Randomized control trials

Infant formula composition affects energetic efficiency for growth: The BeMIM study, a randomized controlled trial

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SUMMARY

Background & aims: Protein source, macronutrient composition and content of long chain-polyunsaturated fatty acids (LC-PUFA) of infant formulae may influence infant growth. We aimed to assess the effect of a modified infant formula on growth.

Methods: In a randomized, double-blind trial, 213 healthy term infants consumed isoenergetic study formulae (intervention formula — IF, control formula — CF) from the first month of life until the age of 120 days. IF (1.89 g protein/100 kcal) contained α-lactalbumin (ALAB) and LC-PUFA, while CF (2.30 g protein/100 kcal) provided standard whey and no LC-PUFA. Anthropometry and dietary intake were regularly assessed. A venous blood sample was obtained on day 120.

Results: Both formulae were well-accepted without significant differences in health related observations. Weight gain was not statistically different between formula groups (IF: 30.2 ± 6.3 vs. CF: 28.3 ± 6.5 g/day, mean ± SD, P = 0.06). Length gain was higher in IF (0.11 ± 0.02 vs. 0.10 ± 0.02 cm/day, P = 0.02). Energy intake from formula was higher in IF at 90 and 120 days (IF: 509 ± 117 and 528 ± 123 vs. CF: 569 ± 152 and 617 ± 169 kcal/day, P < 0.01). Protein intake in CF was significantly higher at each assessment. Growth per energy intake was higher in IF compared to CF for weight (6.45 ± 2.01 vs. 5.67 ± 2.21 g/100 kcal, P = 0.02) and length (0.23 ± 0.08 vs. 0.20 ± 0.08 mm/100 kcal, P = 0.04).

Conclusions: The modified infant formula with reduced protein content with added ALAB and LC-PUFA, meets infant requirements of protein for adequate growth. The increased energetic efficiency of the new infant formula might result from improved protein composition by added ALAB. Apparently minor differences in composition can markedly affect energetic efficiency for growth.

The study was registered at ClinicalTrials.gov (NCT01094080).

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1. Introduction

Infants should be fully breastfed for 6 months whenever feasible.1 Infants benefit from breastfeeding not only via immediate protection against gastrointestinal and respiratory infections,2 but also via a lower risk of obesity and diabetes in adult life.3,4 If full breastfeeding is not possible, safe and suitable infant formulae should be fed.5

During the last decades, infant formulae have been improved for example by the inclusion of long chain-polyunsaturated fatty acids (LC-PUFA)6 and adaptation of content and source of protein.7,8 Protein intake of infants is lower with breast milk than with standard infant formulae9 because of a generally higher formula protein content considering the lower nutritive value of cow’s milk casein
and whey compared to human milk proteins. Differences in the amino acid (AA) composition may require higher amounts of cow’s milk protein to ensure adequate supply of essential AA, which puts additional burden on the renal system because of the higher nitrogen intake. According to the “early protein hypothesis” high protein intake causes rapid early growth, which has been associated with an increased risk of obesity and associated disorders in later life. Thus, lower protein intake in infancy seems beneficial for long term health.

Davis et al. 2008 investigated the use of an infant formula with α-lactalbumin (ALAB). ALAB, the predominant whey fraction in human milk, is the main source of tryptophan (Trp) fraction of breastfed infants and enables the reduction of protein content in infant formulae, while ensuring sufficient supply of Trp and all other essential AA. The composition of milk consumed by infants affects the energy efficiency for growth: Butte et al. 1990 compared the energy efficiency of infants at the ages of 1 and 4 months and found that breastfed infants had an 11% higher weight gain per 100 kcal (energetic efficiency) than formula fed infants.

The inclusion of LC-PUFA into formula aimed to optimize the neuronal development as LC-PUFA are quantitatively and qualitatively important components of nervous tissue and provided by neuronal development. Moreover, there might be interactions with growth as arachidonic acid has been related to growth and LC-PUFA derived prostaglandin may influence adipocyte differentiation.

Since the modification of protein source, macronutrient composition and content of LC-PUFA of infant formulae may influence infant growth and development, modified formula must be tested to ensure they support adequate growth. In present study we compared growth and blood biochemistry of infants fed a modified infant formula with reduced protein content and rich in ALAB as well as added LC-PUFA to a formula with standard protein content and without LC-PUFA, and a reference group of breastfed infants. The randomized controlled trial aimed to assess the suitability of a reduced-protein, ALAB and LC-PUFA containing formula focusing on growth velocities, adverse events, markers of fatty acids and protein status and energetic efficiency in infants until the age of 120 days.

2. Subjects and methods

2.1. Power calculation and randomization

Based on previous findings a weight gain between birth and age 4 months of life of 30 g per day with a standard deviation of 6 g was assumed for sample size estimation. The non-inferiority study assumed a power of 85% and 2.5% risk of α-error to detect a difference of 0.5 standard deviations (one-sided test) as statistically significant required studying 70 infants per formula group. Assuming a total loss to follow-up rate of up to 30% 100 infants were enrolled into each formula arm.

Double blinded randomized allocation of infants to the study formulae was stratified for gender and a block size of four was applied. A random allocation sequence was generated by the study sponsor. The blinded allocation was concealed for participants, support staff and investigators until all laboratory and data analyses had been performed.

2.2. Ethical considerations

The study was approved by the Clinical Center Serbia Ethical Committee. Written informed consent was obtained from all parents prior to study start after the experimental protocol had been explained to them in detail.

The study was registered at ClinicalTrials.gov (NCT01094080).

2.3. Study design

The BeMIM study (Belgrade—Munich Infant Milk Trial), a randomized, double-blind, controlled study with parallel design, was performed with two formula groups (intervention formula — IF and control formula — CF) and a reference group of breastfed infants (BF). Infants were recruited until the age of 28 days. Children of mothers, who could not breastfeed their healthy newborn babies for reasons not related to this study, or who decided—in spite of all benefits of breast milk—to start full formula-feeding within the first 28 days of life, were randomized double blinded into one of the two formula groups. Formula infants (FF) were fully formula fed until the age of 120 days. A reference group of breastfed infants was recruited with intended duration of breastfeeding for at least 4 months. During the first 28 days (Baseline) and at 30, 60, 90 and 120 days of life infants were examined and anthropometric measures were taken. During three days before each study examination parents recorded the volume of consumed formula and completed questionnaires on formula acceptance, consistency and color of stool, occurrence of colic, flatulence, regurgitation, and vomiting.

Weight was determined with a Seca 336 scale (Seca, Hamburg, Germany) equipped with a measuring rod for measuring recumbent length, and head circumference was measured with a tape (Seca 212). All measurements were performed in duplicate and documented with an accuracy of 10 g for weight and 0.1 cm for length and head circumference, respectively. The equipment was regularly checked and calibrated to ensure accuracy of measurements. These checks were done with a calibrated weight of 5 kg every 4 weeks. There was never an aberration of more than 10 g. Therefore, the scales were never reset.

2.4. Study population

From Jan 2010 to May 2011, 505 infants were enrolled verbally at the maternity ward and with flyers providing study information and contact data of the principal investigator at the Institute for Gynecology and Obstetrics of the Clinical Center of Serbia in Belgrade, Serbia. Eligible infants had to be born apparently healthy from singleton pregnancies after 37–41 weeks of gestation, with a birth weight between the 3rd and 97th weight-for-age percentile according to the EURO-Growth charts. Infants with malformations, congenital heart defects, congenital vascular diseases, severe diseases of gastrointestinal tract, kidney, liver, central nervous system, or metabolic disease were excluded from study participation.

2.5. Study diets

Study formulae were provided free of charge to families by HiPP GmbH & Co. Vertrieb KG (Paffenhofen, Germany) in 600 g cartoons and labeled by random numbers. The products were packed in identical white boxes and labeled with the same product name. If complied with the EU-directive of 2006 and CF with the corresponding 1999 EU-directive. The formulas had identical whey/

<table>
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<tr>
<th>Infant formula</th>
<th>Human milk</th>
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<tr>
<td></td>
<td>Intervention</td>
</tr>
<tr>
<td>Energy (kcal/100 mL)</td>
<td>67</td>
</tr>
<tr>
<td>Protein (g/100 kcal)</td>
<td>1.89</td>
</tr>
<tr>
<td>Protein (g/100 mL)</td>
<td>1.3</td>
</tr>
<tr>
<td>Carbohydrates (g/100 mL)</td>
<td>7.5</td>
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casein ratios of 60/40 and energy contents of 67 kcal/100 mL, but differed in protein, fat and carbohydrate content (Table 1). IF had a protein content of 1.89 g/100 kcal and a fat content of 5.3 g/100 kcal, while CF contained 2.20 g/100 kcal and 4.9 g/100 kcal, respectively. IF was rich in ALAB and supplemented with free L-phenylalanine and L-tyrosine to meet required contents (Table 1, supplemental data).28 Egg and fish oils providing arachidonic acid and docosahexaenoic acid (each 10.7 mg/100 kcal) were added to IF (Table 2, supplemental data).

2.6. Laboratory procedures

At 120 days of age venous blood samples was drawn 2 h after the last feeding. Plasma urea, albumin, glucose and creatinine were analyzed at the Clinical Center of Serbia. Plasma aliquots were stored at −80 °C and transported on dry ice to the Dr. von Hauner Children’s Hospital (Munich, Germany) for the analyses of plasma AA and glycerophospholipid fatty acids.

Quantitative AA analysis was performed by LC-MS/MS.30 The samples were deproteinized by methanolic HCl and chemically derivatized with butanolic HCl. Amino acid butyl esters were separated on an XBridge C18 column (Waters, Eschborn, Germany) by ion-pair reversed phase chromatography (HP LC 1100 SL, Agilent, Waldbronn, Germany) and quantified by triple quadrupole mass spectrometry (Sciex API 2000, Applied Biosystems, Darmstadt, Germany).

Glycerophospholipid fatty acids were determined according to Glaser et al. (2010a) with the use of gas chromatography with flame ionization detection (Agilent 7890A, Agilent Technologies, Mülheim an der Ruhr, Germany).31 Derivatization of fatty acids with sodium methoxide was followed by separation of fatty acid methyl esters by gas chromatography using a BPX70 (25 m × 0.22 mm, Phenomenex, Aschaffenburg, Germany) column.

2.7. Calculations

The anthropometric gain (weight, length and head circumference) between 30 and 120 days of age was calculated. For calculation of the anthropometric gain per day, the results were divided by the exact number of days between both visits of each subject. The results were presented as weight: g/d, length: cm/d and head: cm/d and were available for all subjects.

The daily energy and protein intake was calculated as the average intake at 30, 60, 90 and 120 days of age and was available of all four time points for 79 IF and 80 CF infants.

The energetic efficiency was calculated as ratio of the anthropometric gain (weight and length) from 30 to 120 days of age to the average energy or protein intake per day during the study. The monthly energetic efficiency was calculated from the anthropometric gain during one month of life and the energy and protein intake assessed at the end of this month. The energetic efficiency is expressed as weight gain/100 kcal or g protein or as length gain/100 kcal or g protein.

The SD scores (z scores) were calculated by using the anthropometric results relative to the growth standards of the WHO for breastfed children.26

2.8. Data management and statistical analysis

Data were collected in a MS Access database and statistical analyses were performed with Stata/MP 11.0 (StataCorp LP, College Station, TX, USA). Results are presented as mean ± SD or medians and interquartile ranges (IQR, 25th and 75th percentile). Pearson chi-square test was used for statistical comparison of categorical data, t-test for normally distributed continuous variables and Kruskal–Wallis test for non-normally distributed continuous variables. P-values below 0.05 were considered as statistically significant.

The weight gain (g/d) between 30 and 120 days of life was used as primary outcome and was calculated by t-test. For comparison of primary outcome between both formula groups and BF group potential confounder (sex, maternal educational level, smoking during pregnancy) were considered.

The primary (weight gain) and secondary (length and head circumference gain) outcomes were analyzed for intention-to-treat (ITT) and per-protocol (PP) populations. In the ITT analysis, all subjects were considered that received study formula. In the PP analysis, only data from subjects complying with the predefined conditions, no intake of other formula than study formula, a maximum of 50 mL tea intake per day (mean over each 3-day protocol) and less than 3 spoons complementary food per week.

3. Results

A total of 505 infants were recruited (Fig. 1) including 213 FF infants who were randomly allocated to one of the study formulae. Of this group 207 infants finally received study formula from 19 ± 8 days of life onward (no significant difference between groups). During the intervention period forty infants dropped out of the study (19.3%) because of parental refusal (n = 34), medication/illness (n = 4) or loss of contact (n = 2). Three of the 167 infants completing the study were excluded from the analyses because not fulfilling the inclusion criteria (n = 2) or because of implausible values (n = 1). Gender ratio, birth order, maternal age and education, smoking, gestational age, mode of delivery, and anthropometry at birth and at study entry were not significantly different between the randomized formula groups (Table 3, supplemental data).

From the 185 BF infants 93 infants were lost to follow-up (50%) because of parental refusal (n = 76), medication/illness (n = 3) and loss of contact (n = 14). Baseline characteristics were comparable to the FF infants, with the exception of mothers educational level and rate of spontaneous deliveries, which were significantly higher for BF infants than for FF infants (P = 0.002 and P = 0.001, respectively).

3.1. Adverse events

Both formulae were well-accepted and no differences were reported for acceptance as well as consistency and color of stool, colic, flatulence, regurgitation and vomiting. The total number of adverse events (adverse event plus serious adverse event) was 21 in 88 IF infants, 41 in 92 CF infants and 45 in 185 BF infants (Table 4, supplemental data). Thus, a significantly higher rate in adverse events was observed in CF infants compared to IF (P = 0.003) and BF infants (P = 0.001), while IF and BF infants did not differ. The types of adverse events were similarly distributed between formula groups (49% respiratory tract infections, 24% skin infection/-eczema, 10% gastrointestinal problems, 4% urinary tract infections, and 13% others). The number of serious adverse events was 12 in the formula groups (IF = 9, CF = 3) and 4 in the reference group, with one serious adverse event in each formula group considered a potentially association to the study formula (IF: Vomiting, blood in stool, reflux and CF: Vomiting, blood in stool).

3.2. Growth

There were no differences between both formula groups for weight gain from 30 to 120 days of age (Table 2, ITT population). Length gain expressed as cm per day was significantly higher in IF (0.11 ± 0.02 cm/day) compared to CF infants (0.10 ± 0.02 cm/day, P = 0.02). In BF infants weight and length gains were significantly lower than in IF infants (Table 2). No significant differences were
observed for head growth between all groups (0.05 ± 0.01 cm/day for IF and CF; 0.05 ± 0.02 cm/day for BF infants). Determined possible confounders sex, smoking during pregnancy, mother’s age, first pregnancy and gestational age were not different between groups. Additionally, we adjusted for weight or length at 30 days of age to account for the fact that more extreme values tend to have lower or higher gains, respectively. The adjusted estimated daily weight gain for weight from 30 days to 120 days of age was found to be 1.94 g/d (95% CI: 0.04, 3.92; P = 0.055) greater in IF group than in CF group. The adjusted estimated daily length gain for length from 30 days to 120 days of age was found to be 0.05 mm/d (95% CI: 0.0006, 0.10; P = 0.047) greater in IF group than in CF group.

Analyzing PP subjects, weight tended to be higher in IF compared to CF (not significant, Table 5, supplemental data). Mean weight and length gains of IF infants were greater than of BF infants (both P = 0.01).

Results of z-scores are depicted in Fig. 1, supplemental data.

3.3. Nutrient intake

The intake of study formula was similar between the groups at 30 and 60 days of age, but significantly higher in CF infants at 90 and 120 days of age by average 90 and 140 mL, respectively. Energy and protein intakes determined from the 3 day protocols are summarized in Fig. 2. Energy intake in IF and CF infants was identical at 30 and 60 days of age, but at ages 90 and 120 days higher energy intakes were observed for CF infants (569 ± 152 vs. 509 ± 117 kcal/d, P = 0.005 and 617 ± 169 vs. 528 ± 123 kcal/d, P < 0.001, respectively). The protein intake was significantly higher in CF infants than in IF infants (Fig. 2).

The intake of other foods than study formula was generally low. There were no significant differences between the intake of water, tea, other formula than study formula, breastfeeding and complementary feeding between the formula groups during the whole study period.

In the PP population, the same differences in volume, energy and protein intake of IF and CF infants were found as in the ITT population. Neither intake of liquid nor breastfeeding was different between IF and CF infants (data not shown).

3.4. Growth in relation to intake

Weight and length gain from 30 to 120 days of age were related to the average energy intake during the study as a marker for energetic efficiency of the formulae (Fig. 3). Weight gain per 100 kcal was significantly higher in IF infants (6.45 ± 2.01 g/100 kcal) than CF infants (5.67 ± 2.21 g/100 kcal, P = 0.02). A corresponding difference was observed for length gain per 100 kcal (IF 0.23 ± 0.08 mm/100 kcal and CF 0.20 ± 0.11 mm/100 kcal, P = 0.04). The energetic efficiency during the first (birth to 30 days of age) and second (30–60 days of age) month of life in IF and CF infants was identical, but tended to be higher in IF during the third month (60–90 days of age). During the fourth month (90–120 days of age), the energetic efficiency of IF was significantly higher than in CF for weight 5.16 ± 2.96 vs. 4.12 ± 1.64 g/100 kcal and length 0.24 ± 0.23 vs. 0.18 ± 0.11 mm/100 kcal.

Weight gain by protein intake was significantly higher in IF (3.33 ± 1.04 g/g protein) than in CF infants (2.53 ± 0.99 g/g protein, P < 0.001).

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Table 2

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<thead>
<tr>
<th>Formula group</th>
<th>Breastfed group</th>
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<tr>
<td></td>
<td>Mean ± SD</td>
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<tr>
<td>Intervention</td>
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<td>Control</td>
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<tr>
<td>Breastfed</td>
<td>Mean ± SD</td>
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<td></td>
<td>Number of infants</td>
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<td></td>
<td>Length gain (cm/d)</td>
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<td></td>
<td>Head circ. gain (cm/d)</td>
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Data are presented as mean ± SD. Identical letters indicating significant differences between groups (P < 0.05, Student’s t-test).
3.5. Biochemical markers

There were no significant differences between the formula groups for albumin, urea and creatinine. Compared with the BF group, urea and creatinine were higher and glucose was lower in both formula groups (Table 6, supplemental data). All analyzed AA and fatty acid concentrations were within the normal ranges for healthy infants or small children.14,32

Infants receiving IF formula showed a significantly higher plasma total fatty acid concentration of 1225 mg/L (interquartile range: 1108, 1318) than CF infants (1071 mg/L: 988, 1202; \( P < 0.001 \)). Arachidonic acid (8.24 vs. 7.02%, \( P < 0.001 \)) and docosahexaenoic acid (4.51 vs. 1.48%, \( P < 0.001 \)) percentages were higher in IF infants (Table 7, supplemental data). The arachidonic acid level was significantly higher in BF infants compared to both formula groups, while the docosahexaenoic acid level was highest in IF infants, followed by breastfed infants and CF (all group differences \( P < 0.01 \)).

The concentrations of 14 AA were significantly different between groups (Table 8, supplemental data). The total AA concentration were 2907 (2538, 3124) \( \mu \)mol/L in IF and 3053 (2737, 3292) in CF group (\( P = 0.01 \)). The concentrations of Ala, Asn, Asp, His, Ile, Leu, Met, Orn, Pro, Thr and Val were significantly higher in CF in infants, while Phe, Tyr and Trp were significantly higher in IF infants. Branched chain AA concentrations of BF infants were more similar to concentrations in the IF group, while some other AA, including Phe and Trp tended to be more similar between BF and CF infants.

4. Discussion

4.1. Growth, acceptance and tolerance

This randomized, controlled, double-blind intervention study demonstrated that the growth of infants fed a modified infant formula with reduced protein with ALAB and LC-PUFA is similar and within normal ranges for formula fed infants. The absolute growth values of the IF (30.2 \( /6.3 \) g/d) and CF (28.3 \( /6.5 \) g/d) were comparable (in PP and ITT population) and similar to those found in other studies, e.g. 27.8 \( /4.7 \) g/d23 and 28.1 \( /5.4 \) g/d.33 In the BeMIM study, no statistical differences in weight gain were found between the groups, whereas a tendency towards higher weight gain was observed in IF infants. Gain in weight and length was proportional in both study formula groups (IF: \( R = 0.323, P = 0.003 \); CF: 0.391, \( P < 0.001 \)). Weight-for-length \( z \)-score was higher in IF infants compared to CF infants at all time points studied (Fig. 1, supplemental data). This is in contrast to other studies that found lower weight-for-length \( z \)-scores in infants fed a protein reduced formulae.7,33 To assess the long term growth and anthropometry, follow-up visits of these children, specifically at the age of 4 and 6 years are planned.

All infants accepted IF well and for all parameters studied no negative effects were been found. The significantly lower rate in total adverse events (adverse event plus serious adverse event) in the IF group compared to the CF group could be related to the ALAB addition. ALAB was proposed to have immunostimulatory effects and antibacterial properties and thus be protective against infections.16

4.2. Nutrition and satiety

The nutritional intake was adequate from study formulae, did not result in inferior growth and not in compensatory greater intake of formula, complementary diet or liquids compared to BF infants. Our findings in energy and protein intake differed from Fomon et al. 1999.34 In this study infants of the lower protein group (1.7 g protein/100 kcal) consumed significantly more formula and thus significantly more energy than the infants with the higher protein formula. In Fomon’s study, this resulted in a higher BMI of the lower protein group infants suggesting a higher fat
deposition. Thus, compensation for an inadequate protein intake was observed by Fomon et al. 1999 and it was concluded that the formula with 1.7 g protein/100 kcal may not be safe. In BeMIM study, no significantly higher intake in the IF group was found, thus the protein content of 1.89 g/100 kcal can be considered adequate with the protein quality provided. Plasma concentrations of urea depend on mainly dietary protein intake and indicate the level of nitrogen excretion if nitrogen intake exceeds the needs for protein synthesis and growth or dietary proteins are imbalanced. Plasma urea concentrations of IF and CF infants were similar, and both were significantly higher than in BF infants which is consistent with a higher protein intake with both formulae. Different protein intakes have been related to differences in growth between breastfed and formula fed infants.

The energy intake via study formulae was similar to previously reported intakes of healthy term infants. However, IF infants had a significantly lower energy intake than CF infants at 90 and 120 days of age. While volume intake per meal was not different, the lower intake in the IF group was explained by a lower meal frequency, which might indicate a higher satiety. The adequate protein intake and a balanced AA composition of IF were improved by substitution of β-lactoglobulin by ALAB (rich in Trp) and addition of Trp and Phe as free AA. Therefore, a significantly higher Trp to large neutral AA ratio (Trp/large neutral AA) in plasma was achieved in IF (median: 0.11; IQR: 0.10; 0.13) compared to CF (0.10:0.09; 0.11, P < 0.001). While, Trp and large neutral AA compete for transport proteins in the blood-brain-barrier, a higher Trp/large neutral AA ratio results in higher Trp levels in the brain. Trp as precursor of the neurotransmitter serotonin influences the sleep-wake rhythm and is involved in the appetite regulation. According to Halford et al. 2005 supplementation of serotonin precursors, Trp or 5-hydroxytryptophan decreases food intake. Thus, via an increased Trp/large neutral AA ratio, IF may have led to higher satiety and less formula intake in the IF group.

4.3. Energetic efficiency

Both formulae appear safe with respect to infant growth. The apparently minor differences in composition seem to affect energetic efficiency for growth. The higher energetic efficiency of the new infant formula (IF), in spite of its lower protein content, could be due to the improvement of protein composition by the addition of ALAB. Davis et al. 2008 compared the growth of infants, who were fed a formula with replacement of 2.2 g/L β-lactoglobulin by 2.2 g/L ALAB to the growth of infants receiving an unmodified formula (1.3 g/L ALAB). Growth was not statistically different between groups, but tended to be higher in the ALAB group. Furthermore, a slightly lower energy intake was observed in the ALAB-rich formula group. The ALAB-rich formula group had a 0.55 g/100 kcal (calculated from energy intake and weight gain) higher weight gain than the standard formula group. Davis et al. 2008 observed this difference after 8 weeks of intervention. In BeMIM study a difference of 0.78 g/100 kcal was observed after 12 weeks of intervention.

Several beneficial properties have been ascribed to ALAB in infant formula: ALAB is highly digestible and provides a well-balanced AA mixture, while positively affecting mineral absorption, gut microflora and immune function. Furthermore fat absorption might influence energetic efficiency of infant formulae. The fat absorption coefficient in formula fed term infants is about 90%. IF had a markedly higher phospholipid content than CF (no phospholipids) which even exceeded phospholipid content of human milk fat consisting of >98% triacylglycerols, 0.7% phospholipids and 0.5% cholesterol. In BeMIM study, phospholipids from egg oil were used as main source of arachidonic acid resulting in higher plasma levels of this fatty acid in the IF group compared to CF, which did not provide LC-PUFA. The bioavailability of LC-PUFA depends on the chemical form of fatty acid esters. Amate et al. investigated the effect of different LC-PUFA sources, triacylglycerols or phospholipids, on the rate of fat acid absorption in rats. Their results showed, that the fat absorption of some saturated fatty acids and docosahexaenoic acid from egg phospholipids were higher than from triacylglycerols. Sala-Vila et al. (2004), argued that the incorporation of LC-PUFA into plasma phospholipids depends more on the fatty acid composition of the diet fed than on the source (TG or PL) of the dietary LC-PUFA. Although, a positive relation between LC-PUFA, especially arachidonic acid, and growth has been observed by Carlson et al. (1993), we found no correlation between weight gain and plasma levels of arachidonic acid or docosahexaenoic acid in BeMIM study. This is in line with several other studies indicating that arachidonic acid and docosahexaenoic acid supplementation do not significantly influence weight, length or head circumference gain.

Several meta-analyses show the protective effect of breastfeeding on overweight. The inverse relationship between duration of breastfeeding and the risk of overweight has been related to the protein and caloric intake. The multicentric European GROW study, in 1138 healthy, formula fed infants received a protein reduced or a higher protein infant and follow-on formula, has shown that decreasing the protein intake from 11.7 to 7.1% of energy in the infant formula and from 17.6 to 8.8% in the follow-on formula was associated with normalization of early weight gain as compared to breastfed infants. A higher protein intake in formula fed infants results in higher BMI levels than in breastfed infants and thus may increase the risk for later obesity. Thereby, the metabolic programming of later obesity risk seems to occur within one or two years of life. Thus, a high weight gain during this period should be avoided.

A limitation of this study is, that more than one components of the IF have been changed compared to CF. The new modified formula (IF) according to the European directives of 2006 was compared to a standard formula (CF) of European directives of 1999. Thereby, protein source, macronutrient composition as well as content of LC-PUFA differed between the formulas. Thus, we can only assume that ALAB addition and improvement of dietary AA composition was the major reason for improved energy efficiency of IF.

We conclude that an ALAB containing infant formula with a protein/energy ratio of 1.89 g/100 kcal and improved LC-PUFA status provides an adequate intake during the first months of life can be considered safe. This modified infant formula is appropriate for term infants as evidenced by growth velocities, acceptance and tolerance. We consider it most likely that the higher energetic efficiency of the modified infant formula due to the higher content of ALAB and Trp. The results obtained in this trial support the general conclusion that all considerable changes in infant formula composition should be evaluated by clinical trials including assessments of infant growth.

Source of funding

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Statement of authorship

HD, VG and BK were responsible for conception and study design; TN and NL were responsible for recruitment and study visits; BT was responsible for the coordination of the study in Belgrade; MF performed sample analysis, and MF and VG were
responsible for statistical analyses; MF, HD and BK undertook data interpretation; MF, HD were responsible for writing the manuscript. Finally, all authors critically revised the manuscript.

Conflict of interest

All authors have made substantial contributions to the conception and design of the study, or acquisition of data, or analysis and interpretation of data, drafting the article or revising it critically for important intellectual content. Each author has seen and approved the contents of the submitted manuscript. BT was employed by Hipp Gmbh & Co. Export KG, Austria. Otherwise, none of the other authors had any personal or financial conflict of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.clnu.2013.12.007.

References


