



Original article

Longitudinal association of dietary protein intake in infancy and adiposity throughout childhood

Vincent Jen^a, Kim V.E. Braun^a, Leonidas G. Karagounis^{b, c}, Anh N. Nguyen^a,
Vincent W.V. Jaddoe^{a, d}, Josje D. Schoufour^{a, e}, Oscar H. Franco^a, Trudy Voortman^{a, *}

^a Department of Epidemiology, Erasmus University Medical Center, PO Box 2040, 3000 CA, Rotterdam, The Netherlands

^b Nestlé Research Center, PO Box 44, 1000 Lausanne 26, Switzerland

^c Experimental Myology and Integrative Biology Research Cluster, Faculty of Sport and Health Sciences, University of St Mark and St John, Plymouth, Devon PL6 8BH, UK

^d Department of Pediatrics, Erasmus University Medical Center, PO Box 2040, 3000 CA, Rotterdam, The Netherlands

^e Department of Internal Medicine, Erasmus University Medical Center, PO Box 2040, 3000 CA, Rotterdam, The Netherlands



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SUMMARY

Background & aims: Protein intake in infancy promotes growth, but excessive intake may lead to adiposity in children. However, whether this increased adiposity persists throughout childhood and is independent of diet in later life remains unclear. Therefore, we studied the associations of total protein intake and protein from different sources at age 1 year with repeatedly measured growth and body composition up to age 10 years. Additionally, we examined whether these associations are independent of protein intake and overall diet quality at age 8 years.

Methods: We included 3573 children from the Generation R study, a population-based prospective cohort in the Netherlands. Dietary intakes were assessed with food-frequency questionnaires at ages 1 and 8 years and macronutrient intakes were expressed as energy percentages (E%). Height and weight were measured at eight time points between ages 1 and 10 years. Fat and fat-free masses were measured at ages 6 and 10 years with dual-energy X-ray absorptiometry. We calculated body mass index (BMI), fat mass index (FMI) and fat-free mass index (FFMI). Outcomes were standardized for sex and age and expressed as standard deviation scores (SDS). Associations of protein intake with growth and body composition trajectories were examined with multivariable linear mixed models.

Results: After adjustment for confounders, 5E% additional protein intake at age 1 year was associated with a 0.10 SDS higher weight (95% CI 0.04, 0.16), 0.10 SDS higher BMI (95% CI 0.04, 0.16), and 0.07 SDS higher FMI (95% CI 0.01, 0.13) up to age 10 years. These associations were explained by protein from animal sources and not plant sources. Associations were independent of protein intake and overall diet quality at age 8 years, and were independent of whether higher protein was consumed at the expense of carbohydrates or fat in the diet.

Conclusions: Our study suggests that high protein intake in infancy, particularly from animal food sources, is persistently associated with adiposity up to age 10 years. Restricting protein intake in this critical period of development may aid in the early prevention of adiposity in childhood.

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1. Introduction

Nutrition during childhood is an important determinant of health across the life course. In this context, protein is considered a key nutritional component in a well-balanced diet because protein influences growth in early life [1]. The daily protein requirement in the first 2 years of life for maintaining body weight and growth ranges from 0.79 to 1.12 g of protein per kilogram body weight depending on the child's age [2]. Protein intake lower than these

Abbreviations: CI, confidence interval; IQR, interquartile range; SDS, standard deviation score; SD, standard deviation; FFQ, food-frequency questionnaire; DXA, dual-energy X-ray absorptiometry; BMI, body mass index; FMI, fat mass index; FFMI, fat-free mass index.

* Corresponding author. Department of Epidemiology, Erasmus University Medical Center, Office Na-2903, PO Box 2040, 3000 CA, Rotterdam, The Netherlands. Fax: +31 10 70 44657.

E-mail address: trudy.voortman@erasmusmc.nl (T. Voortman).

thresholds may lead to restricted growth [3]. However, protein intake in most developed countries strongly exceeds this recommended intake, even exceeding the recommended safe amount of protein intake, which ranges from 0.97 to 1.31 g/kg body weight per day in the first 2 years of life [2,4].

High protein intake in infancy may enhance secretion of insulin and insulin-like growth factor 1 (IGF1), subsequently increasing the risk of later life obesity [5]. Results from observational studies do not consistently support this hypothesis, with some studies reporting a higher BMI following a higher protein intake in infancy [6–8] and some reporting no association [9,10]. However, findings from a randomized clinical trial showed that children receiving a higher protein content infant formula in the first year of life had a higher weight at their age of 2 years than children receiving a lower protein content formula [11]. These children also had a higher body mass index (BMI) and increased risk of obesity up to the age of 6 years [12]. In addition, we recently observed that this increased BMI due to higher protein intake in early childhood is fully driven by increased body fat mass and not lean mass at 6 years in 2911 children participating in the Generation R Study in the Netherlands [6]. These associations were observed for protein intake from animal sources, but not from plant sources [6]. In line with this, findings from an observational study in Germany showed that higher protein intake during early childhood was associated with higher body fatness at 7 years of age [7,8]. However, there is limited evidence on whether these associations track into later childhood, and whether diet in later childhood can mitigate the harmful effects of high protein intake in infancy on growth and body composition.

Therefore, we explored the longitudinal associations of total protein intake and protein from different sources in infancy with growth and detailed measures of body composition up to the age of 10 years. Additionally, we examined whether these associations were independent of protein intake and overall diet quality in later childhood.

2. Methods

2.1. Study design and population for analysis

This study was embedded within the Generation R Study, a population-based prospective cohort from fetal life onward in Rotterdam, the Netherlands [13]. The study was approved by the Medical Ethics Committee of Erasmus University Medical Center in Rotterdam, the Netherlands. Written informed consent was obtained from parents of all participating children. Children were born between 2002 and 2006 and parents of 5088 children were invited to fill out a questionnaire on their children's diet around the age of 1 year. We received valid dietary intake data for 3629 children (71%) [14]. Of these children, 3573 (98%) had data on anthropometrics (height, weight, BMI) and 3112 (86%) had data on body composition (fat mass and fat-free mass indices) available at one or more time points up to the age of 10 years (Fig. 1).

2.2. Assessment of dietary intake

Children's dietary intake was assessed at a median age of 12.9 months (interquartile range (IQR) 12.7–13.9) using a semi-quantitative food-frequency questionnaire (FFQ) [14]. The FFQ was filled out by the parents and covered their children's diet in the preceding month and consisted of 211 food items with questions on frequency, quantity, type and preparation methods. Food frequencies and quantities were converted into grams per day using standardized portion sizes. Daily energy and macronutrient intakes of the children were calculated with the Dutch Food Composition Table 2006. We separately calculated protein intake from plant and

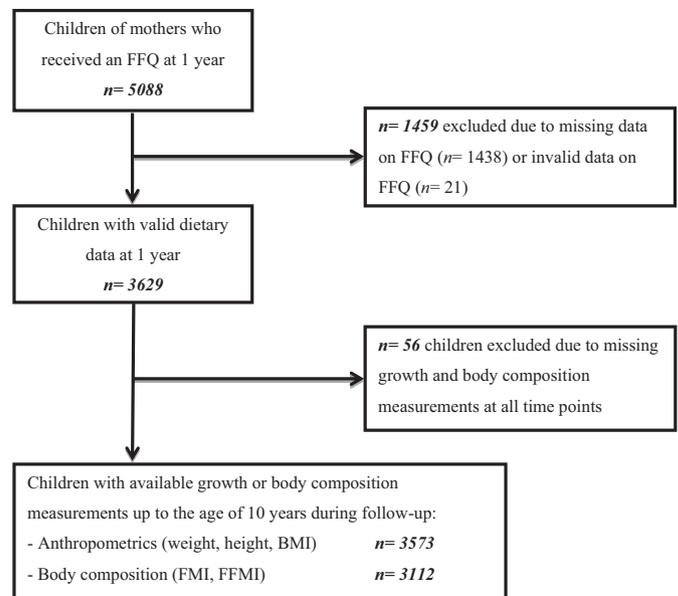


Fig. 1. Flowchart of population for analyses.

animal sources. Animal protein intake was further separated into protein from dairy and non-dairy animal food sources [6]. A small proportion of the children (<10%) were still receiving some breastfeeding during dietary intake assessment. As for other food items, average nutrient and energy content of breastmilk was derived from the Dutch Food Composition Table and protein from breastmilk was categorized as protein from animal sources [6]. The FFQ was previously validated against three 24-h recalls, obtained by trained nutritionists, in a representative sample of 32 Dutch children aged 14 months. This validation showed an intraclass correlation coefficient of 0.7 for total protein intake [14].

2.3. Assessment of anthropometrics and body composition

Height and weight were measured at eight different time points between the ages of 1 and 10 years. Up to the age of 4 years, these measurements were performed during routine visits at the Child Health Centers at median ages (IQR) of 14 (14–15), 18 (18–19), 25 (24–26), 31 (30–31), 37 (36–37), and 46 (45–47) months. At these centers, height and weight were measured without shoes and heavy clothing [13]. In addition, children visited our research center in the Erasmus Medical Center at median ages of 5.9 years (IQR 5.8–6.0) and 9.8 years (IQR 9.6–9.9) for detailed physical examinations [13]. Height was measured without shoes in standing position to the nearest millimeter with a Harpenden stadiometer (Holtain Limited, Dyfed, U.K.) and weight was measured without shoes or heavy clothing using a mechanical personal scale (SECA, Almere, the Netherlands). We calculated BMI (weight (kg)/height (m)²) for all time points. Total body fat and lean mass were measured with a dual-energy X-ray absorptiometry (DXA) scanner (iDXA, Ge-Lunar, 2008, Madison, WI, USA) using enCORE software version 13.6, and we calculated fat mass index (FMI; fat mass (kg)/height (m)²) and fat-free mass index (FFMI; fat-free mass (kg)/height (m)²). For all outcomes, we calculated age- and sex-specific standard deviation scores (SDS) based on the internal study population [6].

2.4. Covariates

We obtained information on several maternal characteristics through self-administered questionnaires when pregnant women

were enrolled in the study. We collected data on maternal age, BMI, educational level (non-higher; higher), household income (<€2400/mo.; ≥€2400/mo., equivalent to <\$2770/mo. and ≥\$2770/mo.), parity (0; 1; ≥2), and folic acid supplement use (start preconceptional; start in the first 10 weeks of pregnancy; never). Smoking and alcohol use during pregnancy were categorized as never; until pregnancy was known; or continued during pregnancy.

Information on child's sex, birth weight, and gestational age at birth was obtained from delivery reports. For birth weight, we calculated sex- and gestational age-specific SD-scores using reference data from a representative sample in Sweden [15]. Ethnicity was defined as Dutch or non-Dutch, on the basis of the countries of birth of the parents [16], which we collected via questionnaires [17]. Information on breastfeeding (exclusively for at least 4 months; partially in the first 4 months; never) was obtained with postnatal questionnaires. Child's sports participation (yes; no) and screen time (television watching or computer use: <2 h/day; ≥2 h/day) at their ages of 6 and 10 years were obtained using questionnaires.

Dietary intake of the children was updated at their median age of 8.0 years (IQR 8.0–8.1) using an FFQ [18]. This FFQ assessed children's habitual dietary intake of the preceding 4 weeks and was previously validated for energy intake using the doubly labeled water method [19]. Dietary intake of fat (E%), carbohydrate (E%) and protein (E%) was calculated based on the reported food intake obtained from the FFQ. We quantified the overall diet quality of all children at the age of 1 year [14] and 8 years [18] using previously defined food-based diet quality scores for children, which measures adherence to dietary guidelines. In short, each diet quality score is the sum of 10 components and each component can score between 0 and 1. Examples of these components are fruits, vegetables, dairy, fish, meat products, and sugar-containing beverages. The overall diet quality scores at age 1 year and 8 years range from 0 to 10 on a continuous scale and a higher score represents a healthier diet [14,18].

2.5. Statistical analysis

We calculated macronutrient intakes in percentages from the total energy intake (5E%) and we used multivariable nutrient density models to examine associations of protein intake (5E%) independent of its energy content and to explore different macronutrient substitution effects [20].

Linear mixed models were used to examine associations of protein intake at age 1 year with trajectories of growth between the ages of 1 and 10 years, and with body composition between the ages of 6 and 10 years. Energy densities of protein intakes were entered as independent variables and longitudinal age- and sex-adjusted SD-scores of height, weight, BMI, FMI, and FFMI up to age 10 years as dependent variables. The linear mixed models incorporated all repeated measurements of the outcomes simultaneously while taking into account that these measurements are correlated within subjects [21]. Likelihood ratio tests were used to determine a suitable random effects structure, random intercepts and slopes, which were used in each of the longitudinal models. For the fixed effect structure, we constructed 3 step-wise adjusted multivariable models. Model 1 included child's sex, ethnicity, age at FFQ and total energy intake. Additional covariates were selected based on previous literature or a ≥10% change in effect estimates when they are entered stepwise in model 1. Following this approach, model 2 was additionally adjusted for birth weight SD-scores [22], breastfeeding [23], diet quality at age 1 year [24], playing sports, household income, maternal BMI, maternal education, folic acid use during pregnancy and smoking during pregnancy. To examine if associations of protein intake in infancy with growth and body composition were independent of diet in later childhood, model 3 was additionally adjusted for protein intake and energy intake at the age of 8

years. In separate models, we additionally adjusted model 2 for the overall diet quality score of children at the age of 8 years [18]. We studied whether dietary protein from different sources was differently associated with growth and body composition by replacing total protein intake with animal and plant protein intakes in all analyses. Models including animal protein were adjusted for plant protein and vice versa. In subsequent models, we further divided animal protein intakes into protein from dairy and non-dairy sources, which were also adjusted for each other and plant protein. We explored different macronutrient substitution effects by adding either intake of carbohydrates (5E%) or fats (5E%) in the abovementioned models. This approach allows us to examine whether protein intake was differently associated with growth and body composition measures when higher protein was consumed at the expense of lower dietary carbohydrates or lower fats [6,25].

The associations of protein intake at 1 year with growth and body composition outcomes were tested for non-linearity using natural cubic splines [26]. We examined sex as a potential effect modifier, by including an interaction term of this covariate with total protein intake in models 1 and 2. Analyses were stratified for boys and girls if the *P*-value for the interaction term was <0.10, two-sided alpha error. To examine whether protein intake at age 1 year modified the velocity of growth and body composition trajectories, we tested for interaction between protein intake and age at growth measurement in the fixed effects structure. As sensitivity analysis, we restricted analyses to children with a Dutch ethnic background since the FFQ was specifically designed for participants from Dutch origin.

Missing data of covariates ranged between 0.0 and 27.2% (for dietary intake at 8 years). To reduce potential bias due to missing values, incomplete data on covariates were multiple imputed ($M = 10$ imputations) using the Fully Conditional Specification method (predictive mean matching) assuming no monotone missing pattern [27]. We reported pooled estimates from the imputed datasets for all analyses. Results were considered statistically significant at $P < 0.05$, two-sided alpha error, except when testing for potential effect modification. Statistical analyses were conducted with SPSS version 21.0 (IBM Inc., Armonk, NY, USA) and R version 3.2.3 (The R Foundation for Statistical Computing, Vienna, Austria).

3. Results

3.1. Subject characteristics

Characteristics of the children and their mothers are presented in Table 1. Most of the children had a Dutch ethnic background (68.1%). Mean ± SD total protein intake at the age of 1 year was 42.1 ± 13.4 g/d, corresponding to approximately 4.1 g/kg/body weight based on study population averages. Mean animal protein intake was 26.5 ± 10.6 g/d, and mean plant protein intake was 15.1 ± 5.9 g/d. The percentage of total energy intake from protein intake increased from 12.9 ± 2.4% at 1 year to 16.5 ± 2.2% at 8 years. At the age of 10 years, the mean height of the children was 141.6 ± 6.5 cm, mean weight was 34.7 ± 6.7 kg, and mean BMI was 17.2 ± 2.5 kg/m². We had on average 6 anthropometric measurements per child.

3.2. Protein intake at age 1 year, growth and body composition

After adjustment for confounders (model 2), linear mixed models showed that 5E% additional total protein intake at 1 year was associated with a 0.11 SDS higher weight (95% CI 0.06, 0.17), and a 0.11 SDS higher BMI (95% CI 0.06, 0.17) up to the age of 10 years (Table 2), but not with height (0.05 SDS; 95% CI -0.01, 0.12). These associations remained after adjusting for protein intake at the age of 8 years for weight (0.10 SDS; 95% CI 0.04, 0.16) and BMI (0.10 SDS;

Table 1
Population characteristics ($n = 3573$).

	Mean \pm SD, median (IQR), or no. (%)
<i>Infant characteristics</i>	
Sex, girls	1820 (50.9%)
Ethnicity, Dutch	2432 (68.1%)
Birth weight (g)	3446 \pm 579
<i>Child characteristics at 1-year dietary assessment</i>	
Age filling out FFQ (months)	12.9 (12.7–13.9)
Total energy intake (kcal)	1321 \pm 413
Protein (E%)	12.9 \pm 2.4
Fat (E%)	28.7 \pm 5.7
Carbohydrates (E%)	58.3 \pm 6.0
Total protein intake (g/d) ^a	42.1 \pm 13.4
Animal protein intake (g/d)	26.5 \pm 10.6
Dairy (g/d)	17.7 \pm 8.3
Non-dairy (g/d)	7.3 (4.8–11.5)
Plant protein intake (g/d)	15.1 \pm 5.9
Diet quality score (0–10) ^b	4.3 \pm 1.4
<i>Child characteristics at 8-year dietary assessment</i>	
Age filling out FFQ (years)	8.0 (8.0–8.1)
Total energy intake (kcal)	1474 \pm 304
Protein (E%)	16.5 \pm 2.2
Fat (E%)	32.6 \pm 4.0
Carbohydrates (E%)	50.9 \pm 5.0
Total protein intake (g/d)	60.7 \pm 15.2
Animal protein intake (g/d)	23.3 \pm 8.2
Plant protein intake (g/d)	37.4 \pm 11.7
Diet quality score (0–10) ^b	4.5 \pm 1.3
<i>Child characteristics at 10-year growth assessment</i>	
Age (years)	9.7 \pm 0.3
Height (cm)	141.6 \pm 6.5
Weight (kg)	34.7 \pm 6.7
BMI (kg/m ²)	17.2 \pm 2.5
Fat mass (kg)	9.3 \pm 4.1
Fat-free mass (kg)	25.2 \pm 3.4

Values are means \pm SD for continuous variables with a normal distribution, medians (IQR) for continuous variables with a skewed distribution, or valid numbers (%) for categorical variables. Missing data of covariates (between 0.0 and 27.2%) were computed with multiple imputation ($M = 10$ imputations).

^a Corresponding to approximately 4.1 g/kg/body weight.

^b based on previously defined quality scores reflecting overall adherence to dietary guidelines on a continuous scale ranging from 0 to 10 [14] [18].

95% CI 0.04, 0.16) (model 3, Table 2) and after adjusting for adherence to dietary guidelines at the age of 8 years (Supplemental Table 1). When examining detailed measures of body composition, we observed a higher FMI (0.09 SDS; 95% CI 0.03, 0.14) and not FFMI (0.03 SDS; 95% CI -0.03 , 0.10) with higher protein intake

Table 2

Associations of protein intake at the age of 1 year with childhood growth measures between the ages of 1 and 10 years ($n = 3573$).

	Weight (SDS)	Height (SDS)	BMI (SDS)
<i>Total protein intake at 1 year (5E%)</i>			
Model 1 (basic)	0.13 (0.07, 0.19)	0.07 (0.01, 0.13)	0.13 (0.07, 0.18)
Model 2 (confounders)	0.11 (0.06, 0.17)	0.05 (-0.01 , 0.12)	0.11 (0.06, 0.17)
Model 3 (protein intake 8 years)	0.10 (0.04, 0.16)	0.05 (-0.02 , 0.12)	0.10 (0.04, 0.16)
<i>Animal protein intake at 1 year (5E%)</i>			
Model 1 (basic)	0.13 (0.07, 0.19)	0.07 (0.01, 0.13)	0.13 (0.07, 0.18)
Model 2 (confounders)	0.12 (0.06, 0.18)	0.06 (0.00, 0.12)	0.11 (0.06, 0.17)
Model 3 (protein intake 8 years)	0.10 (0.04, 0.17)	0.06 (-0.01 , 0.13)	0.10 (0.04, 0.16)
<i>Plant protein intake at 1 year (5E%)</i>			
Model 1 (basic)	0.14 (0.03, 0.24)	0.11 (-0.00 , 0.23)	0.10 (-0.00 , 0.20)
Model 2 (confounders)	0.05 (-0.06 , 0.16)	0.03 (-0.09 , 0.15)	0.04 (-0.06 , 0.15)
Model 3 (protein intake 8 years)	0.11 (-0.11 , 0.14)	-0.08 (-0.22 , 0.07)	0.06 (-0.05 , 0.18)

Values are regression coefficients and 95% CI based on linear mixed models and reflect differences in growth measures (age- and sex-specific SD-scores) per 5E% higher protein intake at the expense of fat and/or carbohydrate. **Bold** values indicate statistical significance ($P < 0.05$). Analyses were repeated to study different protein sources by replacing total protein intake with animal protein and plant protein. Models including animal protein were adjusted for plant protein and vice versa.

Model 1 is adjusted for child sex, ethnicity, age at dietary measurement and total energy intake at 1 year.

Model 2 is additionally adjusted for birth weight SD-score, breastfeeding, diet quality at the age of 1 year (adjusted for protein intake at 1 y), screen time, playing sports, household income, maternal BMI, maternal education, folic acid use during pregnancy and smoking during pregnancy.

Model 3 is additionally adjusted for protein intake and total energy intake at the age of 8 years.

(model 2, Table 3). Also, the association between total protein intake and longitudinal FMI remained after adjustment for protein intake at the age of 8 years (0.07 SDS; 95% CI 0.01, 0.13) (model 3, Table 3) and adherence to dietary guidelines at the age of 8 years (Supplemental Table 1). Different macronutrient substitution models showed that associations of total protein intake with weight, BMI and FMI were similar when protein was consumed at the expense of carbohydrate or at the expense of fat (Table 4).

3.3. Additional analyses

Associations of total protein intake with growth and body composition were driven by protein from animal sources, but not plant sources (Tables 2 and 3). We observed that higher animal sourced protein intake from both dairy and non-dairy sources was associated with higher weight and BMI, but that only non-dairy sourced protein intake was associated with FMI (Supplemental Table 2). Cubic natural splines showed no indications for non-linearity of the associations of protein intake in infancy with growth and body composition. Sex and age at growth measurements did not modify the associations of protein intake with growth or with body composition outcomes (P -values for interaction > 0.10). After restricting our analyses to children with a Dutch ethnic background, we obtained similar effect estimates to those in the total population (*results not shown*).

4. Discussion

In this large population-based prospective cohort, we observed that higher protein intake in infancy, particularly from animal food sources, was associated with higher weight and BMI throughout childhood. These associations were driven by higher fat mass, but not fat-free mass, and were independent of protein intake and adherence to dietary guidelines in later childhood.

4.1. Interpretation of findings and implications

Our finding that a higher protein intake in infancy is associated with increased growth and adiposity is in line with previous studies [7,10,28,29], including previous analyses in our cohort [6,21]. In addition, Koletzko et al. [11] and Weber et al. [12] demonstrated in a randomized controlled trial a causal effect of high protein intake in infancy on increased weight and BMI in early childhood. In the

Table 3Associations of protein intake at the age of 1 year with body composition measures between the ages of 6 and 10 years ($n = 3112$).

	FMI (SDS)	FFMI (SDS)
<i>Total protein intake at 1 year (5E%)</i>		
Model 1 (basic)	0.09 (0.03, 0.15)	0.05 (−0.02, 0.11)
Model 2 (confounders)	0.09 (0.03, 0.14)	0.03 (−0.03, 0.10)
Model 3 (protein intake 8 years)	0.07 (0.01, 0.13)	0.05 (−0.02, 0.13)
<i>Animal protein intake at 1 year (5E%)</i>		
Model 1 (basic)	0.09 (0.03, 0.15)	0.04 (−0.02, 0.11)
Model 2 (confounders)	0.09 (0.03, 0.14)	0.03 (−0.03, 0.10)
Model 3 (protein intake 8 years)	0.07 (0.01, 0.13)	0.05 (−0.02, 0.12)
<i>Plant protein intake at 1 year (5E%)</i>		
Model 1 (basic)	0.00 (−0.12, 0.11)	0.11 (−0.01, 0.23)
Model 2 (confounders)	−0.02 (−0.14, 0.10)	0.07 (−0.06, 0.20)
Model 3 (protein intake 8 years)	−0.01 (−0.13, 0.11)	0.11 (−0.04, 0.25)

Values are regression coefficients and 95% CI based on linear mixed models and reflect differences in body composition measures (age- and sex-specific SD-scores) per 5E% higher protein intake at the expense of fat and/or carbohydrate. **Bold** values indicate statistical significance ($P < 0.05$). Analyses were repeated to study different protein sources by replacing total protein intake with animal protein and plant protein. Models including animal protein were adjusted for plant protein and vice versa.

Model 1 is adjusted for child sex, ethnicity, age at dietary measurement and total energy intake at 1 year.

Model 2 is additionally adjusted for birth weight SD-score, breastfeeding, diet quality at the age of 1 year (adjusted for protein intake at 1 y), screen time, playing sports, household income, maternal BMI, maternal education, folic acid use during pregnancy and smoking during pregnancy.

Model 3 is additionally adjusted for protein intake and total energy intake at the age of 8 years.

Table 4

Associations of different macronutrient substitutions at age 1 year with body composition up to age 10 years.

	Weight (SDS) $n = 3573$	BMI (SDS) $n = 3573$	FMI (SDS) $n = 3112$
5E% higher protein and lower carbohydrate intake	0.10 (0.04, 0.17)	0.10 (0.04, 0.16)	0.07 (0.01, 0.13)
5E% higher protein and lower fat intake	0.13 (0.05, 0.19)	0.11 (0.04, 0.18)	0.07 (0.01, 0.13)

Values are regression coefficients and 95% CI based on linear mixed models and reflect differences in weight, BMI and FMI (age- and sex-specific SD-scores) per 5E% substitution of one macronutrient for another. **Bold** values indicate statistical significance ($P < 0.05$).

Analyses are adjusted for child sex, ethnicity, age at dietary measurement, total energy intake at 1 year (adjusted for protein intake at 1 y), birth weight SD-score, breastfeeding, diet quality at the age of 1 year, screen time, playing sports, household income, maternal BMI, maternal education, folic acid use during pregnancy, smoking during pregnancy, and protein intake and total energy intake at the age of 8 years (model 3 in the main analyses).

current analyses, we extended this evidence by showing that: 1) that the association of high protein intake in infancy with higher BMI is completely explained by body fat, not lean mass, and that this remains up to the age of 10 years, 2) this association was similar when protein was consumed at the expense of either dietary fat or carbohydrate intake, and 3) that the associations of protein intake in infancy with weight, BMI and body fatness were not affected by diet in later childhood. Although the effect estimates were small, a 0.1SDS BMI equates to approximately 0.25 kg/m² in our study population, our findings may be important in the prevention of later adiposity on a population level by optimizing diet in infancy and early childhood. This is further strengthened by observations in other Western populations that showed that, as observed within our study population, the recommended safe level of protein intake is greatly exceeded [4,10,28,29]. Our findings, combined with evidence from previous studies, should be taken into account in the development of new evidence-based age-specific dietary guidelines and in public health strategies. Such approaches may include reformulation of infant formulas and toddler foods to promote a more appropriate intake of dietary protein in order to reduce adiposity on the long term.

4.2. Potential mechanisms

The current study and previous analyses have shown that increased protein intake in infancy and early childhood is associated with a higher body fat mass [6–8]. One possible mechanism behind such an observation could result from the fact that excess protein intake can be converted into glucose through

gluconeogenesis and can subsequently be stored as fat [30]. The effects of protein in infancy specifically could also be explained by the 'early protein hypothesis'. This hypothesis postulates that consuming protein at a level beyond what can be utilized, enhances the secretion of insulin and IGF1 [31]. High values of insulin and IGF1 in specifically early childhood can stimulate growth as well as adipogenesis and may also decrease lipolysis [5,32]. This may have long-term effects on adiposity [33,34], and as observed in our study, this association may be irrespective of diet in later life. Our findings thus support the 'early protein hypothesis' and emphasize a potential long-lasting harmful effect of high protein intake during infancy on later adiposity.

In line with previous studies, the observed associations in our study are driven by protein from animal sources rather than from plant sources [35–37]. These effects of animal sourced protein intake have by some researchers been ascribed to protein from dairy sources in particular, which has been suggested to have specific growth stimulating effects by raising hormonal responses [8,38]. However, our results do not support a specific effect of dairy-sourced protein. After separating animal-sourced dietary protein into dairy and non-dairy sources, the effect sizes were similar for weight and BMI, and for fat mass even slightly stronger for protein from non-dairy sources than from dairy sources.

4.3. Strengths and limitations

A key strength of the current analyses is the longitudinal design, which enabled us to examine the associations of protein intake in infancy on body composition throughout childhood. We studied

these associations while taking into account the role of diet in later childhood. In addition to BMI as a measure of adiposity, we also measured fat and fat-free masses using DXA. We also consider this as a key strength of our study, since BMI alone is a suboptimal predictor of adiposity in children [39]. Another strength of our study is the available information on many child and parental sociodemographic and lifestyle covariates that are included in our analyses. However, residual confounding can never be ruled out in an observational study. For example, we had no data available on puberty status of the children, which would have been useful to examine as a potential confounder or effect modifier, and we only had proxies for physical activity. Another limitation is that dietary intake assessed with an FFQ is prone to measurement error. However, evaluation against 24-h recalls showed good validity to estimate protein intake [14,40]. Another limitation of this study is that the FFQs we used to estimate dietary intake were developed for children with a Dutch ethnic background, whereas our study was conducted in a multi-ethnic study population. However, sensitivity analyses restricted to Dutch children resulted in similar effect estimates as obtained in the whole group, suggesting that no large bias due to ethnicity was present.

5. Conclusions

In this large population-based prospective cohort study, a higher protein intake, mainly from animal food sources, in infancy was persistently associated with adiposity up to the age of 10 years. These associations were independent of protein intake or diet quality in later childhood, which suggests that a diet excessively high in protein in infancy may have a long-lasting impact on adiposity risk. A more appropriate protein intake during this critical period of development may aid in the early prevention of adiposity in childhood.

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Conflict of interest

The authors have no conflicts of interest to report.

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The authors' responsibilities were as follows: VJ and TV designed the research project; VVJ and OHF were involved in the study design and data collection; VJ and TV analyzed the data; KVEB, ANN, JDS, LGK, VVJ, and OHF provided consultation regarding the analyses and interpretation of the results; VJ and TV

wrote the manuscript; VJ and TV had primary responsibility for the final content. All authors read and approved the final manuscript.

Appendix A. Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.clnu.2018.05.013>.

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