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Randomized Control Trials

Long-term effects of a modified, low-protein infant formula on growth and body composition: Follow-up of a randomized, double-blind, equivalence trial

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SUMMARY

Background & aim: High protein intake in early life is associated with an increased risk of childhood obesity. Feeding a modified lower-protein (mLP) infant formula (1.7 g protein/100 kcal) until the age of 6 months is safe and supports adequate growth. The aim of the present study is to assess longer-term anthropometry with BMI at 1 and 2 years as primary outcome parameter and body composition in children fed mLP formula.

Methods: Healthy term-born infants received mLP or control formula (CTRL) (2.1 g protein/100 kcal) until 6 months of age in a double-blinded RCT. A breast-fed (BF) group served as a reference. Anthropometry data were obtained at 1 and 2 years of age. At the age of 2 years, body composition was measured with air-displacement plethysmography. Groups were compared using linear mixed model analysis.

Results: At 1 and 2 years of age, anthropometry, including BMI, and body composition did not differ between the formula groups (n = 74 mLP; n = 69 CTRL). Compared to the BF group (n = 51), both formula-fed groups had higher z scores for weight for age, length for age, waist circumference for age, and mid-upper arm circumference for age at 1 year of age, but not at 2 years of age (except for z score of weight for age in the mLP group). In comparison to the BF group, only the mLP group had higher fat mass, fat-free mass, and fat mass index. However, % body fat did not differ between feeding groups.

Conclusions: In this follow-up study, no significant differences in anthropometry or body composition were observed until 2 years of age between infants fed mLP and CTRL formula, despite the significantly lower protein intake in the mLP group during the intervention period. The observed differences in growth and body composition between the mLP group and the BF reference group makes it necessary to execute new trials evaluating infant formulas with improved protein quality together with further reductions in protein content.

Clinical Trial Registry: This trial was registered in the Dutch Trial Register (Study ID number NTR4829, trial number NL4677). https://www.trialregister.nl/trial/4677.

Abbreviations: BF, breast-fed; BMI, body mass index; CTRL, control; FFM, fat free mas; FFMI, fat free mass index; FM, fat mass; FMI, fat mass index; HCFA, head-circumference-for-age; LFA, length/height-for-age; mLP, modified lower protein; MUAC, mid-upper arm circumference; MUACFA, mid-upper arm circumference-for-age; WFA, weight-for-age; WFL, weight-for-length/height.

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1. Introduction

The prevalence of childhood obesity and overweight has increased tremendously over the last 2 decades. The prevalence among European children aged 2–13 years has been estimated at 21.3% as of 2016 [1]. The first months of life represent an important window for the programming of obesity. Infant feeding may permanently alter the risk of obesity, and has therefore been a key focus of many observational and intervention studies. There is evidence to suggest that protein intake during the first months of life has long-lasting effects on growth, body composition, and obesity risk [2–7]. A potentially effective strategy to target obesity is reduction of protein intake in early life. Protein-reduced infant formulas may reduce the risk of childhood obesity, but to investigate the impact, longer-term follow-up is needed [8].

We have recently shown that feeding a modified lower-protein infant formula (mLP) until the age of 6 months is safe and supports adequate growth [9]. The aim of the present study is to assess the longer-term outcomes in these infants. We hypothesized that children fed mLP formula during their first 6 months of life would have a lower BMI at the ages of 1 and 2 years and a lower body fat percentage at the age of 2 years than children fed a specifically designed control infant formula (CTRL).

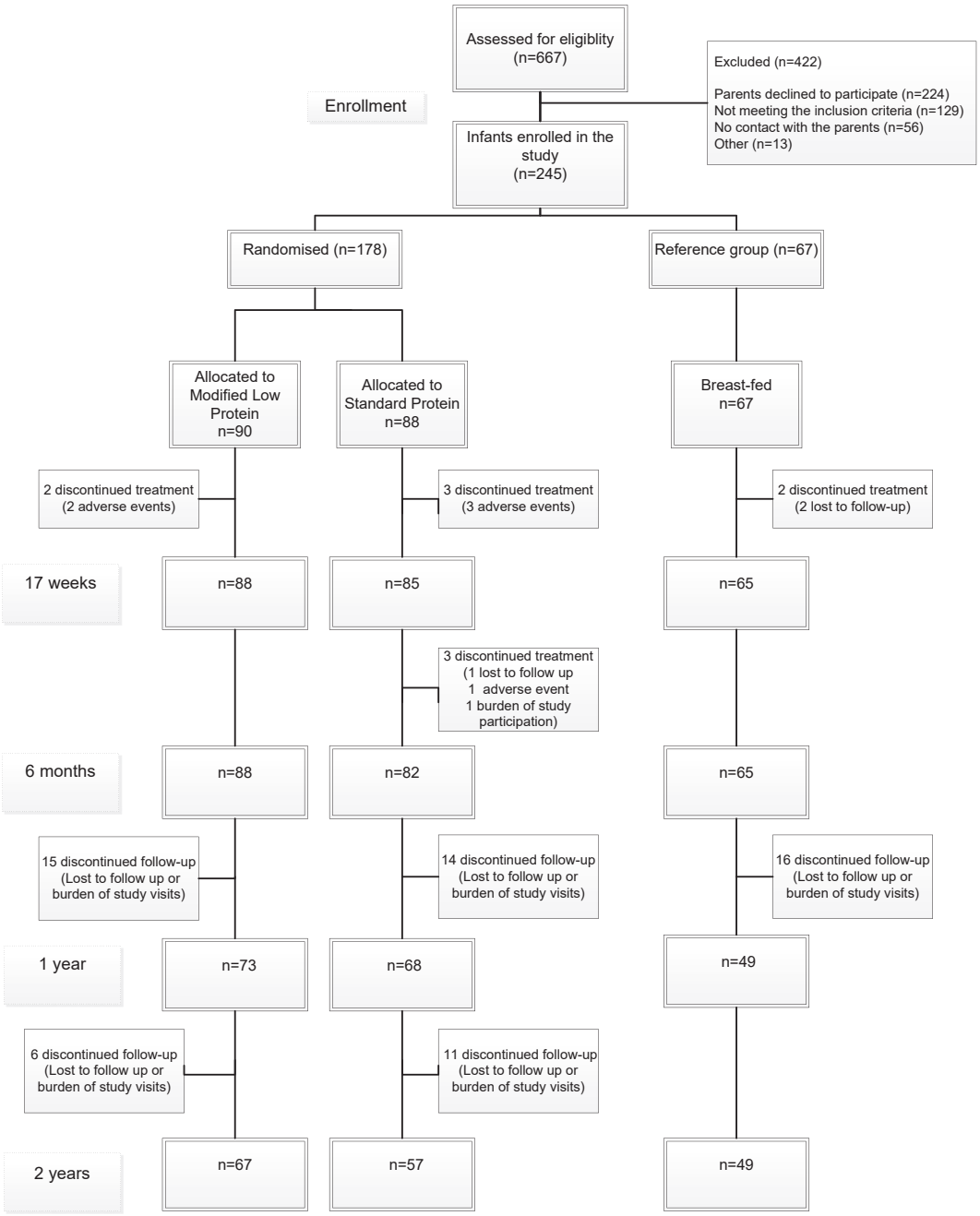

Fig. 1. Flow chart of progression of participants during the study.

2. Material and methods

2.1. Original trial

The ProtEUs study was designed to assess the safety of mLP formula in healthy, term-born infants up to the age of 6 months. Healthy term-born infants received a modified lower-protein (mLP) (1.7 g protein/100 kcal) or a control formula (CTRL) (2.1 g protein/100 kcal) until 6 months of age in a double-blinded RCT. A breast-fed (BF) group served as a reference. The study was conducted at 2 centers: Amsterdam UMC, VU University Medical Center, Amsterdam, Netherlands, and Dr. von Hauner Children's Hospital, Ludwig-Maximilians-Universität, Munich, Germany. A total of 245 infants were enrolled between October 22, 2014, and December 29, 2016. Of these, 90 and 88 infants were randomized to receive mLP formula and CTRL formula, respectively, while 67 breast-fed infants were included as a reference group.

The blinding, randomization, and allocation procedures have been described previously, as were the compositions of the formulas used and the intakes from 2 to 6 months of life [9]. The trial was approved by the institutional review boards of VU University Medical Center Amsterdam and the medical faculty of LMU Munich. The study was conducted according to ICH-GCP and in compliance with the Declaration of Helsinki. Written informed consent was obtained from all participants' parents or guardians.

2.2. Design and outcomes of follow-up study

The primary outcome of this follow-up study was to investigate the differences in body mass index (BMI) at 1 and 2 years of age in children fed mLP formula in comparison to CTRL formula during their first 6 months of life. The secondary outcomes were to investigate the differences in weight, length/height, head circumference, MUAC, waist circumference, and body composition at the age of 2 years. Parents whose infants had participated in the initial study were approached at the end of the intervention period (at 6 months) for their children to take part in the follow-up study.

In cases where informed consent was obtained, parents of formula-fed infants included in the mLP or CTRL group were provided with the same standard commercially available follow-on formula from the age of 6 months to 1 year. All children were fed without feeding restrictions. The breast-fed reference group followed the same visit and assessment schedule as the randomized infants.

Study visits were scheduled at 1 and 2 years of age. During these visits, anthropometry was assessed, and at the age of 2 years, body composition was assessed as well. All measurements were performed by trained study personnel according to standard protocols. Weight was measured to 0.5-g accuracy on a balance scale (MS-4100, MARSDEN, UK). At the age of 1 year, length was measured with a flexible measuring board (Seca 210, SECA, UK).

At the age of 2 years, height was measured with a digital stadiometer (SECA 285). Head circumference, waist circumference, and mid-upper arm circumference (MUAC) were measured in duplicate to 0.1-cm accuracy using a flexible tape measure. The average of two measurements was used in the statistical analysis. At the age of 2 years, body composition was measured with air-displacement plethysmography (ADP, BOD POD Body Composition System; Cosmed, Concord, CA, USA).

The fat mass index (FMI) and fat-free mass index (FFMI) were calculated as FM (kg)/length $(m)^2$ and FFM (kg)/length $(m)^2$ [10], respectively. Nutritional intake at 1 and 2 years was recorded using a 3-d food diary. Information was entered in a web-based tool from The Netherlands Nutrition Centre and in Nutritics (BLS 3.02; German Nutritional Database) to calculate daily intakes of energy, protein, fat, and carbohydrates.

2.3. Statistical analyses

We hypothesized that children fed mLP formula during their first 6 months of life would have a lower BMI at the ages of 1 and 2 years and a lower body fat percentage at the age 2 years than children fed CTRL formula. Before the analyses, all anthropometric data were converted to *z* scores using the World Health

Table 1
Parental and infant characteristics: follow-up population (participation follow-up 1 year and/or 2 years).

	mLP (n = 74)	CTRL (n = 69)	BF (n = 51)
Parental characteristics			
Maternal age (y)	32.8 ± 4.4	32.2 ± 5.1	32.6 ± 4.4
Higher maternal education[a] [n (%)]	42 (57)	36 (52)	39 (76)
Maternal BMI at enrollment (kg/m^2)	26.4 ± 4.5	26.1 ± 5.1	24.8 ± 3.7
Maternal pre-pregnancy BMI[b] (kg/m^2)	24.4 ± 4.4	24.6 ± 5.2	23.4 ± 3.6
Weight gain during pregnancy (%)	23 ± 10	20 ± 10	20 ± 9
Gestational diabetes [n (%)]	7 (10)	4 (6)	2 (4)
Maternal smoking [n (%)]	9 (12)	12 (17)	2 (4)
Paternal BMI (kg/m^2)	26.0 ± 3.3	26.8 ± 4.2	25.7 ± 3.8
Infant characteristics			
Age follow-up 1 year (m)	12.1 ± 0.36	12.1 ± 0.27	12.0 ± 0.27
Age follow-up 2 years (m)	24.2 ± 0.58	24.1 ± 0.36	24.1 ± 0.40
Boys [n (%)]	32 (43)	34 (49)	25 (49)
Caucasian [n (%)]	63 (85)	60 (87)	46 (90)
Gestational age (wk)	39.5 ± 1.3	39.7 ± 1.2	39.9 ± 0.9
Birth weight (gram)	3389 ± 356	3438 ± 379	3474 ± 316
Cesarean delivery [n (%)]	25 (34)	18 (26)	13 (25)
Ever breast-fed [n (%)]	28 (38)	32 (46)	51 (100)
Age at inclusion (d)	29 ± 10	31 ± 10	33 ± 8
Age start intervention (d)	29 ± 10	31 ± 10	NA
Age fully fed with study formula (d)	31 ± 10	32 ± 10	NA
Introduction of complementary feeding during intervention period [n (%)]	68 (95)	60 (92)	40 (95)
Age introduction complementary feeding during intervention period (wk)	19.3 ± 2.4	18.8 ± 2.3	20.5 ± 3.7

Values are mean ± SD or n (%) unless otherwise indicated. BF, breast-fed; CTRL, control formula; mLP, modified Low-protein formula; NA, not applicable.
[a] Defined as higher professional education and university education.
[b] Self-reported.

Organization (WHO) Child Growth Standards [11]. To obtain the differences between the groups at various time points, they were compared with linear mixed model analysis including the group variable (mLP, CTRL, BF), time (the intervention period: 17 weeks, 6 months, and follow-up period: 1 and 2 years), and the interaction between group and time. 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), center, birth-weight z score, and maternal education. Nutritional intake was compared using linear regression analysis. Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 22, and a 2-sided significance level of 5% was used for all comparisons.

3. Results

The original trial was completed by 235 infants whose parents were approached for participation in the follow-up study. Forty-five of them declined to participate or could not be traced, leaving 190 infants (78% of the original children included). At 1 year of age, the feeding groups consisted of 73 children in the mLP group, 68 children in the SP group, and 49 children in the BF group. At the age of two years, 173 children were seen, including 67 children in the mLP group (74% of the randomized cases), 57 children in the SP group (65% of the randomized cases), and 49 children in the BF group (73% of the initial feeding group) (Fig. 1). The characteristics of the children who participated in the follow-up study (at 1 or 2 years of age) are presented in Table 1.

3.1. Anthropometry

At the age of 1 and 2 years, no significant differences were observed in BMI and the BMI-for-age z score between the mLP group and the CTRL group. BMI of the BF group did not differ from that of the formula-fed groups at both ages (Table 2, Table 3, Fig. 2a). No significant differences in anthropometry (body weight, length, head circumference, waist circumference, and MUAC) measured at 1 year and 2 years of age were observed between the formula groups (Table 2, Table 3, and supplementary Table 1). Formula-fed children grew faster than children in the BF group with significantly higher mean body weight at 1 year and 2 years of age. The weight-for-age (WFA) *z* score and length/height were significantly higher in the mLP group than the BF group at both ages. This result was also observed in the CTRL group at 1 year but not at 2 years (Fig. 2b, c, Table 3).

The z scores for waist circumference, mid-upper arm circumference (MUAC), and length for age (LFA) were significantly higher in the formula groups than the BF group at the age of 1 year. This difference disappeared at the age of 2 years (Table 3, Fig. 2c). The z score for arm circumference for age (MUACFA) was significantly higher in the CTRL group than the BF group at the age of 1 year.

3.2. Body composition

No significant differences in body composition were observed between the formula groups (Fig. 3, Table 2, Table 3, supplementary Table 1). In comparison to the BF group, the mLP group had significantly higher total fat mass, total fat free mass, and FMI. Fat mass percentage did not significantly differ between the mLP group and the BF group. In contrast to the mLP group, no significant differences were found between the CTRL group and the BF group (Fig. 3, Table 3, supplementary Table 1).

Table 2
Anthropometric data and body composition by feeding group.

	Feeding groups		
	mLP n = 74	CTRL n = 69	BF n = 51
Anthropometry			
BMI (kg/m2)			
1 year	16.9 ± 1.6	16.9 ± 1.2	16.6 ± 1.2
2 years	16.5 ± 1.4	16.5 ± 1.3	16.3 ± 1.2
BMI for age *(WHO* z score*)*			
1 year	0.16 ± 1.09	0.20 ± 0.85	−0.03 ± 0.81
2 years	0.46 ± 0.99	0.47 ± 0.95	0.32 ± 0.93
Weight (g)			
1 year	9621 ± 1082	9838 ± 995	9334 ± 905
2 years	12493 ± 1472	12481 ± 1240	12233 ± 1174
Length (cm)			
1 year	75.5 ± 2.3	76.3 ± 2.7	75.0 ± 2.3
2 years	86.9 ± 3.1	86.8 ± 2.8	86.5 ± 2.5
Head circumference (cm)			
1 year	45.9 ± 1.8	46.0 ± 1.2	46.1 ± 1.2
2 years	48.6 ± 1.8	48.5 ± 1.3	48.6 ± 1.3
Waist circumference (cm)			
1 year	44.4 ± 3.3	44.4 ± 3.6	43.3 ± 3.3
2 years	47.5 ± 3.0	46.9 ± 3.0	46.9 ± 3.6
MUAC left (cm)			
1 year	15.0 ± 1.1	15.1 ± 1.0	14.7 ± 1.0
2 years	15.8 ± 1.2	15.8 ± 1.0	15.6 ± 1.1
MUAC right (cm)			
1 year	14.9 ± 1.1	15.1 ± 1.1	14.6 ± 0.9
2 years	15.7 ± 1.2	15.8 ± 1.1	15.6 ± 1.0
WFA (WHO z score*)*			
1 year	0.27 ± 0.89	0.42 ± 0.83	0.00 ± 0.76
2 years	0.41 ± 0.94	0.40 ± 0.84	0.23 ± 0.76
LFA (WHO z score*)*			
1 year	0.25 ± 0.85	0.49 ± 1.04	0.03 ± 0.84
2 years	0.12 ± 0.95	0.11 ± 0.88	−0.01 ± 0.77
dWFL (WHO z score*)*			
1 year	0.21 ± 1.05	0.28 ± 0.84	−0.02 ± 0.80
2 years	0.42 ± 0.98	0.42 ± 0.91	0.27 ± 0.89
HCFA (WHO z score*)*			
1 year	0.30 ± 0.96	0.40 ± 0.81	0.43 ± 0.75
2 years	0.57 ± 1.01	0.56 ± 0.86	0.63 ± 0.81
MUACFA left (WHO z score*)*			
1 year	0.49 ± 0.88	0.55 ± 0.84	0.20 ± 0.84
2 years	0.63 ± 0.89	0.61 ± 0.83	0.46 ± 0.85
Body composition	**n = 36**	**n = 29**	**n = 38**
2 years of age			
Fat mass (g)	2831 ± 1246	2673 ± 1274	2456 ± 1040
Fat (%)	21.7 ± 8.1	20.9 ± 8.9	19.7 ± 7.7
Fat free mass (g)	9941 ± 828	9838 ± 1273	9802 ± 925
FMI	3.7 ± 1.5	3.5 ± 1.7	3.3 ± 1.4
FFMI	13.1 ± 1.0	13.1 ± 1.2	13.2 ± 1.2

Values are mean ± SD. BF, breast-fed; BMI, body mass index; CTRL, control formula; FFMI, fat free mass index; FMI, fat mass index; HCFA, head circumference for age; LFA, length/height for age; mLP, modified Low-protein formula; MUAC, mid-upper arm circumference; MUACFA, mid-upper arm circumference for age; WFA, weight for age; WFL, weight for length/height.

3.3. Intake of energy and protein

No significant differences in nutritional intake (energy, protein, fat, carbohydrates) were found between the feeding groups at 1 and 2 years of age (supplementary Table 2).

4. Discussion

We found similar BMI and BMI for age at 1 and 2 years of age in children fed an infant formula with a modified amino acid profile and a protein content of 1.7 g/100 kcal and those fed a control infant formula with a standard amino acid profile and a protein content of 2.1 g/100 kcal. Furthermore, no significant differences in anthropometry or body composition were found between both formula

Table 3
Differences in anthropometry and body composition between the feeding groups.

	mLP minus CTRL			mLP minus BF			CTRL minus BF		
	Difference	CI95%	P value	Difference	CI95%	P value	Difference	CI95%	P value
Anthropometry									
BMI									
1 year	−0.02	−0.41, 0.37	0.93	0.28	−0.15, 0.72	0.19	0.30	−0.13, 0.74	0.17
2 years	−0.05	−0.45, 0.36	0.81	0.23	−0.21, 0.66	0.30	0.28	−0.17, 0.72	0.22
BMI for age (z score)									
1 year	−0.06	−0.32, 0.21	0.66	0.16	−0.13, 0.45	0.29	0.22	−0.08, 0.51	0.15
2 years	−0.05	−0.33, 0.22	0.71	0.14	−0.15, 0.44	0.35	0.19	−0.11, 0.50	0.21
Weight (g)									
1 year	−112	−376, 152	0.41	380	89, 670	**0.01**	492	198, 785	**0.001**
2 years	41	−233, 315	0.77	359	65, 652	**0.02**	318	18, 617	**0.034**
Length (cm)									
1 year	−0.25	−0.86, 0.35	0.41	1.09	0.42, 1.75	**0.001**	1.34	0.67, 2.01	**<0.001**
2 years	0.39	−0.24, 1.02	0.22	0.88	0.21, 1.55	**0.01**	0.49	−0.20, 1.18	0.16
Head circumference (cm)									
1 year	0.06	−0.23, 0.35	0.69	0.13	−0.19, 0.45	0.42	0.07	−0.25, 0.40	0.65
2 years	0.18	−0.12, 0.48	0.25	0.17	−0.16, 0.49	0.31	−0.01	−0.34, 0.32	0.95
Waist circumference (cm)									
1 year	−0.11	−1.03, 0.81	0.81	1.27	0.25, 2.30	**0.02**	1.39	0.35, 2.42	**<0.01**
2 years	0.54	−0.45, 1.52	0.28	0.87	−0.18, 1.92	0.10	0.33	−0.76, 1.42	0.55
MUAC left (cm)									
1 year	−0.01	−0.32, 0.30	0.95	0.37	0.04, 0.71	**0.03**	0.38	0.04, 0.72	**0.03**
2 years	0.07	−0.25, 0.40	0.66	0.32	−0.02, 0.66	0.06	0.25	−0.10, 0.60	0.14
MUAC right (cm)									
1 year	0.02	−0.30, 0.33	0.92	0.47	0.13, 0.81	**<0.01**	0.45	0.11, 0.80	**0.01**
2 years	0.03	−0.30, 0.35	0.88	0.26	−0.08, 0.61	0.14	0.24	−0.12, 0.59	0.19
WFA (z score)									
1 year	−0.08	−0.30, 0.15	0.50	0.32	0.08, 0.57	**0.011**	0.40	0.15, 0.65	**<0.01**
2 years	0.05	−0.18, 0.28	0.66	0.25	0.001, 0.50	**0.048**	0.20	−0.06, 0.45	0.13
LFA (z score)									
1 year	−0.09	−0.33, 0.13	0.40	0.34	0.09, 0.59	**<0.01**	0.48	0.18, 0.69	**<0.001**
2 years	0.14	−0.10, 0.378	0.24	0.24	−0.01, 0.50	0.06	0.10	−0.16, 0.36	0.44
WFL (z score)									
1 year	−0.10	−0.37, 0.17	0.46	0.18	−0.12, 0.48	0.24	0.28	−0.02, 0.58	0.07
2 years	−0.05	−0.33, 0.28	0.71	0.14	−0.16, 0.44	0.36	0.19	−0.11, 0.50	0.22
HCFA (z score)									
1 year	0.09	−0.11, 0.28	0.37	0.12	−0.10, 0.33	0.28	0.03	−0.19, 0.25	0.79
2 years	0.17	−0.03, 0.37	0.10	0.16	−0.06, 0.37	0.16	−0.01	−0.23, 0.21	0.90
MUACFA left (z score)									
1 year	−0.04	−0.31, 0.23	0.75	0.28	−0.01, 0.58	0.06	0.33	0.03, 0.63	**0.03**
2 years	0.02	0.90, −0.27	0.30	0.22	−0.08, 0.52	0.15	0.20	−0.11, 0.51	0.21
Body composition									
2 years of age									
Fat mass (kg)	0.03	−0.27, 0.33	0.86	0.43	0.14, 0.73	**0.004**	0.29	−0.03, 0.61	0.08
Fat (%)	−0.63	−3.06, 1.79	0.61	1.10	−1.16, 3.36	0.34	1.62	−0.83, 4.07	0.19
Fat free mass (kg)	0.19	−0.09, 0.46	0.19	0.22	0.001, 0.45	**0.049**	0.03	−0.21, 0.27	0.82
FMI	−0.01	−0.46, 0.44	0.97	0.45	0.016, 0.87	**0.042**	0.31	−0.16, 0.77	0.20
FFMI	0.15	−0.29, 0.58	0.50	−0.06	−0.43, 0.30	0.74	−0.24	−0.64, 0.15	0.22

Values are differences between feeding groups compared by linear mixed model analysis adjusted for the values measured previously (during the intervention period; at baseline, 17 weeks, 6 months and at 1 year, if applicable) of the particular outcome, for sex, ethnicity, center, birth-weight z score, and maternal education. No significant differences were found between the mLP group and the CTRL group. Significant differences were found between the mLP group and the BF group and the CTRL group and the BF group. Bold font indicates statistical significance ($P > 0.05$). BF, breast-fed (n = 51); BMI, body mass index; CTRL, control formula (n = 69); FFMI, fat free mass index; FMI, fat mass index; HCFA, head circumference-for-age; LFA. Length/height-for-age; mLP, modified Low-protein formula (n = 74); MUAC, mid-upper arm circumference; MUACFA, mid-upper arm circumference-for-age; WFA, weight-for-age; WFL, weight-for-length/height.

groups until the age of 2 years. Previously, we found that infants fed with mLP formula and CTRL formula had significantly higher growth rates, FM, and FFM up until the age of 6 months than breast-fed infants [9].

The current study showed that growth up until the age of 2 years is different in formula-fed infants from that in breast-fed infants, again with higher growth rates in formula-fed infants. Furthermore, higher FM, FFM, and FMI were found in the mLP group than the breast-fed group. The observed differences in growth between the formula-fed groups (particularly the mLP group) and the reference breast-fed reference group support the initiation of new studies evaluating infant formula with even lower protein content.

Two earlier double blinded randomized controlled trials (RCTs) investigated the effect of infant formulas with different protein levels on long-term growth and body composition. In the CHOP study (n = 1138 formula-fed infants), one group of infants was fed high-protein formulas (2.9 g protein/100 kcal for 0–4 months and 4.4 g protein/100 kcal for 4–12 months) for the first year of life, and another group was fed formula with a lower protein level (1.77 and 2.2 g protein/100 kcal respectively). The study found that the infants fed high-protein formulas had a significantly higher BMI from the age of 6 months onwards until the age of 6 years [5]. In line with our results, the EPOCH study demonstrated no differences in BMI from the end of the intervention (1 year of age) up until the age of 5 years between infants fed high-protein formula (2.7 g protein/100 kcal (n = 80)), low-protein formula (1.8 g protein/100 kcal (n = 74)) or breast milk [12].

In addition to BMI, the CHOP study found significant effects of lower protein intake during infancy on weight and WFL in the first

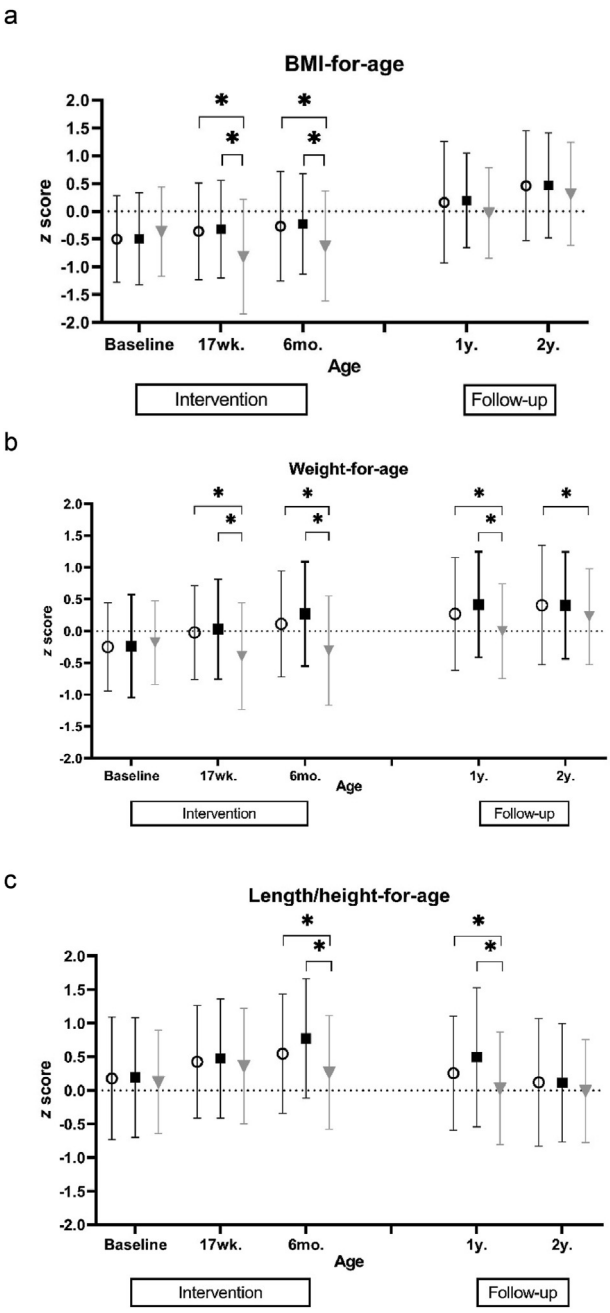

Fig. 2. *a*, b, c: Age-adjusted *z*-scores for BMI, WFA, LFA based on the WHO child growth standards. Data are mean ± SD and compared by using linear mixed model analysis with adjustments for sex, ethnicity, recruitment center, birth-weight z score, and maternal education. Significant differences were found in WFA and LFA between the feeding groups at 1 and 2 years. *$P < 0.05$. ▼, BF (n = 51); ■, CTRL formula (n = 69); ○, mLP formula (n = 74). BF, breastfed; CTRL, control; mLP, modified low-protein.

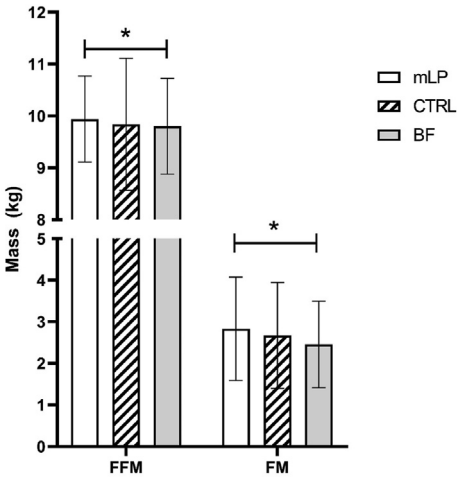

Fig. 3. Fat Free Mass (FFM) and Fat Mass (FM) at 2 years of age. Values are mean ± SD and compared by using linear mixed model analysis with adjustments for sex, ethnicity, recruitment center, birth-weight z score, and maternal education. Significant differences were found between the mLP group and the BF group. *$P < 0.05$. BF, breastfed; CTRL, control; mLP, modified low-protein.

2 years of life without any effect on length or LFA. The differences between the low-protein and high-protein groups were greatest at 12 months of age for weight, WFL, and BMI. Significant differences between the high and low-protein groups were already apparent at the ages of 3 and 6 months.

In contrast to our study findings, both formula groups in the CHOP study had significantly higher BMI and BMI for age than their breast-fed reference group at the age of 1 year. The differences between their formula groups and breast-fed group for body weight, WFA, length, and LFA at the age of 1 year are in line with our findings. However, the latter two differences are only present when compared to the high-protein group (unpublished data).

Furthermore, at the age of 2 years, the higher-protein group in the CHOP study had significantly higher WFA, LFA, WFL, and BMI for age than the breast-fed group, whereas for the lower protein-group, only WFA and LFA were significantly higher than in the breast-fed group. Contrary to our findings, the high-protein group had a significantly higher body fat percentage, FMI, and FFMI than the low-protein group at the age of 2 years. Furthermore, the breast-fed infants in the CHOP study had the highest body fat percentage compared to both formula groups at that age [7]. The EPOCH study found significant differences in length, LFA, head circumference, and HCFA between the low-protein group and the high-protein group at 1 year of age (the end of the intervention period), with lower values for the low-protein group. Next to a significanly lower WFA, these differences were also present at the age of 3 years. There was no follow-up visit at the age of 2 years. Fat mass and fat-free mass (% of body weight) were similar in the formula groups and breast-fed group at the ages of 1 and 3 years. Compared to their breast-fed reference group, the high-protein group had a significantly higher length at the age of 1 year. Furthermore, at the age of 3 years, HCFA was significantly different from the breast-fed reference group, with lower values for the low-protein group.

The duration of the intervention, the difference in protein intake between the intervention group and the control group, the protein quality, and the macronutrient used to make the interventional formula isoenergetic, can influence the different outcomes. The differences found between formula-fed infants and breast-fed reference groups across studies could be partially explained by study design and the use of infant formula with a suboptimal composition. Our intervention started <45 days after birth and ended at the age of 6 months. We investigated the effect of a 20% reduction in protein intake during the first months of life. The window of low-protein intake opportunities might be later or longer than the first 6 months of life.

Several cohort studies found associations between higher protein intake at 12 months of age and greater height, weight, BMI, and FMI at school age, but not FFMI [13–15]. Others found that protein intake at 24 months is associated with an adverse fatness development pattern, an earlier onset of adiposity

rebound, and a higher subsequent BMI level [16,17]. These findings suggest that protein intakes during the toddler period may also have long-lasting effects on growth and body composition, necessitating long-term follow-up.

Not only the amount, but also the type of protein affects infant growth. In an RCT on healthy infants, extensively feeding hydrolyzed-protein formula resulted in a significantly lower weight gain pattern than that of infants fed a standard infant formula with intact (cow's milk) protein [18,19]. It seems to have a long-lasting effect with a significantly lower WFA, WFL, and BMI for age up until the age of 13 months in infants fed extensively hydrolyzed-protein formula. However, in contrast to these findings, the randomized GINI trial, which examined a much larger number of infants, did not find appreciable growth differences in infants fed three infant formulas based on different protein hydrolysates in comparison to a standard cows' milk protein formula [20].

The mLP formula and CTRL formula consisted of 70% intact protein and 30% free amino acids. This balance between intact protein and added amino acids was present in both the mLP and the CTRL formulas. The presence of these two forms of protein, especially the free amino acids, could influence a child's growth and thus the results and outcomes.

Our study has several strengths. This is the first RCT to use this customized blend of essential amino acids. The composition is based on outcomes of clinical trials conducted with healthy, term-born, formula-fed infants. The indirect amino acid oxidation technique was used to assess infants' amino acid requirements. The results of those trials showed that infant formulas are likely to provide an unbalanced excess of essential amino acids [21–26]. Based on these study outcomes, the modified infant formula was developed. The drop-out rate was acceptably low for both the intervention period (4%) and the follow-up period (22%). The protein intake was significantly different between both formula groups during the intervention period, as intended, while there was no difference in protein intake at the ages of 1 and 2 years.

The PEA POD device cannot accommodate infants weighing >10 kg (infants approximately > 6 months of age with body volume of >1.85 L) [27]. In addition, the BOD POD Pediatric Option for the measurement of body composition in children is validated in children aged 2–6 years only [28], and has several practical limitations for children <2 years of age [29]. Therefore, the body composition at the age of 1 year was not assessed, which is an important limitation. Furthermore, the infants were about one month of age at enrollment, and more than 50% of the infants enrolled in the formula groups were fed with breast-milk for some period, with unclear effects on longer-term outcomes.

In conclusion, in contrast to our hypothesis, we did not find differences in growth and body composition between the formula groups up until the age of 2 years. However, we did find significant differences in body composition between the mLP group and the breast-fed group at the age of 2 years. These results may suggest that the adjusted protein quality may allow for even lower total protein levels in infant formula closer to those present in breast milk. Further research should explore the underlying mechanisms and study the optimal amount of protein intake needed in infancy for optimal growth and body composition. The long-term effects of lower protein intake during the first 6 months of life on the development of fat mass and adiposity rebound will be evaluated during further follow-up until the age of 6 years.

Author contribution

SK, JBvG, BK, EvdB, MAB, and BvdH designed the research. SK and NA implemented and conducted the research. SK and JT performed the statistical analyses. SK, MJF, and JBvG interpreted the data and wrote the paper. All authors critically revised the manuscript. SK has primary responsibility for the final content. All authors read and approved the final manuscript.

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Conflict of interest

The study was funded by Danone Nutricia Research. Marieke Abrahamse-Berkeveld and Bert J.*M. van* de Heijning are employees of Nutricia Research. Eline *M. van* der Beek was an employee of Nutricia Research when the study was conducted. They had no role in the execution of the study or in the statistical analyses of the results.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clnu.2021.04.034.

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