversely associated with 3-12 months gains in weight, BMI and adiposity. In contrast, HM %carbohydrate was positively related to weight, BMI and adiposity gains between 3-12 months. Figure 1 shows that the relationships between quintiles of HM %fat or %carbohydrate and adiposity/BMI at 12 months were broadly linear. HM %protein was positively correlated to BMI at 12 months ($p=0.04$), with no association with 12 month weight or adiposity, or 3-12 month gains. Figure 1 also shows adiposity/BMI for 271 exclusively formula-fed CBGS infants at the time of HM sample collection, for comparison. HM %macronutrient contents showed no relationships with infant length at any age.

Sensitivity analyses were carried out, separately by feeding group (exclusive breast-feeding versus mixed feeding at 8 weeks). These showed the same directions of associations as in the total population (full data not shown). For example, in the exclusively breast-feeding subgroup (N=389), the associations with 12 month adiposity were: % HM protein: B 0.02, p=0.3, % carbohydrate: B 0.009, p=0.01, % fat: B -0.007, p=0.02, total calories: B -0.005, p=0.07.

Human milk specific lipid species

Concentrations of ten specific HM lipid species are shown in Table 3. In separate models for each lipid species (adjusted for birthweight, gestational age, sex and exclusive breast- vs. mixed-feeding), all ten lipid species showed inverse associations with infant adiposity at 12 months (data not shown). Using the residual nutrient method, linoleic acid was the only lipid species that remained inversely related to infant adiposity at 12 months (p=0.05).

Discussion

To our knowledge, this study of 614 mother-infant pairs is the largest report describing HM macronutrient contents, and the first extensive study to investigate their relationships with infancy growth. We showed inverse associations between HM total calorie content and subsequent gains in weight and BMI, and also later adiposity. Regarding individual HM macronutrients, %carbohydrate was positively correlated to subsequent infant weight, BMI and adiposity gains, whereas %fat was negatively associated with these infancy outcomes. HM %protein was weakly positively associated with BMI at 12 months but not gains in adiposity.

Associations between HM contents and infancy growth have not been previously reported, largely due to the lack of other large studies. However, in

support of our study design, the observed positive association between HM %protein content and 12m BMI is consistent with experimental evidence from large clinical trials that tested isocaloric infant milk formulas containing high versus usual protein contents. Unfortunately, HM intakes were not assessed in our study and therefore we cannot assess whether the associations observed with HM contents were mediated by nutrient intakes.

Of relevance, a recent study reported an inverse association between fat intake at 2 years of age and body fat, assessed by bioelectrical impedance analysis at 20 years (21), also suggesting that early diet containing greater fat may benefit later body composition, either directly or indirectly. A higher proportion of ingested carbohydrate may promote storage of glycogen and fat. Alternatively, it is possible that infants fed HM with lower %fat may feel less satiated and drink larger volumes of milk, hence gaining more weight. This hypothesis is supported by previous observations that HM %fat was inversely related to the volume of HM intake, whereas %lactose was positively correlated (22), and by older studies reporting that infants consuming formula milk with lower energy, had higher dietary intakes (11).

A recent systematic review concluded that higher protein intake in infancy and early childhood is associated with faster weight gain and greater BMI in childhood (23). We did not have further detailed body composition data, making it difficult to distinguish between gains in lean mass or fat mass. We found relatively less inter-person variability in %protein than in other macronutrients and it may be that larger differences in %protein, such as those seen in formula milk studies (9), are needed to observe significant influences of protein content on infancy weight gain.

It is difficult to directly compare our results with other previous studies of HM constituents, due to differences in the timing of HM collection with pooling of samples, sampling of solely hindmilk, HM assays, and the nature of the populations sampled. A recent systematic review summarised the results of ‘mature’ HM

samples (taken 2-4 weeks postnatally) (7), a total of: 415 for protein, 476 for carbohydrate and 567 for lipids, pooling data from a minimum of 18 studies worldwide, with the largest sample size of 71 in any single study. Our study is therefore far larger than any other reported, allowing informative associations with HM macronutrient contents in the range of the previously pooled meta-analysed values.

Heinig et al (1993) showed that total energy and protein intakes were positively correlated to weight, not only in formula-fed infants (N=46) but also in those exclusively breast-fed (N=73) (24). Specifically, total protein intake was positively correlated with 3-6 month and 6-9 month weight gain in breast-fed infants. Butte et al (2000) reported that intakes of HM protein, fat and carbohydrate, were all positively correlated with weight gain and fat free mass gain (assessed using a multicomponent body composition model) at 3-6 months, but not with fat mass gain, in 40 breast-fed infants and 36 formula-fed infants (25). These studies assessed intakes, not HM content, and were also much smaller cohorts, assessing anthropometry at different time points, with different methods for HM collection and nutrient analysis. Further, larger studies, across different populations, with information on both composition and intakes are needed, using a standardised sampling protocol.

It is interesting to note that the associations between HM macronutrient contents and infant anthropometry in our study were mainly with weight, BMI and adiposity, with no apparent influence on length gains. This is surprising as weight gain and statural growth are closely linked in infancy, hence it may be speculated that other confounders could explain the findings with adiposity. Maternal characteristics could be one source of confounding. Some small previous reports have shown correlations between specific maternal factors and HM fat content including parity (26) and maternal anthropometric status (27). However, these associations have not been extended to all macronutrients or been well replicated,

and we found no associations between such maternal factors and HM nutrient contents. We did not assess maternal diet but other studies have reported no relationship with HM contents (27).

Alternatively, it may be that other constituents in breast milk, such as individual lipid moieties, could explain the relationships seen with HM macronutrients and in particular the inverse relationships between lipid and infancy adiposity. Disentangling the potential independent contribution to growth from individual fatty acids, which are highly correlated with total lipid proved to be difficult. Only the omega-6 fatty acid, linoleic acid showed a consistent independent inverse relationship with later infancy adiposity. Of note, in the literature n-3 and n-6 long-chain polyunsaturated fatty acids have received interest with respect to growth and development, with for example with suggested beneficial effects on growth with alpha-linoleic/DHA supplementation in developing countries, (28), however generally there is an overall paucity of data for n-3 or n-6 LC-PUFAs (15-17). Further detailed LC-PUFA analyses and subsequent studies are required to confirm our finding, and investigate this area further.

The higher total calorie content found in HM from mothers who were exclusively breast-feeding, when compared to those mixed-feeding, is consistent with other observations, maintaining sufficient continued nutrition (29), and suggesting that HM energy content may be down-regulated by infants mixed-feeding. The higher %fat, with lower %protein and %carbohydrate, seen in milk of mothers exclusively breast-feeding, may support our findings of growth associations in indicating that this is a beneficial HM composition with regard to subsequent infant adiposity. It could also be speculated that a higher %fat results in greater infant satiation, resulting in continued breast-feeding, whereas hungrier babies consuming HM with lower fat content are more likely to be given supplementary formula milk.

Alternatively, there may be differences in HM production, regulated by the suckling infant, or even potential confounding by the collection techniques used by mothers expressing milk. Hindmilk contains more fat than foremilk (30) and therefore it is not implausible that the exclusively fed infants consumed more milk, and their HM samples contained relatively more hindmilk. We adjusted for exclusive breast- versus mixed-feeding in our subsequent analytical models, with no interaction seen between feeding type and macronutrient content in analyses, and thus this issue is unlikely to confound the associations with infant growth. Similar trends between HM macronutrient contents and infancy body size/growth were also apparent in the exclusively breast-fed subgroup: although generally less significant in this smaller group, correlations were in the same direction, and with similar effect sizes.

Limitations of our study include the lack of information on HM intakes, and therefore it was not possible to calculate total energy and macronutrient intakes. HM lipid and protein contents are known to vary between individual feeds and with different stages of lactation(7, 8). Mothers were encouraged to pool, over a period of 2 weeks, their collections of expressed hindmilk; however it is possible that systematic differences existed between collections and information on the timings of milk collection was not recorded. Some of these limitations will be tackled and subject of further studies.

Conclusion: In conclusion, in this large study of HM macronutrient content, we found that HM nutrient composition in early infancy differs between exclusively breast-feeding and mixed-feeding mothers. Of note, HM %fat and %carbohydrate, predicted changes in infancy weight and adiposity gains up to age 12 months, with %protein positively related to 12 month BMI. There were no associations with length gains. Although data on milk intakes were unavailable, our findings suggest that

higher HM %fat but lower %carbohydrate may be associated with lower gains in adiposity and BMI.

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Abbreviations

ALA	Alpha-linolenic acid
BMI	Body mass index
CBGS	Cambridge Baby Growth Study
DHA	Docosahexaenoic acid
LC-PUFA	Long chain polyunsaturated fatty acid
HM	Human breast milk
IQR	Interquartile range
m	Month
NMR	Nuclear magnetic resonance
NOESY	Nuclear Overhauser effect spectroscopy
SDS	Standard deviation score
TCC	Total calorie content

369	WHO	World Health Organisation

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Table 1: Description of the study members who provided a breast milk sample, in relation to the wider Cambridge Baby Growth Study cohort. Median & IQR are displayed.

	Mothers providing HM samples (N=614)	**All CBGS mothers exclusively breast- & mixed-feeding (N=924)**
Demographics		
Gestational age (weeks)	40.1 (39.1-41.0)	40.0 (39.1-41.0)
Maternal age (years)	33.9 (31.1-36.5)	34.0 (31.2-36.5)
Maternal BMI (kg/m2)	22.8 (20.9-25.2)	22.7 (20.8-25.2)
Index of deprivation	9.0 (6.9-9.0)	9.0 (6.8-9.0)
Maternal primiparity (%)	43%	42%
White Caucasian (%)	96%	96%
Infant sex (% male)	51%	51%
Exclusive breast-feeding (%)	73%	77%
Growth data		
Birth		
Weight (kg)	3.56 (3.22-3.87)	3.55 (3.22-3.85)
Length (cm)	51.5 (50.0-53.5)	51.5 (50.0-53.3)
Mean skinfold thickness (mm)	6.2 (5.3-7.4)	6.1 (5.2-7.3)
BMI (kg/m2)	13.3 (12.2-14.3)	13.3 (12.2-14.2)
3 months		
Weight (kg)	6.10 (5.60-6.64)	6.09 (5.59-6.62)
Length (cm)	61.2 (59.4-63.0)	61.2 (59.5-63.0)
Mean skinfold thickness (mm)	10.8 (9.4-11.9)	10.7 (9.4-11.9)
BMI (kg/m2)	16.3 (15.4-17.2)	16.2 (15.3-17.2)
12 months		
Weight (kg)	9.85 (9.10-10.60)	9.88 (9.15-10.60)
Length (cm)	75.8 (74.0-77.7)	75.6 (73.9-77.7)
Mean skinfold thickness (mm)	11.0 (9.8-12.5)	11.0 (9.7-12.4)
BMI (kg/m2)	17.1 (16.2-18.0)	17.1 (16.3-18.1)

Table 2: Human milk macronutrient contents and their associations with infancy growth (based on N=614 samples).

	Fat	**Carbohydrate**	**Protein**	**Total calorie content**
*Macronutrient contents**				
Calories (kcal) per 100 mls	23.1 (15.4-32.4)	34.3 (32.9-35.3)	4.6 (4.2-5.1)	
%macronutrient content[1]	37.3 (28.4-48.9)	55.2 (47.6-62.9)	7.5 (6.4-9.0)	
Associations with growth				
Weight SDS at 3 mo	B -0.001, p=0.7	B 0.001, p=0.8	B 0.02, p=0.3	B -0.002, p=0.4
Weight SDS at 12 mo	B -0.005, p=0.1	B 0.006, p=0.2	B 0.03, p=0.1	B -0.005, p=0.1
Delta weight SDS 3-12 mo	**B -0.007, p=0.02**	**B 0.008, p=0.02**	B 0.03, p=0.1	**B -0.006, p=0.02**
Mean skinfolds SDS at 3 mo	B -0.004, p=0.2	B 0.004, p=0.1	B 0.02, p=0.3	B -0.003, p=0.3
Mean skinfolds SDS at 12 mo	**B -0.009, p=0.001**	**B 0.01, p<0.0005**	B 0.03, p=0.1	**B -0.007, p=0.008**
Delta skinfolds SDS 3-12 mo	**B -0.007, p=0.04**	**B 0.008, p=0.03**	B 0.02, p=0.4	B -0.005, p=0.08
BMI SDS at 3 mo	B -0.004, p=0.3	B 0.004, p=0.3	B 0.02, p=0.4	B -0.002, p=0.4
BMI SDS at 12 mo	**B -0.01, p=0.002**	**B 0.01, p=0.002**	**B 0.04, p=0.04**	**B -0.008, p=0.02**
Delta BMI SDS 3-12 mo	**B -0.01, p=0.005**	**B 0.01, p=0.005**	B 0.04, p=0.08	**B -0.008, p=0.01**
Length SDS at 3 mo	B 0.002, p=0.5	B -0.003, p=0.4	B 0.01, p=0.5	B -0.001, p=0.6
Length SDS at 12 mo	B 0.004, p=0.3	B -0.005, p=0.2	B 0.003, p=0.9	B 0.000, p=0.9
Delta length SDS 3-12 mo	B 0.001, p=0.6	B -0.003, p=0.6	B -0.005, p=0.7	B 0.001, p=0.6

*median (IQR)

[1] %macronutrient was calculated as macronutrient energy / total energy content

Models were adjusted for exclusive breast- vs. mixed feeding at 8 weeks, sex, GA, birthweight, duration of sample storage

Table 3: Concentrations of human milk lipid species (mmol/ L) N=614

Lipid species	**Median (IQR) mmol/ L**
Linoleic acid	6.62 (4.39-9.17)
Diglycerides	1.95 (1.19-3.02)
Monoglycerides	0.63 (0.38-0.94)
DHA	0.32 (0.22-0.44)
18:1/16:1	0.77 (0.53-1.08)
Esterified cholesterol	0.18 (0.13-0.24)
Free cholesterol	0.20 (0.14-0.29)
Total cholesterol	0.37 (0.26-0.49)
Omega 3	2.48 (1.62-3.53)
MUFA & PUFA	80.96 (54.66-110.56)

DHA: Docosahexaenoic acid 18:1/16:1 : Oleic/palmitoleic acid MUFA & PUFA: Monounsaturated fatty acid & polyunsaturated fatty acid

Figure 1: Infant adiposity at 12 months by quintiles of human milk macronutrient contents at 4-8 weeks.

a) 12 month SF SDS: skinfold standard deviation score as the mean SDS of measurements at four sites. Error bars indicate group means & 95% confidence intervals.

b) 12 month BMI SDS. Error bars indicate group means & 95% confidence intervals.