

rhBSSL Improves Growth and LCPUFA Absorption in Preterm Infants Fed Formula or Pasteurized Breast Milk

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ABSTRACT

Objectives: Preterm infants often experience suboptimal growth, which can affect organ development. The aim of this study was to improve growth by treatment with bile salt-stimulated lipase (BSSL), naturally present in breast milk, but lost after pasteurization, and absent in formula.

Methods: Two clinical trials were performed with a predefined analysis of combined data to investigate the effects of recombinant human BSSL (rhBSSL) treatment on growth velocity and fat absorption in preterm infants. The studies were randomized and double-blinded comparing 7-day treatment with rhBSSL and placebo, administered in pasteurized breast milk or formula, using a crossover design.

Results: Sixty-three infants were evaluated for safety. At randomization, the mean (standard deviation) weight was 1467 (193) g and mean postmenstrual age was 32.6 (0.5) weeks. Sixty and 46 infants were evaluated for growth velocity and fat absorption, respectively. rhBSSL treatment significantly improved mean growth velocity by $2.93 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ ($P < 0.001$)

compared with placebo (mean 16.86 vs $13.93 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$) and significantly decreased the risk of suboptimal growth ($<15 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$) (30% vs 52%, $P = 0.004$). rhBSSL significantly increased absorption of the long-chain polyunsaturated fatty acids, docosahexaenoic acid, and arachidonic acid by 5.76% ($P = 0.013$) and 8.55% ($P = 0.001$), respectively, but had no significant effect on total fat absorption. The adverse-event profile was similar to placebo.

Conclusions: In preterm infants fed pasteurized breast milk or formula, 1 week of treatment with rhBSSL was well tolerated and significantly improved growth and long-chain polyunsaturated fatty acid absorption compared to placebo. This publication presents the first data regarding the use of rhBSSL in preterms and the results have led to further clinical studies.

Key Words: clinical study, fat absorption, growth velocity, preterm infant, recombinant human bile-salt-stimulated lipase

Received October 16, 2013; accepted February 28, 2014.

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Supplemental digital content is available for this article. Direct URL citations appear in the printed text, and links to the digital files are provided in the HTML text of this article on the journal's Web site (www.jpjn.org).

www.clinicaltrials.gov registration no: NCT00658905 and NCT00659243. The preterm formula study was carried out partially with research funding from the European Community's Sixth Framework Program (the Early Nutrition Programming [EARNEST]) using a formula provided by Ordesa. Swedish Orphan Biovitrum (Sobi) provided the remainder of the funding for the 2 phase II studies and their analysis. O.H., A.L., C.C., V.P.C. are consultants to Sobi for the rhBSSL project. K.T., B.O., and M.V. are employed by Sobi and holders of company shares. The other authors report no conflicts of interest.

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DOI: 10.1097/MPG.0000000000000365

(*JPGN* 2014;59: 61–69)

It is well established that early postnatal growth deficits may have adverse consequences on future growth, body composition, and neurodevelopmental, metabolic, and cardiovascular outcomes (1–3). Suboptimal growth during the preterm period may affect important phases of organ development and differentiation (4). Data suggest that higher growth velocity during neonatal intensive care unit (NICU) hospitalization is associated with a significant effect on later growth and neurodevelopment (1,5). Analysis of data from >24,000 preterm infants has highlighted that the proportion of infants whose weight is <10th percentile at discharge from the NICU increases with the degree of prematurity (5). In addition, in the short term, poor growth is associated with increased vulnerability to infection, and respiratory or intestinal disorders (6). Growth, and in particular sustained growth, is also a factor that positively impacts on the number of days the preterm infant is hospitalized in the NICU (7,8).

A growth velocity for a preterm infant of $15 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ or more is generally regarded as acceptable growth, and is in line with intrauterine growth rate (9,10). Despite efforts to optimize parenteral and enteral feeding, growth in preterm infants is often inadequate (10–12). It is therefore important to explore interventions that can improve the quantity and quality of growth in the early neonatal period.

Studies of the composition of human milk have contributed to the recognition that the long-chain polyunsaturated fatty acids (LCPUFAs), docosahexaenoic acid (DHA; 22:6 n-3), and arachidonic acid (ARA; 20:4 n-6) play an important role for the growing infant. These fatty acids are components of membrane phospholipids and are required for normal growth, immune function, and

visual and central nervous system development (13–16). Increasing awareness of the benefits of breast milk for premature infants have led neonatologists to use banked pasteurized breast milk (PBM; donor milk) if mother's own milk is unavailable or insufficient in quantity. Donor milk is routinely pasteurized to prevent transmission of pathogens. In many NICUs, pasteurization of mother's own milk is also present practice; however, pasteurization destroys important biologically active components, such as the heat-labile bile salt-stimulated lipase (BSSL), also known as carboxyl ester lipase or bile salt-dependent lipase (17,18).

BSSL is a lipolytic enzyme expressed in all species examined to date. It is expressed in the exocrine pancreas and secreted into the intestinal lumen and is primarily acknowledged for its function in facilitating digestion and absorption of dietary fat. BSSL is also found in blood, but its role in the circulation is less clear (19). In the gastrointestinal (GI) tract, BSSL has broad specificity and hydrolyzes a variety of different substrates, for example, tri-, di-, and monoglycerides, cholesteryl and retinyl esters, phospholipids, and ceramides (20–22). The exocrine pancreatic function is not fully developed at birth, and the BSSL production is insufficient in supporting proper fat absorption. In some species, including humans, BSSL is secreted by the lactating mammary gland and provided to the infant through the breast milk, thereby compensating for the poor endogenous production (21,23). Breast milk-derived BSSL, once activated by endogenous bile salts in the upper small intestine, contributes significantly to the efficient use of milk fat in breast-fed infants (18,24). BSSL is therefore believed to be important for the healthy growth and development of the preterm infant (21,25). A recombinant human BSSL (rhBSSL) has been developed as an oral therapeutic strategy to improve growth and lipid absorption, including LCPUFAs, in preterm infants receiving PBM or preterm formula aiming at introducing lipase activity corresponding to that in fresh breast milk.

Two phase II multicenter clinical studies with identical design, except for the type of infant feeding, were performed to investigate the effect of treatment with rhBSSL in preterm infants not fed fresh breast milk. These randomized double-blind placebo-controlled studies compared 7 days' treatment with rhBSSL and placebo using a crossover design, with one study using preterm infant formula study (PF study) and the other using PBM (PBM study). The primary objective in the respective studies was to investigate the coefficient of fat absorption (CFA) in preterm infants following treatment with rhBSSL compared with placebo.

Because the individual studies were not designed to have sufficient power to demonstrate an improvement in growth, a plan was prespecified to conduct analyses of the combined data with the primary objective to demonstrate that rhBSSL improves growth. Hence, the primary endpoint in the analysis of combined data was not the same as that for the individual studies. This is the first presentation of the results of these studies, exploring the effect of rhBSSL intervention on growth and fat absorption in preterm infants fed PBM or formula.

METHODS

Subjects

Infants eligible for these studies were born before week 32 of gestational age (GA) and were <33 weeks of GA at the time of enrollment. Each study planned to enroll 32 infants to obtain 26 evaluable infants. The sample size estimation for the individual studies was based on an anticipated 10% difference in CFA between treatment periods and a standard deviation of 15%, with a power of 90% and a significance level of 5% (18). It was anticipated that a 10% difference in CFA would result in a $2\text{-g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$

difference in growth velocity. The studies were conducted between April 2008 and August 2009 (PF study) in 5 centers in Italy, and between April 2008 and March 2010 (PBM study) in 5 centers in France and 2 in Italy.

Infants were not included if they were to receive any fresh mother's breast milk. Nor were they included if they received parenteral nutrition, mechanical ventilation or required $\geq 30\%$ oxygen, were small for GA, had conditions that may affect growth and development, had hemodynamically significant persistent ductus arteriosus, sepsis or necrotizing enterocolitis, or were treated with corticosteroids, with the exception of hydrocortisone.

Both studies were conducted according to International Conference on Harmonisation-Good Clinical Practice guidelines and the Declaration of Helsinki. They were approved by the appropriate independent ethics committees, and written informed consent was provided by the guardians of each infant. Directive 2001/20/EC: Ethical Considerations for Clinical Trials Performed in Children was consulted.

Feeding Regimens

In the PF study, the infants were required to receive a single preterm formula (formulation developed by Ordesa as part of the Early Nutrition Programming project) (26). Key components in the composition of the formula are listed in Table 1; a more detailed list of components is included in the online-only table (<http://links.lww.com/MPG/A314>). Infants receiving an alternative preterm formula before enrolment were switched to the study formula on the day of enrolment.

In the PBM study, 4 centers used banked milk and 3 centers' pasteurized the mother's own milk. Fortification of the PBM was allowed using a single fortifier (Eoprotin, Milupa, Germany) at a constant concentration throughout the study. Other milk fortifiers had to be discontinued or switched to Eoprotin at least 2 days before randomization. The fortifier provided maltodextrin, protein, vitamins, and minerals; no additional lipid supplementation was permitted.

TABLE 1. Composition for key components of the preterm infant formula used in the PF study

Nutrients	Units	100 mL	100 Kcal
Energy	kcal	81	
	kJ	339	
Protein	g	2.3	2.8
Fat*	g	4.1	5.1
MCT	g	—	—
Linoleic acid	mg	689.0	851.0
α -Linolenic acid	mg	62.0	77.0
ARA	mg	20.5	25.3
DHA	mg	14.4	17.8
Sodium	mg	34.0	42.0
Potassium	mg	94.0	116.0
Chloride	mg	49.0	60.5
Calcium	mg	105.0	129.6
Phosphorus	mg	58.0	71.6
Vitamin A	μg	195.0	240.0
Vitamin D	μg	1.7	2.1
Vitamin E	mg	3.6	4.4
Vitamin K	μg	7.5	9.3

* Source: Martek oil; ARA = arachidonic acid; DHA = docosahexaenoic acid; MCT = medium-chain triglycerides; PF = preterm formula.

For each infant, the investigator selected a target volume between 150 and 180 mL · kg⁻¹ · day⁻¹ and kept the feeding volume at this target level throughout the study. The amount of formula or milk given by bottle or feeding tube was recorded.

Study Design and Treatment Details

Within each study, treatment was administered in a double-blind manner according to a 2-period and 2-sequence randomization scheme. The infants were randomized to receive either rhBSSL or placebo added to their feed (PF or PBM) for the first 7 days. After a 2-day washout period, the infants crossed over to the other treatment and received another 7 days of treatment (Fig. 1). The primary objective of the individual studies was to compare the fat absorption following treatment with rhBSSL or placebo when administered in PBM in 1 study and PF in the other. Secondary objectives were to compare body weight and length following treatment with rhBSSL to that with placebo.

The investigational product, rhBSSL (Sobi, Stockholm, Sweden), is a recombinant glycosylated form of the human BSSL, manufactured in a Chinese hamster ovary cell. The rhBSSL concentration was determined by an optimized enzyme activity assay using 4-nitrophenyl butyrate as substrate. The final product was delivered as a frozen oral solution in glass vials, at a concentration of 15 g/L and a fill volume of 1.3 mL, and was stored frozen (–25°C to –15°C) at each study center. Before administration, the frozen solution was thawed and a 0.9-mL aliquot of the rhBSSL solution was transferred to 90 mL of the preterm formula (PF study) or PBM (PBM study) to give a final concentration in the feed of 0.15 g/L, representative of the physiological concentration found in breast milk (27). Placebo (sterile water) was stored and added to the feeds in the same way.

Growth Assessments

The infants' weight in grams was recorded each day using a standardized scale with an accuracy of at least ±5 g. Whenever possible, body weight was measured at approximately the same time each day. The length of the infant's leg was measured from the knee to the heel using an infant knemometer. Head circumference was not assessed in this study, because the treatment periods (7 days) were considered too short to produce measurable differences between treatments.

Coefficient of Fat Absorption Methodology

The determination of CFA was performed during a 72-hour period toward the end of each treatment period, using a fat-balance

methodology. A carmine red tracer dye was given as a GI transit marker together with a feed on day 4 of each treatment period and collection of stool commenced with the first appearance of the marker in the stool. Collection continued until the appearance of a second marker, administered 72 hours after the first one. (The stool containing the second marker was not collected.) To avoid interference with the CFA evaluation, the use of ointments was prohibited during the stool collection period. The volume of all feeds administered between administration of the GI transit markers was recorded, as was loss owing to regurgitation, spitting, and the like.

Diapers were shipped frozen to a central laboratory (Avantage Nutrition, Marseille, France) for analysis. After addition of a fixed amount of heptadecanoic acid as internal standard, the lipids were extracted by the Folch method (28) and analyzed by gas chromatography. The method was validated for the purpose of the study, and individual fatty acids, including DHA and ARA, were quantified following separation using an Omegawax 250 column (Supelco, Sigma-Aldrich, St Louis, MO); however, because of coelution of DHA with nervonic acid (24:1 n-9), which was only present in the breast milk, samples from infants in the PBM study were also analyzed using a SP-2380 column (Supelco) to separate and quantify the DHA. The weight of total lipid was estimated from the sum of the weights of all individual fatty acids. The same analytical principle was used to determine the lipids present in samples of the PBM and in the preterm formula to estimate fat intake during the fat-balance period.

Adverse Events

Any adverse events (AEs) that occurred during the period from administration of the first dose (day 1) to the follow-up visit (1 week ± 3 days after the last dose of study drug intake) were reported, whether or not the event was considered to be treatment related. No protocol-specific laboratory values were collected. Any routine laboratory values considered by the investigator to indicate a clinically significant deviation were reported as AEs. An independent Data and Safety Monitoring Board assessed unblinded safety data in regular intervals during the study.

Methodology for Statistical Analysis

The number of patients in the individual studies was chosen to ensure 90% power in demonstrating an improvement in total fat absorption assuming a true difference of 10 percentage points and a common standard deviation of 15 percentage points (18). Neither of the studies was expected to have sufficient power to demonstrate an improvement in growth, because of the relatively small number of infants in each study; therefore, a predefined analysis of combined

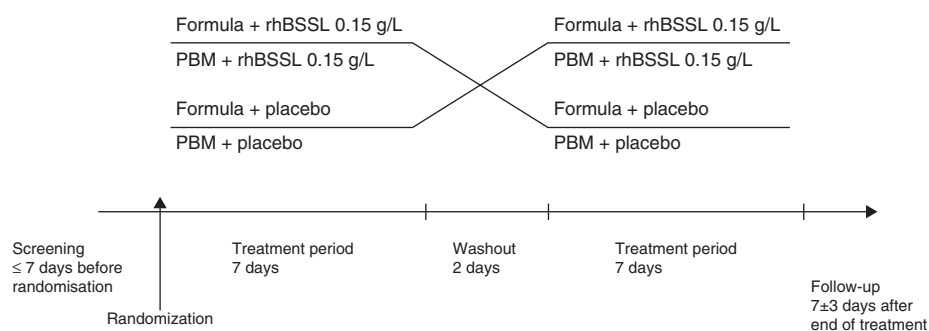


FIGURE 1. Design of the 2 phase II studies on rhBSSL in preterm infants: PF study and PBM study. PBM = pasteurized breast milk; PF = preterm formula; rhBSSL = recombinant human bile-salt-stimulated lipase.

data from the 2 studies was specified, ensuring 80% power in demonstrating improvement in growth assuming a true difference in growth velocity of $2 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ for rhBSSL treatment over placebo.

The statistical analysis plan detailing the analysis of combined data was developed and finalized before database lock and unblinding of the clinical database for either of the 2 clinical studies. Growth velocity, the prespecified primary endpoint for the combined analysis, was defined as the change in weight during the period divided by the weight at the beginning of the period and the number of days exposed. Both the primary efficacy outcome and the secondary efficacy outcome, CFA for total fat, were analyzed by analysis of variance models with treatment, feeding regimen (preterm formula or PBM), treatment sequence, period, and infant nested within feeding regimen and treatment sequence as factors.

Additional secondary outcomes assessed in the combined analysis were the coefficients of absorption (CAs) for ARA and DHA (exploratory efficacy variables in the original studies). A post hoc analysis was made to explore the effect of rhBSSL on reducing the risk of suboptimal growth. Suboptimal growth was defined as growth of $<15 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ during each treatment period, respectively. A logistic regression model with explanatory variables of treatment, feeding regimen, treatment sequence, and infant nested within feeding regimen and treatment sequence was used to estimate the odds ratio (OR) with corresponding 95% confidence interval, and to test the null hypothesis that the OR equals 1 against the alternative that rhBSSL reduces the risk of suboptimal growth.

Safety data were summarized by descriptive statistics. The respective populations used for assessment of safety, primary/secondary clinical efficacy outcomes and CFA are detailed under the Patient Disposition section and in Table 2. Each individual study was analyzed using similar methodology.

RESULTS

Study Population

Patient Disposition

A total of 65 preterm infants were randomized across the 2 clinical studies: 33 infants in the PF study and 32 infants in the PBM study. The number of infants completing and discontinuing during the treatment periods, and the reasons for discontinuation, are shown in Figure 2.

In total, 63 infants received at least 1 dose of study treatment and were evaluated for safety (safety analysis set) (Table 2). Sixty infants, who received at least 1 dose of the randomized study medication and had 1 baseline and at least 1 postbaseline weight

TABLE 2. Infant disposition in combined analysis population by individual study and combined analysis [number (percentage)]

	PF study	PBM study	Total
No. infants randomized	33	32	65
Safety analysis set*	33 (100.0)	30 (100.0)	63 (100.0)
FAS†	33 (100.0)	27 (90.0)	60 (95.2)
PP analysis set‡	26 (78.8)	20 (66.7)	46 (73.0)

FAS = full analysis set; PBM = pasteurized breast milk; PF = preterm formula; PP = per-protocol; rhBSSL = recombinant human bile-salt-stimulated lipase.

* Infants who received at least 1 dose (rhBSSL/placebo).

† Infants who received at least 1 dose (rhBSSL/placebo) and had a baseline and at least 1 postbaseline weight assessment in both treatment periods.

‡ Infants for which complete data on food intake and stool collections were available.

assessment in both treatment periods, were evaluated for clinical efficacy (full analysis set, FAS). The CFA analysis was conducted based on data from the 46 infants for whom complete data on food intake and full stool collections during the required period were available (per-protocol [PP] analysis set). The lower number in this cohort is because of the practical difficulties encountered when performing rigorous food intake measurements and stool collections at the study sites.

Demographics

The mean GA at birth was approximately 1 week higher in the PF study compared with the PBM study (29.18 and 28.13 weeks, respectively). The mean age at randomization (first dose) was lower in the PF study (3.26 weeks) compared with the PBM study (4.39 weeks). As a result, the mean postmenstrual age on the day of randomization was similar in the 2 studies (32.45 and 32.51 weeks, respectively). The sex distribution was similar across the 2 studies (Table 3).

Efficacy Outcomes

Growth Velocity

The predefined analysis using combined data from both studies demonstrated a statistically significant improvement in growth velocity during rhBSSL treatment as compared with placebo ($P < 0.001$) (Table 4). The mean growth velocity was $2.93 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ higher during rhBSSL treatment compared with placebo treatment, corresponding to an improvement of approximately 20%.

In the PF study, there was a statistically significant improvement in growth velocity of $3.74 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ during the rhBSSL treatment period compared with placebo ($P = 0.001$) in the FAS population. In the PBM study, there was an improvement of $1.95 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ with rhBSSL, which did not reach statistical significance ($P = 0.119$) (Table 4). The fat content and energy intake for infants receiving preterm formula were estimated to be $5.5 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ and $130 \text{ kcal} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$, respectively. For the infants receiving PBM, the equivalent estimated intakes were lower: $4.0 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ and $105 \text{ kcal} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$, respectively.

A clinically important observation was that some infants lost weight over the 7-day period when they received placebo, whereas all infants gained weight while receiving rhBSSL (Table 4). In a post hoc analysis of the combined data, rhBSSL significantly decreased the risk of suboptimal growth as compared with placebo (OR 0.19, 95% CI, 0.04–0.66, $P = 0.004$), with 30% of infants having suboptimal growth during rhBSSL treatment and 52% during placebo treatment. The difference in the proportion of infants having suboptimal growth was consistent across both studies, with a decrease during rhBSSL treatment compared with placebo from 42% to 21% in the PF study and from 63% to 41% in the PBM study.

There was a mean increase in knee-to-heel length of 2.36 mm during rhBSSL treatment and 2.19 mm during placebo. The difference was not statistically significant ($P = 0.834$). There was a large within-patient variability owing to difficulties in measuring length in these small infants.

CFA

Fat Intake

As part of the CFA analysis, the total amount of fat ingested between GI transit markers was estimated, together with the total amount of fat in the stools during the same period. The amount of fat ingested was similar during the rhBSSL and placebo treatments; the

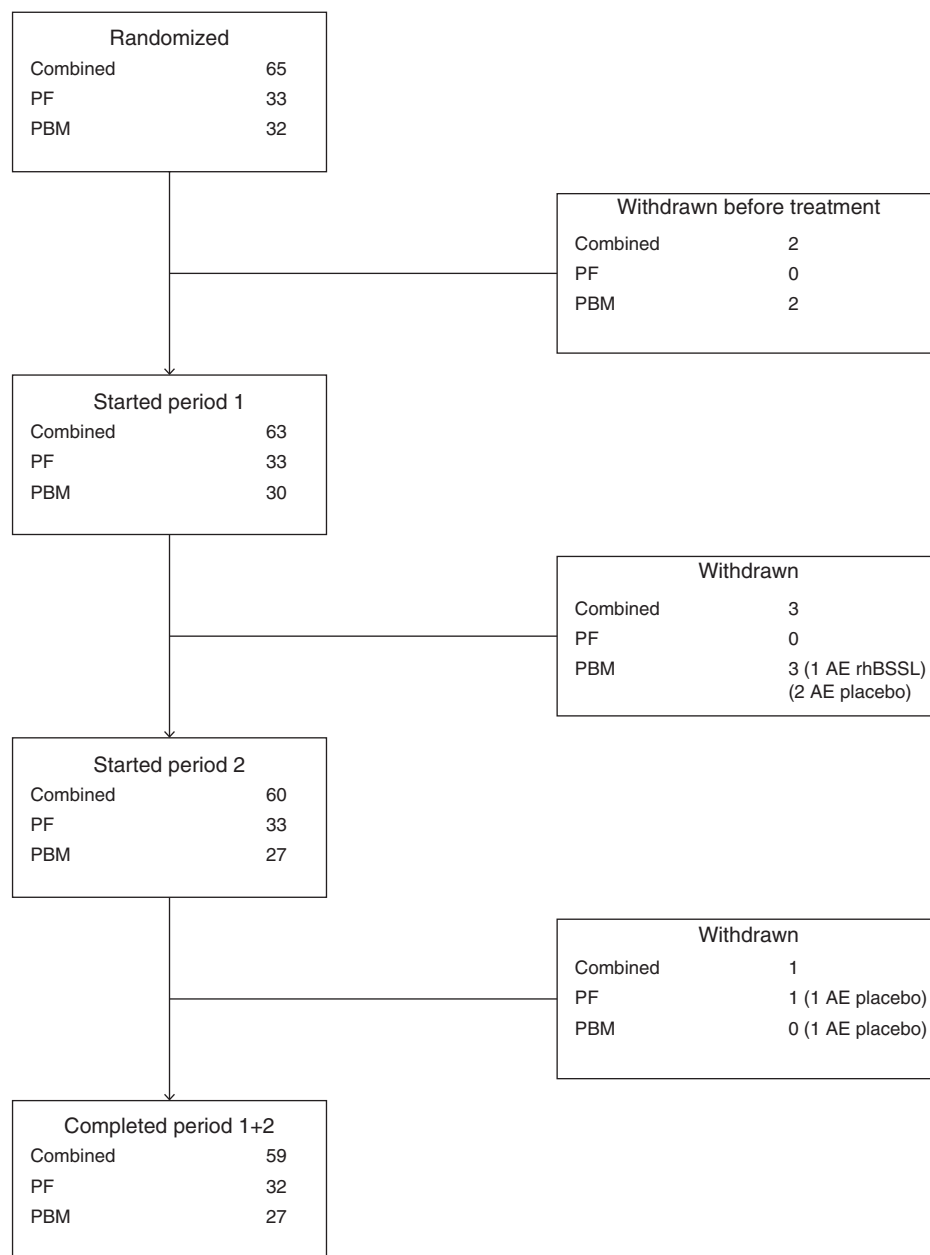


FIGURE 2. Flow diagram of patients included in the 2 phase II studies. AE = adverse event; PBM = pasteurized breast milk; PF = preterm formula; rhBSSL = recombinant human bile salt-stimulated lipase.

mean fat intake in the 72-hour fat-balance period during rhBSSL treatment was $4.84 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ compared with $4.92 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ for placebo (Table 5); however, there was a pronounced difference in fat intake between infants receiving formula and PBM; those fed with formula received $5.5 \text{ g fat} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ on average, whereas those fed with PBM received an average of 3.9 to $4.2 \text{ g fat} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$.

Analysis of CFA

Complete and correct milk/formula intake and stool collection were required for the accurate determination of the CFA; therefore, only infants with reported complete collection were included in this analysis (PP set, $n = 46$). In the combined analysis

of the CFA data, there was a trend toward an improvement in fat absorption after 7 days of treatment with rhBSSL compared with placebo (mean CFA 69.1% vs 65.6%, respectively), with the mean difference in total fat absorption being 3.51% ($P = 0.071$, 95% CI -0.31 to 7.34) (Table 6). In both the PF and PBM studies, numerically higher mean CFAs were observed during treatment with rhBSSL compared with placebo (PF 69.5% vs 67.4, PBM 68.6% vs 63.9%, respectively).

CA for Key Fatty Acids

The absorption of individual fatty acids in the combined analysis showed that treatment with rhBSSL resulted in significantly higher CAs for both DHA and ARA in favor of rhBSSL over

TABLE 3. Infant demographics in combined analysis population by individual study and combined analysis (safety analysis set)

	PF study	PBM study	Combined analysis
No. infants treated	33	30	63
GA at birth, wk			
Mean (SD)	29.18 (1.31)	28.13 (1.67)	28.68 (1.57)
Median (range)	29.10 (25.4–31.4)	28.25 (25.1–31.0)	29.00 (25.1–31.4)
Sex (%)			
Female	16 (51.5)	14 (46.7)	30 (47.6)
Postmenstrual age at randomization, wk			
Mean (SD)	32.45 (0.46)	32.51 (0.54)	32.56 (0.49)
Median (range)	32.60 (31.4–33.0)	32.70 (31.0–33.7)	32.70 (31.0–33.7)
Age at randomization, wk			
Mean (SD)	3.26 (1.19)	4.39 (1.62)	3.87 (1.49)
Median (range)	3.10 (1.3–7.3)	4.00 (1.9–7.6)	3.60 (1.3–7.6)
Weight at randomization, g			
Mean (SD)	1493.5 (195.04)	1437.0 (189.61)	1466.6 (193.02)
Median (range)	1450.0 (1190–1805)	1405.0 (1088–1915)	1440.0 (1088–1915)

GA = gestational age; PF = preterm formula; PBM = pasteurized breast milk; SD = standard deviation.

placebo: 5.76% higher for DHA ($P=0.013$, 95% CI 1.27–10.25) and 8.55% for ARA ($P=0.001$, 95% CI 3.52–13.57) (Table 6). In the PBM study, statistically significant higher mean absorption of both DHA and ARA was observed during treatment with rhBSSL compared with placebo (DHA 78.47% vs 73.86%, $P=0.029$; ARA 72.55% vs 61.97%, $P=0.012$), with the mean difference of 4.62% ($P=0.029$) for DHA and 10.58% ($P=0.012$) for ARA. In the PF study, a trend toward higher mean absorption for DHA and ARA after rhBSSL treatment was observed that did not reach statistical significance (DHA 81.15% vs 74.31%, $P=0.079$; ARA 82.96% vs 75.83%, $P=0.051$). The mean difference was 6.85% ($P=0.079$) for DHA and 7.14% ($P=0.051$) for ARA.

Safety and Tolerability

The combined safety analysis set consisted of the 63 infants who received at least 1 dose of study medication, of whom 61 were exposed to rhBSSL. One infant discontinued treatment during rhBSSL treatment because of grade 2a necrotizing enterocolitis; the treatment was withdrawn after 3 days in the first treatment period of the PBM study. Three infants discontinued placebo owing to AEs (4.8%). The 3 AEs leading to treatment discontinuation during placebo treatment were reported by the investigator as ulcerative colitis (first period, discontinued after 7 days), septic shock (serious AE in the first period, discontinued after 7 days), and meningitis (serious AE resulting in death, occurring in the

second period and treatment discontinued on the third day of the period).

The AEs reported reflected those commonly observed in this patient population and had similar frequencies across treatments in individual studies as well as in the combined data. The most commonly reported events were diaper dermatitis and anemia (Table 7).

DISCUSSION

The 2 studies presented here described the first data investigating the use of rhBSSL treatment in preterm infants. A predefined combined analysis of the studies showed that treatment with rhBSSL in 60 preterm infants (FAS population) fed PF or PBM resulted in approximately 20% higher growth velocity compared with placebo.

It is believed that BSSL stimulates the growth velocity by improving lipid digestion and absorption through its ability to hydrolyze a broad variety of lipids and related substrates found in the PBM and the preterm formula. Although there was a significant increase in the absorption of ARA and DHA after rhBSSL treatment, the total fat absorption did not reach statistical significance. Additional analysis also revealed that there was no statistically significant correlation between the effect of rhBSSL on growth velocity and CFA, indicating that the mechanism of action of rhBSSL may be more complex than just causing an increase in total fat absorption. Emerging preclinical data on the effects of

TABLE 4. Growth velocity ($\text{g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$) for individual studies and combined analysis (FAS)

Treatment	PF study (N = 33)		PBM study (N = 27)		Combined analysis (N = 60)	
	rhBSSL	Placebo	rhBSSL	Placebo	rhBSSL	Placebo
LS mean (95% CI)	18.05 (16.5–19.6)	14.31 (12.8–15.8)	15.58 (13.8–17.3)	13.63 (11.9–15.4)	16.86 (15.7–18.0)	13.93 (12.8–15.1)
Range	9.2–25.5	–4.5 to 23.3	7.5–26.5	–3.1 to 24.6	7.5–26.5	–4.5 to 24.6
Difference (95% CI)	3.74 (1.58–5.90)		1.95 (–0.54 to 4.43)		2.93 (1.35–4.51)	
<i>P</i>	0.001		0.119		<0.001	

CI = confidence interval; FAS = full analysis set; LS mean = least squares mean; PF = preterm formula; PBM = pasteurized breast milk; rhBSSL = recombinant human bile salt-stimulated lipase.

TABLE 5. Amount of fat intake and excretion in the stool during the 3-day fat-balance period, by individual study and combined analysis (PP analysis set)

No. infants	PF study		PBM study		Combined analysis	
	26		20		46	
treatment	rhBSSL	Placebo	rhBSSL	Placebo	rhBSSL	Placebo
Amount of fat ingested in food (formula or breast milk) during 3-day balance period ($\text{g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$)						
Mean	5.56	5.49	3.90	4.17	4.84	4.92
Range	4.80–6.67	4.78–6.58	2.87–5.06	2.92–7.73	2.87–6.67	2.92–7.73
Total amount of fat in stool during 3-day balance period ($\text{g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$)						
Mean	1.67	1.78	1.26	1.53	1.49	1.67
Range	0.71–3.27	0.37–3.55	0.21–2.72	0.36–3.19	0.21–3.27	0.36–3.55

PF = preterm formula; PBM = pasteurized breast milk; PP = per-protocol; rhBSSL = recombinant human bile salt-stimulated lipase.

BSSL on overall digestive efficiency show that undigested lipids in the intestine may cause damage to the villus epithelium in the distal small intestine, which is prevented in the presence of BSSL (29). Thus, positive effects on lipid absorption and epithelial function related to BSSL may secondarily improve overall nutrient absorption. It should also be noted that determining CFA is challenging (30), especially in multicenter studies, which is reflected by the number of incomplete collections in these studies. For the accuracy of the results, it was crucial that all intakes and outputs were carefully collected and handled in a standardized way during the entire 72 hours that the balance study was ongoing. Extracting lipids for analysis from diapers was an additional difficulty.

Growth velocity was higher in the rhBSSL-treated infants in the PF study than in the PBM study (mean 18.05 vs 15.58 $\text{g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$). A likely explanation for the greater growth velocity in infants who received preterm formula is the higher fat and energy intake from the preterm formula, with infants consuming approximately 1.5 $\text{g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ more fat and 25 $\text{kcal} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ more energy compared with PBM. The preterm formula used for the study was a specially optimized formulation created as part of the Early Nutrition Programming project (26); it met the present European Society for Pediatric Gastroenterology, Hepatology,

and Nutrition recommendation, at the time of the study, of 110 – 135 $\text{kcal} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ (31). Despite the special formulation of the preterm formula, 42% of infants had suboptimal growth (<15 $\text{g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$) during placebo treatment; this percentage was reduced to 21% during 7 days of rhBSSL treatment.

Preterm infants have limited endogenous biosynthesis of ω -3 and ω -6 LCPUFAs (32,33), which are required for normal growth, immune function, and maturation of numerous organ systems, most important, the brain and eye (14,34,35). Low blood levels of DHA (an ω -3 fatty acid) appear to be associated with lower visual and neural maturation in both infancy and later childhood (36–38). In the present analysis of combined data, a trend toward better total fat absorption and significantly improved absorption of the particularly important LCPUFAs ARA ($P = 0.001$) and DHA ($P = 0.013$) were observed. This is in line with the observation that BSSL, compared with colipase-dependent pancreatic lipase, more efficiently releases these fatty acids from triglycerides (39). A detailed analysis on the absorption of other LCPUFAs, as well as other fatty acids, will be reported separately.

rhBSSL is an enzyme (lipase), which was administered orally at physiological (breast milk) levels, mixed with either PBM or infant formula. The enzyme is delivered to the intestine

TABLE 6. Effect of rhBSSL and placebo on CA (%) for total fat, DHA, and ARA (PP analysis set)

Statistics	PF study		PBM study		Combined data	
	rhBSSL (N = 26)	Placebo (N = 26)	rhBSSL (N = 20)	Placebo (N = 20)	rhBSSL (N = 46)	Placebo (N = 46)
Total fat						
LS mean	69.46	67.38	68.61	63.87	69.09	65.57
LS mean difference	2.08		4.74		3.51	
95% CI	–3.67 to 7.84		–0.52 to 10.01		–0.31 to 7.34	
P	0.462		0.075		0.071	
DHA						
LS mean	81.15	74.31	78.47	73.86	79.83	74.07
LS mean difference	6.85		4.62		5.76	
95% CI	–0.84 to 14.54		0.53–8.71		1.27–10.25	
P	0.079		0.029		0.013	
ARA						
LS mean	82.96	75.83	72.55	61.97	77.60	69.06
LS mean difference	7.14		10.58		8.55	
95% CI	–0.03 to 14.30		2.63–18.53		3.52–13.57	
P	0.051		0.012		0.001	

ARA = arachidonic acid; CA = coefficient of absorption; CI = confidence interval; DHA = docosahexaenoic acid; LS mean = least squares mean; PP = per-protocol; rhBSSL = recombinant human bile salt-stimulated lipase.

TABLE 7. Total treatment-emergent AEs occurring in >3% of infants in either treatment period (combined safety analysis set)

	rhBSSL, N = 61 (%)	Placebo, N = 62 (%)
Diaper dermatitis	13 (21.3)	13 (21.0)
Anemia	3 (4.9)	6 (9.7)
Bradycardia	1 (1.6)	5 (8.1)
Cardiac murmur	4 (6.6)	2 (3.2)
Hypokalemia	3 (4.9)	2 (3.2)
Thrombocytopenia	0	3 (4.8)
Anemia neonatal	2 (3.2)	1 (1.6)
Urinary tract infection	1 (1.6)	2 (3.2)
Anal fissure	2 (3.2)	0
Apnea	0	2 (3.2)
Retinopathy of prematurity	0	2 (3.2)

If an infant had >1 count for a particular preferred AE term, the infant was counted only once in any treatment period. AE=adverse event; rhBSSL=recombinant human bile salt-stimulated lipase.

in the same way as native BSSL, and exerts its effect locally in the lumen of the GI tract following activation by bile salts in the duodenum. rhBSSL is a large glycosylated protein and, as such, is not expected to be absorbed to any significant degree when administered orally, and it is protected from degradation by intestinal proteases by the bile salts (40). Based on this, no theoretical risks have been identified. The AEs reported in the 2 phase II trials reflected those commonly observed in this patient population and had similar frequencies across treatments.

An increase in growth velocity of approximately 20% during the NICU stay may bring both short- and long-term clinical benefits. It may shorten the length of hospital stay (eg, the time for a 1.0-kg infant to reach 1.8 kg is reduced from 43 to 35 days if the growth velocity is increased from 14 to 17 g · kg⁻¹ · day⁻¹) and it may facilitate development because higher growth rates during the NICU stay have been associated with improved neurodevelopmental outcome (1,41).

Another important aspect in the management of preterm infants is avoidance of growth restriction. Short-term consequences may include increased vulnerability to infection and respiratory or intestinal disorders, and longer-term consequences of growth restriction in preterm infants have been reported to include impaired neurodevelopment, together with cardiovascular, metabolic, and renal concerns (6). A post hoc analysis was made to explore the effect of rhBSSL on decreasing the risk of suboptimal growth (defined as a growth of <15 g · kg⁻¹ · day⁻¹). A significantly lower proportion of patients had suboptimal growth during the rhBSSL treatment period (30%) compared with the placebo period (52%).

The crossover study design was chosen to minimize interpatient variability as infants served as their own controls in the analysis, and to thereby reduce the number of infants required. A 2-day washout between treatment periods was judged to be sufficient to minimize any treatment carryover effects; however, because preterm infants grow quickly, there may well be a treatment sequence effect. This sequence bias was managed by the analysis of variance model analysis.

In summary, results of the analyses of combined data demonstrated that 7-day treatment with rhBSSL significantly improves growth in preterm infants receiving PBM or preterm formula compared with placebo. In addition, rhBSSL improves the absorption of the clinically important LCPUFAs ARA and DHA, which are important factors for healthy infant development. rhBSSL had an AE profile similar to placebo.

Because the encouraging phase II results of short-term treatment with rhBSSL presented here warrant confirmatory

studies, a randomized placebo-controlled phase III study with 4-week treatment in preterm infants is ongoing (<http://clinicaltrials.gov/NCT01413581>).

Acknowledgments: The authors thank the following investigators for their major contributions in the 2 double-blind clinical trials: Italy, Maria Paola Bellagamba (Ancona); Costantino Romagnoli, Simonetta Costa, Mario De Curtis, and Lucilla Managanozzi (Rome); Lino Chianchetti and Giovanna Verlato (Padua); Nicola Laforgia and Grazia Calderoni (Bari); France, Elsa Kermorvant (Paris); Umberto Simeoni, Véronique Millet, and Farid Boubred (Marseille); Elie Saliba and Amélie Favreau (Tours); Nathalie Montjoux (Toulouse). We also acknowledge Maria Rodríguez Palmero, Ordesa, Spain, for providing the preterm infant formula, and the assistance of a medical writer, Josie Sautler, for the initial drafting of the manuscript. Last but not least, we sincerely thank the families who consented to their preterm infants being included in these studies.

REFERENCES

- Ehrenkranz RA, Dusick AM, Vohr BR, et al. Growth in the neonatal intensive care unit influences neurodevelopmental and growth outcomes of extremely low birth weight infants. *Pediatrics* 2006;117:1253–61.
- De Curtis M, Rigo J. The nutrition of preterm infants. *Early Hum Dev* 2012;88 (suppl 1):S5–7.
- Lapillonne A, Griffin IJ. Feeding preterm infants today for later metabolic and cardiovascular outcomes. *J Pediatr* 2013;162 (3 suppl):S7–16.
- Pilling EL, Elder CJ, Gibson AT. Growth patterns in the growth-retarded premature infant. *Best Pract Res Clin Endocrinol Metab* 2008;22:447–62.
- Clark RH, Thomas P, Peabody J. Extrauterine growth restriction remains a serious problem in prematurely born neonates. *Pediatrics* 2003;111 (5 pt 1):986–90.
- Corpeleijn WE, Vermeulen MJ, van den Akker CH, et al. Feeding very-low-birth-weight infants: our aspirations versus the reality in practice. *Ann Nutr Metab* 2011;58 (suppl 1):20–9.
- Ye G, Jiang Z, Lu S, et al. Premature infants born after preterm premature rupture of membranes with 24–34 weeks of gestation: a study of factors influencing length of neonatal intensive care unit stay. *J Matern Fetal Neonatal Med* 2011;24:960–5.
- Merritt TA, Pillers D, Prows SL. Early NICU discharge of very low birth weight infants: a critical review and analysis. *Semin Neonatol* 2003;8:95–115.
- Ehrenkranz RA, Younes N, Lemons JA, et al. Longitudinal growth of hospitalized very low birth weight infants. *Pediatrics* 1999;104 (2 pt 1):280–9.
- Riddle WR, DonLevy SC. Defining the expected growth of the preterm infant. In: Preedy VR, ed. *Handbook of Growth and Growth Monitoring in Health and Disease. Vol 1.* New York: Springer; 2012:625–41.
- Ruth VA. Extrauterine growth restriction: a review of the literature. *Neonatal Netw* 2008;27:177–84.
- Embleton NE, Pang N, Cooke RJ. Postnatal malnutrition and growth retardation: an inevitable consequence of current recommendations in preterm infants? *Pediatrics* 2001;107:270–3.
- Gould JF, Smithers LG, Makrides M. The effect of maternal omega-3 (n-3) LCPUFA supplementation during pregnancy on early childhood cognitive and visual development: a systematic review and meta-analysis of randomized controlled trials. *Am J Clin Nutr* 2013;97:531–44.
- Innis SM. Fatty acids and early human development. *Early Hum Dev* 2007;83:761–6.
- Georgieff MK, Innis SM. Controversial nutrients that potentially affect preterm neurodevelopment: essential fatty acids and iron. *Pediatr Res* 2005;57 (5 pt 2):99R–103R.
- Lapillonne A, Groh-Wargo S, Gonzalez CH, et al. Lipid needs of preterm infants: updated recommendations. *J Pediatr* 2013;162 (3 suppl):S37–47.
- Björkstén B, Burman LG, De Château P, et al. Collecting and banking human milk: to heat or not to heat? *Br Med J* 1980;281:765–9.

18. Andersson Y, Sävman K, Bläckberg L, et al. Pasteurization of mother's own milk reduces fat absorption and growth in preterm infants. *Acta Paediatr* 2007;96:1445–9.
19. Lindquist S, Andersson EL, Lundberg L, et al. Bile salt-stimulated lipase plays an unexpected role in arthritis development in rodents. *PLoS One* 2012;7:e47006.
20. Andersson EL, Hernell O, Bläckberg L, et al. BSSL and PLRP2: key enzymes for lipid digestion in the newborn examined using the Caco-2 cell line. *J Lipid Res* 2011;52:1949–56.
21. Lindquist S, Hernell O. Lipid digestion and absorption in early life: an update. *Curr Opin Clin Nutr Metab Care* 2010;13:314–20.
22. Hernell O, Bläckberg L. Digestion of human milk lipids: physiologic significance of sn-2 monoacylglycerol hydrolysis by bile salt-stimulated lipase. *Pediatr Res* 1982;16:882–5.
23. Leberthal E, Lee PC. Development of functional responses in human exocrine pancreas. *Pediatrics* 1980;66:556–60.
24. Bernbäck S, Bläckberg L, Hernell O. The complete digestion of human milk triacylglycerol in vitro requires gastric lipase, pancreatic colipase-dependent lipase, and bile salt-stimulated lipase. *Clin Invest* 1990;85:1221–6.
25. Montjoux-Régis N, Cristini C, Arnaud C, et al. Improved growth of preterm infants receiving mother's own raw milk compared with pasteurized donor milk. *Acta Paediatr* 2011;100:1548–54.
26. The Early Nutrition Programming Project (EARNEST). Theme 6. www.metabolic-programming.org/theme6.htm. Accessed May 28, 2014.
27. Strömqvist M, Hernell O, Hansson L, et al. Naturally occurring variants of human milk bile salt-stimulated lipase. *Arch Biochem Biophys* 1997;347:30–6.
28. Folch J, Lees M, Sloane-Stanley GH. A simple method for the isolation and purification of total lipides from animal tissues. *J Biol Chem* 1957;226:497–509.
29. Chen Q, Bläckberg L, Nilsson A, et al. Digestion of triacylglycerols containing long-chain polyenoic fatty acids in vitro by colipase-dependent pancreatic lipase and human milk bile salt-stimulated lipase. *Biochim Biophys Acta* 1994;1210:239–43.
30. Howles PN, Stemmerman GN, Fenoglio-Preiser CM, et al. Carboxyl ester lipase activity in milk prevents fat-derived intestinal injury in neonatal mice. *Am J Physiol* 1999;277:G653–61.
31. Agostoni C, Buonocore G, Carnielli VP, et al. Enteral nutrient supply for preterm infants: commentary from the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition Committee on Nutrition. *J Pediatr Gastroenterol Nutr* 2010;50:85–91.
32. Lin YH, Llanos A, Mena P, et al. Compartmental analyses of 2H5-alpha-linolenic acid and C-U-eicosapentaenoic acid toward synthesis of plasma labeled 22:6n-3 in newborn term infants. *Am J Clin Nutr* 2010;92:284–93.
33. Carnielli VP, Simonato M, Verlato G, et al. Synthesis of long-chain polyunsaturated fatty acids in preterm newborns fed formula with long-chain polyunsaturated fatty acids. *Am J Clin Nutr* 2007;86:1323–30.
34. Koletzko B, Agostoni C, Bergmann R, et al. Physiological aspects of human milk lipids and implications for infant feeding: a workshop report. *Acta Paediatr* 2011;100:1405–15.
35. Calder PC, Dangour AD, Diekmann C, et al. Essential fats for future health. Proceedings of the 9th Unilever Nutrition Symposium, 26–27 May 2010. *Eur J Clin Nutr* 2010;64(Suppl 4):S1–3.
36. Innis SM. Dietary omega 3 fatty acids and the developing brain. *Brain Res* 2008;1237:35–43.
37. Larque E, Demmelmair H, Koletzko B. Perinatal supply and metabolism of long-chain polyunsaturated fatty acids: importance for the early development of the nervous system. *Ann N Y Acad Sci* 2002;967:299–310.
38. Escolano-Margarit MV, Ramos R, Beyer J, et al. Prenatal DHA status and neurological outcome in children at age 5.5 years are positively associated. *J Nutr* 2011;141:1216–23.
39. Hernell O. Assessing fat absorption. *J Pediatr* 1999;135:407–9.
40. Bläckberg L, Hernell O. Bile salt-stimulated lipase in human milk. Evidence that bile salt induces lipid binding and activation via binding to different sites. *FEBS Lett* 1993;323:207–10.
41. Ehrenkranz RA. Early nutritional support and outcomes in ELBW infants. *Early Hum Dev* 2010;86(Suppl 1):21–5.