Associations of Measured Protein and Energy Intakes with Growth and Adiposity in Human Milk-Fed Preterm Infants at Term Postmenstrual Age: A Cohort Study

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Abstract

Objective To determine the associations of measured protein, energy, and protein-to-energy (PER) intakes with body composition in human milk (HM)-fed preterm infants.

Study Design Neonates born at < 33 gestational weeks were eligible. Standard fortification method with modular supplements was used and the HM composition was measured. The weight gain velocity was calculated, and body composition was assessed by air displacement plethysmography at 40 weeks' postmenstrual age (PMA). The fat mass percentage and fat mass index were used as indicators of adiposity, with convenience cut-offs ≤ -1 and $\geq +1$ z-scores for low and high adiposity, respectively.

Results Thirty-three infants were included (median [interquartile range] gestational age: 30 [28–31] weeks; birth weight: 1.175 [1.010–1.408] g); 36.4 and 84.8% did not receive the minimum recommended protein and energy intakes, respectively. Weight gain velocity showed positive weak-to-moderate correlations with nutrient intakes. Overall, no correlations between nutrient intakes and body composition were found. Infants with lower adiposity received lower energy, protein, and PER intakes, while those with higher adiposity received lower energy intake but higher PER intake.

Conclusion Overall, no correlations of nutrient intakes with body composition were found; however, differences in nutrient intakes were found between infants with lower and higher adiposity at term PMA.

Keywords
- body composition
- energy intake
- growth velocity
- human milk composition
- protein intake
- very preterm infant

The increasing survival of preterm infants[1] has resulted in the challenge of reducing morbidity in the short, medium, and long term.[2] To achieve this, adequate nutrition is one of the cornerstones.[3]

In extremely and very preterm infants, prevention of in-hospital nutrient deficits may be achieved through optimization of nutritional policies.[4] This include early high parenteral amino acid intakes, early introduction of parenteral lipids, early trophic enteral feeding, and use of fortified human milk (HM), preferably the mother's own milk (OMM) or donor HM (DHM).[5] Standard fortification rarely meets the recommended protein intake, thereby leading to a risk of poor growth and

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neurocognitive impairment.[4,5] Target and adjustable individualized fortifications have been suggested as alternative approaches.[6,7] Moreover, addition of modular protein supplement to standard fortification has been used[8,9] to achieve the recommended protein intakes.[10,11]

A systematic review and meta-analysis found that extremely and very preterm infants at term equivalent age have lower fat-free mass (FFM), greater fat mass percentage (FM%), and are shorter compared with term infants.[12] However, in extremely and very preterm infants, studies evaluating the effects of different hospital nutritional strategies on body composition have reported conflicting results.[13–19] This may be explained by quite different nutritional strategies used in these infants[20] or by unknown factors. While protein and energy intakes are determinant for body composition of growing preterm infants, higher protein-to-energy ratio (PER) seems particularly relevant to avoid excessive adiposity[21] and promote FFM accretion.[18]

Appropriate indicators should be used to assess growth and body composition in growing preterm infants. The exponential weight gain velocity model seems to accurately monitor in-hospital growth, and is not affected by birth weight and length.[22,23] Although body weight has been considered an independent predictor of body composition in infants,[24] it does not provide any information on the different body compartments.[25] In neonates, the body mass index is a poor predictor of adiposity compared with FM%.[26] More recently, FM% has been questioned as an accurate indicator of adiposity, because FM% is a proportion, with FM included both in the numerator and denominator (as component of body mass).[27] Adjusting FM to an unrelated measure of body fat was suggested as a strategy to improve interpretation as indicator of adiposity.[27] The FM index (FMI), in which the FM is adjusted to body length, seems to discriminate adiposity even better than FM%,[27] including in preterm infants.[28]

The primary objective of this study on HM-fed extremely and very preterm infants was to determine the associations between different in-hospital measured protein, energy, and PER intakes and body composition at term (40 weeks) postmenstrual age (PMA). The secondary objective was to determine which protein, energy, and PER intakes associate with lower and higher adiposity at term PMA.

Methods

Study Design and Participants

This cohort study was performed in the neonatal intensive care unit (NICU) of Maternidade Dr. Alfredo da Costa, Centro Hospitalar de Lisboa Central, Lisbon, Portugal. The study period was from birth to 40 weeks' PMA. At 40 weeks' PMA, after discharge, body composition was measured at the Nutrition Laboratory of Hospital Dona Estefânia, Centro Hospitalar de Lisboa Central, Lisbon, Portugal. The study was approved by the hospital ethics committee and is registered at the International Standard Randomised Controlled Trial Number (ISRCTN) (ID: 27916681). Informed written consent was obtained from the parents or legal representative of each infant.

Consecutive inborn neonates with less than 33 weeks of gestation and who were HM-fed at least 80 mL/kg/day, were eligible for inclusion in the study. This minimum enteral intake was used as a convenience criterion for tolerance to enteral feeding. Infants with major congenital malformations and triplets or more were not included. The exclusion criteria were infants with diagnosed inborn errors of metabolism, and those who were subsequently formula-fed more than 12.5% of the enteral volume intake, transferred, deceased, or unavailable for body composition assessment. In our unit, enteral feedings are given every 3 hours (8 times per day); as a convenience criterion, the infants were considered predominantly HM-fed if no more than one out of eight meals (12.5%) was replaced by formula.

The demographic and clinical variables recorded were single or twin pregnancy, sex, gestational age, birth weight, small-for-gestational age (birth weight less than 10th percentile),[29] Neonatal Acute Physiology with Perinatal Extension-II (SNAPPE II) score,[30] use of prenatal and postnatal corticosteroids, diagnosis of late-onset sepsis,[31] severe (grade $\geq$ 3B) necrotizing enterocolitis,[32] severe (grade $\geq$ 3) intraperiventricular hemorrhage,[33] multicystic periventricular leukomalacia,[34] and chronic lung disease.[35,36] The gestational age was determined by an early prenatal ultrasonography, or by the first day of the last menstrual period; in the case of assisted reproductive technology, by adding 2 weeks to the conceptional age.[37]

Nutrition Protocol

Infants were managed according to our NICU nutrition protocol, based on international recommendations for neonatal parenteral nutrition (PN)[38] and enteral nutrition,[5,11] and the national consensus for neonatal parenteral and enteral nutrition.[39,40] Briefly, PN was initiated using a central line within the first two postnatal hours with 2.5 g/kg/day of amino acids and was increased up to 3.8 to 4.0 g/kg/day; parenteral lipids were initiated within the first 24 postnatal hours with 1 g/kg/day and increased up to 3 g/kg/day. Early enteral trophic feeding (10–20 mL/kg/day) was initiated within the first 2 to 4 postnatal days using HM; subsequently, enteral nutrition was increased as the PN was proportionally reduced. Until 35 weeks' PMA, exclusive HM (OMM or DHM) was used. If the OMM was not sufficient after 35 weeks' PMA, formula was used for preterm infants, owing to limited DHM stock. Nutrition was prescribed by physicians in collaboration with a nutritionist.

Human Milk Analysis and Fortification

DHM and OMM were stored frozen in the maternity milk bank. Mothers were advised to collect milk every 3 hours either in the hospital or at home and the samples were identified by date and hour of collection. For each infant, a daily pool of prescribed OMM was obtained from the sequentially collected samples. From this pool, a 3-mL sample was analyzed, which roughly represented the composition of OMM collected along a day. After homogenization, each sample was analyzed using a mid-infrared HM analyzer (Miris AB, Uppsala, Sweden); the DHM composition was always measured. The physicians and nutritionist were blinded to the HM composition during the entire study period. When breastfeeding predominated (unknown volume intake and composition), the OMM composition analysis was suspended. An HM fortifier was used when the HM intake was at least 100 mL/kg/day. The standard fortification method supplemented in a blinded manner with modular protein[8,9] and/or modular medium-chain triglycerides was used, to achieve 1.1 g/dL of protein, assumed to be an average range of reported in preterm OMM during the first 1 to 3 postnatal weeks and 0.8 g/dL thereafter, and always 0.8 g/dL in DHM.[41] The composition of the HM fortifier (Aptamil FMS; Milupa/Danone GmbH, Friedrichsdorf, Germany), modular protein (Aptamil Protein Supplement powder; Milupa/Danone GmbH), and medium-chain triglycerides (MCT OIL; SHS Nutricia/Danone, GmbH, Friedrichsdorf, Germany) used are shown in ►**Table 1**.

The administered volumes of OMM and DHM were used to estimate the energy, protein, and PER intakes, according to the reported macronutrient content of preterm infants' OMM.[41,42] The volumes and powder weights of PN solutions and commercial products were also accounted for in these estimates.

The minimum targeted daily intakes according to body weight, once the daily fluid intake of 140 to 150 mL/kg was reached, were as follows: energy 110 kcal/kg; protein (g/kg) 4.0 if $< 1{,}000$ g, 3.7 if $< 1{,}200$ g, 3.6 if $< 1{,}800$ g, and 3.4 if $> 1{,}800$ g; and PER of 3.6 if $< 1{,}000$ g, 3.2 if $< 1{,}800$ g, and 2.6 if $> 1{,}800$ g.[5,11] Within the first postnatal week, the energy and nutrient intakes were proportional to the relative restriction of fluid intake recommended.[5,11] At discharge, the infants were exclusively breasted ad libitum or alternating with fortified OMM, depending on the weight gain.[43] Clinical evaluation with relevance to the nutritional regimen was focused on weight gain, feeding tolerance, and indications for fluid restriction. Blood urea nitrogen was not routinely measured.

Anthropometry and Body Composition

Anthropometry was performed using the recommended techniques.[25] Body weight was measured daily using electronic scales from birth to discharge; in-hospital weight gain was expressed as the weight gain velocity (g/kg/day), calculated by an exponential model.[23]

Table 1 Energy and nutrient contents of the human milk fortifier (Aptamil FMS), modular protein hydrolysate (Aptamil Protein Supplement), and modular medium-chain triglycerides (MCT OIL SHS) used

Product	Energy (kcal)	Protein (g)	Lipids (g)
Aptamil FMS (per 100 g)	347	25.2	0
Aptamil Protein Supplement (per 100 g)	328.4	82.1	0
MCT OIL SHS (per 100 mL)	855	0	95

Body composition assessment was scheduled after discharge at 40 weeks' PMA using air displacement plethysmography (Pea Pod; Cosmed, Ltd., Concord, CA). This validated method for preterm infants[44] measures body mass (kg), FM, and FFM with a precision of 0.1 g. A constant FM density value of 0.9007 g/mL and age- and sex-specific FFM densities[29,45] were used. Concomitantly, the crown-heel length and head circumference were measured. The FM%, based on the FM and body mass, and the FMI (kg/m^2), based on the FM and length, were calculated.[28] Low and high adiposities,[46] indicated by the FM% and FMI, were defined in this sample using the convenience thresholds of -1 and $+1$ z-scores, respectively.

The correlations of daily protein, energy, and PER cumulative intakes from birth to 35 weeks' PMA with the FM, FFM, FM%, and FMI measured at 40 weeks' PMA were analyzed.

Statistical Analysis

The sample size was calculated to detect a difference of 3.0% in FM% with a standard deviation of 4.4[47] for normally distributed variables, a significance level of 0.05, and an 80% power; thus, a required sample of 70 infants was estimated.

Statistical analysis was performed using R (version 3.4.0) and SPSS (version 13, SPSS Inc., Chicago, IL). The normality of continuous numerical variables was tested using the Shapiro–Wilk test and the data are expressed by adequate central and dispersion measures. Univariable analysis was performed using the Student's *t*-test, the Mann–Whitney U test, Pearson's *r*, or Kendall's tau-b, as appropriate. Categorical variables are described by their absolute values and relative frequencies were compared using the chi-squared or Fisher's exact test. As OMM composition measurements were not always possible, a post hoc analysis for imputation of missing values was performed by referencing a meta-analysis of composition of preterm infants' OMM,[41] in which the true protein and energy changes were nonlinear. Thus, logarithmic transformations of true protein or energy concentrations as dependent variables, using the postnatal days as the fixed effect and each case as a random effect, were used in the two mixed models to predict the missing measurements of OMM true protein and energy between birth and 35 weeks' PMA. Good agreements were found between the curves of reference data (meta-analysis), mixed model-predicted data, and measured *plus* estimated data for true protein (►**Fig. 1**) and energy (►**Fig. 2**). The effect of potential confounding variables was first explored by univariable analysis; associations with $p < 0.10$ were included in the mixed multivariate models, which included FM, FFM, FM%, and FMI as the dependent variables, the average daily protein, energy, and PER intakes up to 35 weeks' PMA and most significant covariables. To determine the associations between lower and higher adiposity and protein, energy, and PER intakes, a nested case–control analysis was performed. Infants with measurements indicating lower and higher adiposity were compared with the remaining infants using nonparametric tests.

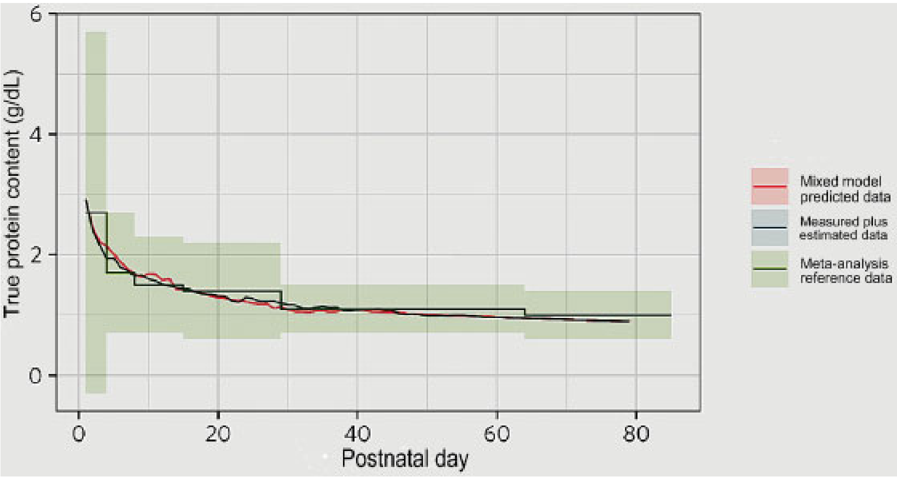

Fig. 1 True protein concentration in own's mother milk: reference data[41] (green line), mixed model-predicted data (red line), and measured plus estimated data (blue line). Lines indicate mean values and shaded areas indicate ± 2 standard deviations.

Results

The study was interrupted before the calculated sample size was reached, owing to logistical constraints in measuring the body composition of the infants. The period of enrolment ran from February 1, 2014 to February 28, 2015 (13 months), during which 156 eligible infants were identified (►**Fig. 3**). Of these, 67 infants were not enrolled owing to the parents refusing to participate in 38 cases, the parents being unable to provide informed consent in 4 cases, and the infants being unavailable for follow-up in 25 cases. Among the 89 enrolled infants, 56 were subsequently excluded because they were formula-fed more than 12.5% of the total volume intake. Thus, only 33 infants completed the study and were analyzed, being 27 very preterm ($\geq$ 28 weeks) and 6 extremely preterm ($<$ 28 weeks); their characteristics and clinical outcomes are summarized in ►**Table 2**. No cases of small-for-gestational age, severe necrotizing enterocolitis, multicystic periventricular leukomalacia, and transferred or deceased infants were recorded. As compared with the 56-excluded formula-fed infants, the 33 infants who completed the study had a lower gestational age (median [interquartile range; IQR] 30 [28–31] versus 32 [30–32] weeks of gestation, $p = 0.002$); a lower prevalence of twins (12% versus 70%, $p < 0.0001$) and stayed longer in the hospital (median [IQR] 51 [35–62] versus 39 [29–51] days, $p = 0.016$).

The infants included received PN during a median (IQR) of 11 (8–16) days. On the third postnatal day, 80% of infants had initiated HM, and on the 28th postnatal day, all infants were exclusively HM-fed. OMM was predominantly used up to 35 weeks' PMA, given by tube or mouth; subsequently, the infants were predominantly breastfed. Fortified HM was started on the seventh postnatal day and generalized to all infants by the 28th postnatal day.

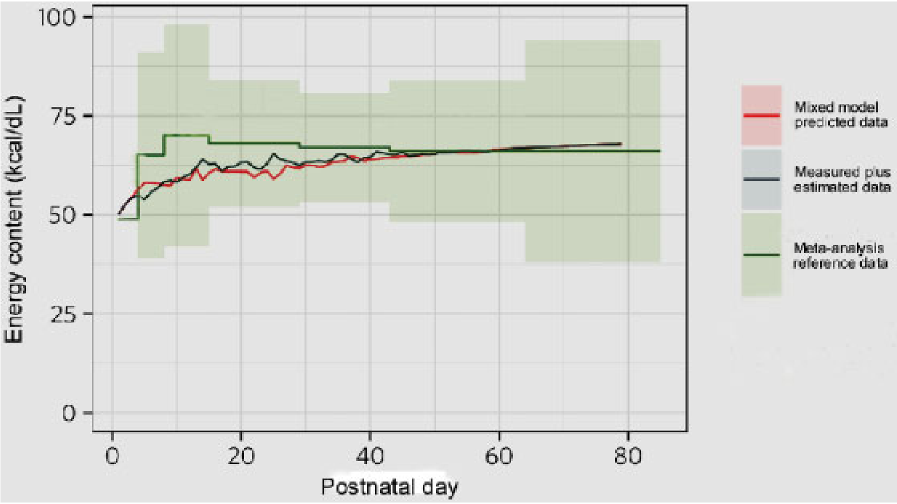

Fig. 2 Energy concentration in own's mother milk: reference data[41] (green line), mixed model-predicted data (red line), and measured *plus* estimated data (blue line). Lines indicate mean values and shaded areas indicate ± 2 standard deviations.

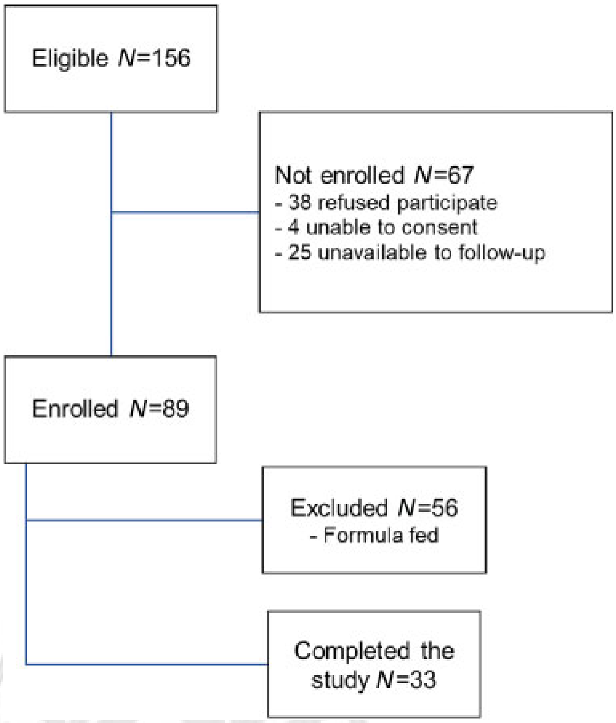

Fig. 3 Consolidated Standards of Reporting Trials (CONSORT) flowchart of the study.

Table 2 Characteristics of studied infants ($N = 33$)

Gestational age (wk), mean (SD)	30 (1.8)
6 extremely preterm, mean (SD)	27.3 (1.4)
27 very preterm, median (IQR)	30.5 (28.7–31.3)
Birth weight (g), median (IQR)	1175 (1010–1408)
Twins, *n* (%)	4 (12)
Antenatal steroids, *n* (%)	33 (100)
Female, *n* (%)	11 (33)
Cesarean section, n (%)	25 (75.8)
SNAPPE II, median (IQR)	13 (0–21)
Small-for-gestational age, *n* (%)	0 (0)
Late-onset sepsis, *n* (%)	4 (12.1)
Chronic lung disease	3 (9.1)
Steroids for chronic lung disease, *n* (%)	1 (3)
Severe necrotizing enterocolitis, *n* (%)	0 (0)
Severe intraperiventricular hemorrhage, *n* (%)	2 (6.1)
Day of full enteral feeding, median (IQR)	12 (9–17)
Multicystic periventricular leukomalacia, *n* (%)	0 (0)
Days with invasive ventilation, median (IQR)	0 (0–6)
Days with supplemental oxygen, median (IQR)	21 (5–42)
Length of stay (d), mean (SD)	48 (18)
Gestational age at discharge, median (IQR)	36 (35–39)

Abbreviations: BW, birth weight; IQR, interquartile range; SD, standard deviation; SNAPPE II, Neonatal Acute Physiology with Perinatal Extension-II score.

Eight hundred thirty-two pooled HM samples were analyzed, representing 65.0% of total HM administered. In the administered OMM, the measured true protein concentration decreased steeply from birth to the 10th postnatal day, after which it gradually decreased and stabilized after the 36th postnatal day (►**Fig. 1**). The measured energy concentration steeply increased within the first two postnatal weeks, after which it gradually increased (►**Fig. 2**).

After disclosing the measured HM composition, the minimum recommended daily nutrient intakes for body weight achieved in at least 75% of the days between the 12th postnatal day (when the fluid intake reached a plateau) and 35 weeks' PMA, was assessed in each infant. It was achieved in 63.6% of infants for protein, 15.2% for energy, and 93.9% for PER (►**Figs. 4** and **5**). The protein, energy, and PER intakes from birth to 35 weeks' PMA are presented in ►**Table 3**. The incidence of infants decreased with gestational age, thus nutrient intakes were evaluated in small numbers of infants at lower gestational ages; at 35 weeks' PMA nutrient intakes were evaluated only in 28 infants, since in 5 the OMM composition was not measured because they were exclusively or predominantly breastfed (►**Table 3**). The median daily protein, energy, and PER intakes ranged from 2.7 to 4.2 g/kg, 53.7 to 109.2 kcal/kg, and 3.4 to 5.6, respectively. Of note, higher PERs were recorded at lower PMAs, reflecting that low protein intakes were associated with very low energy intakes.

After discharge, from 35 to 40 weeks' PMA, the nutrient intakes were neither measured nor estimated. During this period, 21 (63.6%) infants were exclusively breastfed, 10 (30.3%) were breastfed *plus* formula-supplemented, and 2 (6.1%) were formula-fed; in most cases, formula was initiated at or after 39 weeks' PMA.

In the whole sample, the recoup of birth weight occurred at a median (IQR) of 14 (11–17) postnatal days.

The mean (standard deviation; SD) weight gain velocity (g/kg/day) from birth to 35 weeks' PMA, was 10.1 (3.8). Gestational age and weight gain velocity had normal distributions and a significant negative correlation ($r = –0.654$, $p < 0.0001$). No significant differences were found in weight gain velocity, either between sexes or between the extremely preterm and very preterm infants.

Positive weak-to-moderate correlations of daily protein intake ($r = 0.413$, $p < 0.001$), energy intake ($r = 0.288$, $p = 0.018$), and PER intake ($r = 0.371$, $p = 0.002$) with weight gain velocity were found, with coefficients of determination of $r^2 = 0.424$, $r^2 = 0.264$, and $r^2 = 0.217$, respectively. In multivariable analysis with gestational age (weeks) as covariable, only this was negatively associated with weight gain velocity in the three models adjusted for intakes

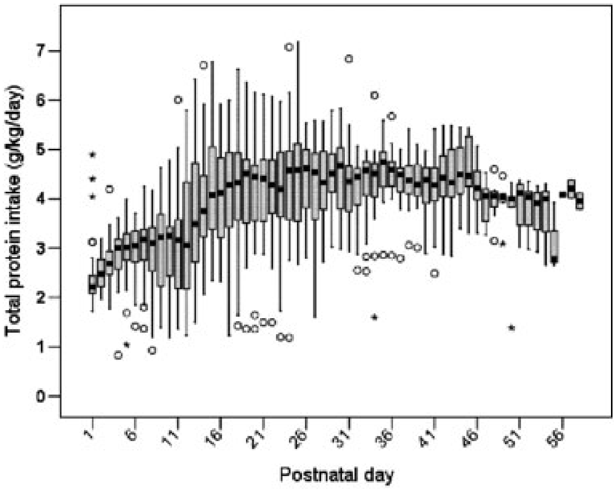

Fig. 4 Daily total protein intake (parenteral *plus* enteral) from birth to 35 postnatal weeks.

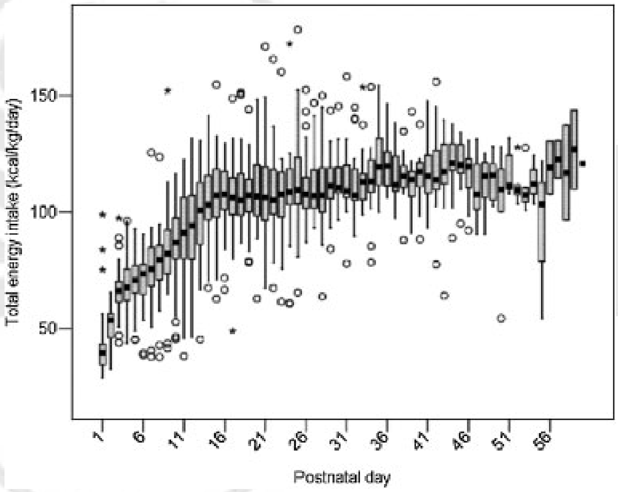

Fig. 5 Daily total energy intake (parenteral *plus* enteral) from birth to 35 postnatal weeks.

of: protein (g/kg/day) (β-estimate –0.65; 95% CI: –0.20, –0.80; $r^2 = 0.41$; $p < 0.0001$), energy (kcal/kg/day) and PER (β-estimate –0.67; 95% CI: –2.0, –0.83; $r^2 = 0.43$; $p < 0.0001$).

Body composition was considered only in 32 infants (10 were females and 6 extremely preterm), at a mean (SD) of 39.9 (1.9) weeks' PMA, since one infant was excluded because the measurement was performed much late. The body composition measurements showed a normal distribution, with a mean (SD) body mass of 2.818 (504) g, FM of 441.5 (184.0) g, FFM of 2.376 (376) g, FM% of 15.3 (4.8), and FMI of 2.0 (0.7). No significant differences were found in any body composition measurement, either between sexes or between the extremely preterm and very preterm infants. No correlations of daily protein, energy, and PER intakes with body composition measurements were also found.

In the univariable analysis, no associations were found between in-hospital nutrient intakes, potential confounding

Table 3 Daily protein, energy, and protein-to-energy ratio intakes from human milk, from birth to 35 weeks' postmenstrual age ($N = 33$)

Postmenstrual age (wk)	26	27	28	29	30	31	32	33	34	35
N	2	6	13	16	22	30	33	33	33	28
Protein intake (g/kg/d), median (IQR)	2.9 (2.4–3.5)	2.7 (2.5–3.6)	3.3 (2.7–3.5)	3.3 (3.1–4.0)	3.9 (2.7–4.7)	3.5 (2.8–4.7)	3.8 (2.9–4.5)	4.2 (3.4–4.6)	3.8 (3.3–4.4)	3.7 (2.6–4.3)
Energy intake (Kcal/kg/d), median (IQR)	53.7 (39.5–67.9)	63.5 (54.9–66.6)	72.4 (54.8–86.4)	78.3 (72.8–99.4)	101.4 (58.1–106.3)	102.5 (63.2–110.8)	104.5 (81.8–109.9)	108.5 (99.1–108.5)	109.2 (103.1–119.5)	106.7 (99.2–116.9)
PER intake, median (IQR)	5.6 (5.1–6.0)	4.6 (4.3–4.9)	4.8 (4.0–5.0)	4.4 (4.1–4.6)	4.2 (3.8–4.6)	4.2 (3.9–4.5)	4.0 (3.6–4.4)	3.8 (3.2–4.1)	3.4 (2.9–4.0)	3.4 (2.5–3.9)

Abbreviations: IQR, interquartile range; PER, protein-to-energy ratio; *N*, number of infants evaluated at each postmenstrual age.

variables, and body composition measurements; therefore, multivariate analysis was not performed.

Seven infants had a FM% of ≤ −1 *z*-score, 7 a FM% of ≥ +1 z-score, 4 a FMI of ≤ −1 z-score, and 8 a FMI of ≥ +1 *z*-score. The nested case–control analysis used to assess differences between infants with higher and lower adiposity, as compared with the remaining infants, showed some significant associations (►**Table 4**). In infants with lower adiposity, a FM % of ≤ −1 *z*-score was associated with lower energy and protein intakes, while a FMI of ≤ −1 *z*-score was associated with a lower PER intake. In infants with higher adiposity, an FMI of ≥ +1 *z*-score was associated with a lower energy intake and a higher PER intake (►**Table 4**).

Discussion

Provision of optimal nutritional support to promote adequate neurocognitive development without increasing the risk of obesity and metabolic syndrome is a current challenge in the care of very preterm infants.[48] In a previous cohort study of preterm infants, it was reported that a relative undernutrition during the neonatal period was associated with beneficial effects on insulin resistance in adolescence.[49] On the other hand, another study found that male preterm infants receiving a high-nutrient diet during the first postnatal weeks had larger caudate volumes and higher verbal intelligence quotients at adolescence.[50]

The present study aimed to determine, in a homogeneous sample of exclusively or almost exclusively HM-fed extremely and very preterm infants, the association between different cumulative in-hospital measured protein, energy, and PER intakes provided by fortified HM and their body composition at term equivalent age. Protein and energy intakes were measured, and a mixed model was used for accurate imputation of missing data. We found that 36.4 and 84.8% of infants did not receive the minimum targeted protein and energy intakes for body weight, respectively, in at least 75% of in-hospital days. This may reflect a poor effectiveness of the fortification method we used, based on the standard fortification method supplemented in a blinded manner with modular protein[8,9] and/or modular fat.

In similar studies measuring[14,15] or estimating[47] nutrient intakes provided by fortified HM and/or formula, insufficient nutrient intakes to achieve the current recommended targets[11] were reported, suggesting suboptimal effectiveness of other HM fortification methods as well.[14,15] However, nutrient intakes in our study are difficult to compare with data from most similar studies, as in these nutrient intakes from HM were estimated,[13,16,17] with exception of two that have measured as we did.[14,15]

In our cohort, the suboptimal nutritional support resulted in a mean weight gain velocity of 10.1 g/kg/day, without significant differences between the extremely preterm and very preterm infants. Significant positive weak-to-moderate correlations between nutrient intakes and weight gain velocity were found; specifically, protein, energy, and PER explaining 42.4, 26.4, and 21.7% of the weight gain velocity variation, respectively. However, in multivariable analysis only gestational age was significantly associated with low weight gain velocity, specifically for each 5-day increase in gestational age a decrease of 1 g/kg/day was observed. The weight gain velocity we found is lower than the velocities of

Table 4 Nested case–control study ($N = 32$), comparing protein, energy and PER intakes in infants with low adiposity (FM% and FMI ≤ −1 *z*-score) and high adiposity (FM% and FMI ≥ −1 *z*-score), with the remaining infants

FM%	≤ −1 *z*-score	> −1 *z*-score	*p*-Value
Protein intake (g/kg/d), median (IQR)	3.8 (3.1–4.4)	4.0 (3.0–4.6)	0.051
Energy intake (kcal/kg/d), median (IQR)	103.3 (88.8–114.6)	107.4 (88.6–118.5)	0.005
PER intake, median (IQR)	3.8 (3.4–4.3)	3.8 (3.2–4.3)	0.73
FMI	**≤ −1 z-score**	**> −1 z-score**	***p*-Value**
Protein intake (g/kg/d), median (IQR)	4.0 (3.0–4.4)	4.0 (3.1–4.6)	0.12
Energy intake (kcal/Kg/d), median (IQR)	108.0 (91.2–119.8)	106.3 (88.0–117.2)	0.16
PER intake, median (IQR)	3.7 (3.2–4.2)	3.9 (3.3–4.3)	0.026
FM%	**≥ +1 *z*-score**	**< +1 *z*-score**	***p*-Value**
Protein intake (g/kg/d), median (IQR)	4.0 (2.9–4.8)	4.0 (3.1–4.5)	0.542
Energy intake (kcal/kg/d), median (IQR)	104.3 (81.5–123.0)	106.8 (91.1–116.5)	0.503
PER intake, median (IQR)	3.9 (3.3–4.2)	3.8 (3.3–4.3)	0.872
FMI	**≥ +1 *z*-score**	**< +1 *z*-score**	***p*-Value**
Protein intake (g/kg/day), median (IQR)	4.0 (3.0–4.8)	4.0 (3.1–4.5)	0.118
Energy intake (kcal/kg/d), median (IQR)	103.3 (80.6–114.4)	107.9 (92.2–118.8)	< 0.0001
PER intake, median (IQR)	4.0 (3.5–4.5)	3.8 (3.2–4.2)	< 0.0001

Abbreviations: FM%, fat mass percentage; FMI, fat mass index; IQR, interquartile range; PER, protein-to-energy ratio.
Note: Mann–Whitney *U*-test.

11.4 and 18.3 g/kg/day reported for very preterm infants in previous studies.[14,19,47] Similarly to our results, a study in preterm infants receiving early low nutrient intakes showed mean velocities varying between 9.4 and 11.3 g/kg/day.[22]

Despite of the low in-hospital weight gain velocity, the body weight (0.139, $p = 0.205$), length (0.247, $p = 0.681$), head circumference (–0.37 cm, $p = 0.358$), and FM% (0.469, $p = 0.589$) of our infants at term equivalent age were similar to the values reported by Roggero et al.[47] In our sample, no significant differences were found in any body composition measurement, either between sexes or between the extremely preterm and very preterm infants. The absence of reported sex differences in body composition[51] may be due to a small sample dimension with an unbalanced sex distribution.

In the whole sample, no correlations between protein, energy, and PER intakes and body composition at term PMA were found. This may have resulted from the study being underpowered due to the small sample size. In line with our results, other authors[13,16,17] found that, in preterm infants, higher estimated protein and energy intakes were associated with better weight gain, but without significant differences in body composition. In contrast, other studies based on estimated or measured nutrient intakes found that, in preterm infants at term equivalent age, specific body compositions were associated with different nutritional strategies. In these studies, higher protein[15,19] and PER[18] intakes were associated with an increase in lean mass[18,19] and decrease in adiposity,[14] while higher fat and energy intakes were associated with increased fatness.[15]

We used a nested case–control analysis to explore the associations between cumulative in-hospital nutrient intakes and extremes of adiposity[46] at term equivalent age. Using a bicompartmental model, adiposity is more accurately estimated than leanness, because FM has a more constant density than FFM which is dependent on its different water content.[45,52] Moreover, FM is more homogenous, comprising predominantly adipose tissue, while FFM is a complex compartment containing not only skeletal muscle, but also bone, organs, and blood.[52] In our infants with lower adiposity (indicated by FM%), both energy and protein intakes were lower (thus not affecting the PER) than the remaining infants, although the lower energy intake was more significant than the lower protein intake (**►Table 4**). In this scenario, impairment of lipogenesis and/or facilitation of lipolysis might have occurred, resulting in low fat reserve.[20,53] In surgical neonates, indirect calorimetry combined with body composition analysis showed that poor energy and protein intakes resulted in preferential fat oxidation and decreased adiposity, suggesting increased lipolysis.[54,55] Our infants with higher adiposity (indicated by FMI) received significantly lower energy intake and higher PER intake, without differences in protein intake, compared with the remaining infants (**►Table 4**). In this context, it may be speculated that preferential protein oxidation in relation to fat oxidation might have occurred in response to poor energy supply, leading to less lipolysis and sparing of the fat reserve.[53,56]

The present study has important strengths that should be acknowledged. First, we examined the association between nutrient intakes and body composition relying on measured protein and energy provided by the HM and not on its assumed composition. Second, a validated accurate method was used to assess body composition.

However, this study also has some limitations. First, the study was interrupted before the estimated sample size was achieved, thus becoming underpowered and precluding the multivariate analysis. Second, a bias of withdrawal exists because enrolled infants completing the study were significantly more immature, were more frequently singletons, and stayed longer in the hospital than those excluded. As the excluded infants were more mature, they needed shorter hospitalization. In addition, when OMM was unavailable they became more frequently formula-fed, owing to DHM preferentially given to more immature infants in case of DHM shortage. In the case of twins, they were more frequently formula-fed due to insufficient breast milk for both. Third, nutrient intakes were not assessed between 35 and 40 weeks' PMA; during this period, only 63.6% of infants were exclusively breastfed, although in partially or exclusively formula-fed infants, formula feeding was generally initiated within 1 week before the body composition was assessed. Finally, convenience cut-offs of ≤ -1 z-score and $\geq +1$ z-score were used as surrogates of low and high adiposity; however, these need validation.

Conclusion

In conclusion, this cohort study on a homogenous sample of exclusively or almost exclusively HM-fed extremely and very preterm infants was prematurely interrupted, which may explain the absence of the hypothesized correlations between cumulative in-hospital nutrient intakes and body composition at term equivalent age. Notwithstanding, significant positive weak-to-moderate correlations of measured protein, energy, and PER intakes with weight gain velocity were found. A nested case–control analysis showed that infants with lower adiposity received significantly lower energy, protein, and PER intakes, while those with higher adiposity, received significantly lower energy intake but higher PER intake, than the remaining infants. The body composition of our infants did not differ significantly from that previously reported for extremely and very preterm infants. However, the measured nutrient intakes were much lower than those recommended. Further studies relying on measured HM nutrient intakes and using targeted HM fortification methods are needed.

Note

This study is part of a PhD thesis in Medicine – Pediatrics of one of the authors (Israel Macedo), supervised by one of the other authors (Luis Pereira-da-Silva) from NOVA Medical School, Universidade NOVA de Lisboa, Lisbon, Portugal.

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Conflict of Interest
None.

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