Original Article
PEDIATRIC OBESITY

Obesity

Intake of Energy and Protein is Associated with Overweight Risk at Age 5.5 Years: Results from the Prospective TEDDY Study

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Objective: The associations of energy, protein, carbohydrate, and fat intake with weight status up to the age of 5.5 years were prospectively assessed in The Environmental Determinants of Diabetes in the Young (TEDDY) study.

Methods: Food record data (over 3 days) and BMI measurements between 0.25 and 5.5 years were available from 5,563 children with an increased genetic risk for type 1 diabetes followed from shortly after birth. Odds ratios (ORs) were calculated for overweight and obesity by previous intake of energy, protein, carbohydrate, and fat with adjustment for potential confounders.

Results: Having overweight or obesity at the age of 5.5 years was positively associated with mean energy intake in previous age intervals (e.g., adjusted OR [95% CI] for overweight: 1.06 [1.04-1.09] per 100 kcal intake at the age of 4.5-5.0 years) and with protein intake after the age of 3.5 and 4.5 years, respectively (e.g., adjusted OR for overweight: 1.06 [1.03-1.09] per 1% of energy intake at the age of 4.5-5.0 years). The respective associations with carbohydrate and fat intake were less consistent.

Conclusions: These findings indicate that energy and protein intake are positively associated with increased risk for overweight in childhood but yield no evidence for potential programming effects of protein intake in infancy.

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Introduction

The prevalence of childhood overweight has been increasing worldwide in recent decades (1). This temporal trend is alarming because overweight in childhood is associated with other long-term health consequences such as overweight, obesity, metabolic syndrome, and cardiovascular disease in adulthood (2). Besides physical activity, nutrition in the first years of life is likely to be a key modifiable factor to prevent both short- and long-term obesity (3). Children's environments at this age are largely under their

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parents' control and are likely to have long-term effects on their risk of overweight (4).

However, there is relatively little evidence on the potential impact of energy and macronutrient intake during childhood on BMI. While it appears likely that greater energy intake is a potential risk factor of overweight (5,6), much less is known about early fat, carbohydrate, and protein intake. Low fat intake has been found to be associated with weight reduction in randomized trials but not consistently in cohort studies (7). Intake of sugar-sweetened beverages seemed to increase the risk of overweight in childhood (8), but studies that investigated the proportion of carbohydrate intake in relation to total energy intake did not report an increased risk of overweight by a greater carbohydrate intake (9,10). There is also limited evidence for a long-term programming effect of a greater protein intake during or after weaning age on overweight later in childhood. A large clinical trial indicated that the consumption of an infant formula with a low content of protein may reduce obesity risk at the age of 6 years (11), but the results from other trials on infant formulas with different protein concentrations were less conclusive (12). A recent twin study suggested that a greater protein intake at the age of 21 months may be associated with increased BMI at the age of 3 to 5 years but found no significant associations with overweight or obesity at these ages (13). Recent observational studies have reported positive associations between childhood overweight and protein intake at different ages (9,14,15).

We investigated the hypotheses that early energy and macronutrient intake is associated with subsequent weight status from shortly after birth up to the age of 5.5 years. For this purpose, we analyzed data from a large, well-characterized, and contemporary prospective cohort study that had not previously been investigated in this context. The use of time-varying covariates and quantile regression allowed us to gain deeper insight into these associations. Further, we investigated potential gene-environment associations using several single-nucleotide polymorphisms (SNPs) that had previously been associated with childhood overweight.

Methods

The Environmental Determinants of Diabetes in the Young (TEDDY) study is an ongoing prospective cohort study funded by the National Institutes of Health with the primary goal of identifying environmental causes of type 1 diabetes. The TEDDY study screened 424,788 newborns for type 1 diabetes-associated human leukocyte antigen (HLA) genotypes between 2004 and 2010 (16), and of these children, 8,676 were enrolled and followed up in six clinical research centers located in the United States, Finland, Germany, and Sweden. Detailed information on study design, eligibility, and methods has been previously published (16-18). Written informed consent was obtained separately for genetic screening and for participation in prospective follow-up for all participants from a parent or primary caretaker. The study was approved by local institutional review boards and has been monitored by an external evaluation committee formed by the National Institutes of Health.

Genotyping

Blood samples of newborn children were obtained in the maternity clinics either as cord blood or dry blood spots. HLA screening was performed by using either a dried blood spot punch or a small volume of whole blood. SNP analysis was performed using the Illumina Immunochip (Illumina Inc., San Diego, California) (19).

Assessment of child's height and weight

Each participant's height and weight were obtained at TEDDY clinics by trained TEDDY personnel at each visit. Clinic visits took place every 3 months between 3 and 48 months of age and biannually thereafter. Each child's height was measured as length before the age of 2 years and as standing height to the nearest 0.1 cm from the age of 2 years using a wall-mounted stadiometer. Body weight was measured in kilograms using regularly calibrated electronic scales. For subjects who could not attend a clinic visit, anthropometric data were copied from their pediatricians' records collected near the TEDDY clinic visit date.

Assessment of diet

The first dietary assessment from children's primary caretakers was carried out by 24-hour recall at the age of 3 months and by 3-day food record every 3 months until 12 months of age, after which dietary assessments were made every 6 months. Every participating family was instructed to keep a consecutive 3-day record of their child's food consumption, ideally including two weekdays and one weekend day. To facilitate the completion of food records, TEDDY staff provided written instructions and examples on how to indicate meal time, meal location, adequate description of foods and beverages, quantity of intake, and use of dietary supplements. Separate food record forms were provided for day cares and for schools that included specific instructions for the personnel on how to record the food consumption of each child during the day. Parents reviewed these records and completed any missing information (e.g., type, amount) by interviewing the caregiver or teacher. To facilitate recalling and recording of infant formula types, the families were provided with a booklet containing pictures of infant formulas available on the market in each country.

Additionally, TEDDY developed a food portion size booklet that contained colorful pictorial illustrations of multi-ingredient composite dishes and black and white shapes and scales to facilitate portion size estimation. In Germany, parents weighed food when keeping the records; the food portion booklet was only used in addition. To calculate the amount of breast milk, the method proposed by the Institute of Medicine based on the age and weight of the child was applied (20).

The records were entered into country-specific food record databases by trained personnel to assess intake of various nutrients. Each country analyzed food records separately to assess the energy and nutrient intake of the children at various ages. The four country-specific food composition databases have been harmonized for the TEDDY study (21). After the nutrient values were calculated, the nutrition coordinator in each country estimated the plausible intake value of nutrients based on the overall distribution of nutrients.

Once the intake amount was verified, the final data were electronically submitted to the data coordinating center in Tampa, Florida, where an inbuilt data error notification system flagged any nutrient value that exceeded the set limits. This data error system automatically notified the clinical center about the suspected error, and the

center then had to either correct the information in the food record or verify it in order to get the data accepted. The electronically submitted final data, including the energy and nutrient intake per child and per age from each country, were merged together into one data set in the data coordinating center in Tampa. The TEDDY study did not provide any recommendations or advice on infant feeding or childhood diet to the families.

Assessment of clinical and sociodemographic covariates

Maternal factors such as maternal age, prepregnancy BMI, gestational weight gain, education, smoking or alcohol intake during pregnancy, and maternal diabetes, as well as the child's birth weight, were obtained by either questionnaires or structured interviews during one of the follow-up visits in the first year of the study. To assess the duration of breastfeeding, families were asked to record the age at weaning and the age at introduction of all new foods in a specific booklet given to the parents at the study entry.

Data transformations

Each child's BMI was calculated as weight (kilograms) divided by height (meters) squared and transformed to standard deviation scores (SDS) using World Health Organization reference values (22,23), which were also used to define overweight (including obesity; BMI SDS >1) and obesity (BMI SDS >2). BMI SDS values below -5 or greater than 5 were deemed implausible and excluded. BMI at the age of 5.5 years was defined as BMI assessed at the 66-month visit, if available, or at the next closest visit between the ages of 54 and 78 months. The intakes of protein, carbohydrate, and fat were calculated as percentages of the total energy (E%). We then calculated mean intake of energy, protein, carbohydrate, and fat at all consecutive visits within 1 year; e.g., intake at the age of 0.25 to 1.0 years was defined as mean intake at the age of 3 to 12 months and intake at 4.5 to 5.0 years was defined as mean intake at the age of 54 and 60 months, respectively. Gestational weight gain was classified as inadequate, adequate, or excessive according to Institute of Medicine guidelines for total weight gain during pregnancy (24). As in a previous analysis of the TEDDY data (25), children's birth weight was transformed to a z score adjusting for country, sex, gestational age, birth type (singleton or multiplet), and maternal height.

Statistical analyses

From the 8,676 children enrolled in the TEDDY study, data for 5,563 children were available for this analysis (Figure 1). Exclusions applied to children whose follow-up lasted for less than 5 years or who had missing or implausible BMI SDS values at the age of 5.5 years. To assess potential attrition bias, included and excluded participants were compared with respect to covariates using χ^2 tests. We calculated odds ratios (ORs) with respective 95% CIs for overweight and obesity at the age of 5.5 years by intake of energy, protein, carbohydrate, and fat at previous ages using separate logistic regression models for each predictor variable and age. Additionally, we determined the age of incident overweight/obesity as the age when overweight/obesity persisting until the age of 5.5 years first occurred. For example, if a child first developed overweight at 4 years of age and the overweight persisted until 5.5 years, the incidence age was set to 4 years, irrespective of whether the child had already had intermittent overweight at an earlier age. We assessed

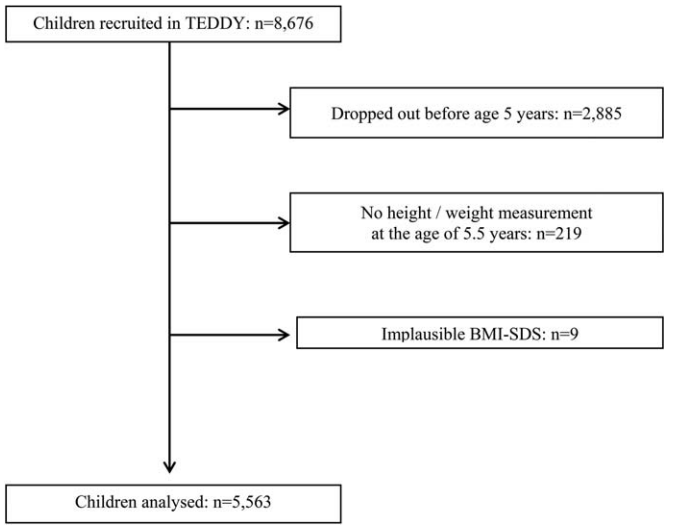

Figure 1 Flowchart of children analyzed.

short-term associations of energy, protein, carbohydrate, and fat intake at each visit with incident overweight and obesity at each following visit using logistic regression with time-varying predictors. For this analysis, we excluded all visits when children were found to have only intermittent overweight/obesity. All models were calculated both unadjusted and adjusted for the potential confounders of sex, country, birth weight z score, maternal age, maternal prepregnancy BMI, gestational weight gain rate, maternal diabetes (yes/no), maternal smoking in pregnancy (yes/no), maternal alcohol intake in pregnancy (any/none), maternal education (high school or lower/more than high school), and duration of any breastfeeding (less/more than 6 months). We further assessed percentile-specific associations between energy, protein, carbohydrate, and fat intake at the age of 4.5 to 5.0 years and BMI SDS at the age of 5.5 years using quantile regression (26). We used interaction terms to explore potential effect modifications of intake at this age with the following genotypes/SNPs, which had previously been found to be associated with childhood overweight risk (25,27,28): HLA-DQ 2/2, rs9939609 (in/near to FTO); rs17782313 (MC4R), rs6548238 (TMEM18), rs10938397 (GNPDA2), rs368794 (KCTD15), rs2568958 (NEGR1), rs925946 (BDNF), rs7647305 (ETV5), rs1421085 (FTO), and rs987237 (TFAP2B). In a sensitivity analysis, we excluded all children who did not have a weight and height measurement between the ages of 63 and 69 months or who had already developed type 1 diabetes at the time of the weight and height measurement that was used to define BMI SDS at the age of 5.5 years, in order to investigate potential bias by reverse causation or potential weight loss due to type 1 diabetes.

For all analyses, the significance level was set to 0.05, with the exception of the genetic interaction analyses, for which we set the significance level to $0.05/11 = 0.0045$ to account for multiple testing by the number of investigated genotypes/SNPs. All calculations were carried out with SAS 9.4 (SAS Institute Inc. Cary, North Carolina) and R-3.3.1 (R Foundation for Statistical Computing, Vienna, Austria).

TABLE 1 Description of the study population ($n = 5{,}563$)

	n missing	*n* (%)/ median (IQR)
BMI SDS at the age of 5.5 y	–	0.28 (−0.31 to 0.92)
Has overweight at the age of 5.5 y	–	1,253 (22.5%)
Has obesity at the age of 5.5 y	–	337 (6.1%)
Males	–	2,862 (51.5%)
Country		
United States	–	2,104 (37.8%)
Finland	–	1,301 (23.4%)
Germany	–	307 (5.5%)
Sweden	–	1,851 (33.3%)
Birth weight (g)	127	3,525 (3175-3860)
Maternal age at birth of child (y)	–	31.0 (28.0-34.0)
Maternal prepregnancy BMI (kg/m^2)	91	23.5 (21.3-27.0)
High maternal education (high school)	92	4,532 (82.8%)
Breastfeeding $\geq$ 6 months	–	3,603 (64.8%)
Excessive total gestational weight gain (according to Institute of Medicine guidelines) (21)	126	2,519 (46.3%)
Maternal diabetes (yes)	172	571 (10.6%)
Maternal smoking during pregnancy (yes)	47	541 (9.8%)
Maternal alcohol intake during pregnancy (yes)	45	1,911 (34.6%)

Values are reported as *n* (% of nonmissing observations) for categorical variables and median (IQR) for continuous variables.
IQR, interquartile range.

Results

Children included in the analyses had a median BMI SDS of 0.28 (interquartile range: 0.31-0.92) at the age of 5.5 years. In our data set, 1,253 children (22.5%) were classified as having overweight (including obesity), and 337 (6.1%) were classified as having obesity at the age of 5.5 years (Table 1). The respective weight and height measurements had been recorded at a median age of 66 (interquartile range: 65-67) months. Not having a BMI SDS measurement at the age of 5.5 years due to loss to follow-up or missing/implausible BMI values was significantly associated with being female ($P = 0.03$), country of residence (only 21% of the Finnish children were excluded compared to 40% of the US children; $P < 0.01$), maternal smoking ($P < 0.01$), and low maternal education ($P < 0.01$), but not with maternal alcohol intake ($P = 0.11$) or prepregnancy overweight (BMI > 25 kg/m^2; $P = 0.23$). Of all children who had overweight/obesity at the age of 5.5 years, 44% and 63% already had persistent overweight from the ages of 2 and 4 years, and 16% and 38% had persistent obesity (Supporting Information Figure S1).

Mean energy intake increased steadily by age, while, relative to E%, protein and carbohydrate intake increased and mean fat intake decreased until the age of 18 months and remained relatively stable thereafter (Supporting Information Table S1). Compared to children with normal weight, subjects who had overweight at the age of 5.5 years had slightly higher mean values of protein intake from the age of 3.5 years onwards and of energy intake over the whole observation period, while there seemed to be no obvious differences for carbohydrate and fat intake.

Accordingly, overweight and obesity at the age of 5.5 years were positively associated with mean energy intake in previous age intervals, particularly during the first year of life. For example, the adjusted ORs (95% CIs) for overweight were 1.47 (1.37-1.58) and 1.06 (1.04-1.09) per 100 kcal intake at the ages of 0.25 to 1.0 and 4.5 to 5.0 years, indicating that an increase in energy intake by 100 kcal elevated the risk for overweight by 47% and 6%, respectively. Increased risks of overweight and obesity were also observed with respect to a greater protein intake after the ages of 3.5 and 4.5 years, respectively, but not at earlier ages (e.g., adjusted OR for overweight: 1.06 [1.03-1.09] per 1% of energy intake at the age of 4.5-5.0 years, Figure 2). The respective associations with carbohydrate and fat intake across ages of intake were less consistent, with significantly increased ORs for obesity (but not overweight) at the age of 5.5 years that correlated to greater fat intake at 2.5 to 3.0 and 3.5 to 4.0 years only and significantly decreased ORs that correlated to a greater carbohydrate intake at the same ages. The observed associations did not change considerably when the data were restricted to those subjects who had a weight and height measurement between the ages of 63 and 69 months and had not already developed type 1 diabetes at the age of 5.5 years (data not shown). Energy and protein intake were also positively associated with both incident overweight and obesity risk in analyses using time-varying predictors (e.g., adjusted ORs for overweight: 1.06 [1.04-1.08] per 100 kcal energy intake and 1.03 [1.02-1.05] per E% protein intake), while carbohydrate and fat intake were not (Table 2).

There was no clear pattern of increasing or decreasing quantile regression estimates across the investigated BMI SDS percentiles at the age of 5.5 years with respect to energy, protein, carbohydrate, or fat intake at the age of 4.5 to 5.0 years, with almost all 95% CIs covering the linear regression estimates (Figure 3), thus not indicating considerable percentile-specific associations. There were also no significant interactions between energy, protein, carbohydrate, or fat intake at age 4.5 to

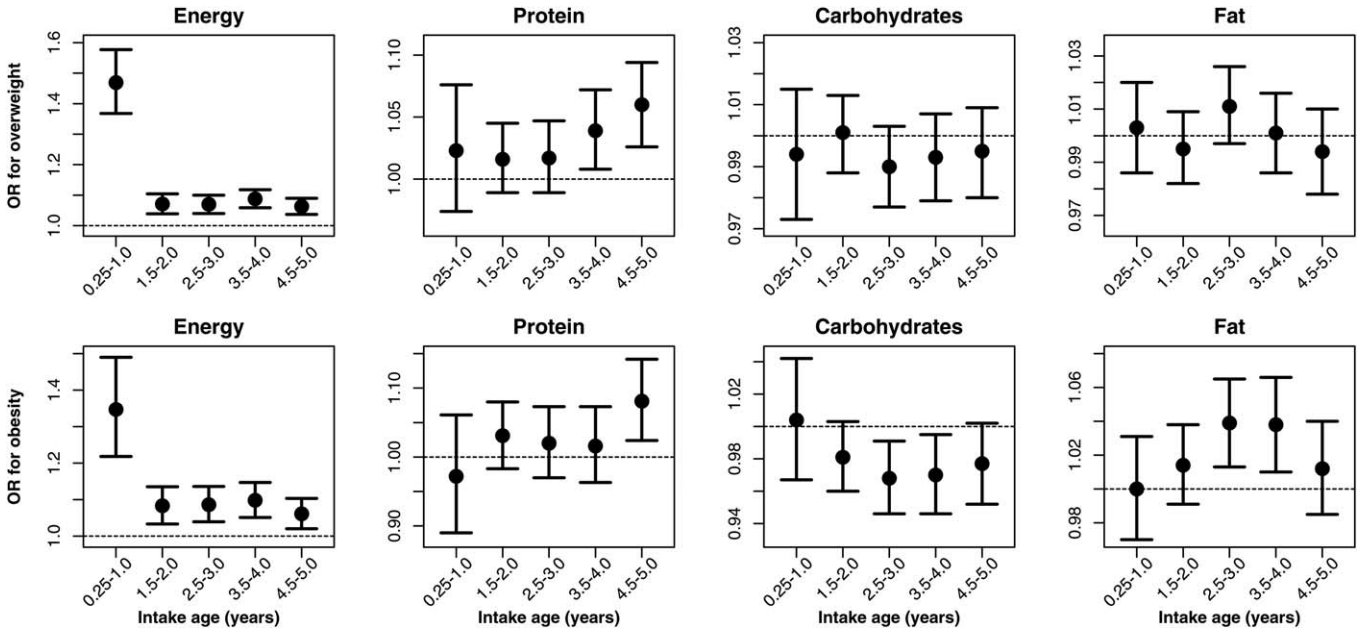

Figure 2 ORs and 95% CIs of overweight and obesity at the age of 5.5 years by age of intake of energy (per kcal/d), protein, carbohydrate, and fat (per 1% of energy intake), adjusted for sex, country, birth weight, maternal age, maternal prepregnancy BMI, gestational weight gain, maternal diabetes, maternal smoking in pregnancy, maternal alcohol intake in pregnancy, maternal education, and duration of breastfeeding. The dashed horizontal lines depict 1.00 as reference.

5.0 years and any of the available overweight risk genotypes/SNPs with respect to overweight at 5.5 years (data not shown).

Discussion

These analyses show that protein intake is positively associated with increased risk for overweight within up to 1 or 2 years but did not yield evidence for early programming effects of a greater protein intake in early infancy with respect to overweight/obesity at the age of 5.5 years. In contrast, we observed that a greater energy intake at any previous age was associated with increased risk for overweight at 5.5 years. We found no consistent associations with respect to carbohydrate and fat intake.

Our findings confirm previous studies that have shown that energy intake is positively associated with higher BMI and overweight risk (29,30). Interestingly, we observed that BMI at the age of 5.5 years was associated with protein intake around the age of 4 to 5 years but not at earlier ages. Further, we observed that the incidence of overweight/obesity persisting until the age of 5.5 years was positively associated with intake of protein about 3 to 6 months prior. Our findings, therefore, seem to indicate that a greater protein intake may be relevant for both initiating and maintaining overweight in children. However, we found no evidence for potential programming effects by protein intake in very early life as suggested by other studies (11,31,32). Instead, a greater energy intake during the first year of life was found to be a strong predictor of overweight at the age of 5.5 years in our data. The OR for overweight by energy

TABLE 2 ORs (95% CIs) for incident overweight/obesity by intake of energy (per 100 kcal/d), protein, carbohydrate, and fat (each per 1% of energy intake) within 6 months after each diet record, modeled as time-varying predictors

	Energy intake		Protein intake		Carbohydrate intake		Fat intake	
Outcome	Unadjusted model	Adjusted model	Unadjusted model	Adjusted model	Unadjusted model	Adjusted model	Unadjusted model	Adjusted model
Overweight	1.06 (1.04-1.07)	1.06 (1.04-1.08)	1.03 (1.01-1.04)	1.03 (1.02-1.05)	0.99 (0.98-1.002)	0.99 (0.98-1.002)	1.00 (0.99-1.01)	1.00 (0.99-1.01)
Obesity	1.14 (1.12-1.17)	1.15 (1.12-1.18)	1.12 (1.08-1.15)	1.12 (1.08-1.16)	1.00 (0.98-1.02)	1.00 (0.98-1.02)	0.97 (0.95-0.98)	0.97 (0.95-0.98)

Models were calculated with and without adjustment for sex, country, birth weight, maternal age, maternal prepregnancy BMI, gestational weight gain, maternal diabetes, maternal smoking in pregnancy, maternal alcohol intake in pregnancy, maternal education, and duration of breastfeeding.

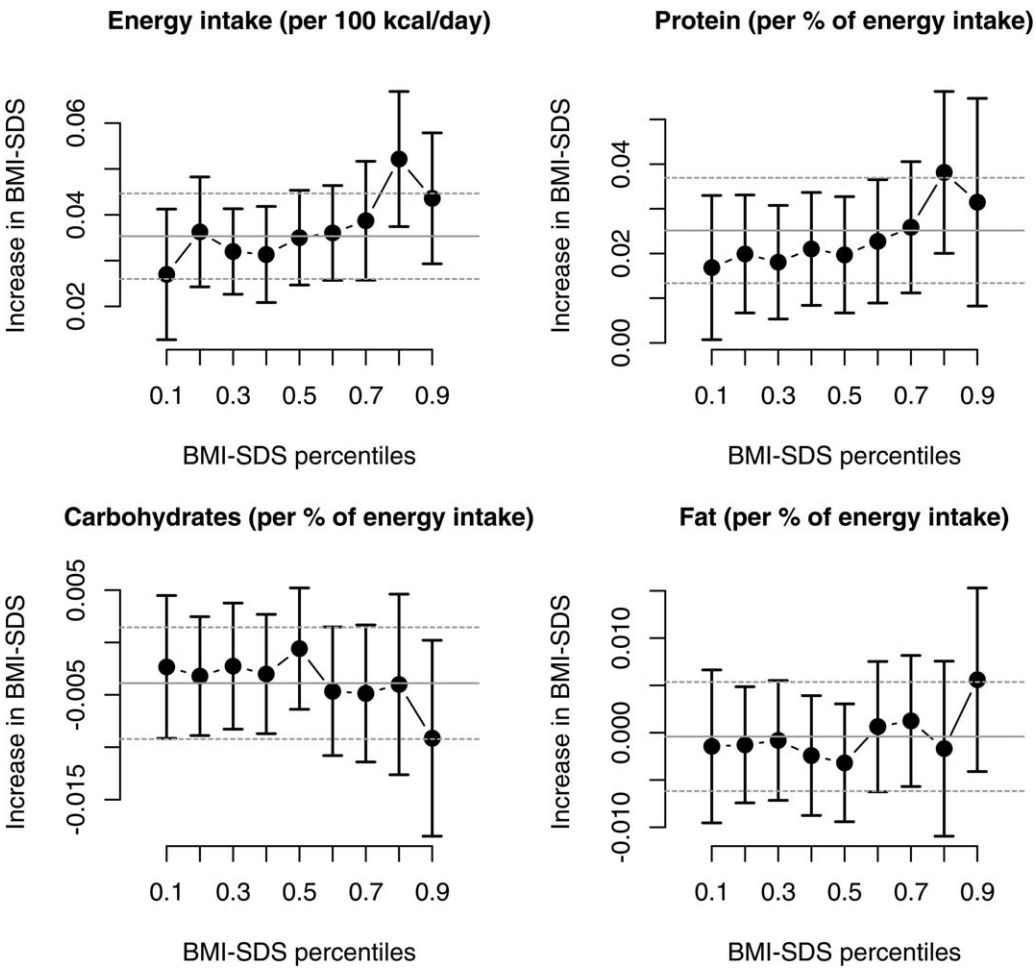

Figure 3 Quantile regression estimates and 95% CIs for differences in sex- and age-specific BMI SDS percentiles (0.1-0.9 deciles) at the age of 5.5 years by intake of energy, protein, carbohydrate, and fat at the age of 4.5 to 5.0 years. The grey horizontal lines represent the linear regression coefficients and their respective CIs. All models were adjusted for sex, country, birth weight, maternal age, maternal prepregnancy BMI, gestational weight gain, maternal diabetes, maternal smoking in pregnancy, maternal alcohol intake in pregnancy, maternal education, and duration of breastfeeding.

intake at this age was by far larger than the respective ORs by energy intake at later ages. This may, however, simply be due to the fact that 100 kcal of energy intake, which was used as the reference unit in all models, is a much larger proportion in relation to the body weight of an infant compared with a toddler or older child. In our models, a relative increase of 50% in the prevalence of overweight at 5.5 years was associated with a difference in daily energy intake of about 100 kcal at the age of 0.25 to 1.0 years and of 700 kcal at the age of 4.5 to 5.0 years.

We observed neither major percentile-specific associations nor evidence for effect modification by increased genetic susceptibility. While we had found corresponding association patterns for several risk factors of childhood overweight, such as maternal overweight, low parental education, or exclusive formula feeding (33), we had already observed less evidence for such patterns or for effect modification by genetic factors with respect to nutritional variables in a previous study (34). Based on these findings, we consider it rather unlikely that the associations of energy and protein intake with childhood overweight are largely driven by genetically susceptible subgroups, although we cannot rule out that other genetic variants that we were not able to investigate here may play a role.

We believe that the quality of the data analyzed is high. Dietary assessment in the TEDDY study was based on 24-hour recalls at 3 months of age and 3-day food records from 6 months of age. The 24-hour recall was conducted at 3 months of age, at which time infants predominantly received breast milk or infant formula and only a few infants were introduced to solid food already (35). The low variability in diet during that age may facilitate recalling; therefore, we do not think that the use of the different dietary assessment methods affected our results. The calculation of nutrients is based on established, harmonized, and up-to-date food databases from each country (21). Although the country-specific recording of foods may affect the comparability of specific nutrients such as soluble fiber (36), the intake of energy and macronutrients has been harmonized to be comparable across countries. The mean values of these nutrients over time as observed in our data appear plausible and are comparable to those from other studies (37).

We used the World Health Organization references for BMI to define overweight and obesity because these had also been established for the age of 0 to 2 years and thus allowed us to use a uniform definition of overweight/obesity across different ages. Further, we aimed to use international references for this multinational cohort.

It should be noted, however, that the TEDDY cohort is not population based, as participation was restricted to infants with increased genetic risk to develop type 1 diabetes. Such children usually have a slightly higher background prevalence of overweight, partly because of exposure to hyperglycemia in utero (38), but apart from that, they are not likely to be considerably different from a comparable population of healthy children. Unfortunately, we were not able to assess associations with BMI during the school age range because some of the TEDDY subjects have not had sufficient follow-up. Follow-up compliance until age 5.5 years was better in children of highly educated, nonsmoking mothers. However, we do not think that this may have biased our findings considerably, as we adjusted our analyses for these factors. We observed that 44% and 16% of all children who were deemed to have overweight/obesity at the age of 5.5 years already had persistent overweight/obesity from the age of 2 years. These proportions are relatively similar to persistence rates of overweight and obesity at comparable ages, as has been observed in other studies (39,40).

In summary, our results indicate that energy and protein intake are positively associated with increased risk for overweight in childhood but yield no evidence for potential programming effects of protein intake in early infancy. O

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