Lower protein in infant formula is associated with lower weight up to age 2 y: a randomized clinical trial[1–4]

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ABSTRACT

Background: Protein intake during infancy was associated with rapid early weight gain and later obesity in observational studies.

Objective: The objective was to test the hypothesis that higher protein intake in infancy leads to more rapid length and weight gain in the first 2 y of life.

Design: In a multicenter European study, 1138 healthy, formula-fed infants were randomly assigned to receive cow milk–based infant and follow-on formula with lower (1.77 and 2.2 g protein/100 kcal) or higher (2.9 and 4.4 g protein/100 kcal) protein contents for the first year. For comparison, 619 exclusively breastfed children were also followed. Weight, length, weight-for-length, and BMI were determined at inclusion and at 3, 6, 12, and 24 mo of age. The primary endpoints were length and weight at 24 mo of age, expressed as length and weight-for-length z scores based on World Health Organization growth standards 2006.

Results: Six hundred thirty-six children in the lower ($n = 313$) and higher ($n = 323$) protein formula groups and 298 children in the breastfed group were followed until 24 mo. Length was not different between randomized groups at any time. At 24 mo, the weight-for-length z score of infants in the lower protein formula group was 0.20 (0.06, 0.34) lower than that of the higher protein group and did not differ from that of the breastfed reference group.

Conclusions: A higher protein content of infant formula is associated with higher weight in the first 2 y of life but has no effect on length. Lower protein intake in infancy might diminish the later risk of overweight and obesity. This trial was registered at clinicaltrials.gov as NCT00338689. *Am J Clin Nutr* 2009;89:1–10.

INTRODUCTION

Rapid weight gain in infancy is associated with an increased risk of later obesity in a large number of observational studies summarized in 3 recent systemic reviews (1–3). Compared with breastfed infants, formula-fed term infants have greater body weight gains in infancy (4–6). The greater weight gain in formula-fed infants than in infants fed breast milk might be explained by different metabolizable substrate intakes (7), particularly protein: protein intake per kilogram body weight is 55–80% higher in formula-fed than in breastfed infants (8). It was proposed that a higher protein intake stimulates secretion of insulin-like growth factor I (IGF-I) and consecutively cell proliferation, which leads to accelerated growth and increased adipose tissue (7, 9). A positive association of protein intake with early growth was seen in 2 observational studies (10, 11); whereas no effect on growth in the first months of life was seen in other studies (12–14). Some observational studies found a higher protein intake in the first 2 y of life that was predictive of overweight in later childhood, whereas energy, carbohydrate, or fat intake was not predictive (9, 10, 15–17).

To test the hypothesis that a higher early protein intake leads to more rapid growth in the first 2 y of life, we performed a multicenter, double-blind intervention trial in infants fed formula randomly assigned to receive infant and follow-on formulas with a lower or higher content of cow milk protein during the first year of life. The growth pattern of formula-fed children was compared with that of breastfed children recruited as an additional observational group. Length and weight at 24 mo were chosen as the primary endpoints.

SUBJECTS AND METHODS

Study design

The study was a double-blind, randomized controlled trial comparing 2 groups of children each fed 2 types (standard and follow-on) of cow milk–based formula with either a lower or higher protein content for the first year of life. Additionally, an observational group of exclusively breastfed children was included, as recommended by the European Society of Pediatric Gastroenterology, Hepatology and Nutrition (18), the European Commission (19), and the US Food and Nutrition Board of the Institute of Medicine of the National Academies (20).

Study population

Eligible for study participation were apparently healthy, singleton, term infants who were born between 1 October 2002 and 31 July 2004. Children of mothers with a hormonal or metabolic disease or illicit drug addiction during pregnancy were not included. Children were recruited in 5 countries (Belgium, Germany, Italy, Poland, and Spain). Anthropometric measurements were made at 11 sites: 2 in Germany (Munich and Nuremberg), 2 in Belgium (Liege and Brussels), 4 in Italy (Milano), 1 in Poland (Warsaw), and 2 in Spain (Reus and Tarragona). Except for Italy and Poland, where one team was responsible for all measurements, different teams were responsible per site in Germany, Spain, and Belgium. A prerequisite for recruitment was that the distance from the place of residence to the local study center was compatible with later study visits.

Infants were enrolled during the first 8 wk of life. Formula-fed infants had to be exclusively formula-fed at the end of the eighth week of life. Breastfed children had to be breastfed since birth. Noncompliance, prompting exclusion and no further follow-up, was determined by maternal interviews at child ages 2, 3, 6, and 9 mo. For the intervention groups, noncompliance was defined as feeding of nonstudy formula or breastfeeding for $>10\%$ of feedings (or >3 bottles/wk) over ≥ 1 wk in the first 9 mo of life. Breastfed children had to be exclusively breastfed ($<10\%$ of feedings or <3 bottles of formula/wk) for the first 3 mo of life. Additional reasons for exclusion were medical conditions that might restrict growth or relocation too far away from the study center to attend visits. The study was approved by the ethics committees of all study centers. Written informed parental consent was obtained for each infant.

Methods

The lower- and higher-protein infant formulas (manufactured and provided free of charge to families by Bledina, Steenvoorde, France) differed in the amount of cow milk protein (7.1% and 11.7% of energy) but had identical energy densities by compensatory adaptation of the fat content (**Table 1**). The source of lipids was a mix of vegetable oils: palm, rapeseed, coconut, and sunflower oils emulsified with soy lecithin. For details of the formula composition, *see* the "Supplemental data" in the online issue.

After introduction of complementary feeding, but not before the start of the fifth month of life, families of randomized infants were provided with follow-on formulas with protein contents of 8.8% and 17.6% of energy, respectively, until the infants reached the age of 12 mo. The composition of protein and fat and the content of all other ingredients were identical (Table 1). The whey-to-casein ratio in all study formulas was 1:4.

The composition of all study formulas complied with the 1991 EU Directive on Infant and Follow-on Formulae (22), and the protein contents represented approximately the lowest and highest levels, respectively, of the range accepted in this Directive.

Recruitment procedures in all centers were designed to promote and support breastfeeding. Introduction of any food other than study formula or breast milk before the age of 4 completed months was discouraged, but no other attempts were made to influence the local and family traditions of introduction of solids into the infants' diet.

Shortly after birth, the parents were approached and invited to participate in a study on obesity prevention, and the influence of dietary protein on infant growth if exclusion criteria did not apply. If the parents had opted for exclusive formula feeding, participation in the intervention group with cost-free provision of formula was offered. In case of consent, children were randomly assigned to treatment, and the first supply of the randomized study formula was distributed with uniform instructions on how to prepare the formula. Inclusion into the study was possible until 8 wk after birth. If a child was not exclusively formula-fed or parents were indecisive, breastfeeding was encouraged and children were included in an observational group of breastfed children if the mother planned to exclusively breastfeed for ≥ 4 mo. Once included in the study, children in the intervention and the breastfed group were followed up identically.

Randomization lists for each country, stratified by sex, were prepared by using random permuted blocks of 8. Two colors each were used to label the lower and higher protein formulas (ie, 4 colors in total). Randomization numbers with respective colors were drawn through an Internet-based platform. The formulas were packaged in otherwise identical cans by the manufacturer and labeled as infant or follow-on formula. The color codes were only disclosed to the statisticians performing the final analysis. All investigators and participating families were kept blinded to allow for blinded follow up until later ages.

Birth weight and length were obtained from hospital data. All other anthropometric measures were obtained at visits to the study centers: at study entry and at the ages of 3, 6, 12, and 24 mo. The study entry visit with baseline anthropometric measurements was scheduled at the time of randomization or as shortly as possible thereafter. All study centers used the same equipment for measuring weight (Seca 336 scales at ≤ 24 mo and Seca 702 scales at 24 mo; Seca, Hamburg, Germany) and recumbent length (Seca 232 until age 6 mo and PED LB 35–107 X scales after 6 mo; Ellard Instruments, Monroe, WA). Standing height was measured in some children at 24 mo (Seca Stadiometer 242). All measurements were performed twice and recorded with an accuracy of 10 g for weight and 0.1 cm for length. The mean of both measurements was used for further analysis. Standing heights were only available for 12 children at 24 mo of age. To make the measurement comparable with recumbent length, 0.7 cm was added in accordance with the procedures established in the World Health Organization (WHO) Growth Multicentre Reference Study (23). Written standard operating procedures were also based on the procedures used in the WHO growth study. Repeated training sessions were performed for all study personnel by experts in anthropometry, with input from the WHO Growth Multicentre Reference Study. Repeated site visits

TABLE 1
Composition of the study formulas, human milk, and commercial formula[1]

| | Study formulas (cow milk–based) | | | | | Formulas used during the study in all countries[3] | |
| | Infant formulas | | Follow-on formulas | | | | |
	Lower protein	Higher protein	Lower protein	Higher protein	Human milk[2] (*n* = 58)	Infant formulas (*n* = 45)	Follow-on formulas (*n* = 94)
Whey:casein ratio	1:4	1:4	1:4	1:4			
Energy (g/100 mL)	69.9	69.8	72.7	72.5	70 ± 6.7	68 (65–74)	72 (64–80)
Proteins (g/100 mL)	1.25	2.05	1.6	3.2	1.2 ± 0.2	1.5 (1.2–1.9)	1.9 (1.6–2.7)
Proteins (g/100 kcal)	1.77	2.9	2.2	4.4	—	2.2 (1.8–2.6)	2.7 (2.2–4.0)
Proteins (% of energy)	7.1	11.7	8.8	17.6	—	8.9 (7.2–10.6)	10.7 (8.9–15.9)
Lipids (g/100 mL)	3.9	3.5	4.0	3.27	3.6 ± 0.7	3.6 (3.1–4.0)	3.3 (2.6–4.4)
Carbohydrates (g/100 mL)	7.5	7.5	7.6	7.6	7.4 ± 0.2	7.5 (6.8–8.9)	8.5 (6.6–10.2)
Lactose (%)	100	100	100	100			
Minerals							
Sodium (mg/100 mL)	27	25	40	47			
Calcium (mg/100 mL)	84	78	117	120			
Iron (mg/100 mL)	0.8	0.9	1.3	1.4			
Zinc (mg/100 mL)	0.5	0.5	0.6	0.7			
Vitamins							
A (μg/100 mL)	55	55	64	64			
D (μg/100 mL)	1	1	1.5	1.5			
E (μg/100 mL)	0.7	0.7	0.8	0.8			
B-6 (mg/100 mL)	0.05	0.05	0.12	0.12			
B-12 (μg/100 mL)	0.26	0.26	0.3	0.3			
Folic acid (μg/100 mL)	9	9	17	17			

[1] The quality of protein, carbohydrate, and fat of the study formulas was identical; the source of lipids was a mix of vegetable oils (palm, rapeseed, coconut, and sunflower oils emulsified with soy lecithin). A mix of synthetic vitamins and vitamin B-12 from a fermentation source was added.

[2] All values are means ± SDs and are from the Darling study (21).

[3] All values are medians (ranges) and are based on all formulas noted by the study participants in 3-d weighted food protocols.

were made by the coordinating center (Munich, Germany) for monitoring and to ensure compliance with the protocol.

Information on the course of pregnancy, medical history, lifestyle and behavior choices, socioeconomic background, and mother's prepregnancy weight was obtained from standardized parent interviews at the baseline visit. Weight and height of the fathers and mothers were measured at study entry and at the 12-mo visit. Parental BMI was calculated from prepregnancy weight and the first available height measurement of the mother and the first available weight and height measurement of the father.

Infant food intakes were recorded by prospective 3-d weighed food records at the infant ages of 3, 6, 12, and 24 mo obtained with food scales (Unica 66006; Soehnle, Murrhardt, Germany) provided to all participating families. Parents were advised on how to record all food and beverages consumed during 2 weekdays and 1 weekend day. Intakes of energy and macronutrients were calculated by using a database that was based on the German BLS II.3 (24) (10652 original food items + 2241 ones added before the study). Food items and recipes not identified in the database were added at each study center according to information from the manufacturers, other databases, or ingredients. Energy intake was not calculated for food records with any breastfeeding, because breastfeeding as intake of breast milk was only measured in a subgroup of infants. Food records with energy intakes >3 SDs of the mean by month and those deemed incomplete and inaccurate or with reported concurrent illness were excluded.

To compare the macronutrient contents of our study formulas with those of other formulas commercially available during the

study period in Europe, we collected compositional data from all infants (recommended before age 4 mo) and follow-on (from 4 mo of age) formulas used by participating families as recorded in the 3-d weighed food records.

The primary endpoints were length and weight at 24 mo, which were expressed as SD scores (*z* scores) for length-for-age and weight-for-length. Weight-for-length shows less variation than weight-for-age and is a better descriptor of body composition in children than weight. We expressed outcomes as *z* scores because this makes the data more comparable with other studies, standardizes for sex, and takes into account the true age at the measurement. Growth up to 24 mo of age was analyzed by adjusting the respective anthropometric measurement for its baseline measurement, as recommended (25). The study followed the recommendations made in the CONSORT guidelines (26).

Statistical analysis

Data management and statistical analyses were carried out with the software packages SAS version 9.2 (SAS Institute Inc, Cary, NC) and Stata version 9.2 (StataCorp LP, College Station, TX). Anthropometric results were expressed as *z* scores relative to the growth standards of the WHO for breastfed children (27), which were calculated by using WHO programs (http://www.who.int/childgrowth/software/en/). Means (±SD) or medians with interquartile ranges (IQR: 25th and 75th percentiles) were used as appropriate. Pearson chi-square and Fisher's exact tests were used for statistical comparison of categorical data, and a *t* test was used for normally distributed continuous data.

Linear regression analysis was applied to adjust the effects of type of feeding on *z* scores at 24 mo for weight, length, weight-for-length, and BMI for the respective baseline values. For pairwise comparison of the effect of each type of formula feeding with the breastfeeding group, potential confounders (sex, mother's educational status, smoking in pregnancy, and country) were also considered. To assess the effect of clustering by 8 different study teams, we used robust cluster variance estimators.

Description of baseline characteristics is based on all children allocated to study formula or to the breastfed group. To test whether there were different effects of the type of feeding between countries, an interaction term between formula and country was added to the linear regression model. Questionnaire data were evaluated for all participating subjects, even if anthropometric data were missing. An intention-to-treat analysis was not performed because children that switched to nonstudy formula (or breastfeeding) after randomization were excluded from further follow-up by study protocol.

Multilevel linear growth models and piecewise-linear-random-coefficient models as described by Singer and Willet (28) and Fitzmaurice et al (29) were applied to model growth differences between the lower- and higher-protein formula groups by using all available measurements from baseline to 24 mo. Both models account for the correlated data structure because of the repeated measurements and use the exact age of measurement. The piecewise-linear-random-coefficient model was chosen to analyze the age-dependent effect of study formula on the anthropometric outcome. The idea of the model is to split the time in fixed segments with different slopes in each segment, in contrast with the usual multilevel linear growth model, which uses one slope over the whole analysis time. The choice of the time segments (0–3 mo, 3–6 mo, 6–12 mo, and 12–24 mo) for this model is based on the measurement points as planned per protocol. Statistical significance of differences between trajectories of the study groups in the piecewise-linear-random-coefficient model was assessed by 95% prediction bands. If the 95% prediction bands of one group (eg, lower protein) does not overlap with the average trajectory of the other study group (eg, higher protein), there is a significant difference between these trajectories with a 5% probability error.

Study size and power calculation

The study was designed to have sufficient power (90%) to detect a difference in length of 0.8 cm at 24 mo of age between the 2 formula groups, with a level of significance of 0.05 and an expected population mean (WHO standard population) of 87.8 ± 3 cm. The necessary number of 296 children in each intervention group was inflated to a target of ≥500 randomized children in each group to meet an expected dropout rate of 30%.

RESULTS

A total of 1138 formula-fed infants were randomly assigned for treatment, and 1090 were allocated to study formula (**Figure 1**). The median age at randomization was 14 d (IQR: 3–30 d) and at the baseline visit was 16 d (IQR: 2–29 d); 249 (23%) children were exclusively formula-fed since birth, and all others gradually switched from breastfeeding to formula feeding within the first 8 wk of life.

After allocation to the study formula, 229 children were lost to follow-up, 5 children were excluded for illness or medication use, and 169 were excluded for lack of compliance (Figure 1). One-hundred eighteen (70%) of the latter children were excluded from further study participation during the first 3 mo of life, 29 by 6 mo of age, and 22 children by 9 mo of age (Figure 1). Eight of these 169 children completed the study up to 24 mo and were excluded according to the study protocol after study termination because questionnaire data or food protocols indicated lack of compliance.

The observational group of breast-fed infants included 619 infants, of whom 349 participated in the follow-up visits at 6 mo, 327 at 12 mo, and 304 at 24 mo. About 7% of all children in the intervention group completed questionnaires at 24 mo but had no anthropometric measurement taken. Thus, the final analysis was confined to 313 (follow-up rate: 58%) children in the lower-protein group, 323 (59%) children in the higher-protein group, and 298 (51%) children in the breastfed group.

Randomization of the infants to the lower- and higher-protein groups was successful: there were no significant differences in descriptors of socioeconomic status, smoking habits, parental anthropometric measures, gestational age, and weight and length at birth and at baseline (**Table 2**). Parents in the observational group of breastfed children had a higher educational level and fewer mothers smoked than in the formula-fed groups.

Parents of children lost to follow-up had a lower educational level, and the children's mothers were more likely to be smokers than were those still in the study at 24 mo, with no difference between the lower- and higher-protein formula groups. In contrast, the 169 children that were excluded for lack of compliance were not significantly different in any of the characteristics considered. They also had no differences in weight or length at baseline or later visits compared with the children staying in the study (data not shown).

Mean weight, length, weight-for-length, and BMI of all participating infants (both formula fed and the breastfed children) were lower at baseline than the median of the WHO Child Growth Standard: average weight of children in the lower-protein group, for example, was 0.42 SDs lower (**Table 3**).

Energy intake in the lower- and higher-protein formula groups was identical at 3, 12, and 24 mo. At 6 mo of age, energy intake in the lower-protein formula group was slightly higher (715 ± 127 kcal) than in the higher-protein formula group (690 ± 121 kcal) (*P* = 0.010) (**Figure 2**). Energy intake in the intervention groups increased from 584 ± 99 kcal/d at 3 mo (*n* = 809) to 1108 ± 236 kcal/d at 24 mo (*n* = 484). The protein intake was significantly different between the 2 formula groups (*P* < 0.001) at all time points until the end of the intervention at 12 mo, but not thereafter (Figure 2). Correspondingly, the fat intake was significantly lower in the higher-protein group at all time points up to age 12 mo, whereas there was no difference in carbohydrate intake (data not shown).

Differences in weight, weight-for-length, and BMI between the formula groups emerged at 6 mo of age and remained relatively stable thereafter with a decreasing tendency toward the end of the study. Length, weight, weight-for-length, and BMI increases were lower for breastfed than for higher-protein formula-fed children between baseline and 12 mo of age (**Figure 3**).

At 24 mo of age, length was not different between the intervention groups. The mean weight attained at 24 mo was 12.42
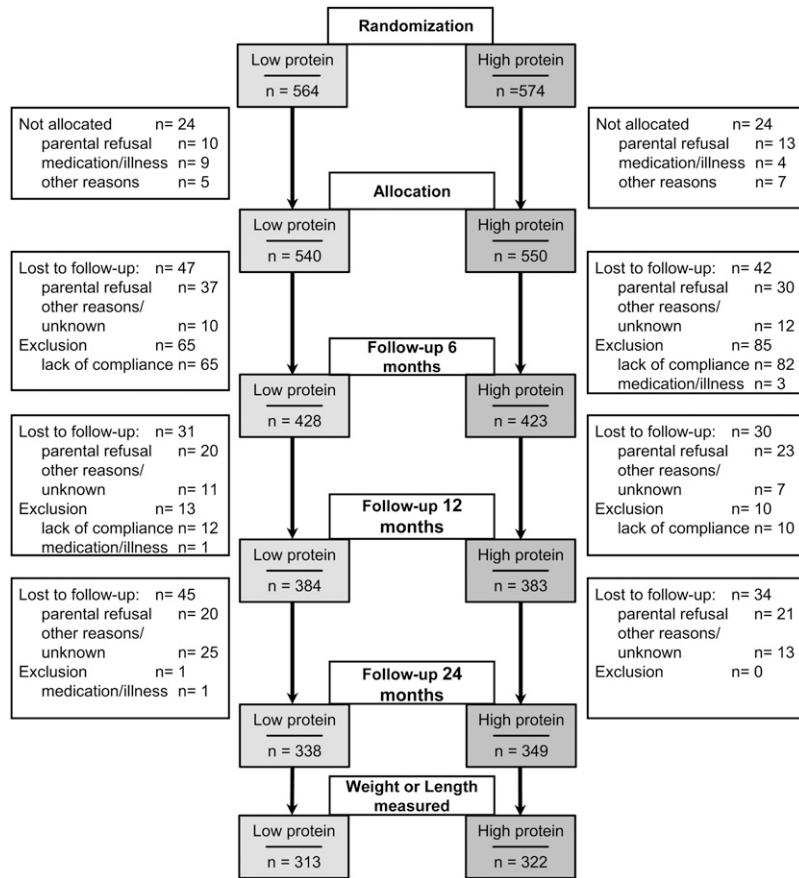
FIGURE 1. Randomization, allocation, and follow-up of study participants in the intervention group.

and 12.60 kg for the lower- and higher-protein groups, respectively (Table 3), without a significant difference in adjusted z scores. At 24 mo the adjusted z score for weight-for-length was found to be 0.20 (95% CI: 0.06, 0.34; $P = 0.005$) greater in the higher- than in the lower-protein formula group (Table 3); the CI was unaffected by clustering by study teams. The differences found in anthropometric z scores at 24 mo of age would translate, eg, for girls, into a difference in length, weight, and BMI (in kg/m^2) of −0.1 (95% CI: −0.6, 0.4) cm, 150 (95% CI: −13, 325) g, and 0.3 (95% CI: 0.1, 0.4), respectively.

Although the anthropometric measures at 24 mo of age differed significantly between countries, the effect of the intervention was not different between the countries; for none of the analyzed anthropometric measures was the interaction between country and type of formula significant. For instance, the baseline-adjusted z scores for weight-for-length at 24 mo for the higher- and lower-protein groups were 0.46 and 0.18 in Spain and 0.23 and −0.05 in Belgium, respectively.

In addition to the analyses at the primary endpoint 24 mo of age, identical analyses were performed at 3, 6, and 12 mo of age. The z scores for weight were significantly higher at ages 3, 6, and 12 mo in the higher-protein group than in the lower-protein group, whereas the z scores for weight-for-length and BMI were significantly higher only at 6 and 12 mo of age. In general, the differences between the lower- and higher-protein groups were greatest at 12 mo of age for weight, weight-for-length, and BMI, indicating the strongest effect of the intervention at this time point (Figure 3).

When modeling the whole growth trajectories of each child over the first 2 y of life with multilevel linear growth models, we saw significant interactions between type of study formula and age, which reflected the above differences in effect of the study formula over age (ie, the strongest effect at 12 mo of age). To depict this age-dependent effect of study formula, we used a piecewise-linear-random-coefficient model. A statistically significant difference between the lower- and higher-protein groups originated at 3–6 mo of age and slightly decreased after 12 mo of age for weight-for-length, BMI, and weight. (*See* Supplemental Figure 4 under "Supplemental data" in the online issue.)

To compare the observational group of breastfed children with children from the intervention group, we adjusted for baseline measurements and potential confounders. Compared with breastfed children, children fed higher-protein formula had significantly higher z scores for weight, length, weight-for-length, and BMI at 24 mo, with a difference of 0.30 (0.15, 0.45), 0.27 (0.12, 0.43), 0.18 (0.02, 0.33), and 0.20 (0.05, 0.36) SDs, respectively. In contrast, there was no significant difference in z scores of weight-for-length and BMI between the lower-protein group and the breastfed group at 24 mo, but the z scores for weight and length were 0.16 (0.01, 0.31) and 0.29 (0.13, 0.45) SDs higher in the lower-protein group than in the breastfed group.

DISCUSSION

This randomized, controlled trial showed that a higher protein content of infant formula is associated with higher growth. Although

TABLE 2
Baseline characteristics for children in the intervention and the observational study groups

| | Intervention group | | | | | Observational group | | |
| | Lower protein | | Higher protein | | | Breastfed | | |
	n (%)	Mean ± SD	n (%)	Mean ± SD	P value[1]	n (%)	Mean ± SD	P value[2]
Country								<0.001
Total	540 (100.0)	—	550 (100.0)	—	—	589 (100.0)	—	—
Germany	88 (16.3)	—	99 (18.0)	—	0.949	94 (16.0)	—	—
Belgium	71 (13.1)	—	67 (12.2)	—	—	117 (19.9)	—	—
Italy	121 (22.4)	—	120 (21.8)	—	—	174 (29.6)	—	—
Poland	103 (19.1)	—	106 (19.3)	—	—	66 (11.2)	—	—
Spain	157 (29.1)	—	158 (28.7)	—	—	137 (23.3)	—	—
Male sex	274 (50.7)	—	290 (52.7)	—	0.544	286 (48.6)	—	0.225
Birth order								0.003
First child	288 (53.6)	—	299 (54.6)	—	0.936	363 (62.3)	—	—
Second child	181 (33.7)	—	185 (33.8)	—	—	166 (28.5)	—	—
Third child	53 (9.9)	—	48 (8.8)	—	—	48 (8.2)	—	—
>Third child	15 (2.8)	—	16 (2.9)	—	—	6 (1.0)	—	—
Mother's educational level								<0.001
Low	173 (32.2)	—	172 (31.4)	—	0.944	78 (13.3)	—	—
Middle	279 (51.9)	—	285 (52.0)	—	—	274 (46.8)	—	—
High	86 (16.0)	—	91 (16.6)	—	—	233 (39.8)	—	—
Mother smoked in past 3 mo before or during pregnancy								<0.001
Yes	257 (47.6)	—	238 (43.3)	—	0.495	145 (24.7)	—	—
Mother smoked beyond 12th wk of gestation								<0.001
Yes	165 (30.7)	—	143 (26.1)	—	0.106	54 (9.2)	—	—
Age of mother (y)	537	29.8 ± 5.2	550	29.8 ± 5.4	0.866	586	31.2 ± 4.5	<0.001
BMI of mother (kg/m^2)	504	23.7 ± 4.6	523	23.7 ± 4.6	0.465	498	22.4 ± 3.6	<0.001
BMI of father (kg/m^2)	507	25.9 ± 3.8	516	26.1 ± 3.5	0.829	535	25.6 ± 3.4	0.041
Birth length (cm)	538	50.6 ± 2.7	548	50.6 ± 2.6	0.839	5884	50.5 ± 2.4	0.625
Birth weight (kg)	539	3.3 ± 0.3	549	3.3 ± 0.4	0.559	585	3.3 ± 0.3	0.008

[1] Chi-square test for categorical data and t test for comparison of means.
[2] For comparison between the intervention and observational group.

there was no significant difference with respect to length, a higher weight, weight-for-length, and BMI emerged within the first 6 mo of life and persisted thereafter, even beyond the intervention period of formula feeding. Because formula groups at 24 mo showed no difference in length, which is correlated with lean body mass (30), the difference in weight-for-length and in BMI is probably due to a difference in body fat or a difference in adiposity.

Our findings are compatible with results of recent cohort studies, which consistently reported lower growth in the first 2 y of life (10, 15, 16) and lower body size (9, 15–17) in children and adolescents fed a lower-protein diet in infancy. Three small randomized trials that compared the growth of children fed formula of different protein contents showed either no (1–3) or some (31) positive effect of higher protein intake on growth in infancy; the different results were potentially due to limited sample sizes.

Interestingly, no effect of the intervention on length growth was observed at any time point over the first 24 mo of life. A potential reduction in length growth had been chosen as an experimental primary endpoint because of suggestions that early length growth might be a novel predictor of later overweight (32). However, many studies (1–3) showed that early weight gain is the best predictor of later childhood overweight, a question explicitly addressed by Toschke et al (33).

The effects observed are likely to be conservative estimates. Many of the study children were partially breastfed during the first 8 wk of life. If all formula-fed children had been exclusively formula-fed from birth on, the effects might have been greater.

The protein contents of the lower- and higher-protein formulas in our study were within the range of commercial infant formulas available in Europe during the study period (Table 1). Traditionally, the protein content in infant formulas has been far higher than in human milk. The protein content of formula was as high as 4 g/100 kcal in the 1970s, decreased to ≈3 g/100 kcal in the 1980s, and tended to decrease further thereafter. Whereas adverse effects of higher protein intakes were not of major concern, worries about the deleterious effects of too low an intake of protein (34) prevailed. Considering that the protein content of human milk varies and tends to have a higher biological value than cow milk protein (19), the protein composition of infant formula was designed to always meet the assumed minimum requirements of protein and indispensable amino acids of infants.

The protein intake provided by the lower-protein formula was still higher than that of the breastfed children or than that recommended for infants (19, 35, 36). For instance, formula-fed children had protein intakes of ≈14 and 20 g/d at 3 and 6 mo, respectively, in our study, whereas breastfed children in the

TABLE 3
Number of children, age at measurement, and anthropometric measures with z scores for the intervention (formula) and observational group (breastfed) at baseline and 24 mo

	Intervention group					Observational group	
	Lower protein		Higher protein		Estimated difference at 24 mo[1]	Breastfed	
	Baseline	24 mo	Baseline	24 mo		Baseline	24 mo
No. of children[2]	539	313	550	322	630	585	298
Age at measurement (d)	16 (2, 30)[3]	733 (730, 737)	15 (2, 28)	733 (729, 737)		12 (3, 21)	734 (730, 738)
Weight							
(kg)	3.72 ± 0.76[4]	12.42 ± 1.32	3.67 ± 0.75	12.60 ± 1.46		3.55 ± 0.61	12.26 ± 1.41
(z score)	-0.42 ± 0.78	0.34 ± 0.85	-0.43 ± 0.75	0.44 ± 0.95	$0.12\ (-0.011, 0.25)$[5]	-0.31 ± 0.78	0.24 ± 0.93
Length							
(cm)	52.1 ± 2.9	88.3 ± 3.1	52.0 ± 3.1	88.1 ± 3.2		51.7 ± 2.5	87.5 ± 3.4
(z score)	-0.25 ± 1.02	0.31 ± 0.94	-0.23 ± 1.00	0.24 ± 1.02	$-0.039\ (-0.18, 0.10)$[6]	-0.02 ± 1.04	0.09 ± 1.06
BMI							
(kg/m^2)	13.6 ± 1.6	16.1 ± 1.2	13.5 ± 1.5	16.4 ± 1.3		13.2 ± 1.4	16.2 ± 1.3
(z score)	-0.47 ± 0.86	0.19 ± 0.89	-0.50 ± 0.82	0.40 ± 0.95	$0.23\ (0.089, 0.36)$[7]	-0.46 ± 0.91	0.25 ± 0.95
Weight-for-length (z score)	-0.48 ± 1.07	0.18 ± 0.86	-0.52 ± 1.05	0.37 ± 0.93	$0.20\ (0.060, 0.34)$[8]	-0.64 ± 1.13	0.21 ± 0.93

[1] Derived from linear regression adjusted for the respective anthropometric baseline value; 95% CIs in parentheses.
[2] Numbers vary slightly between anthropometric measures.
[3] Median; 25th and 75th percentile in parentheses (all such values).
[4] Mean $\pm$ SD (all such values).
[5] $P = 0.072$.
[6] $P = 0.589$.
[7] $P = 0.001$.
[8] $P = 0.005$.

DARLING study (37) had protein intakes of 7 and 8 g/d. Consumption of the lower-protein formula supported normal length growth, and parental reports did not indicate any untoward effects. Given that the supply of total protein and essential amino acids with the lower-protein formula is clearly higher than reference intakes for infants that are regarded as safe (19, 35, 36), one would not expect any untoward effects on growth or functional outcomes, such as neurologic development or immune response. However, further follow-up of the study cohort up to school age is planned to document neurologic and other outcomes.

One of the strengths of our study was the high external validity due to its multinational design. Although the anthropometric measures at 24 mo of age differed in each country, the effect of
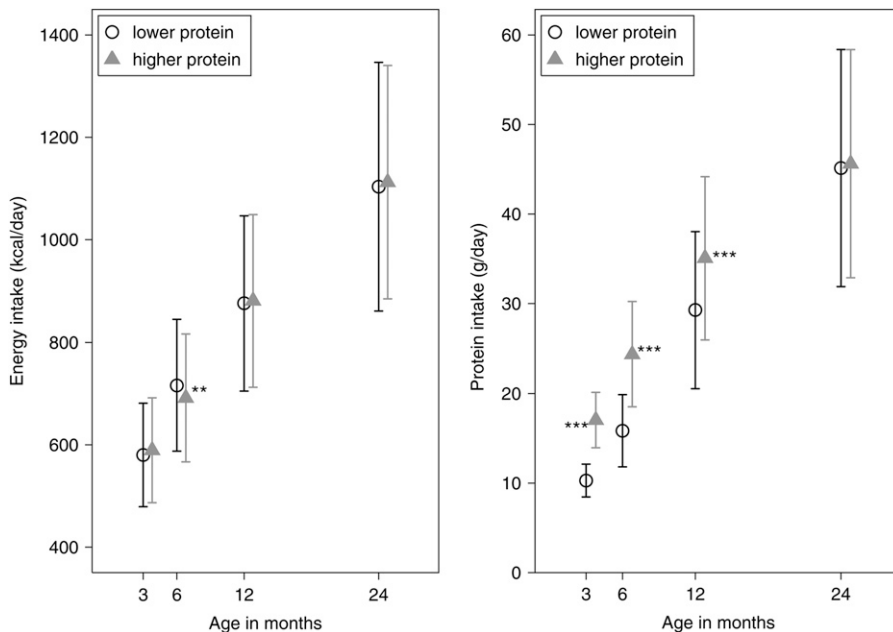

FIGURE 2. Mean ($\pm$SD) daily energy and protein intakes at 3, 6, 12, and 24 mo of age in 456 children in the lower-protein formula group and in 454 children in the higher-protein formula group. **, ***Significantly different from the lower-protein group (t test): **$P < 0.01$, ***$P < 0.001$.
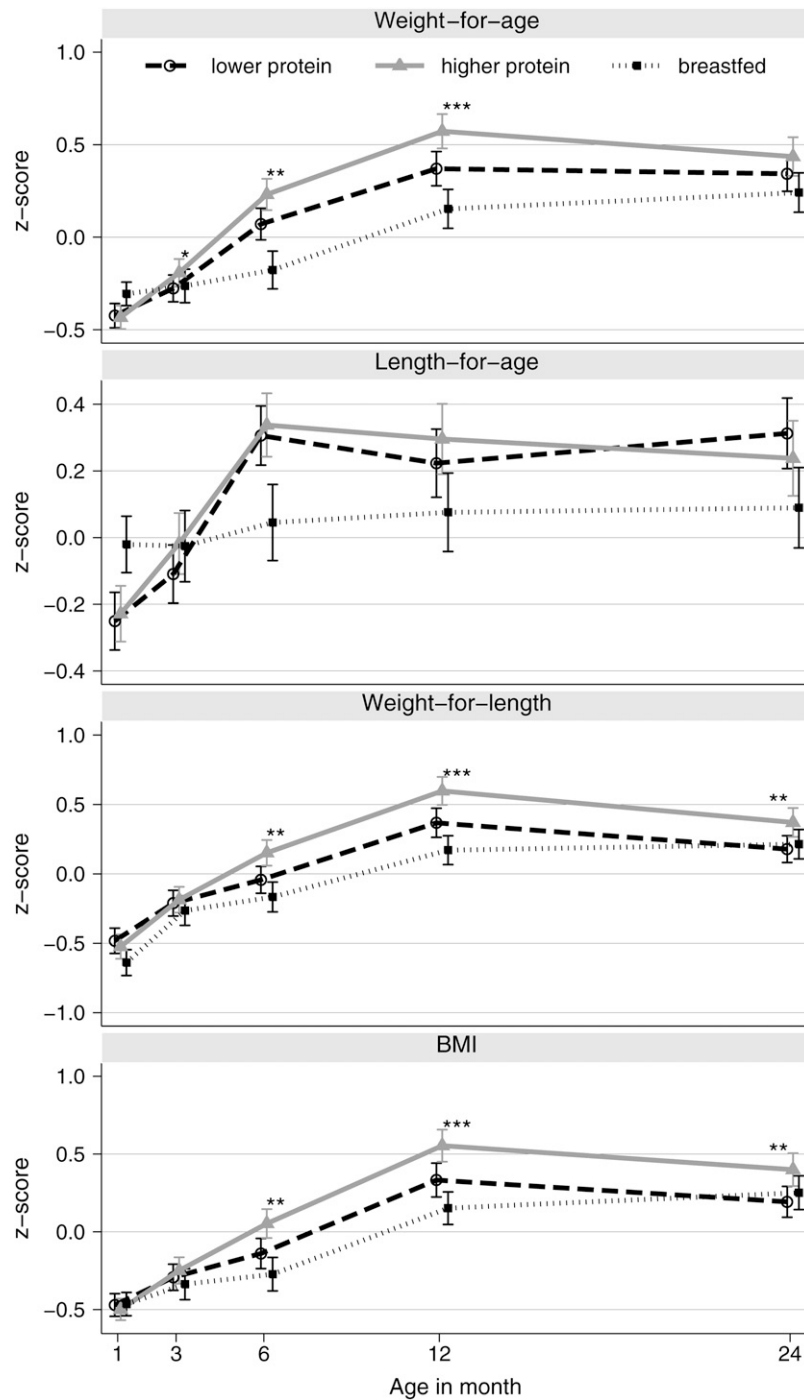
FIGURE 3. Mean *z* scores (with 95% CIs) for length, weight, weight-for-length, and BMI in the lower-protein (*n* = 540) and higher-protein (*n* = 550) groups and in the breastfed (*n* = 588) children at baseline (0–8 wk of age) and at 3, 6, 12, and 24 mo of age. *, **, ***Significantly different from the lower-protein group (ANOVA adjusted for baseline value): *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

the intervention was not different between the countries. A further strength of the study was the documented difference in protein intakes between intervention groups that matches the randomization.

Attrition was higher than expected. This may have been explained by the fact that the questionnaires and visit schedules were demanding and study participation of families with healthy infants was voluntary without any perceived benefit other than

access to free formula. However, because attrition was equal in both arms and randomization was initially successful, attrition likely did not bias our results.

A potentially more serious issue was the exclusion of 169 noncompliant children that should have been included for a proper intention-to-treat analysis. However, exclusion was already fixed in the study protocol, because compliance was not expected to be related to type of study formula or growth. All of the study

formulas could have been marketed as normal infant and follow-on formulae because they complied with European regulatory standards in all aspects, including protein contents. Furthermore, formula-fed infants are known to be frequently switched to another formula whenever parents perceive a potential problem (eg, the infant cries, does not sleep well, or wakes up at night), even though, in most cases, this is not related to the formula used. Importantly, there was no difference in frequency of noncompliance between the 2 study groups, and most (69%) of the noncompliance occurred shortly after randomization within the first 3 mo of life, which rendered it unlikely that noncompliance was related to differences in weight gain between the 2 formula groups.

The proportion of smokers was high: 46% of the mothers in both intervention groups were smokers, and ≈30% smoked after the 12th week of pregnancy. However, the population, for which the data of this intervention trial applies, was the population of nonbreastfeeding mothers only, who are generally more likely to smoke (38, 39). Thus, the high proportion of smoking mothers is expected and unlikely to limit the external validity of the study.

Potential effect of a lower protein diet on obesity

Monteiro et al (40) found an odds ratio of 1.87 (95% CI: 1.10, 3.18) for obesity in 14–16-y-old Brazilian adolescents for every 1-SD change in weight-for-length gain during the first 2 y of life. According to Ong and Loos (3), this is the average effect seen in studies of weight gain in infancy on later obesity. Thus, the observed increase in z score for weight-for-length at the age of 24 mo by 0.19 SDs in the higher-protein formula group compared with the lower protein group would yield an extrapolated odds ratio of 1.13 (95% CI: 1.02, 1.25) [OR = exp(ln(1.87) × 0.19) = 1.13] for being obese in adolescence.

Conclusions

This large randomized controlled trial showed significant effects of a lower protein intake from infant formula on weight, weight-for-length, and BMI in the first 2 y of life. Limiting the protein content of infant and follow-on formula and, more generally, the dietary protein intake during infancy, might constitute a potentially important approach to reducing the risk of childhood overweight and obesity.

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