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Abstract

Background

In several systematic reviews, rapid weight gain in early life has been related to increased risk of later obesity. In line with this finding, the “early protein hypothesis” suggests that reducing early protein intake is a potential lever for obesity prevention.

Objective

To determine whether the variability of protein content of infant formula used in France over the period 2003-2012 is significantly associated with early growth in children.

Methods

A pooled sample of infants from the EDEN (Etude des Déterminants pré et postnatals de la santé et du développement de l’Enfant) mother-child cohort (born in 2003-2006) and the ELFE (Etude Longitudinale Française depuis l'Enfance) birth cohort (born in 2011) (n_{total}=5 846) was used. Protein content of the infant formula received at 4 months was classified into 5 groups. Associations between protein content (or breastfed status) at 4 months and weight-, length- and BMI-for-age z-scores at 6, 12 and 18 months were analyzed by multivariable linear regression.

Results

This analysis showed a positive association between protein content and weight-, length- and BMI-for-age z-scores at 6 months and only for weight-for-age at 12 months. At 6 months, as compared with the intermediate protein-content group (2.1-2.5 g/100 kcal), infants receiving very-high protein content (>2.8 g/100 kcal) had higher BMI-for-age z-score and those from the very-low protein-content group (<2.0 g/100 kcal) had lower BMI-for-age z-score.

Exclusively breastfed infants had lower length and weight z-scores than formula-fed infants at any age.

Conclusions

Our findings show a positive association, under real conditions of use, between protein contents in infant formula still on the market and weight-, length- and BMI-for-age z-scores from 6 to 18 months.

Introduction

From the developmental origins of health and disease (DOHaD) theory, multiple factors or exposures from preconception through early life are now recognized to affect the risk of later non-communicable diseases, such as obesity or cardiovascular diseases [1]. Rapid weight gain in early life (before 2 years of age) has been related to increased risk of later obesity in many studies assessed in different systematic reviews [2-5] and infant feeding practices may have an important role in these growth trajectories [6].

A recent systematic review suggests that exclusive and longer breastfeeding duration is associated with slower growth rates [7]. This potential effect of breastfeeding on growth might be mediated by the lower protein content found in breast milk [8]. Evidence suggests an effect of the amount of protein in early life on growth, also called the early protein hypothesis [8], put forward by Rolland-Cachera [9]. The hypothesis suggests that high early protein intake stimulates the secretion of insulin and insulin-like growth factor I, which can enhance early growth (more so for ponderal than linear growth), and adipogenic activity, which increases the risk of obesity in later life [10]. In animal studies, high protein supply leads to increased adult body fat deposition and adult weight [10]. The plausibility of this mechanism is reinforced by breast milk featuring lower protein content than infant formula (IF) [11,12] and breastfed infants showing lower early growth than non-breastfed infants [13-15].

Several randomized controlled trials (RCTs) have been conducted to investigate this hypothesis in full-term healthy infants [16]. The European CHOP trial is the largest population-based RCT ever implemented on the topic. It showed that consumption of IF with low protein content (1.77 g/100 kcal) was associated with lower weight, weight-for-length, and BMI up to 2 years (closer to the anthropometric measurements of breastfed infants) as compared with consumption of IF with high protein content (2.90 g/100 kcal) [17]. These results have led to a lowering of the maximum level of protein content allowed in European regulations (3 g/100 kcal to 2.5 g/100 kcal for infant and follow-on formulas) [18,19]. This new regulation officially went into force in February 2020 but has been progressively implemented by the food industry since the publication of the CHOP trial results in 2009.

In this context, the aim of this paper was to determine the association between protein content of IF and child's growth up to 18 months, under real conditions of use, by using pooled data from two birth cohorts launched in France before and after major changes in landscape of formula availability on protein content.

Methods

Study design

The analyses were based on the pooled sample from the Etude des Déterminants pré et postnatals de la santé et du développement de l'Enfant (EDEN) mother-child cohort and the Etude Longitudinale Française depuis l'Enfance (ELFE) birth cohort, both carried out in France.

The EDEN mother-child cohort enrolled 2,002 pregnant women attending their prenatal visit before 24 weeks' gestation at Nancy and Poitiers university hospitals between 2003 and 2006 [20]. Exclusion criteria were multiple pregnancies, diabetes history, French illiteracy, and a planned move outside the region in the next 3 years. Gestational age at birth was not a selection criterion. Informed written consent from the parents was obtained at enrollment, and consent for the child was obtained from both parents after the child's birth. The study received approval from the ethics committee (CCPPRB) of Kremlin Bicêtre on December 12, 2002 and from the Commission Nationale Informatique et Liberté (CNIL), the French data privacy institution.

The study ELFE is a nationwide birth cohort including 18,329 children born in 2011 in a random sample of 349 maternity units from mainland France [21]. Inclusion criteria were singleton or twins born after 33 weeks' gestation to mothers aged ≥ 18 years and not planning to move outside of metropolitan France in the next 3 years. Participating mothers had to provide written consent for their own and their child's participation. Fathers signed the consent form for the child's participation when present at inclusion or were informed about their rights to oppose it. The ELFE study was approved by the Advisory Committee for Treatment of Health Research Information (Comité Consultatif sur le Traitement des Informations pour la Recherche en Santé), the National Data Protection Authority (CNIL), and the National Statistics Council.

Variables

Protein exposition

In the EDEN study, data on infant feeding were collected at 4-, 8- and 12-month follow-ups. From these questionnaires, as previously described [22], any breastfeeding duration and age at IF introduction were calculated. At the same follow-ups, infant diet was assessed by food records on three non-consecutive days (two weekdays and one weekend day) when the infant was not sick [23]. The information on infant diet extracted from these records included daily intake of each food (grams or milliliters), as well as the name and brand of all IF consumed during this period. Nutrient intake was then calculated based on two food composition databases: one specific to ready-prepared baby foods from the 2005 French baby foods industry group (SFAE) (not published) and one for common foods from the 2006 French Observatory of Food Nutritional Quality (CIQUAL) [24]. Protein intake from IF, as well as protein intake from complementary foods (CFs), was calculated only for formula-fed infants because protein intake from breast milk was not assessed in breast-fed infants.

In the ELFE study, data on infant feeding were collected during the face-to-face interview during the maternity stay, by telephone interview at the 2-month and 1-year interview, and by internet/paper questionnaire each month from 3 to 10 months after delivery. Up to 10 months, parents reported at each follow-up the mode of feeding (breast or formula milk), the name and brand of the IF used when relevant [25], the daily number of bottles fed and the average quantity consumed by the infant at each bottle feeding. From these data, any breastfeeding duration and age at IF introduction were also calculated as previously described [26].

In both cohorts, the nutritional composition of all infant or follow-on formulas used within the first year was collected. Those details allow to determine the protein content for each IF, expressed in grams of protein per 100 kilocalories (g/100 kcal) and characterized a given IF. All IF were classified into 5 groups according to their protein content. The 2 extreme groups (<2.0 g/ 100 kcal, >2.8 g/100 kcal) were formed to get as close as possible to the protein content of the IF in the CHOP trial (1.77 g/ 100 kcal and 2.9 g/100 kcal), with sufficient sample size. For the group with the lowest protein content, the cut-off was fixed at 2.0 g/ 100 kcal as the suitability of IF with protein content < 2 g/100 kcal had to be demonstrated in the European regulation rule in force up to 2020. Another cut-off was fixed at 2.5 g/ 100 kcal as this is the upper limit of protein content in the European regulation rule in force from 2020. Finally, IF with intermediate protein content (2.0-2.5 g/ 100 kcal) were divided into two groups with comparable sample size. The 5-category classification was then defined as followed: very-low protein content (<2.0 g/100 kcal), low protein content (2.0-2.1 g/100 kcal), intermediate protein content (2.1-2.5 g/100 kcal), high protein content (2.5-2.8 g/100 kcal), and very high protein content (≥ 2.8 g/100 kcal). The protein content constituted the exposition in the main analysis.

Anthropometric and growth variables

In the EDEN study, at each clinical examination (birth, 1, 3 and 5 years), the child's weight and length were measured. At each follow-up (4, 8 and 12 months, 2, 3, 4 and 5 years), weight and length data were collected from self-administered questionnaires and clinical visits when reported by health professionals in the child's health booklet.

In the ELFE study, birth anthropometric measurements were collected from the pediatric medical file. After discharge from the maternity ward, the child's weight and length were collected during phone interviews. During the 2-month interview, parents were asked to report the measurements indicated in the child's health booklet by health professionals for the first and second medical appointments after birth. During the 12- and 24-month interviews, parents reported the measurements indicated in the child's health booklet for the 4-month and 9-month medical appointments. During the 24-month interview, they reported any measurements indicated in the child's health booklet that occurred between 9 and 16 months and between 17 and 24 months.

In both cohorts, individual growth curves for weight and length were predicted by using the Jenss growth curve model as previously described [27]. This method allows for calculating parameters for individual growth patterns, such as weight, length and thus body mass index (BMI). Then z-scores for these predicted values were calculated, based on French standards [28]. In the present study, we used the predicted anthropometric values and French z-scores at 1, 6, 12 and 18 months.

Other variables

In both cohorts, at baseline, we collected paternal BMI (kg/m^2) and maternal pre-pregnancy BMI (kg/m^2), maternal age (years), maternal education level (less than high school diploma, high school diploma, 2-year university degree, at least 3-year university degree), parity (first child, second child or more), maternal smoking during pregnancy (non-smoker, smoker), child's sex (girl, boy), gestational age (weeks), region of residence, size of maternity unit.

Statistical analyses

Comparisons between excluded and included subjects were conducted with chi-square tests for categorical variables and student tests for continuous variables. Exposure to protein content of IF was considered as a 5-category variable to facilitate comparison with RCT data and also as a continuous variable. Unlike in RCTs, in real conditions of use as in cohort study, an infant may be exposed to several protein contents of IF along with IF changes. Using monthly repeated data between age 2 and 10 months for the ELFE study, we assessed the stability of exposure to IF protein content throughout the first year. We used the group-based trajectory method [29] to identify longitudinal trajectories considering all infants with at least two IF protein contents available regardless of the main milk feeding (n=8,724) (PROC TRAJ procedure, SAS software). Four trajectories of IF protein content were identified from age 2 to 10 months (Figure S1): low trajectory (1.85 to 1.95 g/100 kcal) with 28.6% of the population, intermediate trajectory (2.05 g/100 kcal at 2 months to 2.15 g/100 kcal at 10 months) with 52.7% of the population, high trajectory (2.35 to 2.45 g/100 kcal) with 8.9% of the population, and ascendant trajectory (2.10 g/100 kcal at 2 months to 2.75 g/100 kcal at 9 months) with 9.8% of the population. More than 90% of the population used IF with a relatively stable protein content between 2 and 10 months.

These preliminary analyses supported the choice to consider protein content of IF at a given month as a proxy for protein content of IF during first months of life. We considered the 4-month IF protein content because it was the earliest common value for IF protein content in both cohorts. Associations between IF protein content at 4 months and growth at 6, 12 and 18 months were tested by multivariable linear regressions. Potential confounding factors were identified from the literature and selected with the Direct Acyclic Graph method: all models were adjusted for parental characteristics (maternal age, maternal education level, parity, smoking status, pre-pregnancy maternal BMI, paternal BMI) and infant characteristics (sex, the studied growth parameter at 1 month). All models were also adjusted for variables related to study design (region of residence, size of maternity unit).

Secondary analysis considered IF protein content as a linear variable.

All analyses were performed with SAS v9.4 [30]. $P<0.05$ was considered statistically significant.

Sensitivity analyses

A sensitivity analysis was run after excluding infants born small for gestational age (SGA) or large for gestational age (LGA) (n=4,813). We hypothesized that because of their different ante-natal growths, SGA and LGA infants may have also different post-natal growths.

Results

Participants

Among the 2,002 infants included in the EDEN study and the 18,329 infants included in the ELFE study, infants born pre-term or from multiple pregnancies were not considered in the present study because of specific issues regarding both diet and post-natal growth (Figure 1). To have similar inclusion criteria as in the CHOP trial, we included in our formula-fed group only infants who had been breastfed for < 1 month, and in our breast-fed group those who consumed no formula milk up to age 3 months. Of the latter, infant without data on post-natal growth, so as those without enough details on the IF used at 4 months or with missing data on confounding factors were also excluded, thus leading to a final sample of 5,846 infants: 2,574 formula-fed and 3,272 breast-fed infants.

Population description

The characteristics of the population are presented in Table 1. Infants exclusively breastfed at 3 months represented 56.0% of our population. Among formula-fed infants (n=2,574, 44.0%), the mean IF protein content was 2.1 (0.2) g/100 kcal.

As compared with infants included in the present analysis, those excluded were born to younger mothers (30 vs 31 years, p<0.001), who were more frequently overweight (19.5% vs 15.8%, p<0.001) or obese (12.2% vs 8.1%, p<0.001), smoked during pregnancy (27.0% vs 15.8%, p<0.001), and had a lower education level (26.8% vs 46.0% with at least a 3-year university degree, p<0.001). No difference was observed for maternal parity (p=0.26).

Temporal evolution of protein content in IF between EDEN and ELFE

The distribution of IF protein content consumed at age 4 months differed between the two cohorts (Table 1). We found a decrease in IF protein content from the EDEN study (2003-2006, mean [SD] content 2.4 [0.3] g/100 kcal) to the ELFE study (2011, mean [SD] content 2.0 [0.2] g/100 kcal). During 2003-2006 (EDEN study), more than 40% of infants received IF with > 2.5 g/100 kcal protein, whereas less than 4% of infants in 2011 (ELFE study) received this category of IF. Conversely, more than 75% of infants received IF with < 2.1 g/100 kcal protein in the ELFE study as compared with about 25% in the EDEN study.

IF protein content and early growth

In general, IF protein content was positively related to weight-for-age z-score at 6, 12 and 18 months among exclusively formula-fed infants (Figure 2 and Table 2). At 18 months, the association was not significant when IF protein content was considered as a 5-category variable (p=0.12), but we observed a linear trend, and the association was highly significant when IF protein content was considered as a continuous variable (p<0.001). At all ages, exclusively breastfed infants had lower adjusted weight-for-age z-score (Figure 2).

Among exclusively formula-fed infants, adjusted length-for-age z-score at age 6 months was lower for those fed IF with high or very-high protein content than those fed with very-low to intermediate protein content (Figure 2) (p=0.02). When IF protein content was considered as a continuous variable, the association was marginally significant (β [95% CI] -0.08 [-0.16;0.00], p=0.05). The association was reversed at ages 12 and 18 months, even if it was significant only when considering IF protein content as a continuous variable (0.11 [0.00;0.22], p=0.04, and 0.12 [0.00;0.24], p=0.04, respectively). At all ages, exclusively breastfed infants had lower adjusted length-for-age z-score.

We found significant associations with IF protein content as a categorical variable only at 6 months (p<0.001) (Figure 2). IF protein content was positively associated with BMI-for-age z-scores at 6, 12 and 18 months when protein content was considered continuous (Table 2). Infants exclusively breastfed were significantly different at 6 and 12-months.

Sensitivity analyses

The sensitivity analysis excluding SGA and LGA infants showed similar results as the main analysis (Figure S2).

Discussion

This analysis allowed for highlighting the shift that took place between the two cohorts (2003-2006 vs 2011), with a decrease in IF protein content (2.4 to 2.0 g/100 kcal). This pooled analysis showed a positive association between IF protein content consumed at 4 months and weight- and BMI-for-age z-scores at 6, 12 and 18 months. Associations between length-for-age and protein content were less consistent. Weight-, length- and BMI-for-age z-scores were lower for exclusively breastfed infants than exclusively formula-fed infants, even those from the very-low protein-content group, except for BMI at 18 months.

Much of the evidence for the influence of protein content in infant or follow-on formulas is from RCTs. Most RCTs investigated preterm or low-birth-weight infants [31,32]. Among those carried out in healthy full-term infants [16,17,33,34], the CHOP trial, which was the largest, compared two formulas with different protein contents (low protein content: 1.77 g/100 kcal for infant formula and 2.2 g/100 kcal for follow-on formula, vs high protein content: 2.9 and 4.4 g/100 kcal). Infants were recruited during the first 8 weeks of life in 5 European countries. Formula-fed infants (n=1,090) had to be breastfed for < 8 weeks, and breastfed infants (n=589) had to be exclusively breastfed for the first 3 months of life. A positive association was highlighted between high protein content and weight, weight-for-length and BMI up to 2 years of life [17], greater fat mass at age 2 years and excessive body fat at age 6 years [35], and higher BMI and greater risk of becoming obese at 6 years [36]. Our observational findings agree with those from this large RCT. In contrast to the CHOP trial, the BeMIM trial found higher weight-for-length z-scores in infants fed a formula with low protein content (1.89 g/100 kcal) as compared with infants fed 2.2 g/100 kcal protein during the intervention time (up to 120 days) [33], but no difference was observed at age 4 years [37]. However, the range of protein content considered in this trial was lower than in the CHOP trial or in our study. Moreover, the formula with reduced protein content was also enriched in long-chain polyunsaturated fatty acids, so a formal comparison between the two IFs is impossible. In a review conducted in 2015 [16], only RCTs with large differences in protein content (65-70% difference) found discrepancies in growth. If lowering IF protein content is promising for the prevention of overweight, more studies are needed to assess the long-term effects [38].

A few cohort studies investigated the effect of early protein intake and growth in childhood, and most considered protein intake from complementary food at later ages [39,40]. The generation R cohort [39] highlighted a positive association between protein intake at 1 year, assessed by a food-frequency questionnaire, and BMI at 6 years. The association appeared to be driven by fat mass index more than a fat-free mass index and was stronger in girls than boys. Similar findings were found in the DOrtmund Nutritional and Longitudinally Designed (DONALD) study [40], examining daily protein intake from 6 to 24 months and BMI or fat mass at age 7 years. In this study, the association was significant only when considering protein intake from 12 months (and not 6 months). We found no association between protein intake from CF and anthropometric variables, which may be explained because CF at 4-months contributes little to total protein intakes.

This pooled analysis, based on two birth cohorts, offered a unique opportunity to examine a large range of protein content in real conditions of use. In fact, the descriptive statistics underlined a shift in the distribution of IF protein content following the publication of the results from the CHOP trial, even if the new regulation had not yet been officially implemented. In both cohorts, the prospective design limited memory bias, and the monthly data collection in the ELFE study allowed for showing that for most infants, the IF protein content was stable throughout the first year. Therefore, although we only considered the IF reported at age 4 months in the pooled analysis, we assumed that it was a good indicator of the IF protein content throughout the first year. Moreover, the combined analysis between the ELFE and EDEN studies represents a large sample size. However, we were not enabled to investigate the cohort effect because the distribution of protein contents was very correlated to the cohort. Although this was an observational study with structural limitations in causal inference analysis, the opportunity to consider several confounding factors simultaneously and the prospective data collection confirm some results and add nuances and details.

Our findings confirmed the positive association between IF protein content and weight- and BMI-for-age z-scores up to 18 months. Among formula-fed infants, the lowest protein-content group had the lowest anthropometric z-scores, although they remaining higher than those for breastfed infants. These results between protein content and child’s growth should not lose sight of well-known risk factors of sub-optimal growth, such as family socio-economic position and maternal smoking [41-43].

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Conflicts of interest statement

The authors declare no competing financial interests.

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A.C. conceptualized and designed the study, conducted part of the statistical analyses, drafted the initial manuscript, and approved the final manuscript as submitted, agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

C.D.-P., P.S., S.N., B.H., M.-A.C., B.d.L.-G designed the data collection instruments, supervised data collection and data management, conceptualized and designed the study, contributed to the interpretation of the study, reviewed and revised the manuscript, approved the final manuscript as submitted, agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Table 1: Study sample characteristics.

	Pooled sample (n=5846)	EDEN (n=469)	ELFE (n=5377)
Maternal characteristics			
Age at delivery, years	31 (5)	30 (5)	32 (5)
Education level			
Less than high school diploma	4.2% (247)	26.9% (126)	2.3% (121)
High school diploma	25.7% (1501)	19.4% (91)	26.2% (1410)
2-year university degree	24.1% (1410)	22.6% (106)	24.3% (1304)
At least 3-year university degree	46% (2688)	31.1% (146)	47.3% (2542)
Smoker during pregnancy	15.8% (922)	25.2% (118)	15% (804)
Primiparous	43.7% (2554)	61% (286)	42.2% (2268)
BMI before pregnancy			
<18.5 kg/m²	6.6% (385)	6% (28)	6.6% (357)
18.5-24.9 kg/m²	69.5% (4064)	65.7% (308)	69.9% (3756)
25-29.9 kg/m²	15.8% (922)	19.8% (93)	15.4% (829)
≥ 30 kg/m²	8.1% (475)	8.5% (40)	8.1% (435)
Child's characteristics			
Weight-for-gestational-age at birth			
Small for gestational age	7.3% (420)	7.9% (37)	7.2% (383)
Adequate for gestational age	83.2% (4813)	84.7% (397)	83.1% (4416)
Large for gestational age	9.5% (551)	7.5% (35)	9.7% (516)
Exclusively breastfed	56.0% (3272)	24.1% (113)	58.8% (3159)
Formula-fed infants	44.0% (2574)	75.9% (356)	41.2% (2218)
Mean weight at 1 month, g	4238 (438)	4 071 (481)	4 253 (431)
Mean length at 1 month, mm	538 (18)	534 (22)	539 (17)
Specific formula-fed infant's characteristics			
Protein content of the IF, g/100 kcal (n=2,574)	2.1 (0.2)	2.4 (0.3)	2.0 (0.2)
IF with very-low protein content (<2.0 g/100 kcal)	15.6% (910)	10.4% (37)	39.4% (873)
IF with low protein content (2.0-2.1 g/100 kcal)	15.2% (886)	14.9% (53)	37.6% (833)
IF with intermediate protein content (2.1-2.5 g/100 kcal)	9.4% (548)	32.6% (116)	19.5% (432)
IF with high IF protein content (2.5-2.8 g/100 kcal)	3.2% (189)	31.2% (111)	3.5% (78)
IF with very-high protein content (> 2.8 g/100 kcal)	0.7% (41)	11.0% (39)	< 0.1% (2)

Values are % (n) or mean (SD); BMI, body mass index; IF, infant formula.

Table 2. Adjusted associations between 4-month formula protein content and growth parameters from 6 to 18 months, among formula-fed infants (n=2,574).

	Weight-for-age z-score			Length-for-age z-score			BMI-for-age z-score		
	6 months	12 months	18 months	6 months	12 months	18 months	6 months	12 months	18 months
Protein content (g/100 kcal)	0.14 [0.05;0.23] p=0.002	0.21 [0.10 ;0.33] p<0.001	0.21 [0.09;0.33] p<0.001	-0.08 [-0.16;0.00] p=0.05	0.11 [0.00;0.22] p=0.04	0.12 [0.00;0.24] p=0.05	0.25 [0.16;0.34] p<0.001	0.19 [0.07;0.32] p=0.003	0.18 [0.05;0.32] p=0.009

Values are β [95% confidence interval] adjusted for maternal age, maternal education level, parity, smoking status, pre-pregnancy maternal and paternal BMI, infant's sex, the studied growth parameter at 1-month, and study design variables (living region and maternity size).

Figure 1. Study flowchart.

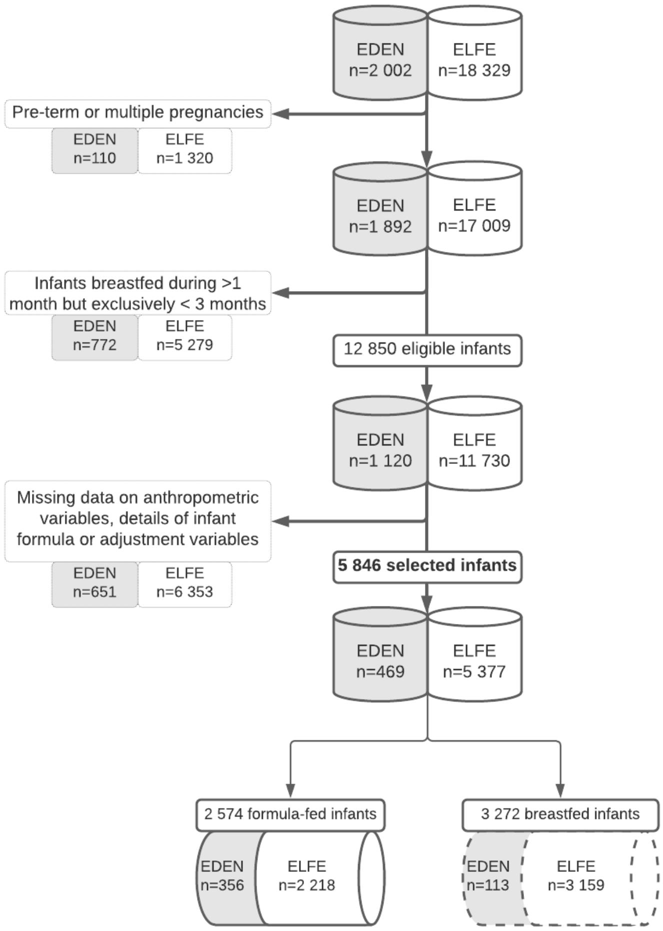
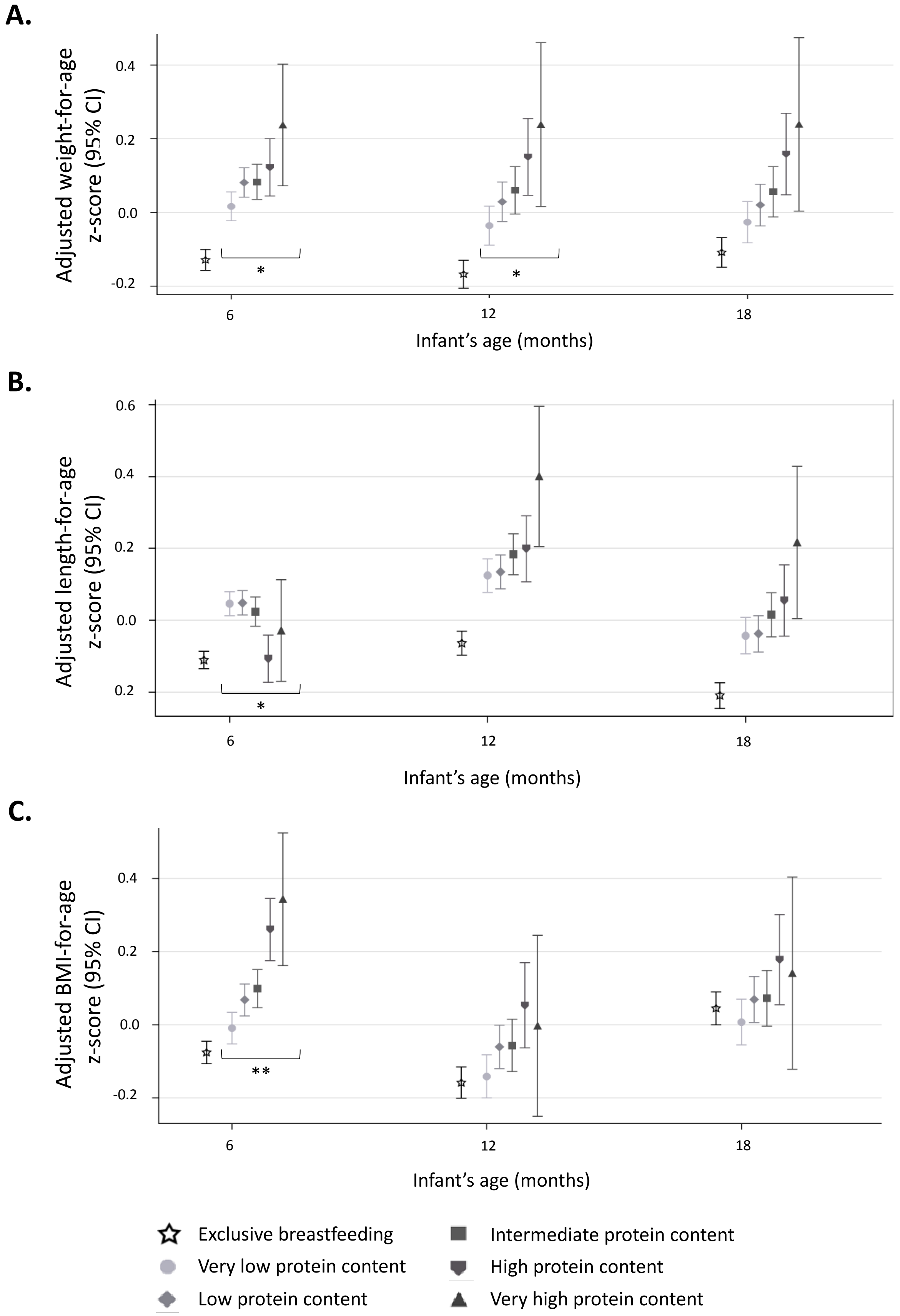


Figure 2. Adjusted means of weight-for-age (part A), length-for-age (part B) and BMI-for-age (part C) z-scores according to 4-month feeding groups (n=5 846).



Legend

Exclusively breastfed infants represented by a star (n=3,272), formula-fed infants (2,574): very-low protein-content group represented by a circle (n=910), low protein-content group represented by a rhombus (n=886), intermediate protein-content group represented by a square (n=548), high protein-content group represented by a pentagon (n=189), very-high protein-content group represented by a triangle (n=41).

Values are means (95% CI) adjusted for maternal age, maternal education level, parity, smoking status, pre-pregnancy maternal and paternal BMI, infant's sex, the studied parameter at 1-month, and study design variables (living region and maternity size). Differences across formula-fed groups were tested by linear regressions. *p<0.05, **p<0.001.