Four-Week Nutritional Audit of Preterm Infants Born <33 Weeks Gestation

Original Manuscript

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Abstract

Introduction Preterm nutritional audits have previously been conducted using assumed milk composition. We audited protein and energy intakes in the first 28 days of preterm life using both assumed milk composition and milk analysis to assess their effect on weight gain and to determine if the recommended reasonable range of intakes (ReasNI) were met.

Methods Parenteral (PN) and enteral (EN) intakes and weight gain were recorded daily for infants (n=63) born <33 weeks gestation, using assumed milk composition. Macronutrient composition was determined by milk analysis for a subset of infants (n=36). Linear mixed models analysis was used to assess the influence of energy and protein intakes on weight gain.

Results [Data median (range)]: Infants (n=63) gestation and birth weight were 30 (24-32) weeks and 1400 (540-2580) g, respectively. Macronutrient milk composition was variable: protein 16.6 (13.4-27.6) g/L, fat 46.1 (35.0-62.4) g/L, lactose 68.0 (50.9-74.8) g/L, energy 3074 (2631-3761) kJ/L. Intakes based on measured composition differed from assumed. Protein intake was significantly associated with weight gain. Compared to infants with longer gestations, those born <28 weeks gestation were fed lower volumes, were more reliant on PN, took an additional seven days to transition to fortified feeds and median weight gain velocity took a fortnight longer to reach targets.

Conclusion Preterm milk composition is variable and routine fortification using assumed composition may result in inappropriate nutrition. Fortification regimens stratified by birth gestation may be necessary to achieve preterm nutrition and growth targets. Milk analysis is required for accurate nutritional audit.

Keywords

Premature birth; clinical audit; nutritional support; human milk; nutritional requirements

What is already known about this topic:

1. Preterm nutritional audits are commonly based on assumed milk composition.
2. Macronutrient composition of human milk is variable.
3. Growth retardation is common in very preterm infants at discharge.

What this article adds:

1. Routinely fortifying milk on assumed composition may result in inappropriate nutrition for preterm infants due to the variable macronutrient composition of preterm human milk.
2. Milk fortification regimens targeted to gestational age groups may better assist in achieving nutrition and growth targets for preterm infants.
3. Safe upper levels of fortification, based on milk analysis, need to be determined.

Introduction

Over the past decade, preterm nutrition research has been directed towards addressing poor growth outcomes that are common at discharge[1] and which are related to nutritional intakes, especially in the first few weeks of life[2].

Routine nutrition practice is to fortify human milk (HM) using an assumed macronutrient milk composition and to audit nutritional intakes using assumed data[3-5]. Prior to 2005, the standard enteral feeding practice in the tertiary neonatal clinical care unit (NCCU) in Western Australia was to preferentially feed infants their own mother's milk (MOM) and to fortify the milk using commercial fortifiers, as directed by manufacturers. Glucose polymer was further added when weight gain velocity was below target. Term and preterm infant formula (IF) was fed when MOM was unavailable.

A review of routine practice revealed that these fortified feeds were unlikely to meet the reasonable nutrient intakes (ReasNI) for protein[6] and energy[7] recommended in 2005 for very preterm infants[8]. Thus, the fortification practice was revised to reflect more accurately the needs of the neonatal population[9], the osmolalities of the feeds were measured[10], and the feeds were integrated into standard clinical practice. In 2006, using assumed macronutrient composition data, the intakes and weight gain of infants achieved

with these feeds during the first 28 days of life was audited. In a subset of infants, samples of milk were measured to determine macronutrient composition, allowing a comparison to be made between assumed and measured protein and energy intakes on days when milk supply permitted sampling of an infant's milk feeds.

Materials and Methods

Infants born <33 weeks gestation, who were admitted to the NCCU at King Edward Memorial Hospital (KEMH) in Perth, Western Australia within the first 24 hours of life and who remained in the nursery for at least seven days, participated in this observational study. Infants with congenital abnormalities were excluded. Milk samples (when available), daily weights and feeding data were prospectively collected for one to four weeks for each infant, depending on length of stay. Informed consent was obtained from each infant's primary carer prior to commencing the study, which was approved by the Ethics Committee at KEMH.

Feeding Protocol

Infants were fed according to the NCCU's 2005 feeding protocol, which was to provide intravenous (IV) glucose on admission to all infants and to progress to parenteral nutrition (PN) (Baxter, Glucose 20% 1L, Baxter Primine™ 10% 1L; Baxter™ Intralipid™ 20% 1L) and initiation of minimal enteral feeds (MEF), usually within two to five days, or, if clinically stable, to progress directly to enteral feeding (HM or IF). Amino acids in parenteral solution and lipid emulsion were initially infused at 0.5 g $kg^{-1}d^{-1}$, with step-wise daily increments until parenteral ReasNI targets were met[8]. If mothers own milk (MOM) was unavailable, formula was provided. Once 150 mL $kg^{-1}d^{-1}$ was achieved, human milk was fortified to Level I (Table I) using an assumed macronutrient composition (protein 12 g L^{-1}, fat 38 g L^{-1}, lactose 70 g L^{-1}, energy 2800 kJ L^{-1}, 20 kcal 30 mL^{-1}). Level I fortification was fed up to a maximum of 180 mL $kg^{-1}d^{-1}$ and Level II fortification was introduced if an infant was growing poorly and was fluid restricted to ≤150 mL $kg^{-1}d^{-1}$. The anticipated protein and energy intakes achieved with these levels of fortification are summarised in Table I. Fortification was ceased near discharge.

HM Feeds and Sampling

Mothers began expressing milk for their infants soon after giving birth (usually within 24 hours). For quality control in the nursery, mothers used one container per milk expression, but in the home, mothers pooled their milk. A mother's milk was delivered to the hospital's central milk room, and depending on stage of lactation and volume, frozen in 14 mL, 50 mL or 200 mL containers until commencement of enteral feeding, upon which time, it was thawed, pooled if necessary, and dispatched to the nursery as

required. The infant's first milk feed was matched with the earliest milk the mother expressed and, if available, frozen milk continued to be fed sequentially for at least the first 14 days of feeding, after which time, mother's fresh or frozen milk was fed. This practice was to ensure infants received the colostrum and high protein content of the preterm mother's early milk[11, 12] and because freezing may reduce the risk of postnatal transmission of cytomegalovirus[13]. On the days when supply permitted, a well-mixed sample (1-3 mL) from each infant's unfortified milk feed was collected in 5 mL polypropylene vials (Disposable Products Pty Ltd, Adelaide, Australia) and frozen in a commercial freezer at -20^0 C until analysed.

Biochemical HM Analysis

Macronutrient composition of milk feed was determined by routine laboratory assay in the Hartmann Human Milk Research Laboratory at The University of Western Australia (Perth, Australia). Each assay has been described previously by Mitoulas et al[14]. ***Protein:*** The protein content of the milk feeds was determined by a modified Bradford method[15] using a commercial protein reagent (Bio-Rad Laboratories, Richmond. CA, USA). The Bio-Rad Protein Assay is a dye-binding assay in which a differential colour change of a dye occurs in response to various concentrations of protein. Protein standards were prepared from an aliquot of human milk and the protein concentration determined by the Kjeldhal method, as described by Atwood and Hartmann[16]. The detection limit of the assay was 0.75 g L^{-1} (n=12) and the inter-assay coefficient of variation (CV) was 2.76% (n=12). ***Fat:*** The fat concentration of unfortified human milk feeds was determined using the spectrophotometric method of Stern and Shapiro[17]. The detection limit of the assay was 0.82 g L^{-1} (n=12) and the inter-assay CV was 4.9 % (n=24). ***Lactose:*** The concentration of lactose in human milk feeds was determined using the modified method of Kuhn[18]. The recovery of a known amount of lactose added to milk samples was 99.04 ± 3.2% (n=10). The detection limit of the assay was 0.98 g L^{-1} (n=12) and the inter-assay CV was 6.5% (n=12). ***Energy*****:** The metabolisable energy content of unfortified milk was calculated using the Atwater conversion factors: protein (16 kJ g^{-1}), fat (37 kJ g^{-1}) and lactose (16 kJ g^{-1}).

Macronutrient Intake Data

Protein and energy intakes for each infant were calculated using assumed macronutrient milk composition data and product nutrient composition data. Data relating to parenteral (separated into nutritional and non-nutritional) and enteral fluid intakes were obtained from the daily observational charts from midnight on day two of life up to four completed weeks. Intake data on day one were excluded as these were not representative of a complete 24-hour period. If breastfed, and the feed was recorded as 'breastfeed without top-up', the volume consumed during a breastfeed was estimated to be equal to the infant's prescribed feed volume. If a top-up was required, the amount given was subtracted from the prescribed

volume and the balance estimated to be the volume of milk consumed during the breastfeed. The composition data obtained from the protein, fat and lactose assays were used to recalculate nutrient intakes for comparison between measured and assumed intake.

Growth Data

In keeping with nursery protocol, infants requiring intensive care (NICU) were weighed daily either in their incubator or with digital scales (g; SECA, Germany 10/20 kg) and those in special care were weighed twice weekly, with daily weight derived by interpolation between the two time points. Daily weight gain velocity (g $kg^{-1}d^{-1}$) was calculated each week of the audit using an exponential model that has been validated in preterm infants: $[1000*Ln(W_n/W_1)]/(D_n - D_1)]$, where *Ln* is the natural logarithm, *W* is the weight in grams, *D* is day, *1* is the beginning of the time interval and *n* is the end of the time interval[19, 20]. Birth weight was converted to z-score using Australian national birth weight data[21].

Statistical Analysis

Descriptive statistics for continuous data were based on medians, interquartile ranges (IQR) and ranges (R) or mean and standard deviation, according to normality. Categorical data were summarised using frequency distributions. Univariate comparisons between gestation groups, <28 weeks *vs.* ≥ 28 weeks, were made using Mann-Whitney tests for continuous data and Chi-square or Fisher exact tests for categorical outcomes. Linear mixed models regression analysis was used to determine the association of weight gain with nutritional intake across the four weeks of the audit. Candidate predictors of growth included energy and protein intakes and clinical factors such as respiratory support, antibiotics and days to full enteral feeds were also assessed for their influence on growth. Adjustment was made for gestational age, birth weight z-score and days to fortification of feeds. All tests were two-sided and p-values <0.05 were considered statistically significant. SPSS© 14.01 statistical software was used to analyse the data.

Results

Subjects

Seventy-two infants born <33 weeks were admitted to the NCCU during the two-month recruitment period between 1 October and 30 November 2006. Within the first week of life, five infants died and four infants were transferred to the NCCU's surgical unit at Princess Margaret Hospital for Children, Perth. The median (IQR; R) gestation and birth weight of infants (n=63) were 30 weeks (27-32; 24-32) and 1400 g (965-1750; 540-2580), respectively. Their clinical data, stratified by gestational age (<28 weeks; ≥ 28 weeks), are described in Table II.

Feeding

Infants received nutrition from various combinations of sources [parenteral nutrition (PN), intravenous dextrose (IV), human milk (HM), infant formula (IF)]: PN, IV & HM (n=30), PN, IV & IF (n=2), IV & HM (n=12), IV, HM & IF (n=18) and IV & IF (n=1). Parenteral nutrition was the predominant source in the first two weeks of life for infants born <28 weeks gestation and continued to provide over 40% and 20% of nutrition to these infants during the third and fourth weeks of life, respectively. Conversely, no more than 20% of the nutrition provided to older infants in the first week of life came from PN and by week two, infants born ≥ 28 weeks gestation received over 80% of their nutrition enterally (Figure I). Relative to older infants, those born < 28 weeks gestation were delayed in commencing (minimal) enteral feeds by two days, took an additional 12 days to achieve full enteral feeds and an additional seven days before transitioning to fortified feeds (Table II).

Milk Composition

Three hundred and forty-one samples of human milk feeds were collected for 36 infants. The number of samples collected for each infant ranged from 1 to 17, and sampling was dictated by a mother's milk supply, the number of days an infant was enterally milk-fed in the first 28 days of life and by the number of weeks the infant participated in the audit. The macronutrient compositions of the milk feeds were variable over the four-week audit period and median values for protein and fat (and therefore energy) were higher than the assumed values (Table III).

ASSUMED Protein and Energy Intakes and Weight Gain

The assumed protein and energy enteral and combined intakes of infants during each week of the audit are described in Table IV. Based on the assumed macronutrient composition of milk feeds, median estimated enteral and combined energy and protein intakes of infants born <28 weeks gestation did not meet recommended ReasNI for any week of the audit. Furthermore, fluid intakes were low in weeks two to four, relative to levels to which fortification was targeted, to the levels achieved by older infants and to those recommended[8]. The median protein energy ratio (PER) of enteral feeds did not fall within the recommended range until week four but the PER achieved with combined nutrition was within the range recommended for all weeks.

Conversely, after week one, older infants were mostly enterally fed and although fluid intakes did not reach the levels to which fortification was targeted, fluid and energy intakes, and the PER were within the recommended ranges of ReasNI. Infants met the ReasNI for protein by week three.

Infants born <28 weeks gestation did not reach the third trimester fetal rate of weight gain until week four of the audit [week 1: -2.7 (-24.1-10.8 g, week 2: 8.1 (-8.8-29.5) g, week 3: 12.0 (-7.1-25.0) g and week 4: 17.2 (0.0-28.9) g, whereas the weight gain of infants born ≥ 28 weeks gestation approached the fetal rate by week two (week 1: -10.0 (-28.2-9.2) g, week 2: 14.6 (2.2-25.2) g, week 3: 15.0 (-2.9-30.4) g and week 4: 16.6 (1.5-22.2) g].

MEASURED Protein and Energy Intake

The measured enteral intakes of 36 infants, calculated on days when milk samples were available, were compared to assumed intakes on corresponding days (Table V). Only one of these 36 infants transitioned to Level II fortification during the audit period. Generally, measured enteral protein and energy intakes were greater than those assumed for infants in both gestational age groups. Infants born <28 weeks achieved the ReasNI for enteral protein in week three but not week four of the audit, and the energy ReasNI was met by week three. An enteral PER of at least 2.8, which is within the recommended range, was achieved throughout the four-week audit period. Conversely, older infants met the ReasNI for energy in week two of the audit and exceeded it in weeks three and four. In these latter weeks, protein ReasNI was met and from week two, the achieved PER was within the range recommended (Table V).

The combined measured macronutrient intakes for these 36 infants were modelled against their weekly weight gain. Combined measured protein intake was found to have a positive effect on weight gain, after adjustment was made for gestational age, birth weight z-score and day of fortification; i.e. for every g increase in total protein intake there was an associated average 1.0 g $kg^{-1}d^{-1}$ increase in weight gain (95% CI 0.07-1.84, p=0.035).

Discussion

To our knowledge, this is the first Australian audit[22] to assess the influence of protein and energy intakes on weight gain in the first four weeks of preterm life using measured macronutrient milk analyses.

The mean macronutrient composition of the milk feeds was higher than the assumed values upon which our routine fortification was based, including protein, which was 5.1 g/L (42%) higher than the assumed value. This disparity in the mean value for protein is not surprising, as the assumed value more closely represents the protein content of preterm milk expressed after two[23] to three[24] months of lactation or of term milk[25], rather than of milk feeds made from milk expressed in the early weeks after preterm delivery, which was when the milk used for the feeds measured in this study was expressed. The measured protein content of the milk feeds, was in close agreement with that of others who have measured the composition of preterm human milk during the first 15[23, 26-28] to 30[23, 28] days of lactation.

Lai[23] measured the macronutrient content of preterm mothers' individual milk samples from left and right breasts of each expression within a 24-hour period on several days spanning the first 60 days of lactation, and found it varied considerably between and within mothers, between milk expressions and between breasts. Similar variations were found in the 24-hour unfortified milk intakes of the infants in this study which were made up from their own mothers' individual and pooled collections of expressed milk and may have included milk from different days. This variation in the composition of the feeds has implications for some infants if the milk feeds are then routinely fortified using an assumed composition as this study has shown. Nutritional intakes were not always within recommendations and measured intakes were different from those that were assumed. In this audit, the majority of infants prescribed Level I consumed lower fluid volumes than anticipated. It is noteworthy that at least 25% of protein intakes in infants ≥ 28 weeks gestation were at or above the upper limit of the reasonable recommended range; thus, had fluid intakes reached anticipated upper targets the potential existed for infants to consume protein in amounts exceeding requirements and possibly, even metabolic capacity.

The risk of over-feeding may be avoided by fortifying milk on measured milk composition and new methods have been evaluated[29-31] and are now available to facilitate its easy measurement. However, milk analysis is a time-consuming task and, in a busy tertiary neonatal unit, its clinical application in routine practice may be limited. Adjusting protein fortification on blood urea nitrogen (BUN) and using assumed macronutrient composition has proven a relatively simple and successful strategy for fortifying milk and for ensuring the metabolic capacity of infants is not exceeded; however, retrospective milk analysis, in a trial utilising this method, revealed that infants often received less protein than recommended and intakes were lower than assumed[32]. Safe upper limits of fortification need to be

determined and then easily implemented and safe fortification regimens need to be developed to facilitate clinicians in maximising fortification to achieve nutrition and growth targets.

It is noteworthy that in the first week of life, infants born <28 weeks lost a smaller amount of weight than older infants. This disparity is not entirely unexpected given the infants' clinical history and the Unit's nursing and feeding protocols. Younger infants are more likely to be nursed in humidified incubators and to have a delayed diuresis[33]. They are also more likely to be fed PN in the first week of life, compared to older infants. In the last trimester of pregnancy, the age-matched fetus accrues protein at a rate of approximately 1.5 g $kg^{-1}d^{-134}$ to 2.0 g $kg^{-1}d^{-135, 36}$, and most of the nitrogen reaching the fetus is supplied as amino acids. Approximately 1% of body protein stores is lost daily if no exogenous nitrogen is fed in the days following birth[37]. In this study, infants born <28 weeks gestation were fed at a higher PER and their nitrogen intakes, mainly from the amino acids in parenteral solutions, were higher than those of older infants, whose N intakes were mainly from the protein and free amino acids in unfortified HM. The amino acid preparation in the parenteral solution was an attempt to mimic the amino acid concentration in the cord blood of the last trimester of pregnancy (Baxter Primene™ 10%). Early high dose amino acid infusions soon after birth (targets: day 1: 3.5 g $kg^{-1}d^{-138}$; on day 2: 2.4 g $kg^{1}d^{139}$ or 2.5 g $kg^{-1}d^{-140}$) have been shown to reverse negative nitrogen balance without adverse effect[38-40] and improve short-term growth outcomes[41-43]. Indeed, data suggest that early parenteral nutrition of only a few days may influence later cognition[42, 44]. In this audit, over 30% of total fluid intake in the first week of life for all infants came from intravenous fluids other than PN, mostly due to the delay in prescribing PN or to ensure fluid targets were achieved whilst upgrading to full enteral feeds. Adopting recent recommendations to commence PN earlier and more aggressively[45] may improve growth of infants during the first week of life, avoiding the need for catch-up growth and the concerns relating to it[46-49], including increased risk of metabolic alterations and later chronic health outcomes[50-52].

The short audit period limited the capacity of this study to determine the influence of macronutrient intake and PER on discharge and longer-term preterm growth outcomes, including body composition. Given that the rate of weight gain of some infants did not match the fetal rate, it is possible that the preterm infants in this audit may have had an altered phenotype at term corrected age, compared to the infant born at term[53]. Further studies of longer duration are required to assess this outcome. However, based on milk analysis, routinely fortifying on assumed milk composition may result in inappropriate

nutritional intakes for some preterm infants, due to the variable macronutrient composition of preterm human milk. Safe upper levels of fortification, based on milk analysis, need to be determined and milk fortification regimens stratified by gestational age may better assist in achieving nutrition and growth targets.

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Table I Routine Fortification Practice and Targeted Nutrient Intakes, Based on Assumed Milk Composition

FORTIFICATION	
Level 1 – target volume 160-180 mL $kg^{-1}d^{-1}$	HMF (4g) + Promod (0.3 g)
Level 2 – target volume 130-150 mL $kg^{-1}d^{-1}$	HMF (4 g) + Promod (0.8 g) + Duocal (3 g)

EXPECTED NUTRIENT INTAKES BASED ON ASSUMED COMPOSITION
(Protein: 12 g L^{-1}; Energy: 2800 kJ L^{-1})

$kg^{-1}d^{-1}$	**Energy (kJ)**		**Protein (PER) (g)**	
	Fluid restricted 120 to ≤150 mL	**Non-fluid restricted** >150 to 180 mL	**Fluid restricted** 120 to ≤150 mL	**Non-fluid restricted** >150 to 180 mL
EBM	335-419	448-507	1.4-1.8 (1.9)	1.9-2.2 (1.9)
Level 1	n/a	557-624	n/a	3.9-4.4 (2.9)
Level 2	502-628	n/a	3.4-4.2 (2.8)	n/a

Table II Clinical history

	<28 weeks n=15			*≥28 weeks n=48*			
Variable	**n (%)**	**Median**	**(IQR, R)**	**n (%)**	**Median**	**(IQR, R)**	**p-value**
Male	7 (47%)			26 (54%)			0.905
Gestation (wk)		26	(25-27; 24-27)		31	(30-32; 28-32)	<0.001
Birth weight (g)		740	(635-965; 540-1185)		1545	(1263-1917; 775-2580)	<0.001
Discharge weight (g)		2850	(2360-3060; 1910-3280)		2050	(1832-2361; 1430-2935)	<0.001
Corrected discharge gestation (wk)		39	(37-41; 36-54)		35	(34-36; 32-40)	<0.001
Days in neonatal unit (d)		85	(68-114; 57-215)		25	(15-36; 9-73)	<0.001
Days requiring ventilation and/or CPAP <28 w n=14; ≥ 28 w n=17		48	(27-65; 1-90)		11	(3-21; 1-34)	0.001
Courses of antibiotics ≥ 2 (n (%))	11 (73%)			7 (15%)			<0.001
Days on parenteral nutrition <28 w n=15; ≥ 28 w n=18		19	(8-26; 4-39)		7	(4-12; 1-23)	0.003
Days to minimal enteral feeds		4	(3-8; 3-11)		2	(2-3; 1-10)	<0.001
Days to full enteral feeds		17	(10-28; 8-40)		5	(3-9; 2-41)	0.001
Days to fortification of feeds <28 w n=11; ≥ 28 w n=42		16	(14-22; 10-27)		9	(6-14; 1-46)	0.008

Table III Composition of Unfortified Milk Feeds Fed During the First Four-Weeks of Life

	Measured (n=341 feeds)	**Assumed**
	Mean (SD) ***Median (IQR; R)***	**Mean**
Protein (g L^{-1})	17.1 (2.6) *16.6 (15.4-18.2; 13.4-27.6)*	12
Fat (g L^{-1})	46.4 (6.2) *46.1 (42.2-50.9; 35.0-62.4)*	38
Lactose (g L^{-1})	68.1 (4.4) *68.0 (66.4-71.1; 50.9-74.8)*	70
Energy (kJ L^{-1})	3080 (255) *3074 (2913-3193; 2631-3761)*	2800
Energy (kcal 30 mL^{-1})	22 (2) *22 (21-24; 19-28)*	20
PER (1 g:419 kJ)	2.4 (0.3) 2.3 (2.2-2.5; 1.8-3.2)	1.8

Values - Mean (SD) and Median (range), PER - protein to energy ratio; 4.184 kJ = 1 kcal

Table IV Estimated intakes for all infants (n=63) calculated using assumed milk composition, compared with ReasNI for transitional and growing periods

<28 Weeks Gestation	Audit week	Infants (n)	Fluid (mL $kg^{-1}d^{-1}$)	Energy (kJ $kg^{-1}d^{-1}$)	Protein (g $kg^{-1}d^{-1}$)	PER (g protein:100 kcal)
Transition						
Parenteral ReasNI			**90-140**	**314-356**	**3.5**	
Enteral ReasNI			**90-140**	**377-419**	**3.5**	
Enteral Feed	1	15	1 (0-18)	4 (0-50)	0.0 (0.0-0.2)	0.6 (0.0-1.5)
Combined Nutrition Sources			134 (116-165)	199 (145-255)	1.4 (0.6-2.4)	2.4 (1.4-4.2)
Stable-Growing						
Parenteral ReasNI			**140-180**	**440-482**	**3.5-4.0**	**3.0-3.8**
Enteral ReasNI			**160-220**	**545-629**	**3.8-4.4**	**2.5-3.4**
Enteral Feed	2	15	8 (0-133)	5 (0-100)	0.1 (0.0-2.6)	1.5 (0.3-2.5)
Combined Nutrition Sources			140 (124-162)	333 (237-476)	2.2 (1.6-4.1)	3.0 (1.8-5.0)
Enteral Feed	3	15	90 (1-170)	295 (3-591)	1.5 (0.0-4.1)	1.8 (0.8-2.9)
Combined Nutrition Sources			141 (101-170)	421 (222-591)	3.3 (1.5-4.3)	2.9 (1.7-5.2)
Enteral Feed	4	15	139 (0-159)	477 (0-550)	3.2 (0.0-3.8)	2.8 (0.3-2.9)
Combined Nutrition Sources			139 (120-159)	477 (304-550)	3.4 (2.2-4.0)	2.9 (2.7-5.5)
≥28 Weeks Gestation						
Transition						
Parenteral ReasNI			**90-140**	**251-293**	**3.5**	
Enteral ReasNI			**90-140**	**314-377**	**3.5**	
Enteral	1	48	74 (8-93; 0-125)	210 (1-393)	1.0 (0.0-2.2)	1.8 (0.0-2.6)
Combined			128 (101-145)	271 (172-405)	1.2 (0.1-2.2)	1.4 (0.1-3.2)
Stable-growing						
Parenteral ReasNI			**120-160**	**377-419**	**3.2-3.8**	**3.2-4.2**
Enteral ReasNI			**135-190**	**461-545**	**3.4-4.2**	**2.6-3.8**
Enteral	2	40	149 (8-171)	482 (24-586)	2.7 (0.1-4.0)	2.3 (1.5-2.9)
Combined			153 (121-171)	485 (270-586)	2.9 (0.9-4.0)	2.6 (0.1-4.2)
Enteral	3	32	151 (37-169)	518 (105-584)	3.5 (0.4-4.1)	2.8 (1.5-2.9)
Combined			152 (124-169)	518 (259-584)	3.5 (1.2-4.1)	2.9 (1.8-3.3)
Enteral	4	19	153 (3.0-166)	525 (8-576)	3.6 (0.1-4.0)	2.9 (1.0-2.9)
Combined			153 (128-166)	525 (223-576)	3.6 (1.7-4.0)	2.9 (2.5-2.9)

Combined intake (parenteral, enteral (assumed composition) and IV); PER protein to energy ratio; ReasNI Reasonable nutrient intakes[8]; 4.18 kJ = 1 kcal; Data are median (range).

Table V **Comparison of enteral intakes of a subset of infants (n=36), using assumed *vs.* measured macronutrient milk composition**

	Week	Infants (n)	Milk Samples (n)	Fluid (mL $kg^{-1}d^{-1}$)	Energy (kJ $kg^{-1}d^{-1}$)	Protein (g $kg^{-1}d^{-1}$)	PER (g protein:100 kcal)
Assumed	1	2	2	23 (9-38)	64 (22-107)	0.3 (0.1-0.5)	1.8 (1.8-1.8)
Measured					54 (18-90)	0.4 (0.1-0.7)	3.2 (3.0-3.4)
Assumed	2	8	33	111 (3-156)	319 (9-486)	1.4 (0.0-3.4)	1.8 (1.8-2.9)
Measured					314 (8-565)	1.9 (0.1-4.0)	2.8 (2.5-3.9)
Assumed	3	8	30	147 (17-170)	504 (48-588)	3.5 (0.2-4.1)	2.9 (1.8-2.9)
Measured					571 (48-642)	3.9 (0.2-4.4)	2.8 (1.9-3.1)
Assumed	4	11	52	143 (99-159)	491 (277-550)	3.4 (1.2-3.8)	2.9 (1.8-2.9)
Measured					574 (283-592)	3.7 (1.2-4.4)	2.8 (1.8-3.3)
Infants ≥ 28 weeks gestation							
Assumed	1	6	9	73 (4-148)	205 (10-474)	0.9 (0.0-2.8)	1.8 (1.8-2.5)
Measured					256 (12-703)	1.4 (0.1-4.3)	2.3 (2.0-4.1)
Assumed	2	23	77	146 (3-169)	474 (10-580)	2.4 (0.0-4.1)	2.2 (1.8-2.9)
Measured					527 (9-762)	3.3 (0.1-5.6)	2.8 (2.3-3.8)
Assumed	3	18	70	152 (73-171)	513 (206-593)	3.5 (0.9-4.2)	2.9 (1.8-2.9)
Measured					610 (258-693)	4.1 (1.2-5.6)	2.9 (1.9-3.5)
Assumed	4	14	63	155 (131-166)	538 (501-578)	3.7 (3.3-4.1)	2.9 (2.7-2.9)
Measured					592 (517-699)	4.1 (3.7-4.8)	2.8 (2.4-3.3)

PER - protein to energy ratio; ReasNI - Reasonable nutrient intakes[8]; 4.18 kJ = 1 kcal; Data are median (range)