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Body composition in very preterm infants before discharge is associated with macronutrient intake

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Abstract

Very preterm infants experience poor postnatal growth relative to intra-uterine growth rates but have increased percentage body fat (%fat). The aim of the present study was to identify nutritional and other clinical predictors of infant %fat, fat mass (FM) (g) and lean mass (LM) (g) in very preterm infants during their hospital stay. Daily intakes of protein, carbohydrate, lipids and energy were recorded from birth to 34 weeks postmenstrual age (PMA) in fifty infants born <32 weeks. Clinical illness variables and anthropometric data were also collected. Body composition was assessed at 34–37 weeks PMA using the PEA POD Infant Body Composition System. Multiple regression analysis was used to identify independent predictors of body composition (%fat, FM or LM). Birth weight, birth weight *z*-score and PMA were strong positive predictors of infant LM. After adjustment for these factors, the strongest nutrient predictors of LM were protein:carbohydrate ratios (102–318 g LM/0·1 increase in ratio, $P = 0{\cdot}006$–$0{\cdot}015$). Postnatal age (PNA) and PMA were the strongest predictors of infant FM or %fat. When PNA and PMA were accounted for a higher intake of energy (–1·41 to –1·61 g FM/kJ per kg per d, $P = 0{\cdot}001$–$0{\cdot}012$), protein (–75·5 to –81·0 g FM/g per kg per d, $P = 0{\cdot}019$–$0{\cdot}038$) and carbohydrate (–27·2 to –30·0 g FM/g per kg per d, $P = 0{\cdot}012$–$0{\cdot}019$) were associated with a lower FM at 34–37 weeks PMA. Higher intakes of energy, protein and carbohydrate may reduce fat accumulation in very preterm infants until at least 34–37 weeks PMA.

Key words: **Preterm infants: Macronutrients: Lean mass: Fat mass**

Preterm infants at term equivalent age have increased adiposity (percentage body fat (%fat) and fat mass (FM)) and reduced lean mass (LM) compared with infants born at term[1]. This rapid accumulation of fat commences by 32–33 weeks postmenstrual age (PMA) and may be initiated by events associated with birth[2].

Potential adverse impacts of alterations in body composition in early infancy have been reported in a number of recent observational studies. These include the consistent observation that higher adiposity during the first 2 years of infancy in infants born at term is associated with an increased risk of obesity and the metabolic syndrome (type 2 diabetes, adverse cardiovascular outcomes) in later life[3,4]. Adults born preterm also have increased rates of cardiometabolic disease, and this has also been associated with early rapid fat accumulation[5,6]. Equally important in preterm infants has been the observation that a restriction in lean body mass growth has a stronger association with adverse neurodevelopment than does overall weight restriction[7]. It is possible that nutrient intake that is not matched to the requirements for normal growth drives both these alterations in body composition and the adverse neurologic and cardiometabolic outcomes in preterm infants.

There is limited evidence regarding the effect of macronutrient intake on fat accumulation and LM growth during the period between very preterm birth and term equivalent age. Therefore, the aim of the present study was to assess the association between energy, protein, fat and carbohydrate intakes and body composition in infants born at <32 weeks gestation and measured before term equivalent age.

Methods

Subjects

Preterm infants were recruited from the Royal Brisbane and Women's Hospital, Australia, between 1 April 2011 and 11 July 2016. Infants were considered eligible if their birth gestational age was less than 32 weeks, they were medically stable, required no supplemental O_2 or respiratory support (constant

Abbreviations: %fat, percentage body fat; BW, birth weight; BWZ, birth weight *z*-score; FM, fat mass; LM, lean mass; PMA, postmenstrual age; PNA, postnatal age.

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positive airway pressure, or high flow nasal cannulae), were receiving <50 ml/kg per d intravenous therapy at the time of body composition measurement and had more than 21 d of nutritional data available. When infants were still receiving intravenous infusions of <50 ml/kg per d, their intravenous lines were flushed and capped during measurements. Exclusion criteria were the presence of chromosomal anomalies, a known enteral malabsorption, major gastrointestinal surgery or cyanotic congenital heart disease. The present study was conducted according to the guidelines laid down in the Declaration of Helsinki, and the Human Research Ethics Committees of the Royal Brisbane and Women's Hospital (Protocol number HREC 10/QRBW/475) and The University of Queensland (Project number 2011000426) approved all procedures involving human subjects/patients. Written informed consent was obtained from the parents of all subjects.

Body composition measurement

Body composition was measured at 34^{+2}–37^{+1} weeks PMA. Body composition measurements were performed using the PEA POD Infant Body Composition System (Cosmed). Infant preparation for measurement followed manufacturer's recommendations. Hospital identification bracelets, umbilical cord clip, nasogastric tubes and electrocardiogram (ECG) electrodes, when present, were tared before undertaking mass and volume measurements. Infants were naked during all measurements. Infant mass was assessed using the integrated scale which is accurate to 0·1 g. Infants were then placed in a closed chamber to determine body volume by measuring changes in pressure within the chamber. Whole-body density was derived by dividing body mass by body volume. Assuming a two-compartment model, the proportion of fat and lean (fat-free) compartments was derived from body density by algorithms integral to the PEA POD system. Anthropometric measurements included weight (using the PEA POD scale), crown-heel length to 0·1 cm (using a supine length board) and maximal occipital-frontal head circumference to 0·1 cm.

Macronutrient intake

The nursery nutritional protocol for very preterm infants included establishing parenteral nutrition in the first 6 h after birth and introduction of trophic enteral breast milk in the first day. Enteral feeds for this gestational group, in all but one infant, were exclusively breast milk. Forty of the fifty infants studied were given mothers own milk for more than 90 % of the study period. Where mother's own milk was not available, pasteurised donor human milk was used. Six infants received exclusively donor milk on more than 30 % of days during the study period. No infants received exclusively donor milk for more than 60 % of the study period. Human milk fortification with a multicomponent cows' milk-based supplement S26 Human Milk Fortifier (Wyeth) half or full strength was used by individual consultant judgement based on infant tolerance. The full strength fortifier provided an additional 1·0 g/100 ml of protein, 2·3 g/100 ml of carbohydrate and 0·15 g/100 ml of lipids. Nineteen infants received partial or fully fortified breast milk and thirty infants received unfortified breast milk exclusively.

Daily nutrient intakes were calculated from birth until infants were 34 weeks PMA when intake measurement became less precise due to significant suck feeding at the breast. Daily intake volumes of all parenteral and enteral nutrition were obtained from the infant medical records commencing at 24.00 hours on the day of birth. The total daily intake of energy, protein, lipids and carbohydrate in kJ or g/kg per d was calculated using published nutrient composition for each solution or enteral feed. As expressed breast milk macronutrient composition is known to vary depending on the postnatal day of expression and the proportion of hind milk in the sample, assumed macronutrient values for breast milk were derived from recent published average values for breast milk expressed from 2 to 8 weeks postpartum[8]. These values were 274 kJ energy/100 ml, 1·27 g protein/100 ml, 3·46 g lipids/100 ml and 7·34 g carbohydrate/100 ml. Protein was defined as bioavailable N multiplied by the general conversion factor 6·25[8]. The effects of pasteurisation, freezing and thawing on the macronutrient content of donor human milk were based on the published estimation of a reduction of 5·5 % in lipid content and 3·9 % in protein content when compared with fresh expressed breast milk[9]. The daily intake of energy, protein, lipids and carbohydrate in kJ or g/kg per d was calculated. The average daily intake was also calculated for the following periods: days 1–3, days 1–7, days 1–14, days 8–14, days 15–21, the 34th week and day 1–34 weeks. Protein:energy ratios were calculated using both total energy and non-protein (residual) energy. Protein:carbohydrate ratios were also calculated. The fraction of total and enteral protein intake derived from human milk (mother's own milk and donor milk) or cows' milk was recorded as was the proportion of days that infants received only human milk with no additional protein (parenteral or fortifier).

Other measures

Weight and head circumference *z*-scores at birth and at body composition measurement were calculated for each infant using the PediTools Growth Parameter calculator (https://peditools.org/fenton2013/) based on Fenton[10]. FM and LM *z*-scores were calculated using the calculator provided by Demerath *et al.*[11]. Weight velocity in g/kg per d was calculated using Patel's equation[12]:

$$\text{Weight velocity} = (1000 \times \ln(W_n/W_1))/(D_n - D_1),$$

where W_n is the weight at body composition measurement, W_1 is the birth weight (BW) and $D_n - D_1$ are days from birth to measurement.

Statistical analysis. Data were analysed using the Statistical Package for Social Sciences (SPSS), version 25 (IBM). Potential predictors fell into five categories:

- factors relating to maturity and size at birth (BW and birth weight *z*-score (BWZ)),
- factors relating to age at measurement (PMA and postnatal age (PNA)),
- factors relating to severity of illness (sex, infant morbidity including hours on supplemental O_2, hours of intubation,

hours on constant positive airway pressure, episodes of septicaemia (defined as blood culture positivity and treated with >72 h of antibiotics) and necrotising enterocolitis defined as Bell stage 2 or greater[13]),
- maternal factors (pre-pregnancy BMI, maternal age at delivery, diagnosis of type 1 diabetes, type 2 diabetes, gestational diabetes, pre-pregnancy hypertension or pre-eclamptic toxaemia), and
- macronutrient intakes.

Univariate analyses using Pearson's coefficient were used to identify the variables correlated with growth and body composition outcomes. Potential predictors were identified from those with $P < 0{\cdot}2$ in the univariate analysis. Where multiple factors were intercorrelated (e.g. BW and birth gestation), only the one with the highest R value was included in the regression analysis. In addition, all models initially included at least one factor from each category (even if $P > 0{\cdot}2$ in univariate analysis), so that the influence of all areas could be considered. Backward stepwise regression was used to identify the best model for predicting body composition. Macronutrient intakes were significantly intercorrelated, and so each nutrient was examined separately in a multiple regression analysis adjusted for other independent predictors. Potential interactions between macronutrient predictors were examined using ratios including protein:energy and protein:carbohydrate. Significance was accepted when $P < 0{\cdot}05$.

Table 1. Infant characteristics at birth and time of body composition assessment
(Mean values and standard deviations; numbers and percentages)

Infant cohort (*n* 50)	Mean	SD
Birth gestation (weeks)	28·7	1·9
Birth weight (g)	1175	296
Birth weight *z*-score	0·07	0·86
Number born SGA (<10th percentile)		
n	3	
%	6	
Birth head circumference (cm)	26·5	1·8
Birth head circumference *z*-score	0·31	1·21
Sex: male		
n	24	
%	48	
When measured at 34–37 weeks		
Postmenstrual age (weeks)	35·6	0·8
Postnatal age (weeks)	6·9	2·1
Weight (g)	2172	363
Weight *z*-score	−0·96	0·75
Head circumference (cm)	31·6	1·7
Head circumference *z*-score	−0·36	0·90
Change in weight *z*-score	−1·04	0·58
Change in head circumference *z*-score	−0·67	1·18
Weight velocity (g/kg per d)	13·05	2·52
Percentage body fat	17·3	5·7
Fat mass (g)	384	155
Fat mass *z*-score	+1·10	1·04
Lean mass (g)	1788	278
Lean mass *z*-score	−1·77	1·16

SGA, small for gestational age.

Results

Descriptive data for the fifty infants studied are shown in Table 1. Weight *z*-score fell by an average of 1·04 and head circumference *z*-score fell by 0·67 SD between birth and body composition assessment at 34–37 weeks representing significant postnatal growth restriction.

The intakes of energy and lipids were lower than recommended levels in the first week after birth but were within the recommended range from days 8 to 14 and from birth to 34 weeks PMA (Table 2). Average protein intakes did not reach recommended levels at any time point (Table 2). Average carbohydrate intakes were above the recommended range after day 3 (Table 2). Protein:energy ratios were significantly lower than consensus recommendation over the whole study period, and protein:carbohydrate ratios were approximately half of those recommended.

There was no difference in weight, weight *z*-score or body composition at 34–37 weeks between infants who received fortified breast milk and those who received unfortified breast milk. There was also no difference in these outcomes between infants who received predominantly mother's own milk compared with those who received exclusively donor milk for more than 30 % of the study period, although this may be due to lack of power resulting from the small number of infants in the latter group.

Predictors of body composition

LM (g) at 34–37 weeks was significantly and positively associated with BWZ (R 0·703, $P < 0{\cdot}001$), BW (R 0·636, $P < 0{\cdot}001$) and PMA (R 0·315, $P < 0{\cdot}026$). All analyses of nutritional predictors of LM were adjusted for these three factors. While there was some collinearity between BW and BWZ, the combination of these variables produced a model that was stronger than that with either variable alone. Collinearity statistics indicated that collinearity was not a problem in the models with a variance inflation factor value between 1·0 and 2·0. Energy intake was not a significant predictor of LM at any time point studied. Higher protein and lipid intakes were associated with higher LM in the first week but not at later time points (Table 3). There were no significant associations between carbohydrate intake and LM. The most consistent and strongest nutritional predictor of LM was protein:carbohydrate ratio. Higher ratios at 1–3 d, 1–7 d, 1–14 d, week 34 and averaged across the whole period from birth to 34 weeks were significantly associated with higher LM (Table 3). A higher amount (g) of protein originating from breast milk as a proportion of the total enteral protein was significantly associated with a lower LM (Table 3). There were very few nutrient predictors of LM index. Early protein:carbohydrate ratio remained a significant predictor, but the model was not as strong as for LM.

FM at 34–37 weeks was significantly and positively associated with PMA (R 0·563, $P < 0{\cdot}001$) and PNA (R 0·559, $P < 0{\cdot}001$). Markers of illness (hours on supplemental O_2, hours of intubation, hours on constant positive airway pressure) were also positively associated with FM but did not remain significant when adjusted for PMA and PNA. There was no association between maternal BMI and very preterm infant FM. All analyses of nutritional predictors of FM were adjusted for PMA and PNA. Higher protein, carbohydrate and total energy intake at 15–21 d, week

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Table 2. Average macronutrient intake for postnatal periods studied, and intakes recommended by the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN)[23] (Mean values and standard deviations; ranges)

Postnatal period	Energy (kJ/kg per d)*		Protein (g/kg per d)		Carbohydrate (g/kg per d)		Lipids (g/kg per d)		Protein: total energy (g/100 kJ)		Protein:NP energy (g/100 kJ)		Protein: carbohydrate (g/g)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Days 1–3	230	63	1·10	0·74	8·9	1·7	1·7	0·9	0·378	0·225	0·437	0·272	0·10	0·07
Days 1–7	360	54	2·07	0·68	13·2	2·3	2·6	0·7	0·495	0·148	0·569	0·186	0·14	0·05
Days 8–14	518	42	2·83	0·55	16·1	2·6	5·4	1·0	0·545	0·086	0·602	0·110	0·17	0·02
Days 15–21	502	33	2·57	0·46	13·9	1·5	6·1	0·7	0·507	0·062	0·557	0·076	0·18	0·02
Week 34	502	46	2·60	0·56	13·8	1·5	6·1	0·2	0·511	0·072	0·562	0·088	0·19	0·02
Day 1 to 34 weeks	473	29	2·51	1·44	14·1	1·3	5·3	0·4	0·509	0·069	0·566	0·088	0·17	0·02
ESPGHAN recommendation for enteral feeding (ranges)	460–565		<1 kg: 4·0–4·5 1–1·8 kg: 3·5–4·0		11·6–13·2		4·8–6·6		<1 kg: 0·86–0·98 1–1·8 kg: 0·76–0·86		Not stated		<1 kg: 0·34–0·38 1–1·8 kg: 0·30–0·34	

NP, non-protein.

34 and over the whole study period (day 1–34 weeks) were all significantly associated with a lower very preterm infant FM (Table 3). Predictors of %fat were similar to those for FM. Predictors of FM index were almost identical to those for FM, but the models were not as strong as for FM.

The relationship between weight velocity and body composition was investigated to determine whether rapid growth rates are the result of LM or FM accumulation. Weight velocity was significantly and negatively associated with BW (R –0·613, $P < 0·001$) and BWZ (R –0·587, $P < 0·001$). It was also associated with sex (females had higher weight velocity), although sex did not make a significant contribution when entered in the multiple regression. All analyses of the relationship between weight velocity and body composition were adjusted for BW and BWZ. When considered together, higher values of LM and FM contributed similarly to increased weight velocity (Table 3). However, when the distribution between these compartments was considered by including %fat or percentage LM, weight velocity was positively associated with %fat and negatively with percentage LM, suggesting that the infants with the fastest weight velocity were those with the highest %fat and the lowest percentage LM (Table 3).

Discussion

The present study has identified nutritional and other predictors of LM, FM and %fat in fifty very preterm infants before term equivalent age.

The strongest predictors of LM at 34–37 weeks PMA were BW and BWZ – babies born with the lowest weight z-score had the lowest LM at 34–37 weeks. After adjustment for BW, BWZ and PMA, the most significant nutritional predictors of LM were protein:carbohydrate ratios during the first 2 weeks after birth and averaged over the period from birth to 34 weeks. Protein: carbohydrate ratios were stronger predictors of LM than protein intake alone. This finding is consistent with the findings of McLeod *et al.*[14] who could only detect a significant relationship between nutrition and LM when both protein and carbohydrate intakes were included in the model. They reported a positive association between LM and overall protein intake and a negative association with carbohydrate intake. A similar study in infants born slightly larger and about 3 weeks later than our cohort also found that BWZ was the strongest predictor of LM at discharge but was unable to identify nutritional predictors of absolute LM, possibly because the period of nutritional data collection was very short (<3 weeks), and they did not report carbohydrate or lipid intakes[15]. They did report that higher protein:energy ratios were associated with a decreased risk of LM >2 SD below reference values[15,16].

A randomised trial of increased protein intake in the first days after birth in very preterm infants reported no effect on non-adipose mass by MRI at 37–44 weeks[17]. Several studies of nutrition after term corrected age have similarly reported that higher protein intakes or higher protein:carbohydrate ratios were associated with greater LM gain[18,19]. Growth of LM rather than just overall weight gain is critical because LM gain is a stronger predictor of better neurodevelopmental outcome than weight gain[7]. Together these results suggest that in very preterm infants

Table 3. Models of very preterm infant body composition in relation to macronutrient intakes and growth (Effect sizes and model characteristics)

Outcome variable	Nutrient intake	Nutrient effect size	*P*	*R* for overall model
LM (adjusted for BWZ, BW and PMA)	Protein			
	Days 1–3	83·6 g LM/g per kg per d	0·027	0·860
	Lipids			
	Days 1–3	67·8 g LM/g per kg per d	0·029	0·860
	Days 1–7	74·5 g LM/g per kg per d	0·032	0·859
	Protein:carbohydrate			
	Days 1–3	102 g LM/0·1	0·006	0·869
	Days 1–7	137 g LM/0·1	0·015	0·863
	Day 1–34 weeks	318 g LM/0·1	0·009	0·866
	Human milk protein/all enteral protein	–0·28 g LM/0·1	0·042	0·857
FM (adjusted for PNA, PMA)	Energy			
	Day 1–34 weeks	–1·41 g FM/kJ per kg per d	0·012	0·706
	Days 15–21	–1·61 g FM/kJ per kg per d	0·001	0·737
	Protein			
	Day 1–34 weeks	–81·0 g FM/g per kg per d	0·038	0·690
	Week 34	–75·5 g FM/g per kg per d	0·019	0·699
	Carbohydrate			
	Days 15–21	–27·2 g FM/g per kg per d	0·019	0·700
	Week 34	–30·0 g FM/g per kg per d	0·012	0·706
Weight velocity (adjusted for BW and BWZ)	FM	0·006*	<0·001	0·791
	LM	0·006*	<0·001	0·830
	Percentage body fat	0·117*	0·015	0·748
	Percentage LM	–0·117*	0·015	0·748

LM, lean mass; BWZ, birth weight *z*-score; BW, birth weight; PMA, postmenstrual age; FM, fat mass; PNA, postnatal age.
* Value for FM, LM, percentage body fat or percentage LM.

during the period before term corrected age that it may be necessary to manipulate both protein and carbohydrate intakes in order to increase LM and improve neurodevelopment.

After adjustment for PMA and PNA, the strongest predictors of FM and %fat at 34–37 weeks in very preterm infants were energy, protein and carbohydrate intakes from birth to 34 weeks and in the later weeks of the study period. The strong association between PNA and FM is possibly due to an adaptive mechanism that provides protection against hypothermia and intermittent nutrient availability following separation from the constant nutrient supply provided by the placenta, as discussed in our previous publication[2]. The lack of association between FM or %fat and maternal BMI may indicate that the influence of maternal BMI on infant FM at birth in term babies may occur late in pregnancy or that this influence has been overwhelmed by postnatal factors in very preterm infants.

The associations between nutrient intake and FM were strongest for energy and carbohydrate and were negative, indicating that lower intakes were associated with higher FM and % fat. This surprising finding is consistent with several published studies (described below), although other studies report different findings. In a randomised trial of individualised fortification of breast milk for very preterm infants, those with protein intakes below 3·4 g/kg per d had higher %fat than those with intakes >3·4 g/kg per d[20]. However, in an earlier study from the same group, higher lipid and energy intakes were associated with a higher FM[14]. A small observational study of body composition at term in very preterm infants found that those infants with the highest adiposity had received lower energy intakes than those with average adiposity, but those with lower adiposity had also received lower energy as well as lower protein intakes[21]. However, a randomised trial of increased protein intake in the first days after birth in very preterm infants reported no effect on adipose mass[17], and Simon *et al.*[15] were not able to identify nutritional predictors of FM in very preterm infants before term. These small studies may have lacked the power to detect a relationship. Several studies have reported that lower protein intakes after term corrected age were associated with higher %fat at 1 month corrected age or higher %fat and FM gain at 6 months corrected age[18,19].

In our study, FM was not associated with lipid intake suggesting that excess fat accumulation is not due to high lipid intake. It has been suggested that excessive glucose intake may lead to increased fat deposition[22]. Carbohydrate intake was above ESPHGAN recommendations in our cohort; however, we report a significant negative association between carbohydrate intake from birth to 34 weeks and FM suggesting that higher carbohydrate intake in this period does not contribute to greater fat accumulation. Agostoni *et al.* suggested that a low protein intake in association with high-energy intake will lead to higher fat deposition[23]. This was partially true in our study, where a lower protein intake was associated with a higher fat deposition. However, the negative association with energy and a lack of relationship with protein:energy ratios do not support the suggestion that high-energy intakes contribute to early fat accumulation.

An alternative explanation could be the phenomenon of 'catch-up fat' – the preferential deposition of fat when weight is gained after a period of nutrient restriction[24]. This is thought to be an adaptive mechanism that will protect against future periods of nutrient restriction. In our cohort, there was a period of nutrient restriction – protein, lipid and energy intakes were well below recommended intakes in the first week after birth. This was followed by a period of improved nutrition where lipid

and energy intakes were within the recommended range and protein intake was significantly higher than during the first week, but still below recommended intakes. This idea is also supported by the observations that in very preterm infants a greater loss of weight *z*-score between days 1 and 5 was associated with increased FM at discharge[15]. While our previous results suggest that an early drive to accumulate an energy reserve may be obligatory[2], our present results suggest that it may be intensified by a programmed response that is triggered by inadequate nutrient intakes. This could explain the negative associations between nutrient intake and FM and may also explain the increased risk for later adiposity and associated disease seen in adults born preterm[6].

A concerning possibility is that the drive to accumulate fat (due to a normal adaptive mechanism triggered by birth[2] and exacerbated by early nutrient restriction) is so strong that fat deposition may occur at the expense of LM growth. The possibility is supported by our observation that LM *z*-score at 34–37 weeks was –1·77 – almost double the deficit in weight, but at the same time point, FM *z*-score was +1·10. Thus, the very significant deficit in LM in our cohort of very preterm infants (who were on average appropriate for gestational age at birth, BWZ 0·07) may be due to both direct effects of nutrient restriction and also the redirection of nutrient resources to fat accumulation. If this is the case, early rapid fat accumulation in very preterm infants may contribute to both their adverse neurodevelopmental outcomes and their increased risk of poor metabolic and cardiovascular outcomes[6,25,26]. This possibility underlines the urgent need for a better understanding of the way that nutrient intakes influence the growth of both LM and FM.

The associations between infant body composition and growth velocity highlight the potential for misleading conclusions when weight alone is measured. While both LM and FM growth contribute to weight velocity, the fastest weight velocities were seen in those with the highest %fat and the lowest percentage LM. Similarly, another study in very preterm infants has reported that faster postnatal growth from day 5 to discharge was associated with a higher FM at discharge[15]. In very preterm infants studied from term equivalent age to 1 month corrected age, a higher protein intake was associated with higher percentage LM but lower overall weight gain[18]. Thus, it should not be assumed that rapid weight gain means equally rapid growth of LM – it may just indicate rapid fat deposition. This may explain why many trials of nutritional interventions have produced an increase in growth without an improvement in neurodevelopmental outcome[27].

Our study suggests that nutrient intakes in the first week after birth, particularly protein:carbohydrate ratios, have the strongest influence on LM at discharge or close to term corrected age. In contrast, macronutrient intakes over the whole study period or later in the study period have a greater association with FM.

There have been some suggestions that human breast milk proteins support better growth than protein from other sources[28,29]. We have been unable to confirm this suggestion. In fact, our results could suggest the opposite as a higher proportion of breast milk protein/total protein in enteral intakes was associated with lower LM. However, this may be a reflection of lower total protein intakes in babies fed breast milk only with no fortification, rather than an effect of protein quality. We did not find any differences in outcome between infants who received fortified breast milk compared with those who did not. However, the majority of infants receiving fortification received only half strength fortifier or did not receive fortification for the whole study period. This level of fortification may not have been sufficient to alter growth or body composition.

Strengths of the present study include the sample size, continuous rather than intermittent nutrient data collection and cessation of nutrient data collection at 34 weeks or when significant intake was obtained directly from the breast. Thus, data do not include errors associated with unknown breast milk intake. Nutrient data include lipid and carbohydrate intakes as well as the commonly studied protein and energy intakes, and we were able to examine the influence of different time periods. To our knowledge, this is the largest study of infant body composition with continuous nutrient data from birth to 34 weeks PMA including protein, carbohydrate, lipid and energy intakes and analysis over multiple time periods.

The study was limited by the lack of body composition measurements at birth and therefore the inability to calculate LM or FM trajectories. However, it is not possible to obtain body composition measurements at birth in very preterm infants as the PeaPod requires subjects to have no respiratory support or parenteral infusions, excluding the majority of very preterm infants from measurement until later in their course. Ideally, we would have measured all subjects at the same PMA. However, variable ages at which babies were able to be measured and variable ages at discharge made this impossible. We have made assumptions about the composition of breast milk that do not account for variation between individuals and between days and times. However, the values used are based on average values obtained from multiple studies and are the same as those used in similar studies[8,15]. Our cohort had low-protein intakes, although these were similar to other studies[15], and so results may not be generalisable to groups with higher intakes.

In conclusion, our study supports the hypothesis that in very preterm infants, the accumulation of fat may be an obligatory response triggered by birth to improve survival during intermittent nutrient supply after separation from the placenta. This drive to accumulate fat could be intensified when nutrient intakes are low. Increased growth of LM may require both protein and carbohydrate intakes to be manipulated to reduce the protein: carbohydrate ratio.

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B. E. L., T. J. D. and P. B. C. formulated the research questions and designed the study. N. T., T. J. D. and B. E. L. conducted infant measurements and data collection. B. E. L., N. T. and Y. A. E. analysed the data. B. E. L. and N. T. drafted the manuscript. All authors reviewed and revised the manuscript and approved the manuscript for publication.

The authors declare that there are no conflicts of interest.

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