

Lower protein content in infant formula reduces BMI and obesity risk at school age: follow-up of a randomized trial^{1–5}

Martina Weber, Veit Grote, Ricardo Closa-Monasterolo, Joaquín Escribano, Jean-Paul Langhendries, Elena Dain, Marcello Giovannini, Elvira Verduci, Dariusz Gruszfeld, Piotr Socha, and Berthold Koletzko for The European Childhood Obesity Trial Study Group

ABSTRACT

Background: Early nutrition is recognized as a target for the effective prevention of childhood obesity. Protein intake was associated with more rapid weight gain during infancy—a known risk factor for later obesity.

Objective: We tested whether the reduction of protein in infant formula reduces body mass index (BMI; in kg/m²) and the prevalence of obesity at 6 y of age.

Design: The Childhood Obesity Project was conducted as a European multicenter, double-blind, randomized clinical trial that enrolled healthy infants born between October 2002 and July 2004. Formula-fed infants (*n* = 1090) were randomly assigned to receive higher protein (HP)– or lower protein (LP)–content formula (within recommended amounts) in the first year of life; breastfed infants (*n* = 588) were enrolled as an observational reference group. We measured the weight and height of 448 (41%) formula-fed children at 6 y of age. BMI was the primary outcome.

Results: HP children had a significantly higher BMI (by 0.51; 95% CI: 0.13, 0.90; *P* = 0.009) at 6 y of age. The risk of becoming obese in the HP group was 2.43 (95% CI: 1.12, 5.27; *P* = 0.024) times that in the LP group. There was a tendency for a higher weight in HP children (0.67 kg; 95% CI: –0.04, 1.39; *P* = 0.064) but no difference in height between the intervention groups. Anthropometric measurements were similar in the LP and breastfed groups.

Conclusions: Infant formula with a lower protein content reduces BMI and obesity risk at school age. Avoidance of infant foods that provide excessive protein intakes could contribute to a reduction in childhood obesity. This trial was registered at clinicaltrials.gov as NCT00338689. *Am J Clin Nutr* doi: 10.3945/ajcn.113.064071.

INTRODUCTION

Several metabolic and endocrine exposures during pregnancy and early childhood have been associated with the later risk of obesity and associated disorders (1, 2). One possible mechanism of metabolic programming is represented by the “early protein hypothesis” driven by the observation of moderate but consistent protective effects of breastfeeding as compared with formula feeding against later obesity (3–6). The lower supply of protein from human milk (9 and 10 g/d at ages 3 and 6 mo) as compared with formula (14 and 18 g/d, respectively) (7) might attenuate both early weight gain and later obesity (8). One of the best predictors of later obesity risk is weight gain during the first year of life (9–11). The more rapid weight gain in formula-fed infants

might be mediated through an enhanced secretion of insulin and insulin-like growth factor I (IGF-I)⁶. Generally, insulin and IGF-I concentrations are greater in formula-fed than in breastfed infants (12). Whereas protein quantity is an important factor for the observed differences, protein quality may also be important (13). IGF-I concentrations in early life have been linked to concentrations in later childhood and adulthood (14). Total protein intake in infants and young children, independent of feeding practices, have been associated with later obesity in several observational studies, for which exact comparison of protein quality and quantity is difficult (15, 16). Overall, effects of protein intake on early growth mediated through hormonal status might have a significant effect on growth patterns throughout childhood and on the later risk of obesity and associated disorders.

¹ From the Division of Metabolic and Nutritional Medicine, Dr. von Hauner Children’s Hospital, University of Munich Medical Centre, Munich, Germany (MW, VG, and BK); the Paediatrics Research Unit, Universitat Rovira i Virgili, Reus, Spain (RC-M and JE); Centre Hospitalier Chrétien St Vincent, Liège-Rocourt, Belgium (J-PL); the Department of Paediatrics, University Children’s Hospital Queen Fabiola, Université Libre de Bruxelles, Brussels, Belgium (ED); the Department of Paediatrics, San Paolo Hospital, University of Milan, Milan, Italy (MG and EV); the Children’s Memorial Health Institute, Neonatal Intensive Care Unit, Warsaw, Poland (DG); and the Children’s Memorial Health Institute, Department of Gastroenterology, Hepatology and Eating Disorders, Warsaw, Poland (PS).

² MW and VG contributed equally as first authors.

³ The contents herein do not necessarily reflect the views of the Commission of the European Union and in no way anticipate the future policy in this area.

⁴ The studies reported herein were partially supported by the Commission of the European Community, specific RTD Programme “Quality of Life and Management of Living Resources,” within the 5th Framework Programme (research grant nos. QLRT-2001-00389 and QLK1-CT-2002-30582); the 6th Framework Programme (contract no. 007036); and the European Union’s Seventh Framework Programme (FP7/2007-2013), project EarlyNutrition under grant agreement no. 289346.

⁵ Address reprint requests and correspondence to B Koletzko, Division of Metabolic and Nutritional Medicine, Dr. von Hauner Children’s Hospital, University of Munich Medical Centre, Lindwurmstrasse 4, D-80337 Munich, Germany. E-mail: office.koletzko@med.uni-muenchen.de.

⁶ Abbreviations used: HP, higher protein; IGF, insulin-like growth factor; LP, lower protein.

Received April 12, 2013. Accepted for publication February 20, 2014.

doi: 10.3945/ajcn.113.064071.

The European Childhood Obesity Project is a multicenter, randomized trial funded by the European Commission, to examine the effect of protein intake in formula-fed infants during the first year of life on growth and later obesity risk. We have already shown that infants fed a formula containing higher protein (HP) gained more weight during the first year of life and were heavier at 2 y of age than were infants fed a lower-protein (LP) formula (17). Here we report on the follow-up of the cohort to 6 y of age.

SUBJECTS AND METHODS

Study design

Details of the design and results of the primary study of the first 2 y of life were reported previously (17). Briefly, a double-blind randomized intervention trial was performed with 2 sets of formulas that differed in protein content. Breastfed infants were followed as a reference group (18). Infants were enrolled and randomly assigned during the first 8 wk of life in 5 countries (Belgium, Germany, Italy, Poland, and Spain). The follow-up study reported here spans from 2.5 y of age until 6 y of age and was completed in August of 2010.

Subjects

Apparently healthy term infants who were appropriate for gestational age from uncomplicated singleton pregnancies were eligible for enrollment between the 1 October 2002 and 31 July 2004. Subjects with pregnancies involving gestational diabetes, a known familial history of metabolic or hormonal diseases, or any disease interfering with metabolism or growth of the child were excluded. After parents decided to formula-feed, infants were randomly assigned to receive formula with an HP or LP content. Block randomization was performed with a block size of 8, stratified by sex and study center. Formula groups were coded by 4 colors—2 colors per intervention group. Color codes were kept by the manufacturer to ensure double-blinding. Blinding of study personnel with direct child contact continues to be maintained. The pseudonymized database was unblinded only for data analysts after plausibility checks for the primary endpoint of the 2-y follow-up.

Breastfed infants were included if parents expressed the intention to exclusively breastfeed for ≥ 3 mo. Breastfeeding was encouraged in all study centers following a standard protocol for recruitment. All families attending the 2-y visit and all families, which did not withdraw their consents, were asked to continue to participate in the follow-up study until 6 y of age.

The study was conducted according to the principles expressed in the Declaration of Helsinki. The local ethics committees of each study center approved all study procedures: Belgium (Comit  d'Ethique de L'Hopital Universitaire des Enfants Reine Fabiola; no. CEH 14/02), Germany (Bayerische Landes rzttekammer Ethik-Kommission; no. 02070), Italy (Azienda Ospedaliera San Paolo Comitato Etico; no. 14/2002), Poland (Instytut Pomnik-Centrum Zdrowia Dziecka Komitet Etyczny; no. 243/KE/2001), and Spain (Comit   tico de investigaci n cl nica del Hospital Universitario de Tarragona Joan XXIII). Written informed consent was obtained from all parents before enrollment.

Intervention

The study formula (manufactured by Bledina) was distributed free of charge to infants from the baseline visit until 12 mo of age. Infant formulas were replaced by follow-up formulas with the introduction of complementary feeding from about the fifth month of life onward. The formulas differed in the content of cow milk protein (2.05 compared with 1.25 g/dL in infant formula and 3.2 compared with 1.6 g/dL in the follow-up formula, respectively) but had identical energy contents achieved by adjustment of total fat content (Table 1 in reference 17; also see Supplemental Table 1 under "Supplemental data" in the online issue for more details). All other compositional aspects of the 2 types of formula were similar. The composition followed European legislation at the time (19).

Total energy and nutrient intakes were assessed by weighed 3-d food protocols. Energy intake did not differ between intervention groups at 3, 12, and 24 mo of age, whereas the HP group showed a slightly lower energy intake than did the LP group at 6 mo (17). Protein intake was ~ 1 g/kg body weight higher in the HP group at 3 and 6 mo of age and ~ 0.5 g/kg body weight higher at 12 mo of age (all $P < 0.001$). At 18 and 24 mo, dietary energy and protein intakes and the amount of formula consumed were not significantly different between groups (17). These findings were unchanged when only the subsample of children who could be followed to 6 y of age was considered (see Supplemental Table 2 under "Supplemental data" in the online issue).

Methods

Anthropometric measurements were performed at study centers at the baseline visit; at 3, 6, 12, and 24 mo of age; and thereafter every 6 mo until 6 y of age. The time of the visits was planned to be within 14 d of the targeted age until the 2-y visit and within 3 mo for all later time points. Standard operating procedures (see Supplemental Method 1 under "Supplemental data" in the online issue) based on the WHO Multicentre Growth Reference Study (20) were established, and study personnel were trained repeatedly during the study. The same equipment was used in all centers. All measurements were taken twice, and their means were taken for analysis.

Data regarding the course of pregnancy, maternal prepregnancy weight, the child's medical history, and the family's socioeconomic status were collected by questionnaire, and the paternal weight and height of parents were measured by study personnel at the baseline visit.

In the intervention groups, intake of nonstudy formula or breastfeeding during the first 9 mo exceeding 10% of feedings (3 bottles/wk) was considered to be noncompliance (as assessed by questionnaire and monthly weighed 3-d food protocols). Breastfed infants had to be exclusively breastfed for ≥ 3 mo. Noncompliant infants in the intervention and breastfed groups were excluded from further study participation per protocol from the moment children violated the compliance criteria. Children were considered lost to follow-up if parents could no longer be contacted, could not be traced, or refused further participation.

We tried to contact all originally enrolled children who did not visit the study centers at 6 y of age, including those excluded for noncompliance or lost to follow-up, by telephone to either reactivate their participation or at least collect current height and weight as reported by the parents. All data were introduced in a common, Web-based, remote data entry tool with embedded

plausibility checks. Our study followed the guidelines published in the Consolidated Standards of Reporting Trials statement (21).

Statistical analysis

The focus of statistical analyses was the comparison of the 2 randomized groups. The breastfed group was included as an observational, comparison group only. The main outcome measure was BMI (in kg/m^2) at 6 y of age; secondary outcomes were weight, height, and obesity. Obesity was defined by using the International Obesity Task Force criteria (22, 23): girls and boys were classified as obese at 6 y of age if they had a BMI >19.7 or >19.8 , respectively. Weight (kg), height (cm), and BMI (kg/m^2) were transformed to age- (in d) and sex-specific z scores according to the WHO growth standards (20, 24) (<http://www.who.int/childgrowth/software/en/>, <http://www.who.int/growthref/tools/en/>).

Continuous data are presented as means \pm SDs or medians and IQRs. Primarily, linear regression was applied to estimate the effect of formula type on the anthropometric outcome (eg, BMI) at the age of 6 y, including the respective z score at baseline in the model (eg, BMI z score). Obesity, as a binary outcome, was compared by using logistic regression. Analyses were at first performed without any further adjustment, as generally recommended for randomized trials. Potential confounders, including sex, exact age at measurement, country, highest educational level of mother and father, smoking during pregnancy, and mother's and father's BMI were included for adjustment. Crude and adjusted ORs for the risk of obesity between feeding groups are reported.

We also depicted the 50th, 85th, 90th, and 95th percentiles of BMI over the duration of the study to see the development of the upper tails of the BMI distribution. Quantile regression was applied to quantify the effects of formula feeding on different quantiles at 6 y of age and to look for any increased effects toward upper tails. Furthermore, to check for the effect of missing data, analyses at 6 y of age were also performed on a data set including weight and height (and calculated BMI) as reported by parents in telephone interviews. Finally, we performed a further sensitivity analysis on data that were imputed by chained equations (*see* Supplemental Method 2 and Supplemental Table 3 under "Supplemental data" in the online issue).

Statistical significance was assumed at a maximum error probability of 0.05. Data management was carried out with SAS 9.3 (SAS Institute Inc) and statistical analyses with R 2.15.3 (The R Foundation for Statistical Computing) and Stata 12.1 (StataCorp).

RESULTS

Study population

Of the randomized 1090 formula-fed children, 518 (48%) were followed until 6 y of age. Dropout numbers and the reasons were not significantly different between the randomized groups (**Figure 1**). About 16% ($n = 169$) of all randomized, formula-fed children were excluded for noncompliance during the first 9 mo of life. Loss to follow-up was higher in the observational breastfed group, especially during the first 2 y of life. Of the 588 breastfed children allocated, 237 (40%) were followed up to 6 y of age; 14% ($n = 81$) were excluded for noncompliance.

The attendance rate at the biannual study visits from 2 to 6 y of age ranged from 82% to 88% among those still participating in the study. Data on BMI at 6 y of age were available for 448 ($n = 221$ HP and 227 LP) of 518 formula-fed and 209 of 237 breastfed children. The overall median age at the 6-y visit was 6.02 (IQR: 6.00, 6.05) y; only 11 ($n = 6$ LP, 2 HP, and 3 breastfed) of 657 children came >3 mo after their sixth birthday (maximum: 6.55 y).

For 362 additional children ($n = 112$ HP, 98 LP, and 152 breastfed), height and weight at 6 y of age was obtained by telephone interviews. One hundred two of 250 (41%) formula-fed and breastfed children who were excluded from further study participation because of noncompliance were able to be re-contacted, and weight and height measurements at 6 y of age were reported by parents. Thus, 60% (658/1090) of the randomized children in the intervention and 61% (361/588) in the breastfed group provided BMI data at 6 y of age.

Baseline characteristics of all study participants at study entry were published previously (17); characteristics of the population that was followed until 6 y of age are presented elsewhere (*see* Supplemental Table 2 under "Supplemental data" in the online issue). There were slight differences from the original population. Children with a higher parental education or nonforeign parents were more likely to stay enrolled in the study. Differences between formula-fed and breastfed infants, such as socioeconomic status, smoking behavior, and parental BMI and obesity, persisted. However, no differences were observed between the randomized groups.

Anthropometric measurements at 6 y of age

Anthropometric outcomes at baseline and at 6 y of age by study group are shown in **Table 1**. BMI at 6 y of age was significantly higher in the HP than in the LP group, assessed by linear regression adjusted for baseline BMI z score; the estimated difference in BMI of 0.51 (95% CI: 0.13, 0.90; $P = 0.009$), was reduced to 0.40 (95% CI: 0.03, 0.77; $P = 0.034$) after adjustment for sex, exact age at measurement, country, highest educational level of mother and father, smoking during pregnancy, and mother's and father's BMI. There was a tendency for higher weight in the HP group, whereas height remained unaffected by the intervention (Table 1). The estimated difference in BMI between the HP and LP groups was not significantly different between countries (data not shown).

The difference between the 2 intervention groups was estimated to be slightly lower in the data set including BMI obtained from telephone interviews (Table 1); adjustment did not change the effect size (0.45; 95% CI: 0.11, 0.79; $P = 0.010$). Including imputed data, the estimated difference in BMI between the HP and LP groups was lower and no longer significant (0.30; 95% CI: -0.01 , 0.61; $P = 0.058$) (Table 1).

The median and 90th and 95th percentiles of the BMI distribution from 3 mo to 6 y of age are shown in **Figure 2**. BMI is clearly higher in the HP group from 3 to 12 mo of age. The difference between the HP and LP groups' tracks attenuated from 12 to 24 mo of age but then remained stable from 24 to 36 mo of age. From ~ 42 mo of age onward, the HP group deviated to higher BMI values, particularly in the upper tails of the BMI distribution. Thus, the estimated difference in BMI between the HP and LP groups increased significantly from 0.29 (95% CI:

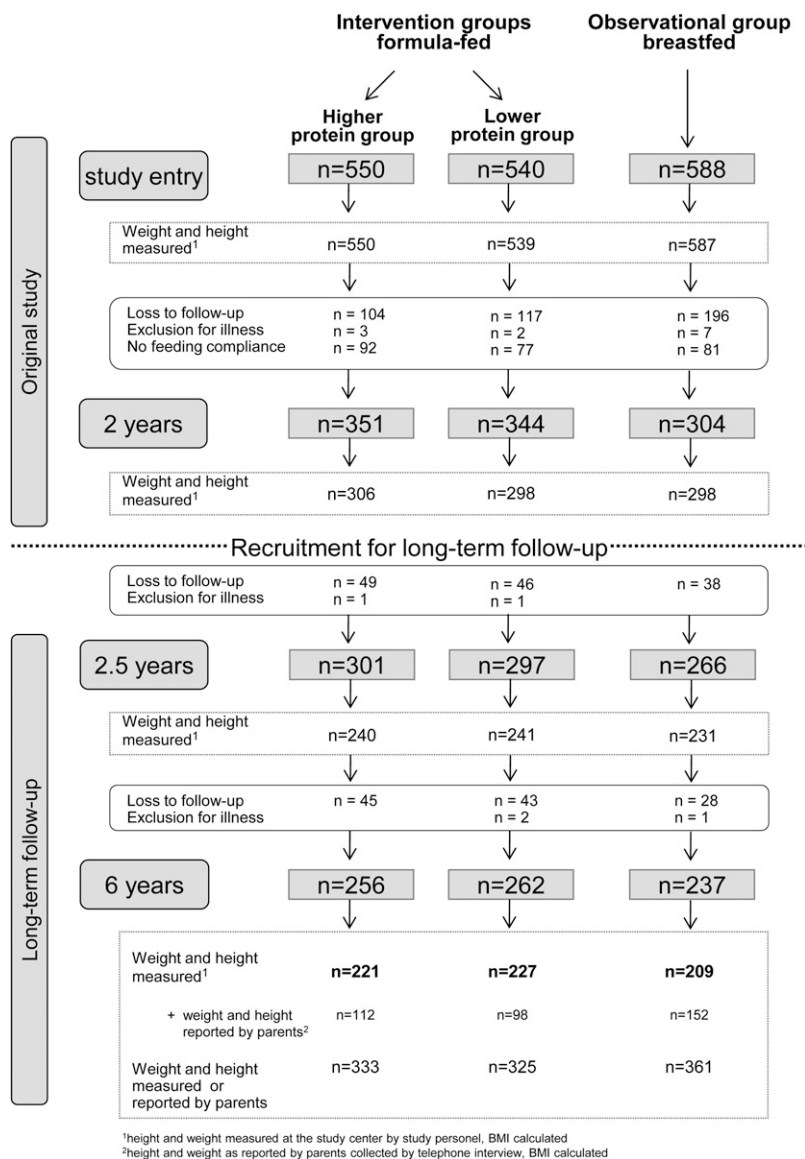


FIGURE 1. Flowchart of children from study entry to 6 y of age by study group. Telephone interviews were done on all originally included study children who did not attend the 6-y visit, unless the parents refused further study participation.

–0.00, 0.59) at the median to 2.50 (95% CI: 0.50, 4.50) at the 95th percentile [Table 2; estimated difference of intervention effect for the median compared with the 95th percentile: 2.21 (95% CI: 0.26, 4.16); $P = 0.027$], although significant effects for HP compared with the LP groups were observed only at the 95th percentile. When the data reported by parents were included, all upper quantiles (85th, 90th, and 95th) were significantly affected by the intervention (Table 2). Adjustment for confounders, as used in the linear regression model, resulted in somewhat smaller effect estimates. The tendency for increased differences in the upper tails was still observed, but the model estimation results were unstable at the tails because of the small number of children (Table 2): only 6 children (2.6%) in the LP group compared with 17 children (7.7%) in the HP group had BMI values above the 95th percentile of this cohort-specific BMI distribution.

Regarding weight, the baseline-adjusted difference between the HP and LP groups at the median, the 90th percentile, and the 95th percentile were 0.46 (95% CI: –0.15, 1.08; $P = 0.141$), 2.38

(95% CI: 0.06, 4.69; $P = 0.044$), and 3.32 (95% CI: –0.63, 7.26; $P = 0.100$) kg, respectively. No effect of the intervention on distribution quantiles of height was observed.

Obesity at 6 y of age

The overall prevalence of obesity in formula-fed children was 7.1% (32 children) (Table 3). The prevalence of obesity was 5.6 percentage points (95% CI: 0.9, 10.4) higher in the HP than in the LP group, which resulted in an estimated increase in risk of obesity at 6 y of age by HP intake in infancy of 2.43 (95% CI: 1.12, 5.27; $P = 0.024$); adjustment for confounders resulted in a somewhat higher OR of 2.87 (95% CI: 1.22, 6.75; $P = 0.016$; model summary in Supplemental Table 4 under “Supplemental data” in the online issue).

Obesity prevalence in the imputed data set was lower in the HP group and slightly higher in the LP and breastfed group (HP: 8.0% compared with 10.0%; LP: 4.6% compared with 4.4%; breastfed:

TABLE 1
Number of children, age at measurement, and anthropometric measurements of the intervention groups and the breastfed group at baseline and 6 y of age

	Higher protein		Lower protein		Estimated differences: higher protein compared with lower protein ¹			Observational group: breastfed	
	Baseline	6 y	Baseline	6 y	Estimate (95% CI)	P value	Baseline	6 y	
Children followed to 6 y of age									
No. of children	550	221	539	227			587	209	
Age at measurement: baseline (d)/6 y (mo) ²	15 (2, 27)	72.2 (71.9, 72.6)	15 (2, 29)	72.2 (72.0, 72.7)			12 (2, 21)	72.2 (72.0, 72.6)	
Weight									
(kg)	3.65 ± 0.73 ³	22.47 ± 4.28	3.70 ± 0.75	21.88 ± 3.46	0.67 (-0.04, 1.39)	0.064	3.55 ± 0.60	21.61 ± 3.46	
(z score)	-0.43 ± 0.76	0.53 ± 1.20	-0.41 ± 0.79	0.38 ± 1.01	0.18 (-0.03, 0.38)		-0.29 ± 0.79	0.29 ± 1.03	
Height									
(cm)	51.89 ± 3.03	116.86 ± 4.75	51.96 ± 2.92	117.25 ± 4.52	-0.19 (-1.01, 0.63)	0.654	51.65 ± 2.45	116.78 ± 5.26	
(z score)	-0.22 ± 1.00	0.23 ± 0.94	-0.24 ± 1.02	0.30 ± 0.88	-0.03 (-0.20, 0.13)		-0.01 ± 1.05	0.22 ± 1.03	
BMI									
(kg/m ²)	13.32 ± 1.38	16.36 ± 2.29	13.52 ± 1.50	15.86 ± 1.87	0.51 (0.13, 0.90)	0.009	13.30 ± 1.42	15.78 ± 1.76	
(z score)	-0.50 ± 0.83	0.55 ± 1.27	-0.46 ± 0.86	0.25 ± 1.12	0.30 (0.09, 0.52)		-0.46 ± 0.92	0.21 ± 1.07	
Children followed to 6 y of age, including children with measured weight and height as reported by parents									
No. of children		333		325				361	
Age at measurement: baseline (d)/6 y (mo) ²		72.2 (72.0, 72.9)		72.3 (72.0, 73.0)				72.3 (72.0, 73.0)	
Weight									
(kg)		22.75 ± 4.40		22.14 ± 3.77	0.64 (0.03, 1.25)	0.041		21.72 ± 3.60	
(z score)		0.57 ± 1.21		0.42 ± 1.09	0.16 (-0.01, 0.34)			0.29 ± 1.07	
Height									
(cm)		117.37 ± 5.63		117.54 ± 5.46	-0.14 (-0.95, 0.66)	0.726		116.91 ± 5.68	
(z score)		0.28 ± 1.10		0.32 ± 1.06	-0.04 (-0.20, 0.12)			0.20 ± 1.10	
BMI									
(kg / m ²)		16.43 ± 2.34		15.99 ± 2.18	0.45 (0.11, 0.79)	0.010		15.81 ± 1.81	
(z score)		0.57 ± 1.32		0.30 ± 1.30	0.27 (0.07, 0.47)			0.21 ± 1.08	
All children: missing data imputed by chained equations									
No. of children		546		535				580	
BMI (kg/m ²)		16.25 ± 2.58		15.97 ± 2.72	0.30 (-0.01, 0.61)	0.058		15.74 ± 2.43	

¹ Derived from linear regression adjusted for the respective anthropometric baseline z score (weight, height, and BMI z score, respectively).

² Values are medians; 25th and 75th percentiles in parentheses.

³ Mean ± SD (all such values).

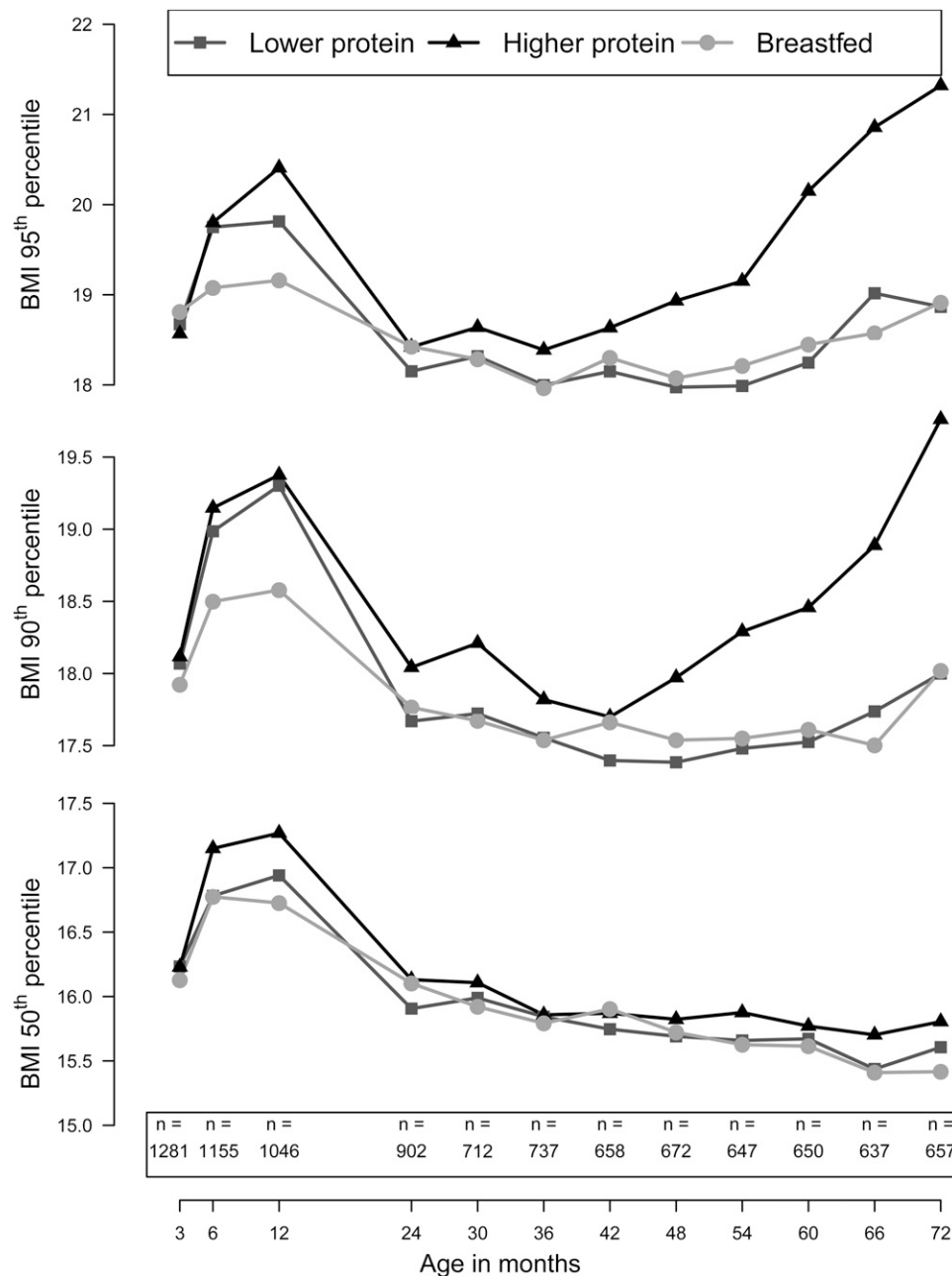


FIGURE 2. Median and 90th and 95th percentiles of BMI by study group from 3 mo to 6 y of age and the number of children.

3.4% compared with 2.9% for the data set including only data from the study visit compared with the data set with multiple imputations, respectively). Therefore, the estimated risk ratio for HP compared with LP was lower (OR: 1.85; 95% CI: 1.05, 3.25; $P = 0.034$) but remained significant; adjustment resulted in a slightly higher OR of 1.92 (95% CI: 1.06, 3.48; $P = 0.032$) for imputation analyses.

Formula-fed and breastfed children

No significant difference was found between the LP and breastfed children in mean BMI or obesity risk. Compared with breastfed children, weight and BMI were significantly higher in the HP group in unadjusted comparisons. Adjustment for

confounders such as socioeconomic status, smoking in pregnancy, country, and parental BMI considerably attenuated these effects, which were no longer significantly different; the estimated difference (HP compared with breastfed) in BMI was 0.60 (95% CI: 0.22, 0.98; $P = 0.002$) in the unadjusted analysis and 0.24 (95% CI: -0.14, 0.63; $P = 0.218$) in the adjusted analysis. The BMI distribution in breastfed children was more similar to the LP than to the HP group (Table 2, Figure 2); the prevalence of obesity at 6 y of age was considerably lower in breastfed children (2.9%) than in formula-fed children (Table 3). The adjusted odds of being obese at 6 y of age tended to be 2.84 (95% CI: 0.94, 8.70; $P = 0.063$) times that in the HP than in the breastfed group.

TABLE 2
 BMI at the 50th, 85th, 90th, and 95th percentiles by study groups and respective estimated differences between the intervention groups obtained from quantile regression models

	Intervention groups								Observational group: breastfed
	BMI		Estimated differences: higher protein compared with lower protein				BMI		
	Formula fed ³	Higher protein	Lower protein	Crude model ¹		Adjusted model ²		P value	
<i>kg/m² (n; n)</i>	<i>kg/m²</i>	<i>kg/m²</i>	Estimate (95% CI)	P value	Estimate (95% CI)	P value	<i>kg/m²</i>	<i>kg/m²</i>	
Children followed to 6 y of age									
Median	15.71 (118; 106)	15.81	15.61	0.29 (-0.00, 0.59)	0.053	0.08 (-0.28, 0.44)	0.655	15.42	
85th percentile	17.55 (41; 27)	18.09	17.35	0.77 (-0.08, 1.63)	0.074	0.51 (-0.34, 1.35)	0.238	17.39	
90th percentile	18.48 (28; 17)	19.76	18.00	1.49 (-0.21, 3.18)	0.085	0.43 (-0.70, 1.57)	0.455	18.02	
95th percentile	20.61 (17; 6)	21.32	18.87	2.50 (0.50, 4.50)	0.014	1.22 (-0.30, 2.46)	0.056	18.91	
Children followed to 6 y of age, including children with BMI as reported by parents									
Median	15.86 (176; 153)	15.94	15.71	0.25 (-0.06, 0.56)	0.107	0.30 (-0.03, 0.63)	0.073	15.49	
85th percentile	18.11 (60; 39)	18.48	17.53	1.00 (0.40, 1.59)	0.001	0.43 (-0.18, 1.03)	0.167	17.39	
90th percentile	18.86 (43; 23)	19.88	18.34	1.58 (0.26, 2.89)	0.019	0.36 (-0.40, 1.11)	0.350	18.00	
95th percentile	20.71 (24; 9)	21.37	19.68	1.96 (0.65, 3.26)	0.003	0.68 (-0.39, 1.74)	0.213	18.99	

¹ Estimated from quantile regression adjusted for baseline BMI z score.

² Estimated from quantile regression adjusted for baseline BMI z score, sex, parental BMI, smoking in pregnancy, and parental highest educational level.

³ Values in parentheses represent the number of children with a BMI value at 6 y of age above the respective BMI distribution quantile of all formula-fed children (no. higher protein; no. lower protein).

TABLE 3
Prevalence of obesity in the intervention groups at 6 y of age and estimated crude and adjusted ORs between the higher-protein and lower-protein formula groups

	Intervention group						Observational group: obesity	
	Obesity		Risk difference ¹ (95% CI)	Crude model ¹		Adjusted model ²		Breastfed <i>n/N</i> (%)
	Higher protein <i>n/N</i> (%)	Lower protein <i>n/N</i> (%)		OR (95% CI)	<i>P</i> value	OR (95% CI)	<i>P</i> value	
Children followed up to 6 y of age	22/221 (10.0)	10/227 (4.4)	5.6 (0.9, 10.4)	2.43 (1.12, 5.27)	0.024	2.87 (1.22, 6.75)	0.016	6/209 (2.9)
Children followed up to 6 y of age, including children with BMI reported by parents	35/333 (10.5)	17/325 (5.2)	5.3 (1.2, 9.4)	2.14 (1.17, 3.90)	0.013	2.60 (1.33, 5.10)	0.005	13/361 (3.6)
All children: missing data imputed by chained equations	44/546 (8.0)	25/535 (4.6)	3.4	1.85 (1.05, 3.25)	0.034	1.92 (1.06, 3.48)	0.032	20/580 (3.4)

¹ Estimated from binary logistic generalized linear regression adjusted for baseline BMI z score.

² Estimated from binary logistic generalized linear regression adjusted for baseline BMI z score, sex, parental BMI, smoking in pregnancy, and parental highest educational level.

DISCUSSION

Main findings

Optimal infant nutrition is of major importance because it lays the foundation for future health. Our results show that protein intake through infant formula affects BMI and obesity risk at school age. The intervention led to an increased protein intake of ~6 to 8 g/d in the HP formula group during the first year of life. BMI in the HP group was 0.51 kg/cm² higher at 6 y of age, and the risk of obesity was 2.43 times higher. Reducing the protein content of infant formula to a level similar to that found in human milk leads to early weight gain and BMI at 6 y of age that resembles that of breastfed children.

Comparison with other studies and implications

The early protein hypothesis postulated that differences in protein supply between human milk and infant formula play an important role in early programming and are causative for a more rapid weight gain in the first 2 y of life and for the increase in obesity risk observed in formula-fed than in breastfed children (8). The difference in protein intake provided by our intervention equated to one found in an observational study between formula-fed and breastfed children in Germany (7). We showed the relation between protein intake and more rapid weight gain until 2 y of age in a previous publication of the Childhood Obesity Project study (17). The weight z score at 1 y of life was 0.25 SD higher in the HP than in the LP group. In line with this, Axelsson et al (25) showed in a small intervention study that an HP formula given between the ages of 4 and 6 mo led to an excessive weight gain in the same age period. Besides this intervention study, several observational studies have shown associations of protein supply assessed in the first 2 y with more rapid growth in infancy. Several studies have reported long-term effects of early protein intake on anthropometric markers in early school age (26–30). The studies vary in method, time point, and frequency of dietary assessment. Protein intake during the period of complementary feeding (6–18 mo of age) was positively associated with BMI at 4 and 7 y by Öhlund et al (29) and Günther et al (28), respectively. Protein intake at 1 y of age was shown to increase weight at 4 y (29), overweight at 5 y, and BMI at 6 y of age in boys (30). Dietary data in the first year of life is rare in observational studies. Therefore, direct comparisons with our results are not possible. Most of the cited studies were underpowered and did not see consistent effects in both sexes or on all anthropometric markers: the number of children studied was ~200. Furthermore, dietary data from observational studies might not be directly comparable with our intervention during the first year of life. Nevertheless, most observational studies report positive associations of protein intake in the first 2 y of life with both more rapid weight gain in infancy and later weight or BMI (27, 29–31).

Rapid, early weight gain has been consistently shown to be a risk factor for later obesity (10, 11, 32, 33). On the basis of an individual meta-analysis of data for 47,661 study participants, Druet et al (11) reported that an increase of 1 SD in weight z score in the first year of life is associated with a 2-fold risk of childhood obesity and a 23% higher risk of obesity in adulthood. Transferring this information to our finding of a 0.25-SD higher weight gain during the first year in the HP than in the LP group

(2) resulted in an extrapolated 1.18 increased risk of obesity at school age. The risk ratio of 2.43 that we observed was markedly higher. This might suggest that early weight gain is not the only mechanism behind the early protein hypothesis, but that there are other metabolic programming factors associated with protein intake, eg, the increase in IGF-I concentrations observed in the HP group of our study population (13).

Some discrepancies existed between the modest effect of protein intake observed on mean BMI and the strong effects on obesity risk. The intervention had a more pronounced effect on the upper tails of the BMI distribution. Stronger effects of, for instance, breastfeeding, on distribution tails of BMI were also reported by other investigators (34). Some interaction with epigenetic or genetic predisposition or other environmental and lifestyle factors might lead to an increased sensitivity to protein intake in certain subgroups only. Thus, the results of a study can differ considerably depending on the choice of outcome, mean BMI or obesity, based on BMI cutoff values, which should, in general, both be reported.

Earlier adiposity rebound is associated with an increased risk of later obesity (35). Rolland-Cachera et al (36) reported significant associations of protein intake at 2 y of age with time of adiposity rebound and with BMI at 8 y. However, others did not reproduce these effects (28, 37). In our cohort, we saw a trend toward an earlier adiposity rebound in the HP group; the upper tails of the BMI distribution departed to higher BMI values as early as 4 y of age—a trend that was not seen in the LP or breastfed group (Figure 2). However, adiposity rebound has not yet occurred at 6 y in more than half of our children.

Our results may offer new opportunities for public health promotion concerning early nutrition with long-term consequences. Adapting the protein intake during the first year of life to recommended intakes (38) has the potential to markedly reduce later obesity risk. Formula feeding and complementary feeding have led and currently lead to protein intakes that are ~1.5–2.5 times higher than recommendations (7, 39–41). Our data support the conclusion that breastfeeding, which naturally provides protein intakes lower than in current formula feedings and feeding formulas with LP contents, has a protective effect on later obesity risk. Compositional factors of formula, such as extensive protein hydrolyzation (42, 43) or the source of protein (15), were shown to affect anthropometrical measurements.

Study strengths and limitations

The major strengths of this trial were its randomization, large sample size, multinational design, and long follow-up period. Anthropometric measurements were well standardized, and major potential confounding factors were taken into consideration. Although we have shown that early protein intake affected early weight gain and later BMI in our randomized trial cohort, the generalizability of our study was limited and extrapolation of the presented effect sizes was not straightforward. The current US regulation limits the protein content of formulas to be between 1.8 and 4.5 g protein/100 kcal (44), whereas follow-up formulas do not exist. Since the study start in 2002, the European regulations on protein contents in infant and follow-up formulas changed in 2006: the lower limit was reduced from 2.25 to 1.8 g/100 kcal for both infant and follow-up formulas; the upper limit for follow-up formulas was lowered to 3.5 g/100 kcal compared

with 4.5 g/100 kcal beforehand. Therefore, the differences in protein supply caused by our intervention were probably higher than the differences found nowadays. Nevertheless, even with current formulas containing LP, formula-fed children will have a clearly higher total protein intake than will exclusively breastfed children.

Attrition was of concern. Nonetheless, we were still able to obtain anthropometric data through measurements by study personnel 6 y after the trial was initiated in ~48% of children, and data are available for 60% of children if we also include reported data. Attrition was highest during the first 2 y of life. Within the formula-fed groups, many infants were excluded for switching to nonstudy formula [118 children (70%) of all non-compliant children were excluded within the first 3 mo]. Because compliance was not thought to be related to the type of study formula or associated with growth, those children were excluded. Study formulas complied with European regulatory standards (19) in all aspects, including protein content, and could have been marketed as normal infant and follow-up formulas.

Furthermore, although high follow-up rates are desirable, they are more difficult to achieve in long-term follow-up studies of healthy infants (45). The participants in our study had no perceived benefit from study participation except for the distribution of formula, which was free-of-charge during the first year of study. Parental expectations of infant feeding differ from real life. Exclusive feeding of human milk or study formula may be anticipated at baseline but is not feasible for a variety of reasons later on.

We found no indication of bias: dropout rates and reasons for dropout were similar in the 2 randomized groups and were not associated with anthropometric measurements (*see* Supplemental Tables 3 and 5 under “Supplemental data” in the online issue). On the basis of all measured BMI values from baseline until dropout, the mean BMI trajectory of dropouts and children followed until 6 y of age were similar (*see* Supplemental Figure 1 under “Supplemental data” in the online issue).

Furthermore, when we replaced missing values by data from telephone interviews and multiple imputations, the estimated effects decreased (Tables 1 and 3). However, the inclusion of BMI from telephone interviews also included measurement error and reporting bias. The latter was especially problematic for obese persons (46, 47), who are generally more likely to underreport weight. Overall, additional analyses of BMI indicate that the estimated effects of protein intake on mean BMI might be lower than those deduced from the main analysis. Besides the fact that median, 85th and 90th quantiles were shifted upward in the HP group, only the 95th percentile showed significant results. The prevalence of obesity in the whole cohort was low, at 5.8%, and the reported risk difference between the HP and LP groups (5.6%) might be viewed as small. However, the number of obese children in our cohort was similar to the current number of obese children in the respective European countries (<http://www.iaso.org/iotf/obesity/?map=children>).

In addition to crude estimates, we reported adjusted estimates for our main findings. Especially in the logistic regression model for obesity, the increased adjusted OR of 2.87, as compared with 2.43 in the unadjusted analysis, raised some concern of over-adjustment. Nevertheless, common confounders such as smoking in pregnancy (OR: 2.38) and higher compared with lower parental

educational level (OR: 0.66) were in line with those of other studies (*see* Supplemental Table 4 under “Supplemental data” in the online issue) (48). Moreover, the results of adjusted linear regression analysis on BMI for HP compared with LP groups were not substantially different from the unadjusted estimates. However, as expected, our observational breastfed group was different from formula-fed children with regard to several confounders (*see* Supplemental Table 2 under “Supplemental data” in the online issue). Adjustment for these factors completely attenuated the observed differences between breastfed and formula-fed children in weight and BMI at 6 y of age.

To increase the protein content in the infant and follow-up formulas without affecting the total energy content, the fat content had to be changed. However, the fat composition was the same in both formula groups. Because there is no biological model that demonstrates that lower fat intakes during infancy increase weight gain and later obesity risk, we assumed that the observed effect was not attributable to the difference in fat content (7, 31, 36, 37).

Conclusion

This randomized intervention trial with long-term follow-up provides strong evidence that infant feeding choices affect BMI and obesity risk at school age. Targeting dietary protein intake during infancy should be considered a valuable approach to reducing excessive early weight gain. Reducing infant protein intakes by promoting breastfeeding and by reducing the protein content of infant formulas may effectively contribute to the prevention of childhood obesity.

We thank the participating families and all project partners for their enthusiastic support of the project, Hans Demmelmair for his continued work on the study and his helpful comments and input for this manuscript, and Melissa Theurich for proofreading. The European Childhood Obesity Trial Study Group: Philippe Goyens, Clotilde Carlier, Joana Hoyos, Pascale Poncelet, and Elena Dain (Université Libre de Bruxelles–Brussels, Belgium); Françoise Martin, Annick Xhonneux, Jean-Paul Langhendries, and Jean-Noel Van Hees (CHC St Vincent–Liège-Rocourt, Belgium); Ricardo Closa-Monasterolo, Joaquin Escribano, Veronica Luque, Georgina Mendez, Natalia Ferre, and Marta Zaragoza-Jordana (Universitat Rovira i Virgili, Institut d'Investigació Sanitària Pere Virgili, Taragona, Spain); Marcello Giovannini, Enrica Riva, Carlo Agostoni, Silvia Scaglioni, Elvira Verduci, Fiammetta Vecchi, and Alice Re Dionigi (University of Milano, Milano, Italy); Jerzy Socha and Piotr Socha (Children's Memorial Health Institute, Department of Gastroenterology, Hepatology and Immunology, Warsaw, Poland); Anna Dobrzańska and Dariusz Gruszfeld (Children's Memorial Health Institute, Neonatal Intensive Care Unit, Warsaw, Poland); Anna Stolarczyk and Agnieszka Kowalik (Children's Memorial Health Institute, Department of Pediatrics, Warsaw, Poland); Roman Janas and Ewa Pietraszek (Children's Memorial Health Institute, Diagnostic Laboratory, Warsaw, Poland); Emmanuel Perrin (Danone Research Centre for Specialized Nutrition, Schiphol, the Netherlands); Rüdiger von Kries (Division of Pediatric Epidemiology, Institute of Social Pediatrics and Adolescent Medicine, Ludwig Maximilians University of Munich, Munich, Germany); Helfried Groebe, Anna Reith, and Renate Hofmann (Klinikum Nürnberg Sued, Nürnberg, Germany); and Berthold Koletzko, Veit Grote, Martina Weber, Peter Rzehak, Sonia Schiess, Jeannette Beyer, Michaela Fritsch, Uschi Handel, Ingrid Pawellek, Sabine Verwied-Jorky, Iris Hannibal, Hans Demmelmair, Gudrun Haile, and Melissa Theurich (Division of Nutritional Medicine and Metabolism, Dr von Hauner Childrens Hospital, University of Munich Medical Centre, Munich, Germany).

The authors' responsibilities were as follows—RC-M, MG, PS, J-PL, and BK: designed the research; MW, VG, JE, ED, EV, and DG: conducted the

research; MW and VG: analyzed the data and wrote the manuscript; and MW, VG, and BK: had primary responsibility for the final content. All authors read and approved the final manuscript. All authors declared no support from any organization for the submitted work, no financial relationships with any organizations that might have an interest in the submitted work in the previous 3 y, and no other relationships or activities that could appear to have influenced the submitted work. The participating company had no decisive role in the conduct and analysis of the study. The formula for the study was produced by Bledina (Villefranche-sur-Saône Cédex, France, part of Danone Baby Nutrition), who operated as a partner of this EU project and received a grant from the EU Commission for this task. No funding bodies had any role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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