A modified low-protein infant formula supports adequate growth in healthy, term infants: a randomized, double-blind, equivalence trial

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ABSTRACT

Background: A high protein intake in early life is associated with a risk of obesity later in life. The essential amino acid requirements of formula-fed infants have been reassessed recently, enabling a reduction in total protein content and thus in protein intake.

Objectives: We aimed to assess the safety of an infant formula with a modified amino acid profile and a modified low-protein (mLP) content in healthy term-born infants. Outcomes were compared with a specifically designed control (CTRL) infant formula.

Methods: In this double-blind, randomized controlled equivalence trial, infants received either mLP (1.7 g protein/100 kcal; $n = 90$) or CTRL formula (2.1 g protein/100 kcal; $n = 88$) from enrollment (age $\leq$ 45 d) to 6 mo of age. A breastfed group served as a reference ($n = 67$). Anthropometry and body composition were determined at baseline, 17 wk (including safety blood parameters), and 6 mo of age. The primary outcome was daily weight gain from enrollment up until the age of 17 wk (at an equivalence margin of $\pm$3.0 g/d).

Results: Weight gain from baseline (mean $\pm$ SD age: 31 $\pm$ 9 d) up to the age of 17 wk was equivalent between the mLP and CTRL formula groups (27.9 and 28.8 g/d, respectively; difference: −0.86 g/d; 90% CI: −2.36, 0.63 g/d). No differences in other growth parameters, body composition, or in adverse events were observed. Urea was significantly lower in the mLP formula group than in the CTRL formula group (−0.74 mmol/L; 95% CI: −0.97, −0.51 mmol/L; $P < 0.001$). Growth rates, fat mass, fat-free mass, and several essential amino acids were significantly higher in both formula groups than in the breastfed reference group.

Conclusions: Feeding an infant formula with a modified amino acid profile and a lower protein content from an average age of 1 mo until the age of 6 mo is safe and supports an adequate growth, similar to that of infants consuming CTRL formula. This trial was registered at www.trialregister.nl as Trial NL4677. *Am J Clin Nutr* 2019;00:1–13.

Keywords: infants, infant nutrition, growth, body composition, safety, protein quality, protein quantity, protein intake, amino acids

Introduction

The mean BMI and prevalence of obesity in children and adolescents are increasing worldwide. In 2016, an estimated 124 million children were obese and an additional 213 million children and adolescents were overweight (1).

Obese children are at risk of a wide range of health problems such as breathing difficulties, hyperlipidemia, hypertension,

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Danone Nutricia Research had no role in the execution of the study or in the statistical analyses of the results.

Supplemental Tables 1–4 are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/ajcn/.

Data described in the article, code book, and analytic code will be made available upon request pending application and approval.

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Abbreviations used: AE, adverse event; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CTRL, control; FFM, fat-free mass; FFMI, fat-free mass index; FM, fat mass; FMI, fat mass index; IAAO, indirect amino acid oxidation; ITT, intention-to-treat; mLP, modified low-protein; MUAC, midupper arm circumference; PP, per-protocol.

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glucose intolerance, sleep apnea, psychosocial problems, orthopedic complications, and, in adolescent girls, polycystic ovary syndrome (2). Overweight and obese children have an increased risk of becoming overweight as adults (3, 4) and may experience an earlier onset of chronic diseases like type 2 diabetes (5). It is critical to prevent childhood obesity early in life as articulated by the "Developmental Origins of Health and Disease" concept (6). In a meta-analysis, breastfeeding was associated with a lower risk of childhood obesity than formula-feeding (7). However, a cluster-randomized trial conducted in the Republic of Belarus demonstrated that a greater breastfeeding duration and exclusivity of breastfeeding did not prevent obesity in childhood or adolescence (8).

Apart from potential demographic differences, several mechanisms may account for the lower obesity risk in breastfed infants such as the differences in appetite regulation, early growth patterns, leptin, and gut microbiome.

The protective effect of breastfeeding could at least be partially attributed to the lower protein content of breast milk than of infant formula (9–13). Consequently, the quantity of protein present in infant formula has gained much attention. However, a systematic review of the long-term effects, including obesity risk, of infant formula and follow-on formula with different protein contents demonstrated that the current evidence to support the use of low-protein formulas is insufficient and conflicting (14). Securing a sufficient supply of essential amino acids is necessary for healthy growth—particularly in early life when growth velocity is high. Consequently, both the protein quantity and the protein quality in infant formula should be considered.

The determination of dietary essential amino acid requirements in infants is challenging. To date, the amino acids provided by infant formula have been roughly based on the average amino acid content in human milk (15). In a series of studies, we used the indirect amino acid oxidation (IAAO) technique to assess infant amino acid requirements and showed that infant formulas are likely to provide an excess and unbalanced amount of essential amino acids (16–21). Based on these outcomes, a modified infant formula was developed with an optimized amino acid composition that facilitated a protein content reduction of 20%. It was postulated that this infant formula would provide a more balanced supply of amino acids, supporting a healthy growth and metabolic development, bringing the physiological properties of infant formula closer to those of human milk, i.e., reducing overweight risk and associated metabolic diseases (22, 23). However, first a stringent evaluation of adequacy and safety is required.

Previously, the safety of this nutritional concept was evaluated in 2 piglet studies (24, 25): no detrimental effects on growth or maturation were observed. The aim of this study was to assess the adequacy and safety of this new modified low-protein (mLP) infant formula in healthy term-born infants. We hypothesized that an infant formula with an optimized amino acid composition and lower protein amounts during the first 6 mo of life is safe and results in adequate infant growth.

Methods

Study design and participants

This double-blind randomized controlled trial (ProtEUs study; NL4677) was conducted in 2 centers: Amsterdam UMC, location VU University Medical Center, Amsterdam, Netherlands; and Dr. von Hauner Children's Hospital, Ludwig-Maximilians-Universität, Munich, Germany. Healthy, term-born, formula-fed infants were randomly assigned to receive either the mLP infant formula with a protein content of 1.7 g/100 kcal or a control (CTRL) infant formula with a protein content of 2.1 g/100 kcal. Breastfed infants were included as a reference group. Eligible infants were born at a gestational age of ≥37 wk with a normal birth weight (i.e., between the 3rd and 97th percentiles) and were ≤45 d old at enrollment. Exclusion criteria were being part of a multiple birth, current or previous conditions, illnesses, interventions, or congenital disease or malformations that could interfere with the study. We also excluded those requiring a special diet other than a standard cow milk–based infant formula as well as those currently or previously participating in any other study involving investigational or marketed products. The trial was approved by the Institutional Review Boards of VU University Medical Center Amsterdam and of the Medical Faculty, Ludwig-Maximilians-Universität, Munich. The study was conducted according to International Council for Harmonisation (ICH) and Good Clinical Practice (GCP) guidelines and in compliance with the principles of the Declaration of Helsinki. An independent data safety monitoring board monitored trial safety. Written informed consent was obtained from all participants' parents or guardians.

Randomization and blinding

A computer-generated randomization scheme with a random block size (maximum of 8) was used to assign infants to 1 of the study groups in a 1:1 ratio with stratification according to sex. The formula was blinded using 4-letter codes by the manufacturer (Nutricia). Both the families and the investigators were blinded to group allocation until data analysis was complete. Unblinding (to investigators only) occurred after statistical analyses of the primary outcome.

Procedures

Formula-fed infants eligible for participation were randomly assigned to receive either the mLP or CTRL formula from baseline up to the age of 6 mo. All infants consumed ad libitum throughout the study period. Parents were allowed to introduce complementary feeding no earlier than after the age of 17 wk in line with national guidelines. Breastfed infants were included in the reference group and followed the same visit and assessment schedule as the randomly assigned infants including completion of monthly 3-d food diaries.

The parents were given study product and diaries at the first visit. Parental weight and height were self-reported. Subsequent study visits were scheduled at 17 wk (visit 2) and 6 mo (visit 3). During these visits, anthropometry and body composition measurements were performed by trained study personnel according to standard protocols. Venous blood sample from a hand vein was obtained on the second visit after ≥3 h of fasting.

Weight was measured to 0.5-g accuracy on a baby scale (MS-4100, Marsden). Length was measured with a flexible measuring board (Seca 210, Seca). Head circumference, waist circumference, and midupper arm circumference (MUAC) were measured to 0.1-cm accuracy using a flexible tape. The mean of 2 measurements was used in the statistical analysis.

Body composition was measured with air-displacement plethysmography (PEA POD Infant Body Composition System, Cosmed).

The fat-free mass (FFM) index (FFMI) and the fat mass (FM) index (FMI) were calculated using the following equations: FMI = FM (kg)/length (m)^2 and FFMI = FFM (kg)/length (m)^2 (26).

The parents recorded the infants' health status and study product intake every month using a questionnaire combined with a 3-d food diary.

We assessed formula intake using 3 complementary methods:

1) Parents were asked to record study product intake in the 3-d food diary every month: total milk, protein, and energy intakes were calculated using these diaries.
2) At the end of the study period, we recorded the number of study formula tins used.
3) In a subgroup of 20 randomly assigned infants, milk intake was measured using deuterium dilution at the age of 17 wk (before the introduction of complementary foods).

The occurrence of (serious) adverse events (AEs), the use of medication and nutritional supplements, as well as compliance with the study product were recorded by the parents and discussed with the investigator during the visit.

Laboratory analysis

Venous blood was collected in a 2.5-mL serum tube and a 0.5-mL heparin tube. This was divided into 6 different aliquots after centrifugation (10 min; 1800 × *g*; 20°C). Samples were stored at −80°C and thawed only once just before analysis. Blood parameters were analyzed on a Beckman Coulter AU5800/AU680 in serum. Urea, alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were assessed by a kinetic UV test. Creatinine was assessed by kinetic colorimetric assay. Amino acids were detected by LC-tandem MS and analyzed with a 110 HPLC (Agilent) and AP12000 tandem mass spectrometer (AB Sciex).

Study formulas

The study formulas were isoenergetic (67 kcal/100 mL) and had an identical nutritional composition except for protein and lactose; the latter was used to make the formula isoenergetic (**Table 1**). The protein concentration of the mLP formula (1.7 g/100 kcal) was below the range minimum of the European legal standards for infant formula (range: 1.8–2.25 g/100 kcal).

TABLE 1 Macronutrient contents of the study formulas[1]

	mLP formula	CTRL formula
Energy, kcal	67/100	67/100
Protein equivalent	1.12/1.70	1.40/2.12
Added amino acids	0.33/0.53	0.42/0.66
Casein	0.35/0.52	0.47/0.70
Whey	0.44/0.65	0.51/0.76
Carbohydrates	7.7/11.5	7.3/11.0
Lactose	7.3/10.9	7.0/10.5
Glucose + starch	0.4/0.6	0.3/0.5
Fibers/oligosaccharides (scGOS/lcFOS 9:1)	0.8/0.5	0.8/0.5
Fat	3.4/5.1	3.4/5.1

[1]Values are grams per 100 mL/per 100 kcal. CTRL, control; lcFOS, long-chain fructo-oligosaccharides; mLP, modified low-protein; scGOS, short-chain galacto-oligosaccharides.

The amino acid composition of the mLP formula (**Table 2**) is based on the estimated infant requirements as assessed in a series of IAAO studies performed in healthy term-born infants (16, 19, 20). No suitable single or mix of different intact protein sources could provide the right composition and amount of amino acids. Hence, the required composition was met by combining intact cow milk protein (70%) with a specific mixture of free amino acids (30%). Infants in the CTRL formula group received an infant formula with a standard amino acid composition currently on the market. However, the CTRL formula was adapted to comprise the same ratio of intact protein and free amino acids as the mLP formula to avoid possible differences in nitrogen utilization and growth between the formula groups due to differences in essential amino acid bioavailability. Hence, it must be noted that in the current study, safety and adequacy of the mLP formula were not evaluated compared with a standard, commercially available formula, but compared

TABLE 2 Amino acid composition of the study formulas[1]

	mLP formula	CTRL formula
Essential		
L-Isoleucine	77/117	69/104
L-Leucine	102/155	143/217
L-Lysine	96/145	121/185
L-Methionine	28/41	31/46
L-Phenylalanine	43/65	56/85
L-Threonine	50/74	76/116
L-Tryptophan	12/19	76/36
L-Valine	80/122	74/112
Nonessential		
L-Hystidine[2]	30/45	35/53
L-Alanine	24/37	42/63
L-Arginine	24/37	35/53
L-Aspartic acid/L-asparagine	101/153	132/200
L-Cyst(e)ine[2]	35/53	25/38
L-Glutamic acid/L-glutamine	202/306	284/432
Glycine	19/29	27/40
L-Proline	67/101	80/155
L-Serine	56/84	102/116
L-Tyrosine[2]	76/115	55/83

[1]Values are milligrams per 100 mL/per 100 kcal. CTRL, control; mLP, modified low-protein.
[2]Conditionally essential.

with a CTRL formula specifically adapted to allow specific evaluation of the impact of amino acid composition and total protein.

Outcomes

The primary outcome was daily weight gain (g/d) from enrollment to age 17 wk. Secondary outcomes were formula intake, protein intake, body weight, length, head circumference, MUAC, waist circumference, and body composition at 17 wk and 6 mo of age. Other secondary outcomes were blood parameters at age 17 wk and AEs.

Statistical analyses

The primary outcome was equivalence in weight gain in grams per day from baseline to age 17 wk in the per-protocol (PP) population. The predefined equivalence margin of 3 g/d is based on the advice of the American Academy of Pediatrics (27) and supported by the European Commission's Scientific Committee on Food (28) which recommended that growth studies conducted for the purpose of assessing infant formula should be able to detect a difference of 3.0 g weight gain/d during the first 3–17 wk of life. Based on these numbers, 70 infants per arm were required (for 80% power to detect a difference at a significance level of 0.05). Before the study, we assumed 25% dropouts and 10% protocol violations or deviations/noncompliance. Hence, a sample size of 216 formula-fed infants (108 infants per arm) was needed to answer the primary objective of the equivalence analysis.

Equivalence of weight gain was assessed in the PP population (primary analysis) and in the intention-to-treat (ITT) population. Selecting the PP population and the use of 90% CI (instead of 95% CI) are methods generally used within bioequivalence trials (29, 30). Infants were excluded from the PP population if another infant formula was introduced, if complementary feeding started before the age of 17 wk, and when infants were breastfed more than once a day. Before analyses, all anthropometric data were converted to z scores using the WHO Child Growth Standards (31). The differences in outcome between the groups were analyzed with linear regression analysis. Secondary growth parameters were assessed in the ITT population and analyzed with linear mixed-model analysis. Interactions were added to obtain the differences between the groups at different time points. The analyses were adjusted for the baseline value of the particular outcome. Besides the crude analyses, adjusted analyses were also performed in which adjustments were made for sex, ethnicity (Caucasian/other), recruitment center, maternal weight gain during pregnancy, maternal BMI, paternal BMI, maternal smoking, gestational diabetes, and maternal education. The residuals of all outcomes were normally distributed except for creatinine. Therefore, creatinine was dichotomized (group 1 = 26.5 μmol/L, $n = 39$ and group 2 = 35.4 μmol/L, $n = 93$ and 44.2 μmol/L, $n = 1$). ORs and Fisher's exact test were used to compare the feeding groups. The differences in the number of (serious) AEs between the feeding groups were analyzed with Poisson regression analysis. Statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) version 22 (IBM), and a significance level of 5% was used for all comparisons.

Interim analyses and stopping guidelines

Blinded interim analyses were conducted after the inclusion of 10, 40, and 70 randomly assigned infants per arm. All interim analyses were used to monitor safety: the trial would have been stopped prematurely had there been safety concerns. Continuation of the study was advised by the Data Safety Monitoring Board at all time points.

Results

A total of 245 infants (of the 667 screened for eligibility) were enrolled between 22 October, 2014, and 29 December, 2016: 178 were formula-fed and 67 were breastfed. Ninety infants were randomly assigned to the mLP group and 88 infants to the CTRL group (**Figure 1**). The number of included infants was lower than the calculated sample size owing to the low dropout rate of only 2.8% (5 of 178) at 17 wk. Three infants dropped out after the age of 17 wk. Two infants were lost to follow-up in the breastfed group. The dropout rate and protocol violations together were 14% at the age of 17 wk and 18% at the age of 6 mo. This resulted in a PP population (primary outcome) that included 77 infants in the mLP group and 76 in the CTRL group. In the breastfed reference group, 83% (54 of 65) and 65% (42 of 65) of the infants were exclusively breastfed at 17 wk and 6 mo, respectively. Baseline characteristics are presented in **Table 3**.

Primary outcome

In the PP population, weight gain from baseline (mean age: 31 d) up until the age of 17 wk was 27.9 g/d in the mLP group and 28.8 g/d in the CTRL group (**Figure 2**). Equivalence in daily weight gain was demonstrated between the mLP and the CTRL group given that the observed difference in means of −0.86 g/d and its CI (90% CI: −2.36, 0.63 g/d) lay within the predefined equivalence margins of ±3 g/d. Equivalence in daily weight gain was also confirmed for the ITT population (mLP: 27.9 g/d, CTRL: 28.3 g/d; difference: −0.35 g/d; 90% CI: −1.77, 1.08 g/d). Moreover, the equivalent daily weight gain (with margins of ±3 g/d) was demonstrated for both boys and girls separately until the age of 17 wk (PP population and ITT population). Adjustment for baseline body weight did not change these results (data not shown). As a reference, infants in the breastfed group gained less weight during the same period: 23.8 g/d, which is significantly lower than the mLP group ($P < 0.001$) and the CTRL group ($P < 0.001$).

Secondary outcomes

Intake of study formula.

The questionnaires showed that the daily intake of study formula was similar between the formula groups in both the ITT and the PP population (**Supplemental Table 1**). Consequently, the mean daily protein intake was lower in the mLP group,

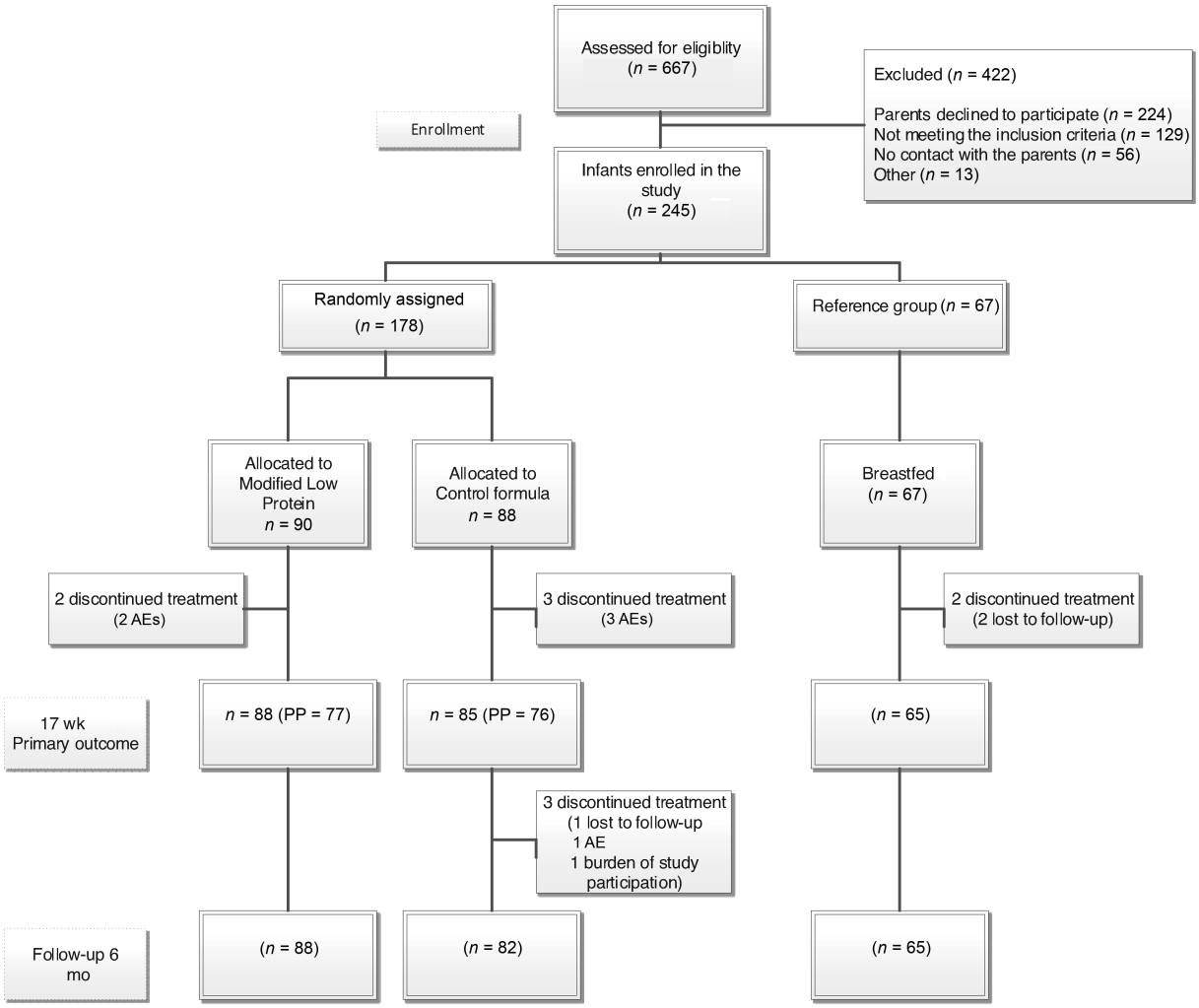

FIGURE 1 Flowchart of progression of infants during the study. AE, adverse event; PP, per-protocol.

but the mean daily energy intake was similar (**Figure 3**). In addition, the mean ± SD number of study formula tins supplied during the intervention period did not differ significantly between groups (mLP: 59.0 ± 8; CTRL: 57.7 ± 10; $P = 0.33$). Similar findings were obtained with the deuterium oxide method, showing that mean ± SD daily formula intake at 17 wk of age was similar between groups, with values of 1007 ± 207 mL in the mLP group and 985 ± 151 mL in the CTRL group ($P = 0.81$).

Growth.

No significant differences in growth parameters (body weight, length, head circumference, waist circumference, and MUAC) measured at 17 wk and at 6 mo of age were observed between the formula groups (**Tables 4**, **5**, **Figure 4**, **Supplemental Table 2**). In general, the formula-fed infants grew faster than the breastfed group with significantly higher mean body weight and mean weight-for-age z scores at 17 wk and 6 mo. At 6 mo, the body length and length-for-age z scores were significantly higher in the formula groups than in the breastfed group. The MUAC and waist circumference were significantly larger in both formula groups than in the breastfed group at both time points (Table 5, Supplementary Table 2).

Body composition.

No significant differences in body composition were observed between the formula groups (Tables 4, 5, Supplemental Table 2) except for FFMI at age 17 wk, which was significantly lower in the mLP group (−0.24; 95% CI: −0.47, −0.003; $P = 0.047$) after adjustment for potential confounders. This result was not observed at 6 mo of age.

In comparison with the breastfed group, the total FM at 17 wk was significantly higher in both formula groups. This persisted for the CTRL group until 6 mo of age and was paralleled by a higher FMI value. FM percentage, however, did not significantly differ between the formula groups and the breastfed group at both time points. In addition, total FFM and FFMI were also significantly higher in the formula groups than in the breastfed group.

TABLE 3 Parental and infant characteristics: intention-to-treat population[1]

	mLP ($n = 90$)	CTRL ($n = 88$)	BF ($n = 67$)
Parental characteristics			
Maternal age, y	32.9 ± 4.3	32.2 ± 5.0	32.2 ± 4.5
Maternal higher education,[2] %	58.9	55.7	77.6
Maternal BMI at enrolment, kg/m^2	26.2 ± 4.7	25.9 ± 5.2	24.6 ± 3.6
Maternal prepregnancy BMI,[3] kg/m^2	24.2 ± 4.4	24.5 ± 5.2	23.2 ± 3.5
Weight gain during pregnancy, %	22.4 ± 8.9	20.6 ± 10.5	20.4 ± 8.6
Gestational diabetes	7 (8.8)	5 (6.0)	3 (4.6)
Paternal BMI, kg/m^2	26.0 ± 3.2	26.6 ± 3.9	25.6 ± 3.8
Infant characteristics			
Boys	41 (46)	41 (47)	31 (46)
Caucasian	76 (84)	77 (89)	60 (90)
Gestational age, wk	39.6 ± 1.2	39.6 ± 1.2	39.9 ± 1.1
Birth weight, g	3422 ± 371	3429 ± 391	3415 ± 339
Cesarean delivery	31 (34)	26 (30)	16 (24)
Ever breastfed	40 (45)	40 (45)	67 (100)
Age at inclusion, d	30 ± 9	31 ± 10	32 ± 8
Age at start of intervention, d	30 ± 9	31 ± 10	NA
Age when fully consuming study formula, d	31 ± 9	32 ± 10	NA
Introduction of complementary feeding during intervention period	81 (96)	72 (92)	52 (91)
Age at introduction of complementary feeding during intervention period, wk	19.4 ± 2.4	18.9 ± 2.2	20.4 ± 3.8

[1] Values are mean ± SD or *n* (%) unless otherwise indicated. BF, breastfed; CTRL, control; mLP, modified low-protein; NA, not applicable.
[2] Defined as higher professional education and university education.
[3] Self-reported.

Blood parameters.

At 17 wk of age, we collected 53 samples in the mLP group, 45 in the CTRL group, and 36 in the breastfed group. All mean values of the blood parameters were within the normal ranges for infants in this age category. Two infants had values above the upper reference value of urea (0.3–5.0 mmol/L): 1 infant in the breastfed group and 1 infant in the CTRL group. Twelve infants had values above the upper reference margin for AST (<64 U/L): 10 infants in the breastfed group and 2 infants in the mLP group. Eighteen infants were above the upper margin for ALT (<55 U/L): 9 infants in the breastfed group, 3 infants in the CTRL group, and 6 infants in the mLP group. The urea concentration was significantly lower in the mLP group than in the CTRL group (−0.74 mmol/L; 95% CI: −0.97, −0.51; $P < 0.001$) and closer to that of the breastfed group. We found no significant differences in creatinine, AST, and ALT concentrations between the formula groups (**Table 6**). AST was significantly higher in the breastfed group than in the formula groups. ALT was significantly higher in the CTRL group than in the breastfed group.

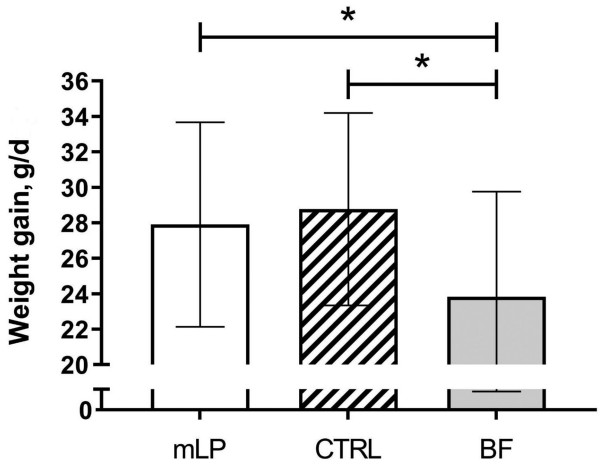

FIGURE 2 Weight gain (g/d) from baseline through 17 wk of age (per-protocol population). Data are mean ± SD and compared by using linear regression analysis. The weight gain rate of the mLP group did not differ from that of the CTRL group. The weight gain rates of the mLP group and CTRL group were significantly different from that of the BF group. *$P < 0.001$. BF, breastfed ($n = 65$); CTRL, control ($n = 76$); mLP, modified low-protein ($n = 77$).

Plasma amino acids were analyzed at the age of 17 wk. Most of the essential amino acids were significantly different between the formula groups. This was in line with the differences in amino acid composition of the formulas. Furthermore, there were several differences between the formula groups and the breastfed group. The plasma amino acids of the mLP group were all within the reference ranges (centiles 0.10–0.90) for 4-mo breastfed infants except for isoleucine, valine, and tyrosine (32). However, the values were within the reference ranges (centiles 0.90–0.95) for 4-mo formula-fed infants, except for valine (32) (**Supplemental Table 3**).

AEs.

In total, 517 AEs in 213 infants were recorded during the intervention period. In general, there was great variability in the incidences and severity of AEs. Eight serious AEs were recorded in the mLP group, 6 in the CTRL group, and 2 in the breastfed group (**Supplemental Table 4**); all were due to hospitalization. The total number of AEs during the intervention period was not significantly different between the formula groups (rate ratio: 1.05; 95% CI: 0.86, 1.28; $P = 0.62$); the occurrence and severity were comparable (**Table 7**).

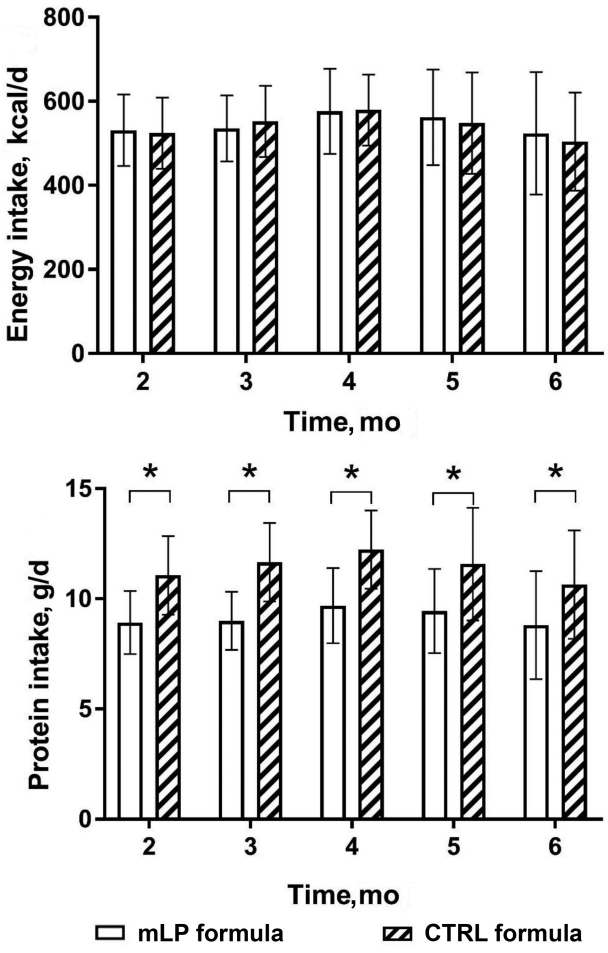

FIGURE 3 Protein intake (g/d) and energy intake (kcal/d) derived from infant formula during the intervention period as recorded by the parents. Data are mean ± SD and compared by using Student's *t* test. The protein intake of the mLP group ($n = 77$–84) was significantly different from that of the CTRL group ($n = 69$–86) at all time points. There was no difference in energy intake. $^{*}P < 0.001$. CTRL, control; mLP, modified low-protein.

Discussion

We showed that the daily weight gain of infants consuming an infant formula with a modified amino acid profile and a protein content of 1.7 g/100 kcal was equivalent to that of infants consuming CTRL infant formula (with a standard amino acid profile and a protein content of 2.1 g/100 kcal) from baseline to age 17 wk. The weight gain rate can be used as a safety parameter to assess infants' growth and health (27). Both the mLP formula and the CTRL formula result in growth, body composition, and metabolic outcomes different from those in breastfed infants.

The current study primarily focused on the evaluation of growth adequacy of infants that consumed the mLP formula. This formula was specifically designed and produced for this clinical study using a tailored blend of amino acids (30%) and intact dairy proteins (70%). As a proof of principle, to allow a true evaluation of the impact of amino acid composition and protein concentration and to avoid possible differences in nitrogen utilization based on nitrogen sources, the CTRL formula was adapted to comprise the same ratio of intact protein and free amino acids. Hence, caution is warranted when comparing outcomes to previous safety studies studying the impact of protein quality and quantity on infant growth outcomes. Although the current study lacks a "standard" infant formula group, the inclusion of the breastfed reference group as well as evaluation of growth outcomes using the WHO growth standards provided appropriate guidance for growth adequacy evaluation.

Numerous reports have shown that infants can control their macronutrient intake by regulating volume of intake (33–35). In this study we used 3 independent assessments and concluded that the volume of intake in both formula groups was similar, and no compensatory volume of intake was observed in the mLP group. This confirms that the targeted difference in protein intake was established between the mLP and CTRL formula groups.

Over the past few decades, several studies have been investigating the safety of infant formulas with reduced protein amounts.

A recent report showed that infants consuming an infant formula with a protein amount of only 1.43 g/100 kcal resulted in a significantly lower weight gain rate than for formula containing 1.9 g or 2.18 g protein/100 kcal during the first 4 mo of life (36). Although the investigators reported normal and adequate growth, no broad conclusions can be made regarding the safety of this very-low-protein infant formula. Furthermore, the study formulas were not isoenergetic, and the subjects' body composition, volume intake, and blood parameters were not measured.

A study investigating the safety of an infant formula with a protein amount of 1.9 g/100 kcal found no differences in growth parameters, body composition, and AEs from <3 wk of age up to the age of 4 mo compared with a control formula (protein amount of 2.5 g/100 kcal) (37). However, protein intake and energy intake both differed between the study groups because the formulas were not isoenergetic. Therefore, the results could not be attributed to only a difference in protein intake.

Lastly, in a meta-analysis, growth of infants consuming an infant formula with a protein amount of 1.8 g/100 kcal as well as breastfed infants was evaluated by comparing the anthropometric *z* scores against the WHO child growth standards (38). In line with our findings, they observed that infants consuming lower-protein formula resulted in healthy early growth close to the WHO standards. However, in line with our observations, they also observed a different growth pattern between breastfed infants and those consuming the lower-protein formula. Further research should indicate if even lower amounts of protein can be administered.

Two larger studies have also investigated the long-term effects of total protein intakes of (isoenergetic) infant formulas. The EU Childhood Obesity Programme was a large, double-blind, randomized controlled trial ($n = 1138$ formula-fed infants). It showed that the intake of infant formula and follow-on formula during the first year of life with a higher protein amount (2.9 for 0–4 mo and 4.4 g protein/100 kcal for 4–12 mo) led to a higher body weight and an increased weight-for-length and BMI from 6 mo up to 2 y of age without an effect on length compared with formulas with lower protein amounts (1.77 and 2.2 g protein/100 kcal, respectively) (39). The effect of the different protein intakes via the different study formulas during the first year of life induced marked differences in mean BMI at the age of 6 y and FM deposition at 2 and 6 y of age (40, 41). The findings obtained in this study did not offer insight into whether differences in

TABLE 4 Anthropometric data and body composition by feeding group[1]

	mLP	CTRL	BF
Anthropometry	$n = 88–90$	$n = 82–88$	$n = 65–67$
Weight, g			
Baseline	4147 ± 524	4207 ± 538	4289 ± 546
17 wk	6655 ± 632	6706 ± 636	6381 ± 771
6 mo	7738 ± 809	7900 ± 771	7369 ± 861
Length, cm			
Baseline	54.4 ± 2.3	54.6 ± 2.1	54.6 ± 2.1
17 wk	63.7 ± 2.0	63.8 ± 2.1	63.6 ± 2.1
6 mo	67.9 ± 2.1	68.4 ± 2.2	67.2 ± 2.3
Head circumference, cm			
Baseline	36.8 ± 1.4	37.1 ± 1.4	37.3 ± 1.1
17 wk	41.3 ± 1.2	41.4 ± 1.0	41.3 ± 1.1
6 mo	43.1 ± 1.3	43.4 ± 1.3	43.2 ± 1.3
Waist circumference, cm			
Baseline	33.0 ± 2.8	33.2 ± 2.9	34.0 ± 3.0
17 wk	40.5 ± 2.5	40.6 ± 2.4	39.2 ± 2.7
6 mo	41.8 ± 2.7	42.2 ± 2.8	40.7 ± 2.7
MUAC left, cm			
Baseline	11.0 ± 0.7	11.1 ± 0.8	11.1 ± 0.9
17 wk	13.5 ± 0.8	13.8 ± 0.9	13.1 ± 1.1
6 mo	14.4 ± 1.0	14.6 ± 1.1	14.0 ± 1.1
MUAC right, cm			
Baseline	10.9 ± 0.7	11.1 ± 0.9	11.1 ± 0.9
17 wk	13.5 ± 0.9	13.8 ± 1.0	13.1 ± 1.0
6 mo	14.3 ± 1.0	14.6 ± 1.1	13.9 ± 1.1
WFA, *z* score			
Baseline	−0.3 ± 0.7	−0.2 ± 0.8	−0.2 ± 0.7
17 wk	−0.01 ± 0.8	0.05 ± 0.8	−0.4 ± 0.9
6 mo	0.1 ± 0.8	0.3 ± 0.8	−0.3 ± 0.9
LFA, *z* score			
Baseline	0.16 ± 0.8	0.2 ± 0.9	0.1 ± 0.7
17 wk	0.5 ± 0.9	0.5 ± 1.0	0.4 ± 1.0
6 mo	0.6 ± 1.0	0.9 ± 1.0	0.3 ± 0.9
HCFA, *z* score			
Baseline	0.04 ± 1.0	0.2 ± 1.0	0.3 ± 0.7
17 wk	0.2 ± 0.9	0.3 ± 0.7	0.2 ± 0.7
6 mo	0.3 ± 0.9	0.5 ± 0.8	0.3 ± 0.9
Body composition	$n = 82–87$	$n = 73–83$	$n = 61–64$
Fat mass, g			
Baseline	699 ± 202	751 ± 252	753 ± 263
17 wk	733 ± 350	754 ± 422	624 ± 446
6 mo	1996 ± 461	2092 ± 491	1924 ± 494
Fat, %			
Baseline	16.3 ± 3.4	17.2 ± 4.4	16.9 ± 4.2
17 wk	25.6 ± 3.7	25.6 ± 4.6	24.8 ± 4.7
6 mo	25.5 ± 4.0	26.2 ± 4.6	25.7 ± 4.7
Fat-free mass, g			
Baseline	3543 ± 394	3535 ± 384	3611 ± 381
17 wk	5009 ± 431	5056 ± 431	4838 ± 489
6 mo	5777 ± 495	5839 ± 530	5495 ± 596
FMI			
Baseline	2.3 ± 0.6	2.5 ± 0.8	2.5 ± 0.8
17 wk	0.7 ± 0.7	0.7 ± 0.2	0.7 ± 0.2
6 mo	4.3 ± 1.0	4.5 ± 1.0	4.2 ± 1.0
FFMI			
Baseline	11.9 ± 0.8	11.8 ± 0.7	12.1 ± 0.9
17 wk	12.3 ± 0.8	12.4 ± 0.7	12.0 ± 0.9
6 mo	12.6 ± 0.8	12.5 ± 0.8	12.1 ± 0.9

[1] Values are mean ± SD. BF, breastfed; CTRL, control; FFMI, fat-free mass index; FMI, fat mass index; HCFA, head circumference-for-age; LFA, length-for-age; mLP, modified low-protein; MUAC, midupper arm circumference; WFA, weight-for-age.

TABLE 5 Differences in anthropometry and body composition between the feeding groups[1]

	mLP minus CTRL			BF minus mLP			BF minus CTRL		
	Difference	95% CI	*P*	Difference	95% CI	*P*	Difference	95% CI	*P*
Anthropometry									
Body weight, g									
17 wk	−16	−219, 187	0.88	−352	−570, −133	<0.01*	−368	−582, −153	<0.01*
6 mo	−126	−329, 77	0.22	−424	−642, −206	<0.001*	−550	−765, −335	<0.001*
WFA, *z* score									
17 wk	−0.04	−0.27, 0.19	0.74	−0.44	−0.69, −0.19	0.001*	−0.48	−0.72, −0.23	<0.001*
6 mo	−0.12	−0.35, 0.11	0.30	−0.49	−0.73, −0.24	<0.001*	−0.61	−0.85, −0.36	<0.001*
Length, cm									
17 wk	0.20	−0.31, 0.71	0.44	−0.53	−1.07, 0.02	0.06	−0.33	−0.87, 0.21	0.24
6 mo	−0.30	−0.79, 0.23	0.28	−0.89	−1.44, −0.35	<0.01*	−1.17	−1.72, −0.63	<0.001*
LFA, *z* score									
17 wk	0.06	−0.18, 0.30	0.62	0.22	−0.48, 0.04	0.09	−0.16	−0.42, 0.10	0.22
6 mo	−0.15	−0.39, 0.09	0.22	−0.39	−0.65, −0.13	<0.01*	−0.54	−0.80, −0.28	<0.001*
Head circumference, cm									
17 wk	0.0002	−0.28, 0.28	1.00	−0.20	−0.50, 0.10	0.19	−0.20	−0.49, 0.09	0.18
6 mo	−0.15	−0.43, 0.12	0.28	−0.11	−0.41, 0.19	0.48	−0.26	−0.56, 0.03	0.08
HCFA, *z* score									
17 wk	0.02	−0.18, 0.23	0.81	−0.18	−0.70, 0.04	0.10	−0.16	−0.37, 0.05	0.15
6 mo	−0.12	−0.32, 0.08	0.25	−0.11	−0.32, 0.11	0.34	−0.23	−0.44, −0.01	0.04*
Waist circumference, cm									
17 wk	−0.23	−1.07, 0.60	0.58	−1.69	−2.60, −0.77	<0.001*	−1.92	−2.82, −1.01	<0.001*
6 mo	−0.61	−1.45, 0.23	0.15	−1.37	−2.28, −0.46	<0.01*	−1.98	−2.88, −1.07	<0.001*
MUAC left, cm									
17 wk	−0.11	−0.42, 0.19	0.47	−0.50	−0.83, −0.18	<0.01*	−0.62	−0.94, −0.30	<0.001*
6 mo	−0.19	−0.49, 0.12	0.23	−0.52	−0.84, −0.19	<0.01*	−0.70	−1.03, −0.38	<0.001*
MUAC right, cm									
17 wk	−0.08	−0.38, 0.21	0.58	−0.55	−0.86, −0.23	<0.01*	−0.63	−0.94, −0.32	<0.001*
6 mo	−0.13	−0.43, 0.17	0.40	−0.52	−0.83, −0.20	<0.01*	−0.64	−0.96, −0.33	<0.001*
Body composition									
Fat mass, g									
17 wk	−8.05	−147.42, 131.32	0.91	−154.78	−305.67, −3.89	0.04*	−162.83	−312.03, −13.63	0.03*
6 mo	−61.18	−202.47, 80.11	0.39	−116.43	−267.27, 34.40	0.13	−177.61	−327.85, −27.38	0.02*
Fat, %									
17 wk	0.25	−1.12, 1.63	0.72	−1.21	−2.70, 0.27	0.11	−0.96	−2.44, 0.52	0.20
6 mo	−0.14	−1.54, 1.26	0.84	−0.17	−1.66, 1.31	0.82	−0.32	−1.81, 1.17	0.68
Fat-free mass, g									
17 wk	−68.56	−191.70, 54.58	0.27	−201.06	−335.73, −66.38	<0.01*	−269.62	−402.66, −136.58	<0.001*
6 mo	−98.24	−223.78, 27.30	0.13	−333.37	−468.32, −199.02	<0.001*	−431.91	−566.29, −297.53	<0.001*
FMI									
17 wk	0.04	−0.19, 0.26	0.76	−0.10	−0.35, 0.15	0.42	−0.067	−0.31, 0.18	0.59
6 mo	−0.17	−0.41, 0.07	0.16	−0.16	−0.40, 0.09	0.22	−0.32	−0.57, −0.07	0.01*
FFMI									
17 wk	−0.24	−0.47, −0.003	0.047*	−0.31	−0.56, −0.05	0.02*	−0.54	−0.80, −0.29	<0.001*
6 mo	−0.11	−0.35, 0.13	0.37	−0.44	−0.70, −0.18	0.001*	−0.55	−0.81, −0.29	<0.001*

[1] Values are differences between feeding groups compared by linear mixed-model analysis adjusted for baseline body weight, baseline length, baseline head circumference as appropriate, for sex, ethnicity, center, weight gain during pregnancy, maternal BMI, parental BMI, maternal smoking, gestational diabetes, and maternal education. No significant differences were found between the mLP group and the CTRL group except for FFMI at the age of 17 wk. Significant differences were found between the mLP group and the BF group and between the CTRL group and the BF group. *$P < 0.05$. BF, breastfed ($n = 65$–67); CTRL, control ($n = 82$–88); FFMI, fat-free mass index; FMI, fat mass index; HCFA, head circumference-for-age; LFA, length-for-age; mLP, modified low-protein ($n = 88$–90); MUAC, midupper arm circumference; WFA, weight-for-age.

formula protein supply during early infancy or later infancy were particularly relevant to the observed effects.

A second double-blind randomized controlled trial (the Early Protein and Obesity in Childhood study) was conducted in healthy infants to examine the effect of a different protein intake on insulin-like growth factor-1 concentration and growth until the age of 60 mo. Infants consumed a low-protein formula containing 1.8 g/100 kcal ($n = 74$) or a high-protein formula that contained 2.7 g/100 kcal ($n = 80$) until the age of 1 y. This study showed significantly lower growth parameters in the low-protein group than in the high-protein group at several time points (35). Body composition measured with Pea Pod and DXA was similar in the formula groups during the first 60 mo of life. Although the majority of growth parameters were within the normal range of the WHO child growth standards, head circumference at 36, 48, and 60 mo of age was significantly lower in infants consuming the low-protein formula than in breastfed infants. Hence, it was debatable whether the low-protein infant formula provided an adequate amount of essential amino acids. Furthermore, despite the use of isoenergetic formulas, volume of intake (mL/d) and energy intake (kcal/d) were significantly higher between 2 and 6 mo of life in the infants consuming the high-protein formula than in the infants consuming low-protein formula. These findings emphasize the importance of investigating the protein quality of infant formulas, especially the blend of essential amino acids and detailed intake volumes, before lowering the protein concentration.

We found no differences in safety blood parameters between the formula groups except for lower urea in the mLP group. Urea is an indicator of protein oxidation activity and negatively correlates to protein synthesis. The mLP and CTRL groups differed in urea, and the lower urea concentration in the mLP

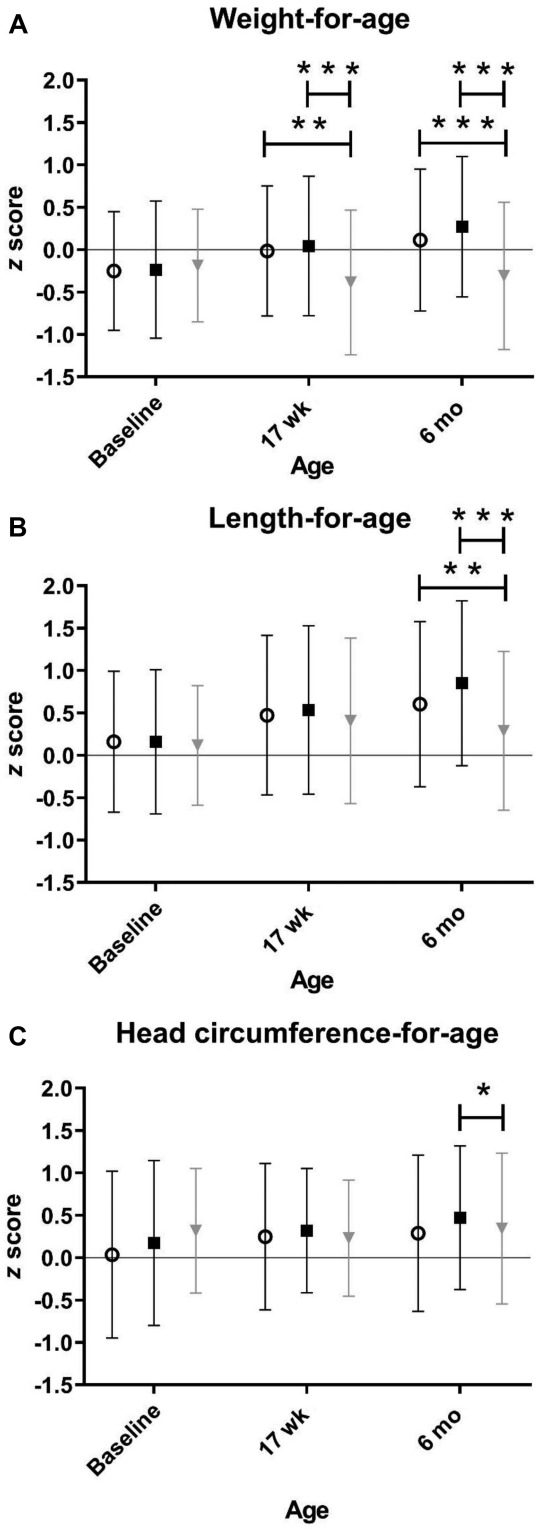

FIGURE 4 Age-adjusted *z* scores for weight (A), length (B), and head circumference (C) based on the WHO child growth standards. Data are mean ± SD and compared by using linear mixed-model analysis with adjustments for sex, ethnicity, recruitment center, maternal weight gain during pregnancy, maternal BMI, paternal BMI, maternal smoking, gestational diabetes, and maternal education. Significant differences were found between the mLP group and the BF group and between the CTRL group and the BF group. No significant differences were found between the mLP and the CTRL group. *,**,***Significant difference: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. ▼, BF ($n = 65$–67); ■, CTRL formula ($n = 82$–88); ○, mLP formula ($n = 88$–90). BF, breastfed; CTRL, control; mLP, modified low-protein.

TABLE 6 Safety blood parameters at 17 wk of age by feeding group[1]

	mLP ($n = 53$)	CTRL ($n = 44$–45)	BF ($n = 36$)	mLP minus CTRL			BF minus mLP			BF minus CTRL		
				Difference	95% CI	*P*	Difference	95% CI	*P*	Difference	95% CI	*P*
Urea, mmol/L	2.1 ± 0.5	2.9 ± 0.6	2.0 ± 0.7	−0.74	−0.97, −0.51	<0.001*	−0.09	−0.34, 0.15	0.45	−0.83	−1.08, −0.58	<0.001*
Creatinine, μmol/L	33.0 ± 3.9	32.6 ± 4.6	32.9 ± 4.0	0.68‡	0.29, 1.66	0.51	0.90‡	0.36, 2.42	1.00	1.37‡	0.52, 3.51	0.63
AST, U/L	48.5 ± 15.9	45.1 ± 7.0	56.6 ± 15.7	3.34	−2.14, 8.81	0.23	8.08	2.29, 13.88	<0.01*	11.42	5.39, 17.45	<0.001*
ALT, U/L	39.9 ± 21.1	35.8 ± 11.1	44.03 ± 18.3	4.03	−2.99, 11.05	0.26	4.18	−3.31, 11.66	0.27	8.21	0.46, 15.96	0.04*

[1] Values are mean ± SD compared by linear regression. Urea of the mLP group was significantly different from that of the CTRL group. Urea of the CTRL group was significantly different from that of the BF group. AST was significantly higher in the mLP and CTRL groups than in the BF group. ALT was significantly different between the CTRL and the BF group. Creatinine did not differ between the feeding groups. * $P < 0.05$. ‡OR and its 95% CI; *P* values calculated with Fisher's exact test. ALT, alanine aminotransferase; AST, aspartate aminotransferase; BF, breastfed; CTRL, control; mLP, modified low-protein.

TABLE 7 Number of AEs during the intervention period by feeding group[1]

AEs	mLP ($n = 90$)	CTRL ($n = 88$)	BF ($n = 65$)
Infections			
Fever of unknown origin	41*	33*	24*
Meningitis	1	0	0
Viral infection	12	17*	5
Respiratory infection	49*	59*	41*
Gastrointestinal problems			
Reflux	10	8	5*
Vomiting	7*	3	1
Diarrhea	12	16*	4
Gastroenteritis	8	9*	4*
Constipation	4	3	1
Infant colic	7	9	0
Dermatosis			
Eczema	11	6	5
Candidiasis	9	5	11*
Other	29	27	21
Total	200	195	122

[1] *AE reported more than once in the same infant. AE, adverse event; BF, breastfed; CTRL, control; mLP, modified low-protein.

group was comparable with the breastfed group. This result—specifically in combination with the similar growth rates and body composition data—suggest that the protein utilization was more efficient in the mLP group than in the CTRL group. Apparently, fewer amino acids were oxidized and a relatively higher amount of amino acids were used for protein synthesis. Lower urea (or blood urea nitrogen) concentrations in infants consuming low-protein formula than in those consuming high-protein formula were confirmed in several studies at different ages (42–45). Interestingly, and in contrast to most of these other studies, the urea concentrations of the mLP group and our breastfed reference group were similar. This supports the idea that the efficiency of protein utilization in the mLP group is closer to that of breastfed infants than is the CTRL group.

Our study has several strengths and limitations. As far as we know this is the first randomized controlled trial that used this customized blend of essential amino acids. The composition is based on outcomes of clinical trials conducted in healthy term-born formula-fed infants. Our study had a very low dropout rate and the study formulas were well-tolerated. Another strength of our study is that the formulas were comparable in terms of energy content and that energy intake was similar between the formula groups throughout the intervention period. Furthermore, we did not introduce any other ingredients to our experimental formula, so our findings can be attributed solely to a difference in protein intake (e.g., quantity and quality). We also used a CTRL formula with a protein amount and quality based on those of a standard infant formula currently on the market.

An important limitation was that the mLP, and hence also the CTRL formula, consisted of 30% free amino acids. This is because there was no intact protein to provide the right composition of essential amino acids for the mLP formula. The infants were ~1 mo of age at enrollment, and >50% of the infants enrolled in the formula groups consumed breast milk for some period. The effects of these first weeks on the outcomes are unclear.

In conclusion, from an average age of 1 mo until the age of 6 mo, feeding an infant formula with a modified amino acid composition and 1.7 g protein/100 kcal appears to be safe and supports adequate growth and body composition development. The lower urea blood concentration, similar volume intake, and comparable growth in the mLP formula group compared with the CTRL formula group indicate that protein metabolism is more efficient in infants consuming the mLP formula.

Infants that consumed mLP formula and CTRL formula had significantly higher growth rates, FM, and FFM than breastfed infants. Furthermore, the plasma amino acids, including the branched-chain amino acids, were significantly different from those in breastfed infants. Although these findings are in line with outcomes of other studies (35, 45, 46), the observed (short-term) differences in growth, body composition, and metabolic outcomes between the formula-fed groups and our reference breastfed group support new studies with even lower protein intakes.

The primary focus of the current study was on the evaluation of growth adequacy and safety when consuming the mLP formula. The potential impact will be assessed in a follow-up study that is currently ongoing.

Our findings suggest that the minimum protein amount of the current European legal standards for infant formula, 1.8–2.5 g protein/100 kcal, can be adjusted to lower amounts if protein quality is further improved. This approach might lead to an infant formula with a protein quantity and quality that supports healthy growth and could potentially reduce the later risk of childhood obesity.

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