Received: 16 December 2022 | Revised: 15 June 2023 | Accepted: 17 July 2023

DOI: 10.1111/apa.16910

ACTA PÆDIATRICA
NURTURING THE CHILD
WILEY

ORIGINAL ARTICLE

High protein content in breast milk from South African mothers of preterm infants

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Funding information
No funding received

Abstract

Aim: Macronutrient and energy content of human milk are largely assumed for fortification practices. The aim was to explore macronutrient and energy content of transition and mature human milk from South African mothers of preterm infants with a birth weight <1800 g. Secondary objectives compared day to night milk; and explored associations with selected innate factors.

Methods: In this single-centre, observational study macronutrient and energy content of day, night and mixed samples of transition (first 14 days of life) and mature (from Day 15 of life) human milk were analysed with mid-infrared spectroscopy.

Results: In total, 116 samples (38 days; 37 night; 41 mixed) from 47 mothers were retained for statistical analysis. Mean true protein, carbohydrate, fat and energy content of mixed samples per 100 mL were 1.5 ± 0.4 g, 7.2 ± 0.7 g, 3.5 ± 0.9 g and 69.4 ± 9.9 kcal, respectively. Mixed transition milk ($n = 9$) had 1.9 ± 0.3 g protein and 67.4 ± 9.6 kcal and mixed mature milk ($n = 32$) 1.4 ± 0.4 g protein and 70.0 ± 10.1 kcal, per 100 mL.The protein content of transition ($p = 0.004$) and mature ($p = 0.004$) milk were significantly higher than published data. Transition milk: 1.5 g protein, 65 kcal; mature milk: 1.2 g protein, 72 kcal per 100 mL. Night samples had less fat ($p = 0.014$) and energy ($p = 0.033$) than day samples. With increasing day of life protein content declined ($p = 0.003$).

Conclusion: The protein content of human milk from South African mothers of preterm babies differs from published data and has implications for human milk fortification practises.

KEYWORDS
human milk, low-to-middle income countries, macronutrient analysis, preterm infant, transition milk

Abbreviations: GA, gestational age; HMA, human milk analysis; LMIC, low-to-middle income country; MUAC, mid-upper arm circumference; PMA, post-menstrual age; VLBW, very low birth weight.

1 | INTRODUCTION

Human milk is the preferred feed for all infants and due to numerous advantages[1–4] has been described as the 'only milk for premies'.[1] Human milk macronutrient composition is dynamic and influenced by innate, for example, the mother's age, parity, nutritional status and the infant's gestational age (GA) and nursing frequency, and methodological factors, for example, sampling technique, storage and chemical analysis.[4–6] It is not clear whether the same factors apply to mothers and infants in low-to-middle income country (LMIC) countries. A South African study indicated differences in macronutrient content between milk from HIV-infected and uninfected mothers.[7]

Mother's milk is unique in its capability to adapt to the infant's nutritional needs; this is especially true for preterm infants whose mothers' milk has been shown to be higher in protein and fat, especially in the first few weeks of life.[4,8] Three recent systematic reviews[9–11] indicated protein content as high as 2.7 g/100 mL in colostrum/transition milk from mothers of preterm infants. They and others[4,5,8] reported declining protein content over time with values as low as 1.0 g/100 mL by week 10–12 of life. The energy content, which is lower in the first few days of life, seemed to vary more and could be related to the variability in the fat content. With declining protein content, human milk may not meet the increased requirements of the growing preterm infant, *especially* the very small, very immature preterm infant. To have all the advantages of human milk but also meet nutritional requirements, the addition of a multinutrient human milk fortifier may be needed.[1,12–14]

In South Africa, where 8% of babies are born prematurely,[15] fortification is practiced routinely for infants weighing less than 1500 g, that is, very low birth weight (VLBW)[16] and often for infants with a birth weight of up to 1800 g, in accordance with international guidelines.[12,14] With standard fortification, the protein, fat and carbohydrate content of human milk are largely assumed *and* based on studies from high income countries. When quantifying the intake of preterm infants receiving fortification, an estimation of the nutrient content of human milk is needed and the question arises as to which values to use. Manufacturers of the two commercially available human milk fortifiers in South Africa use different reference values in their calculations of standard fortification: Nestlè (FM85®) base their recommendations on Tsang et al.[17] that is, 1.6 g protein and 67 kcal energy per 100 mL, whereas Sanulac (S26 Alula HMF Gold®) refer to Boyce et al.[9] i.e., 1.3 g protein and 66 kcal per 100 mL.

To standardise the reporting of neonatal research, Cormack et al.[18] recommended using preterm transitional milk values, that is, 1.5 g protein and 65 kcal per 100 mL up to Day 14 of life, and mature milk values, that is, 1.2 g protein and 72 kcal per 100 mL from Day 15 onwards to 'improve the comparability of studies and the likelihood of finding optimal protein and energy intakes for preterm babies'.

Data are lacking on the energy and macronutrient content of human milk for South Africa and other LMIC countries. In the systematic review and meta-analysis by Gidrewicz and Fenton[11] data from these countries were explicitly excluded, 'in an attempt to exclude mothers with suboptimal nutritional status'.

Key Notes

- Macronutrient content of milk from mothers who delivered prematurely in a low-to-middle income country cannot be assumed to be the same as published data.
- According to point of care mid-infrared spectroscopy, protein content of transition and mature milk was higher than international references with implications for standard human milk fortification in comparable settings.
- Day of life was inversely correlated with protein content of milk from mothers of preterm infants.

It is hence not known what the intakes of premature infants are when standard fortification is practiced. The aim of our study was to explore the macronutrient (primarily protein) and energy content of transition and mature human milk from mothers who gave birth to preterm infants, that is, less than 37 weeks completed gestation, with a birth weight of 1800 g or less in a tertiary academic hospital serving a resource-limited community in Gauteng, South Africa and to compare it to published data. As part of a larger study[19] on growth in preterm infants receiving fortified human milk based on the values recommended by Cormack et al.[18] this sub-study investigated the clinical implications for the care of preterm infants in LMIC when using high income country nutrient values for human milk.

Our secondary objectives were to explore differences in the macronutrient and energy content of day and night samples, and the association between nutritional content and selected innate factors. Day and night samples each referred to a mixture of two milk samples taken at daytime and at night respectively, whereas a mixture of these four collections was taken to represent a 24 h sample. The innate factors included maternal and infant characteristics and took transition and mature milk into consideration. Transition milk was defined as milk up to Day 14 of life and mature milk from Day 15 onwards as per the Cormack et al.[18] definition.

2 | METHODS

2.1 | Study design and setting

A single-centre observational study nested in a larger investigation, namely 'Growth of preterm infants receiving fortified human milk',[19] was designed, following the STROBE guidelines.[20] The setting reflects real-life *practices* at the study hospital where lodging facilities and rooming-in for mothers are limited. Mothers of preterm babies would therefore not necessarily stay at the hospital but would come in daily and spend the day on the premises to provide breast milk (day feeding times: 9:00, 12:00, 15:00, 18:00) and leave expressed

milk for night feeds (night feeding times: 21:00; 24:00; 03:00; 06:00). The 3200-bed urban hospital, including 185 neonatal beds, serves mostly lower income communities.

2.2 | Study population and sampling

The study population consisted of mothers of preterm infants (less than 37 weeks completed GA) with a birth weight of 1800 g or less who were expressing milk for their infants in the neonatal wards of the hospital between June 2018 and September 2018. Convenience sampling was done and only mothers who reported to have sufficient milk for their infants, were included.

The same human milk sample collection methods applied for the day *and* night samples: each consisted of *two* samples of approximately 5 mL each collected at *two* different feeding times (Day: 9:00, 12:00 *or* 15:00; Night: at the mother's discretion) and mixed in one bottle to make up a 10 mL sample. All samples were hand expressed in a 10 mL plastic sample collection bottle with a screw-on lid (as specified by MIRIS™ for collection and storage of human milk[21]) *after* the mother had expressed sufficient milk for her infant – thus 'hind' milk. The day sample bottles were kept in the ward refrigerator (4°C) in-between collections and the mothers were requested to follow the same procedure overnight. The sample collection bottles were taken immediately after the second sample was added and frozen at −18°C until the analyses were performed. The 'Night' collection sample bottles were collected the following morning and frozen at −18°C until the time of analysis.

Biographic information of the mother and infant including birth, anthropometric and feeding data was collected through self-report during an interview. The mother's mid-upper arm circumference (MUAC) was taken by either the researcher or the research assistant with a flexible, non-stretchable measuring tape according to standardised techniques.[22] The MUAC is an easy and non-invasive measure of the mothers' protein-energy status recommended by the South African Department of Health[23] for pregnant and lactating women in LMIC. The mothers' pre-pregnancy weights were not available and pre-pregnancy BMI could therefore not be calculated.

2.3 | Nutritional content

To have samples representing 24 h, 'Day' and 'Night' samples were mixed as follows: after defrosting at room temperature, equal volumes of milk (lightly swirled before measured with a 2cc B/BRAUN Injekt® syringe) from the day and night collection bottles of each participant were mixed in a new collection bottle marked with the study number and 'Mixed'. For those providing sufficient milk, it resulted in three sample bottles per mother. In some cases, insufficient milk was provided, leading to only a 'Day' or a 'Night' analysis *or* a 'Mixed' analysis. Figure 1 details the mothers enrolled, and samples included in the spectroscopic and statistical analyses.

Human milk analysis (HMA) with mid-infrared transmission spectroscopy (MIRIS HMA™ [Uppsala, Sweden]) followed standard operating procedures after in-depth in-person training of the researcher (JEK) by the manufacturer. Samples were defrosted at room temperature, heated in a MIRIS waterless bath to 40°C and homogenised with the MIRIS Ultrasonic Processor before analysis. A sample of known content, that is, MIRIS control™, was analysed once daily/every time that the equipment was used to validate internal calibration.[21] In most cases, samples were analysed twice or thrice, depending on the volume of sample obtained, and the average of the values was used.

2.4 | Data analysis

After data were cleaned the coding was done by two independent persons and compared for consistency. Data from the macronutrient analysis of human milk were only used if it did not include 'out-of-range' values, even if it applied to only one of the analysed components, that is, fat, crude protein, true protein, carbohydrate, total solids and energy. 'True protein' values, which refers to protein nitrogen only,[21] were used for statistical comparisons.

Data were summarised by treatment and reported as follows: For continuous variables descriptive statistics with 95% confidence intervals and two-sample *t* tests are presented. For categorical variables, data frequencies and proportions were reported, followed by Fisher's exact test. A one-sample *t*-test was used to compare the analysed content of human milk to published values. A sub-analysis limited to mothers for whom both day and night values were available, was done to compare 'Day' to 'Night'. Regression analysis namely, Pearson's product–moment correlation coefficient and Point biseral correlation was performed for continuous and categorical parameters, respectively, to determine the association between the content of human milk and selected factors, that is, maternal age, HIV status and MUAC; birth weight, sex and GA of infant; day of life, post-menstrual age (PMA) and infant weight when milk sample was taken. Preliminary analysis showed that gravida, mode of delivery and birth weight category were not related to human milk content. These factors were hence not further analysed.

Testing was done at the 0.05 level of significance. Data analysis was done using STATA/IC 15.1 for Windows Windows Revision 15 October 2018 (StataCorp LLC) statistical software.

2.5 | Ethical considerations

Ethics approval was obtained from the University of Pretoria, Faculty of Health Sciences Research Ethics Committee (Reference no 286/2017); the University of the Witwatersrand Health Research and Ethics Committee (Clearance certificate no M170546); and the Medical Advisory Committee of the hospital (Letters dated 17 May and 9 October 2017). Each mother provided written informed consent before providing human milk samples.

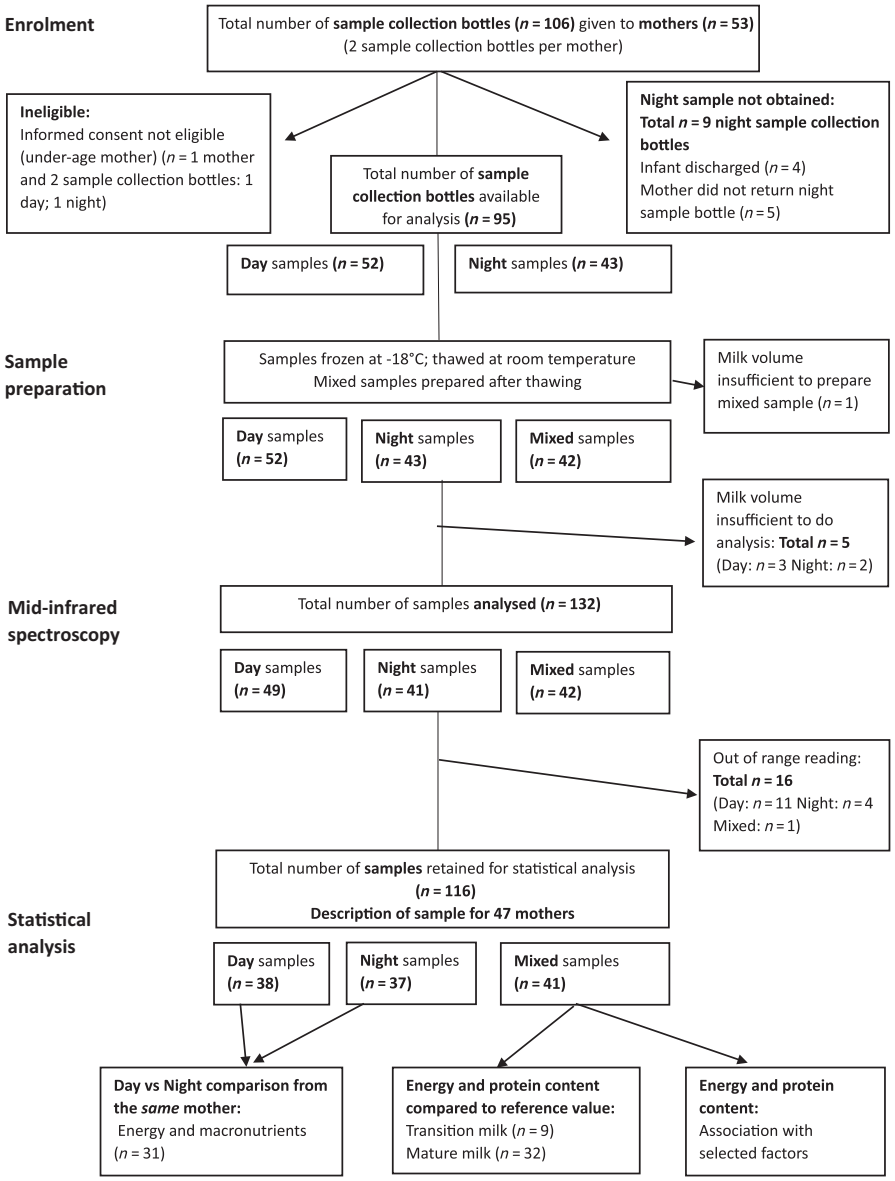

FIGURE 1 Flow diagram of enrolment, sampling and analyses.

3 | RESULTS

3.1 | Study population

The human milk samples retained for statistical analysis came from 47 mothers (Figure 1) whose characteristics, as well as those of their infants, are depicted in Table 1.

3.2 | Macronutrient and energy content

Mean protein, carbohydrate, fat and energy content of mixed samples ($n = 41$) per 100 mL were 1.5 ± 0.4 g, 7.2 ± 0.7 g, 3.5 ± 0.9 g and 69.4 ± 9.9 kcal, respectively (Table 2). In comparison to Cormack et al.[18] (Table 3), the protein content of the mixed sample of our study pointed to significant differences with higher values in terms of transition ($p = 0.004$) and mature ($p = 0.004$) human milk. The energy content of both transition and mature milk showed no differences.

Macronutrient and energy content of the day and night samples are compared in Table 4. Fat ($p = 0.014$) and energy ($p = 0.033$) pointed to significant differences with lower content in the night samples when comparing samples from the same mother.

3.3 | Factors associated with nutritional content

The information presented in Table 1 shows that among the factors investigated, only the day of life when the sample was taken, pointed to a significant relationship with human milk content: a decreasing protein content correlated with an increase in day of life ($r = -0.460$; $p = 0.003$).

4 | DISCUSSION

In this exploratory study, the nutritional content of milk from South African mothers with preterm babies and living in a

TABLE 1 Mother, infant characteristics and association with macronutrient and energy content of mixed[a] sample. [Correction added on 02 August 2023, after first online publication: Table 1 was updated in this version.]

Mother and infant characteristics ($n=47$[b])			Association with macronutrient and energy content of mixed[a] sample ($n=41$)							
			Protein[c]		Fat		Carbohydrate		Energy	
	Mean ± SD	95% CI	*r*-Value[d]	*p*-Value	*r*-Value[d]	*p*-Value	*r*-Value[d]	*p*-Value	*r*-Value[d]	*p*-Value
Mother										
Age, years	27.4 ± 6.3	25.6; 29.3	−0.0359	0.8261	−0.0537	0.7421	−0.0249	0.8786	−0.0588	0.7184
MUAC, cm ($n=45$)	29.4 ± 4.3	28.1; 30.7	−0.0892	0.5994	0.0254	0.8669	0.0435	0.7980	−0.0002	0.9992
HIV status										
Positive	$n=14$ (30%)		−0.1133	0.4807	0.1291	0.4211	−0.0843	0.6001	0.0630	0.6956
Negative	$n=33$ (70%)									
Infant										
GA, weeks	29.7 ± 2.6	28.9; 30.4	0.2159	0.1752	0.1716	0.2834	0.0046	0.9771	0.2076	0.1928
Birth weight, g	1190 ± 240	1121; 1259	0.2754	0.0813	0.0885	0.5824	0.0152	0.9250	0.1466	0.3605
Sex										
Female	24 (51%)		−0.0581	0.7182	−0.1456	0.3635	0.0820	0.6101	−0.1257	0.4336
Male	23 (49%)									
When human milk sample was taken										
Day of life	26.4 ± 17.9	21.3; 31.6	−0.4603	0.0025	0.0829	0.6046	0.0641	0.6906	−0.0227	0.8880
PMA, days	33.1 ± 3.2	32.2; 34.1	−0.2124	0.1824	0.1851	0.2467	0.0513	0.7499	0.1261	0.4320
Infant weight, g ($n=45$)	1401 ± 323	1304; 1497	−0.0926	0.5857	0.1094	0.5191	−0.1772	0.2942	0.0226	0.8945

Abbreviations: GA, gestational age; HIV, human immunodeficiency virus; MUAC, mid-upper arm circumference, PMA, post-menstrual age.

[a]Day and night samples mixed.

[b]Unless otherwise specified.

[c]True protein values.

[d]Pearson's product–moment correlation coefficient (for continuous parameters) and Point biseral correlation (for categorical parameters).

TABLE 2 Macronutrient and energy content of mixed[a] sample.

	Human milk composition of mixed[a] sample per 100 mL ($n=41$)	
	Mean ± SD	95% CI
Crude[b] protein, g	1.9 ± 0.5	1.7; 2.0
True[c] protein, g	1.5 ± 0.4	1.4; 1.6
Fat, g	3.5 ± 0.9	3.2; 3.8
Carbohydrate, g	7.2 ± 0.7	6.9; 7.4
Total solids[d], g	12.5 ± 2.2	11.8; 13.2
Energy, kcal	69.4 ± 9.9	66.3; 72.5

Abbreviations: CI, confidence interval; g, gram; kcal, kilocalorie; SD, standard deviation.

[a]Day and night samples mixed.

[b]Includes both protein and non-protein nitrogen (e.g. oligosaccharides, urea).[21]

[c]Excludes non-protein nitrogen.[21]

[d]Total solids: Dry matter, including carbohydrate, fat, protein and minerals.[21]

resource-constrained setting differs from published data. As part of a larger study[19] on growth in preterm infants receiving fortified human milk based on the values recommended by Cormack et al.[18] this sub-study investigated the clinical implications for the care of preterm infants in LMIC when using high income country nutrient values for human milk.

In comparison to the Cormack group's recommendation,[18] the protein content of both mature and transition milk was higher. In the systematic review by Boyce et al.[9] the protein content of mature milk (1.3 g/100 mL) falls in-between the values found in our study (1.4 g/100 mL) and those recommended by the Cormack group[18] (1.2 g/100 mL). The protein content of transition milk in our study was the same as the value reported by Boyce et al.[9] (1.9 g/100 mL). It should be noted though, that Boyce's et al.[9] definition of transition milk (lactation week 1) and mature milk (lactation weeks 2–8) differs slightly from ours, which were based on Cormack's et al.[18] recommendations. Also, Boyce et al.[9] included only two studies where mid-infrared spectroscopy was used for measuring protein. Comparisons to other reviews are complicated by different classifications in terms of lactation days, yet Mimouni et al.[10] indicated protein values of 1.98 g/100 mL to 2.57 g/100 mL in the first 2 weeks of life compared to Gidrewicz and Fenton's[11] 1.5 g/100 to 2.7 g/100 mL.

Several factors can possibly explain the difference in protein content in our study compared to previous research. In a study by Gates et al.[24] milk from self-identified black mothers of preterm infants had 29% more protein on Day 28 of life than their white counterparts. Even though a direct comparison to the Gates study[24] is

TABLE 3 Comparison of current study and published[18] protein and energy content of transition and mature milk.

	Human milk composition per 100 mL					
	Transition[a] milk			Mature[b] milk		
	Current study mixed[c] sample ($n=9$)			Current study mixed[c] sample ($n=32$)		
	Mean ± SD	Published data: Cormack et al.[18]	*p*-Value[d]	Mean ± SD	Published data: Cormack et al.[18]	*p*-Value[d]
Protein, g	1.9 ± 0.3[e]	1.5	0.004	1.4 ± 0.4[e]	1.2	0.004
Energy, kcal	67.4 ± 9.6	65	0.238	70.0 ± 10.1	72	0.866

Abbreviations: g, gram; HMA, human milk analysis; kcal, kilocalorie; mL, millilitre; SD, standard deviation.

[a]Up to Day 14 of life.

[b]Day 15 of life onwards.

[c]Day plus night milk.

[d]One-sample *t* test.

[e]True protein values.

TABLE 4 Human milk composition: Comparison of day and night samples from the same mother.

	Human milk composition per 100 mL				
	Samples from the same mother ($n=31$)				
	Day[a] samples		Night[b] samples		
	Mean ± SD	95% CI	Mean ± SD	95% CI	*p*-Value[c]
Crude[d] protein, g	1.8 ± 0.5	1.6;2.0	1.9 ± 0.4	1.7;2.0	0.845
True[e] protein, g	1.5 ± 0.4	1.3;1.6	1.5 ± 0.4	1.4;1.7	0.884
Fat, g	3.9 ± 1.2	3.5;4.3	3.3 ± 1.3	2.8;3.8	0.014
Carbohydrate, g	7.0 ± 0.7	6.8;7.3	7.2 ± 0.7	6.9;7.4	0.920
Total solids[f], g	13.1 ± 1.4	12.5;13.6	12.5 ± 1.6	12.0;13.1	0.040
Energy, kcal	72.3 ± 11.5	68.1;76.5	67.4 ± 12.8	62.7;72.1	0.033

Abbreviations: CI, confidence interval; g, gram; kcal, kilocalorie.

[a]Day samples represent milk expressed at two collection times during the day.

[b]Night samples represent milk expressed at two collection times during the night.

[c]Paired *t* test.

[d]Includes both protein and non-protein nitrogen (e.g. oligosaccharides, urea).[21]

[e]Excludes non-protein nitrogen.

[f]Total solids: Dry matter, including carbohydrate, fat, protein and minerals.

not possible, all mothers in our study were observed to be of black race/ethnicity and the protein values we found were higher than expected. Gates et al.[24] do however note that causality of race/ethnicity in explaining the milk composition differences cannot be concluded and in subsequent analyses found no difference between black and white mothers within any day. It does appear that black mothers produce lower volumes of milk than their white counterparts and therefore protein values are higher when compared to white mothers. The dilutional effect of protein – the more milk the mother produces, the lower the protein content[4]—may therefore be applicable to our study but our method of milk sampling did not allow for such an analysis.

Another factor relevant to the protein content of human milk that did emerge in our study, is the decline in protein over time. This effect has been reported in many studies: not only day of life/postnatal age[10,11] and lactation week[8,25] but also PMA[5,9] correlated negatively with protein content. In our study no other maternal or infant factor was significantly related to milk composition.

As previously stated, the comparisons to the systematic reviews[9–11] should be done with caution due to different analytical methods. Furthermore, all three systematic reviews[9–11] only included studies with 24-h samples (in the case of Gidrewicz and Fenton[11] this applied to fat and energy only) and thus would have included foremilk as well. Even though we attempted to represent a 24-h sample period, our samples consisted of hind milk only. We were faced with the ethical dilemma of getting 24-h samples from mothers often not producing sufficient milk for their preterm babies. Our context-specific approach to make up a 24-h milk sample requires validation. The comparison of day to night samples point to the importance of within-mother and within a 24-h period sampling. Fenton and Elmrayed[26] recently again emphasised the need for 24-h human milk samples and indicated the importance of the degree of

breast emptying to get all the fat in hind milk. If a 24-h milk sample is not feasible, Leghi et al.[27] recommended the collection of pre- and post-feed samples (with pooling prior to analysis) for all feeds across a 24-h period as the most appropriate alternative.

To our knowledge there is only one recent study[7] on the macronutrient content of human milk of South African mothers of preterm infants. In that study a single sample of hind milk was taken on day seven of lactation, thus assuming transition milk and infrared analysis of macronutrients was performed. Protein levels in the milk of HIV-infected and uninfected mothers were 1.95 g/100 mL and 1.78 g/100 mL respectively ($p = 0.04$).[7] Even though there were differences in methodology between the Fouche study[7] and our study, for example in terms of sample collection and spectrometers used, the protein content is comparable. The energy content in the Fouche study[7] was 69.8 kcal/100 mL and 66.6 kcal/100 mL in HIV-infected and uninfected mothers, respectively ($p = 0.27$). In contrast to the Fouche[7] study where statistically significant differences were seen in protein, carbohydrate and fat content between milk from HIV-infected and uninfected mothers, no such differences were seen in our study, admitting that our study was not designed to determine such differences.

When day and night values from the same mother were compared, the only significant difference was in the lower fat and energy content in the night samples. Since fat contributes approximately 50% of the energy in human milk, fat and energy content are highly correlated. Fat is the most variable macronutrient in human milk and hind milk may be two to three times higher in fat than foremilk.[4] Ballard and Marrow[4] reported a lower fat content at night and in the mornings, when compared to afternoon and evening samples. In our study 'Day' samples were taken at 9:00, 12:00 or 15:00 feeding times and 'Night' samples between 21:00 and 06:00 h. The loss of fat during storing, freezing and thawing[28] may be additional considerations, however, all our samples were collected in the prescribed MIRIS™ bottles and frozen and thawed in the same way, with the only exception being the storage of the night samples before the mothers delivered it to the researcher in the morning. Even though the mothers were requested to refrigerate the samples in-between the two collection times during the night, this may not have been the case.

Our study has made a clinical contribution by being the first of its nature in South Africa and can hence be a starting point for future research in comparable settings. The meticulous way in which human milk samples were collected and analysed is a strong point of our study since good clinical laboratory practices are needed when using human milk analyzers.[29] We reported 'true protein' values, as crude protein would also include non-digestible proteins, for example, urea and oligosaccharides, and could skew the results.[11] Even though the accuracy of protein as measured with the MIRIS HMA™ is within 10% of the Kjeldahl standard,[21] a more robust method would have been the removal of non-protein nitrogen from samples before measuring protein.[5] As an exploratory study with financial constraints such methods were not available to us.

We reported only data form HMA that did not include 'out-of-range' values, even if the 'out-of-range' value applied to only one of the components analysed. Even though this strengthened the reliability of the data we reported, the high number of 'out-of-range' readings that had to be excluded, reduced our sample size. The small sample size of the present study and especially those of transition milk is a limitation, however the small variation in composition suggests that this did not compromise the estimation. Apart from expanding on the sample size, additional factors that may influence nutritional content of human milk could also have been investigated. Based on MUAC measurements, the mothers in our study did not have a poor protein-energy status, but their pre-pregnancy body mass index[6] and diet,[4,30] could have added additional insight.

The higher protein content found in our study has implications for fortification strategies in South Africa and other LMIC. When using current nutritional values, for example, those from Cormack et al.[18] in standard fortification calculations, protein may be given at higher levels than currently recommended.[12] In institutions where S-urea is available, this may guide protein intake calculations, and adjustable fortification could be an option.[14] Since HMA is both expensive and labour-intensive, target fortification is usually not a viable option in LMIC. If the higher protein values found in our study are confirmed in larger studies, the use thereof in standard fortification calculations may be advisable.

5 | CONCLUSION

When assessed with mid-infrared spectroscopy, the protein content of human milk of mothers with preterm babies differs from international figures from high income countries. This requires follow-up in other LMIC settings, with larger sample sizes and ideally with complete 24-h milk samples, using robust analytical methods. Such data would provide the evidence necessary for extrapolating recommended human milk fortification practices to LMIC settings where the problem of preterm birth is most prevalent.

AUTHOR CONTRIBUTIONS

Johanna Elizabeth Kemp had primary responsibility for protocol development, patient screening, enrolment, data collection, outcome assessment, preliminary data analysis and writing the manuscript. Piet Becker had primary responsibility for statistical analysis and contributed to the writing of the manuscript. Friedeburg Anna Maria Wenhold supervised the design and execution of the study and contributed to the writing of the manuscript.

ACKNOWLEDGEMENTS

The authors acknowledge the support and resources provided by dr Marie Ekholm from MIRIS™, Uppsala Sweden. We would also like to thank Mrs Amanda van Zyl for her assistance with data collecting and processing.

FUNDING INFORMATION

The researcher (JEK) used the mid-infrared spectrometer and other equipment (excluding consumables) on a loan-agreement from MIRIS™, Uppsala Sweden. No funding for this research project was received by any of the three authors of the manuscript.

CONFLICT OF INTEREST STATEMENT

None declared.

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How to cite this article: Kemp JE, Becker P, Wenhold FAM. High protein content in breast milk from South African mothers of preterm infants. Acta Paediatr. 2023;112:2129–2136. https://doi.org/10.1111/apa.16910