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Original article

Evidence for the protein leverage hypothesis in preschool children prone to obesity

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S U M M A R Y

Background & aims: The protein leverage hypothesis (PLH) proposed that strict regulation of protein intake drives energy overconsumption and obesity when diets are diluted by fat and/or carbohydrates. Evidence about the PLH has been found in adults, while studies in children are limited. Thus, we aimed to test the PLH by assessing the role of dietary protein on macronutrients, energy intake, and obesity risk using data from preschool children followed for 1.3 years.

Methods: 553 preschool children aged 2–6 years from the 'Healthy Start' project were included. Exposures: The proportion of energy intake from protein, fat, and carbohydrates collected from a 4-day dietary record. Outcomes: Energy intake, BMI z-score, fat mass (FM) %, waist- (WHtR) and hip-height ratio (HHtR). Power function analysis was used to test the leverage of protein on energy intake. Mixture models were used to explore interactive associations of macronutrient composition on all these outcomes, with results visualized as response surfaces on the nutritional geometry.

Results: Evidence for the PLH was confirmed in preschool children. The distribution of protein intake (% of MJ, IQR: 3.2) varied substantially less than for carbohydrate (IQR: 5.7) or fat (IQR: 6.3) intakes, suggesting protein intake is most tightly regulated. Absolute energy intake varied inversely with dietary percentage energy from protein ($L = -0.14$, 95% CI: −0.25, −0.04). Compared to children with high fat or carbohydrate intakes, children with high dietary protein intake (>20% of MJ) had a greater decrease in WHtR and HHtR over the 1.3-year follow-up, offering evidence for the PLH in prospective analysis. But no association was observed between macronutrient distribution and changes in BMI z-score or FM%.

Conclusions: In this study in preschool children, protein intake was the most tightly regulated macronutrient, and energy intake was an inverse function of dietary protein concentration, indicating the evidence for protein leverage. Increases in WHtR and HHtR were principally associated with the dietary protein dilution, supporting the PLH. These findings highlight the importance of protein in children's diets, which seems to have significant implications for childhood obesity risk and overall health.

1. Introduction

Childhood obesity is an important public health issue worldwide. From 1975 to 2016, the prevalence of overweight or obesity among children and adolescents increased more than four-fold from 4% to 18% globally [1]. Children with obesity are at risk of later developing somatic and psychological issues, like type 2

Abbreviations: AIC, Akaike information criterion; BMI, body mass index; FM, fat mass; HHtR, hip-height ratio; IQR, Interquartile range; MJ, megajoule; PLH, protein leverage hypothesis; SD, standard deviations; RMT, right-angled mixture triangle; WHtR, waist-height ratio.

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diabetes [2], cardiovascular disease [2], non-alcoholic fatty liver disease [3], and mental health disorders [4], which all have negative impacts on their quality of life and lifespan. In evaluating the role of diet on obesity, the conventional nutrition approaches have typically focused on single nutrients. Such approaches have provided the foundations of nutrition science, but they fail to capture the multidimensional essence of nutrition [5]. Diets are more than the sum of specific nutrients; they are complex mixtures of nutrients and other constituents. Changing the concentration of a specific nutrient in the diet can alter the character of the entire blend [6]. Thus, a narrow focus on the individual effects of single nutrients ignores the interaction between nutrients in diets and their implications on health outcomes. A multidimensional modelling framework called nutritional geometry was developed from nutritional ecology to explicitly account for nutrient interactions within diets and quantify the health effects of different diet compositions [7].

Based on nutritional geometry, studies ranging from locusts to primates [8–10] demonstrated that protein intake is more tightly regulated than fat and carbohydrates, where protein intake is relatively stable, while fat, carbohydrates and total energy intake vary depending on dietary protein concentration. This pattern of macronutrient regulation, known as "protein prioritization", forms the basis of a novel hypothesis for human obesity, the protein leverage hypothesis (PLH). According to the PLH, the interaction between dietary protein dilution by fat and carbohydrates and the strong human protein appetite leads to excessive energy intake and obesity [11]. The PLH is supported among adults by substantial evidence from randomized controlled trials (RCTs) [12–15] and large national diet surveys [16–18]. In several RCTs, individuals were experimentally confined to imbalanced diets and showed that protein intake is prioritized, resulting in calorie overconsumption on protein-dilute diets [12–15]. These experimental findings are consistent with surveillance population data that emphasized the central role of protein in the obesity epidemic, in which low-protein and highly processed foods led to high energy intake due to a biological response to macronutrient imbalance triggered by a dominant appetite for protein [16]. However, studies examining the effect of protein prioritization on energy intake and obesity risk in children are still limited.

Here we applied nutritional geometry to data on preschool children followed for 1.3 years to test the PLH. We hypothesized that in children, i) protein intake is tightly regulated within a narrow range, compared with fat or carbohydrate intakes, ii) energy intake is an inverse function of the dietary protein concentration, and iii) longitudinal adiposity-related changes over 1.3 years are inversely associated with children dietary protein.

2. Material & methods

2.1. Study design

The present study used data from the 'Healthy Start' project as a cohort study. The 'Healthy Start' study was conducted between 2009 and 2012 among children aged 2–6 years prone to obesity. Children were born between 2004 and 2007 in 11 selected municipalities from the greater Copenhagen area. Details of this study have been described elsewhere [19]. At recruitment, to be eligible for the study, the child had to meet at least one of the following criteria that put the child at risk of developing overweight and obesity: 1) a high birth weight (>4000 g), 2) a mother with a pre-pregnancy body mass index (BMI) > 28 kg/m^2, or 3) a mother with low education (≤10 years of schooling) (subgroup only, from administrative birth forms in one of the 11 municipalities).

We included all children that participated in the 'Healthy Start' study (n = 635) and excluded participants who had not provided complete information on diet intake at baseline (n = 82), leaving 553 eligible children for further analyses (Fig. 1). Regarding anthropometric information at baseline, 553 children had height and weight, 333 children had body composition data, and 470 and 467 children, respectively, had waist and hip circumferences measured. After a follow-up period of 1.3 years, the sample sizes for children's weight and height, body composition, and waist and hip circumferences were 379, 212, 323 and 320, respectively.

The 'Healthy Start' study was conducted according to the guidelines laid down by the Declaration of Helsinki. The Danish Data Protection Agency approved using the data obtained in the Healthy Start primary prevention intervention (journal number: 2015-41-3937). The Scientific Ethical Committee of the Capital Region in Denmark decided that the project was not a bio-ethics project and consequently did not need approval from the Danish Bioethics Committee (journal number H-A-2007-0019). Written informed consent to use the collected data for research purposes was obtained from all parents [20]. The ClinicalTrials.gov identifier for 'The Health Start' study is NCT01583335.

2.2. Assessment of macronutrients and energy intake

Dietary intake data were collected at baseline using a 4-day dietary record [19], filled in by one of the parents. Parents were asked to keep track of their children's dietary intake from Wednesday to Saturday, allowing information to be collected on both weekday and weekend days. The food diaries were accompanied by a picture book including seventeen photo series with foods and portion sizes [21] to help estimate food and portion sizes. All food records were entered into the Dankost 3000 software program for energy and macronutrient analysis. The software was based on the Danish Food Composition Databank, version 7.01,

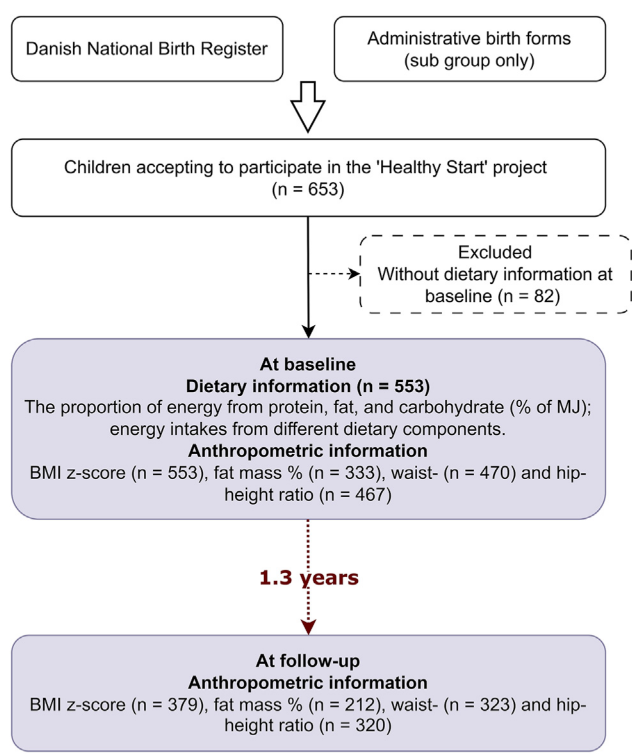

Fig. 1. Flowchart of the study population.

released 2009-03-02 (http://www.foodcomp.dk/), developed by the National Food Institute at the Technical University of Denmark. Afterwards, total energy intake (MJ) and proportion of energy intake from each macronutrient (% of MJ) were estimated from the dietary record for each child. In addition, animal and plant protein intake was expressed as a percentage of total energy consumption (% of MJ). Major sources of animal protein include processed and unprocessed red meat, poultry, dairy products, fish, and eggs. Major food contributors to plant protein include fruit, vegetables, rice, pasta, and potatoes.

2.3. Anthropometric measurements

All anthropometrics were measured every time by health consultants trained in dietetics and nutrition in the 'Healthy Start' project [19]. Height to the nearest 0.1 cm was measured using a stature meter (Soehnle, 5,002 or Charter ch200P). Bodyweight was measured to the nearest 0.1 kg using a mechanical or beam-scale type weight (TanitaBWB-800 or SV-SECA 710). BMI was computed as the ratio of weight (kg) and squared height (m^2). BMI z-score was generated using the Lambda-Mu-Sigma (LMS) method, which summarized the changing distributions of the dependent variable (e.g., BMI) by the median, and the coefficient of variation and skewness were expressed as Box–Cox power [22]. Using z-score enables comparisons between measured BMI and adequate gender- and age-specific reference values from Danish national reference data [23]. It was chosen to apply a national reference of BMI z-score to the study population, and thus, a power transformation of 0.1 years of age was used [22]. Waist circumference was measured to the nearest 0.5 cm midway between the lowest rib and the iliac crest. Hip circumference was measured to the nearest 0.5 cm, where the circumference was the largest, seen from the frontal and medial angles. Both waist circumference and hip circumference were measured in triplicate, and a mean was calculated. Waist-height ratio (WHtR) or hip-height ratio (HHtR) were calculated as waist circumference (cm) or hip circumference (cm) measurement divided by height measurement (cm), respectively. Changes in WHtR (%) and HHtR (%) were calculated as measurements at follow-up minus measurements at baseline × 100%.

Bioelectrical impedance was measured to estimate the child's percentage of body fat at resistance 50 kHz (using SEAC Multiple Frequency Bio Impedance Meter (model SFB3 and SFB2 version 1.0), RJL or Animeter (BIA-101 and BIA-103)), which was taken twice, and a mean was calculated [19]. Bioelectrical impedance was measured at a resistance of 50 kHz using a SEAC Multiple Frequency Bioimpedance Meter (model SFB3 and SFB2 version 1.0), RJL or Animeter (BIA-101 and BIA-103). Electrodes were positioned on the child's right side for the measurement. On the hand, the first electrode was positioned directly beneath the joint of the middle finger, and the second electrode was positioned midway between the two bones on the dorsal side of the wrist. On the dorsal side of the foot, the first electrode was positioned beneath the joint of the third toe, and the second electrode was positioned between the two large bones of the ankle. Readings of bioelectrical impedance were taken twice, and the mean was calculated [19]. Fat mass (FM) was calculated using an equation described by Goran et al. (1996) in young children [24]. FM% was computed as the ratio of FM (kg) and body weight (kg) × 100%.

2.4. Possible confounders

Possible confounders that might influence the association of dietary protein percentage with energy intake and obesity risk were selected a priori based on the existing literature [25–27]. Information on these variables was obtained from a parental questionnaire completed by parents at baseline and from the Danish Medical Birth Registry. Information on children's sex (boys or girls) and age (years) was obtained from the Danish Medical Birth Registry. Physical activity level was collected using the parental questionnaire, based on a single question: 'How physically active is the child compared to other children at the same age', and grouped into two groups: 'very active' and 'not very active'. Information on maternal educational levels was obtained from the parental questionnaire and grouped into three levels [28]: low education level ('elementary/High school', 'upper secondary', 'one or more short courses', or 'skilled worker'), medium education level ('short-term further education three years' or 'medium higher education three to four years'), and high education level ('long higher education over four years' or 'research level'). Information on fibre intake (g/day) was collected from the 4-day dietary record and calculated by the Dankost 3000 programme. Breastfeeding duration (exclusively breastfeeding ≥4 months versus <4 months) was obtained through linkage to the Danish Health Visitor's Child Health Database, where infant feeding was registered by health nurses four times from a few days after birth to approximately 10 months of age [29]. All models that included changes in outcomes were further adjusted for the baseline value of outcomes.

2.5. Statistical analysis

Descriptive statistics are presented as mean (standard deviations [SD]) or number (%) according to the type of variable. Characteristic differences across tertile groups of protein intake were assessed using the ANOVA test for continuous variables and Chi-square for categorical variables. As a measure of intake variability, interquartile ranges (IQRs) of macronutrient intake distribution were calculated as the difference between the 75th and 25th intake percentiles (quartile 3 - quartile 1).

To test for protein prioritization, power functions were fitted to predict the strength of leverage from the proportion of energy intake from protein toward total energy intake, as the equation: $E = P \times p^{L}$, where E is total energy intake, p is the proportion of energy contributed by protein in the diet, P is a constant, and L is the strength of leverage [11,30]. Complete protein leverage would be indicated by an exponent (L) $= -1$. Partial protein leverage is indicated when the exponent in the equation is ($-1 < L < 0$). L = 0 indicates that protein and non-protein energy are regulated equally and that there will be no relationship between p and E (i.e., no protein leverage).

The Effects of macronutrient composition (percentage of energy from macronutrients) on energy intake and anthropometric changes were analyzed using mixture models and displayed as surface plots in the right-angled mixture triangle (RMT) (Supplementary Fig. 1) [8,31]. The mixture models used in this study provide a method for statistical and graphical analysis of complex associations between macronutrients and health markers [8]. This includes the evaluation of nonlinear associations and nutrient interactions in a manner that complements and extends traditional nutritional epidemiology approaches that focus on a single nutrient or isocaloric nutrient replacement. The first three mixture models from Lawson and Willden [32] were developed to test for linear and nonlinear associations between the percentage of energy derived from macronutrients (protein, carbohydrates, and fat) and energy intake and weight-change outcomes. Model 1 was the null model (no dietary association), Model 2 was the linear model (the 'partition substitution model' from nutritional epidemiology), and Model 3 was the quadratic model. Each model was fully adjusted for age, sex (boys or girls), fibre intake, physical activity (very active or not very active), and maternal education (low, medium, or high education level). Since the BMI z-score was sex- and age-standardized, the model with BMI z-score as the outcome was only adjusted for fibre intake, physical activity, and maternal education level. The baseline value of each anthropometric was additionally incorporated into models that focused on anthropometric changes. We used the Akaike information criterion (AIC) to compare models, where smaller values (beyond a margin of two points) indicate a better model fit [8,33]. The effects of macronutrient composition on outcomes were visualized on RMT as response surfaces predicted by the AIC-favored model. Response surfaces are interpreted like a topographic map, with red areas representing the high and blue areas representing the low values of outcomes. By evaluating the predicted values in conjunction with the location on the RMT where all individual points equal 100% by adding protein (x-axis) + fat (y-axis) + carbohydrate, the associations between dietary macronutrients and outcomes can be inferred. Carbohydrates are represented by diagonal lines, with higher intake closer to the origin.

To test the robustness of our results, we further conducted two sensitivity analyses. Firstly, to evaluate the effect of protein quality, we examined two major types of protein by food sources (i.e., animal and plant protein) and analyzed the association of animal and plant protein (% of MJ) with energy intake. Second, considering that the early feeding modality may affect later dietary behaviour and weight gain, we conducted a subgroup analysis by early feeding modality (exclusively breastfed ≥4 months versus <4 months). All statistical analyses were conducted using the statistical software R, version 4.0.2 (R Foundation for Statistical Computing). A 2-tailed test with a p-value <0.05 was considered statistically significant.

3. Results

3.1. Children's characteristics

Participants' characteristics according to proportional energy from protein are shown in Table 1. Children in the highest versus lowest category of protein (≥16.5 vs < 14.6% of MJ) were younger, with ages (years, mean ± SD) 3.7 ± 1.1 vs 4.3 ± 1.0, and had slightly higher fibre intake (12.7 ± 4.3 vs 12.5 ± 3.7, although not statistically significant). Sex, physical activity, and maternal education level were unrelated to protein intake in children.

3.2. Variability of macronutrient intake

Macronutrient intake distributions as a percentage of energy are presented in Fig. 2. The protein intake range was the narrowest compared to the carbohydrate and fat intake ranges. Quartile 1 and quartile 4 intakes were <14.0 and >17.2% of MJ for protein, <26.6 and >32.3% of MJ for carbohydrate, and <51.7 and >58.0% of MJ for carbohydrate. The IQR intake distribution was smallest for protein (3.2), followed by fat (5.7) and carbohydrates (6.3). The mean proportional energy from protein (% of MJ) was 15.7 ± 2.3, carbohydrates (% of MJ) were 54.8 ± 4.7, and fat (% of MJ) was 29.5 ± 4.5 (Table 2).

3.3. Protein leverage

Figure 3 shows the relationships between the proportional energy from macronutrients and absolute intakes of various dietary components. Model 3 (quadratic model) was favoured by AIC for all forms of energy intake (except for energy density, where a linear model was favoured) (Supplemental Table 1), suggesting nonlinear and complex associations between the dietary macronutrients and energy intake, where there were significant 2-way associations within macronutrients (p-value <0.05) (Supplemental Table 2). Virtually from the response surface (Fig. 3), the intakes of combined fat and carbohydrate (A) and total energy (B) increased with decreasing proportional energy from protein, and their values were high when protein concentration was low, supporting protein leverage. Increasing energy intake on low-protein diets resulted from both high carbohydrate (C) and fat (D) consumption. As expected, diets with a high proportion of fat had the highest energy density (E), as fat has twice the energy density of carbohydrates and protein. As predicted by the protein leverage model, intakes of all components increased with decreasing dietary protein except absolute protein intake (F).

Table 3 demonstrates that all these associations were highly significant and that the leverage strength (the L value) varied between components. Proportional energy from protein was inversely associated with combined fat and carbohydrate (MJ) (L = −0.33), total energy (MJ) (L = −0.14), absolute carbohydrate (MJ) (L = −0.35), absolute fat (MJ) (L = −0.29), and energy density (MJ/kg) (L = −0.50). Meanwhile, it was directly related to absolute protein intake (L = 0.86) (Fig. 4).

3.4. Macronutrient composition and obesity risk

The response surfaces for macronutrient composition and weight-related outcomes are presented in Fig. 5. In mixture models examining the effects of macronutrient composition on anthropometrics, the linear model was favoured by AIC for WHtR and HHtR, indicating a linear relationship between macronutrients and WHtR and HHtR. AIC favoured the null model for BMI z-score and FM%, suggesting dietary macronutrient composition was not a significant determinant of BMI z-score and FM% (Supplemental Tables 3 and 4). Based on a visual analysis of response surfaces (Fig. 5), changes in WHtR and HHtR were primarily associated with the proportional energy from protein but not with fat or carbohydrates. Compared to children with high fat or carbohydrate intakes, high dietary protein content (>20% of MJ) was associated with greater reductions in WHtR and HHtR after a 1.3-year follow-up.

Sensitivity analyses on the association of protein by the source of intake (animal and plant) with energy intake from different dietary components showed that although there was a slight difference, both animal and plant protein (% of MJ) was significantly associated with intakes of combined fat and carbohydrate (MJ) (L = −0.33 and −0.14, respectively), total energy (MJ) (L = −0.14 and −0.12, respectively), total carbohydrate (MJ) (L = −0.35 and −0.11, respectively), total fat (MJ) (L = −0.29 and −0.18, respectively), protein energy (MJ) (L = 0.86 and −0.05, respectively), and energy density (MJ/kg) (L = −0.50 and −0.07, respectively) (Supplemental Table 5). Breastfeeding duration (exclusively breastfeeding for ≥4 months versus <4 months) was not significantly associated with total energy intake (MJ) in power function models at baseline (Supplemental Table 6).

4. Discussion

Using nutritional geometry, we found evidence consistent with the PLH in preschool children: (i) Protein intake was less variable than carbohydrates or fat, indicating protein is the most tightly regulated macronutrient; (ii) Energy intake was inversely related to dietary protein concentration, as the protein leverage mechanism predicted, suggesting that a dominant protein appetite can interact with protein dilution in the diet and drive excessive energy intake; (iii) Compared to children with high fat or carbohydrate intakes, children with a high dietary protein intake (>20% of MJ) experienced a bigger decrease in WHtR and HHtR over a 1.3-year follow-up, providing evidence for the PLH. However, no association was found between macronutrient distribution and BMI z-score or FM%.

Table 1
Characteristics of study participants according to proportional energy from protein.

	All children	Proportional energy from protein by percentile (% of MJ)			p-value
		Tertile 1 (8.6– < 14.6)	Tertile 2 (14.6– < 16.5)	Tertile 3 (16.5–23.8)	
	(n = 553)	(n = 184)	(n = 181)	(n = 188)	
Age (y)	4.0 ± 1.1	4.3 ± 1.0	4.0 ± 1.1	3.7 ± 1.1	<0.001
Fibre intake (g)	12.7 ± 3.9	12.5 ± 3.7	12.8 ± 3.7	12.7 ± 4.3	0.58
Sex					
Boys	314 (57)	103 (56)	113 (62)	98 (52)	0.13
Girls	239 (43)	81 (44)	68 (38)	90 (48)	
Physical activity					
Not very active	219 (41)	78 (43)	67 (39)	74 (41)	0.87
Very active	313 (59)	102 (57)	106 (61)	105 (59)	
Maternal education level					
Low	117 (22)	37 (21)	39 (23)	41 (23)	0.88
Medium	283 (54)	100 (57)	93 (55)	90 (52)	
High	121 (23)	40 (23)	37 (22)	44 (25)	

Mean ± standard deviation for all such values, and n (percentage, %) for all such values and. P values evaluated by ANOVA for continuous variables and $\chi 2$ test for categorical variables.

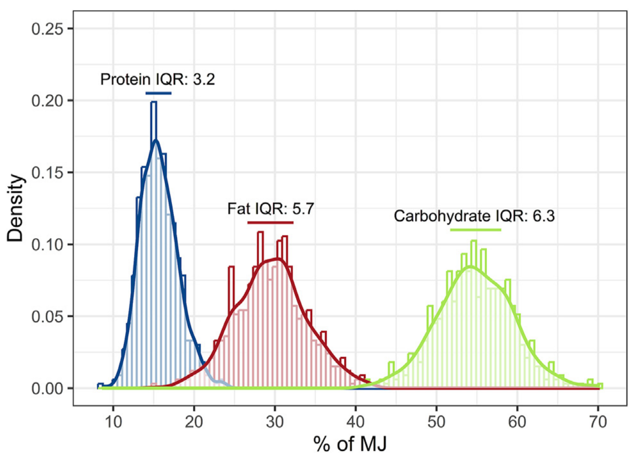

Fig. 2. Distribution of macronutrient intake among preschool children. The distribution of protein had a narrower and steeper peak, and smaller tails, compared with the fat and carbohydrate distributions. IQR, interquartile range.

Table 2
Macronutrients intake of study participants.

	Protein	Fat	Carbohydrate
Mean proportional intake (% of MJ)	15.7 ± 2.3	29.5 ± 4.5	54.8 ± 4.7
Mean absolute intake (MJ/day)	0.8 ± 0.2	1.4 ± 4.0	2.6 ± 5.9
Distribution of intake (% of MJ)			
Intake quartile 1	<14.0	<26.6	<51.7
Intake quartile 2	14.0–15.5	26.6–29.5	51.7–54.7
Intake quartile 3	15.6–17.2	29.6–32.3	54.8–58.0
Intake quartile 4	>17.2	>32.3	>58.0
IQR	3.2	5.7	6.3

Mean ± standard deviation for all such values. Interquartile ranges (IQRs) of macronutrient intake distribution were calculated as the difference between the 75th and 25th intake percentile (quartile3 - quartile1) as a measure of variability of intake.

These findings highlight the importance of protein in children's diets. More research expanding to larger cohorts is needed to identify the optimal dietary protein and macronutrient composition on the risk of obesity and overall health.

Our findings corroborate the results from previous studies using nutritional geometry, suggesting the possible role of dietary protein driving energy intake [16,18,25,34], consistent with protein leverage, indicating a strong human appetite for protein drove the energy overconsumption, weight gain, and obesity in protein-dilute diets. However, the main body of the preceding evidence was primarily derived from studies in adult humans [16,18,25], laboratory animals [10,35], and nonhuman primates in the wild [9]. Data using nutritional geometry on the role of dietary protein in obesity risk in children were, until now, limited. Although some attempts have been made to study the role of dietary protein on weight gain and adiposity among children, most research has focused solely on the role of dietary protein, ignoring the interaction between macronutrients within diets [36–38]. Such oversight has contributed to inconsistent results in many previous studies. To our knowledge, the present study is the first to date to show a robust association between dietary protein and prospective obesity risk based on nutritional geometry in young children. Our findings indicate that compared with children with high fat or carbohydrate intakes, children with high protein intake (>20% of MJ) had lower energy intake and a bigger decrease in WHtR and HHtR after a 1.3-year follow. These findings gain support from the previous two cross-sectional studies on children and adolescents [26,39], indicating an inverse association between dietary protein and energy intake using nutritional geometry. However, no association was observed between dietary protein and weight status, possibly due to the cross-sectional design causing difficulties in establishing causal relationships between diet and obesity risk.

Although the crucial underlying mechanisms remain unclear, many potential mechanisms have been proposed to explain the protein appetite. One potential driver of increased energy intake in response to dietary protein restriction is fibroblast growth factor (FGF)-21. FGF-21, a predominantly liver-derived hormone, represents an endocrine signal of protein restriction and is activated during periods of reduced protein intake [40]. Circulating FGF-21 acts on the brain to alter macronutrient preference, maintaining protein intake in the face of dietary protein restriction [41,42]. An RCT on lean healthy adults demonstrated that reduced dietary protein intake from 25% to 10% over a period of 4 days was associated with a 6-fold increase in fasting circulating plasma FGF-21 levels and 14% increased energy intake [15]. In addition, the effect of dietary protein on appetite regulation is also plausible through circulating branched amino acids (BCAAs). BCAAs, essential amino acids, are primarily in animal protein, like meat, chicken, fish, dairy products, and eggs [43]. BCAAs influence the release of appetite-regulating hormones from the intestines and the hypothalamus [44]. Increased blood BCAA levels are associated with increased body fat and lean body mass [45]. As our analyses showed that both animal and plant protein was significant drivers of energy intake,

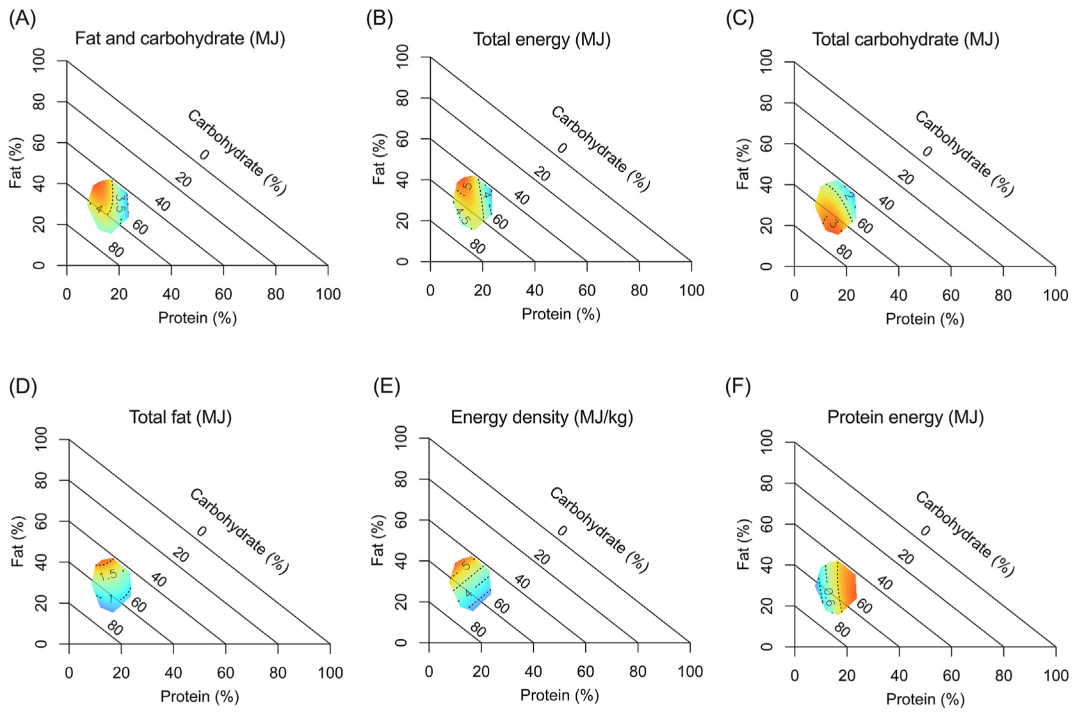

Fig. 3. Right-angle mixture triangle for dietary macronutrient distribution and energy intake from different dietary components. Response surface shows the predicted energy intake from different dietary components superimposed onto a dietary macronutrient composition triangle where cool colours represent the lowest values and warm colours represent the highest values of energy intake. All points on the triangle represent 100% of dietary energy, being the sum of protein (x-axis) + fat (y-axis) + carbohydrate. Carbohydrate is shown as diagonal lines with higher intake closer to the origin. The response surface has been trimmed to display predictions for values observed in the dataset. Energy intake from different dietary components is shown as **(A)** Fat and carbohydrate (MJ), **(B)** Total energy intake (MJ), **(C)** Total carbohydrate (MJ), **(D)** Total fat (MJ), **(E)** Energy density (MJ/kg), **(F)** protein energy (MJ). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 3
The exponent (L) from power regression testing protein prioritization.

	L	95% CI	
		Lower	Upper
Fat and carbohydrate (MJ)	−0.33	−0.43	−0.22
Total energy (MJ)	−0.14	−0.25	−0.04
Total carbohydrate (MJ)	−0.35	−0.46	−0.24
Total fat (MJ)	−0.29	−0.45	−0.13
Energy density (MJ/kg)	−0.50	−0.63	−0.38
Protein energy (MJ)	0.86	0.75	0.96

Power functions were fitted to predict the strength of leverage from the proportion of energy intake from protein (% of MJ) toward and energy intake from different dietary components. L coefficients were derived from the log−log regression analysis, adjusted for sex, age, physical activity, fibre intake, and maternal education level. L indicates strength of leverage for each macronutrient (−1 signifies complete leverage, 0 means no leverage). L, leverage; CI, confidence interval; MJ, megajoule.

further studies about protein quality and amino acid sources are needed to explore the underlying mechanisms of protein sensing, improving our understanding of dietary protein impacts health.

4.1. Strengths and limitations

The major strength of our study is the use of data with the prospective study design among preschool children. It allowed us to assess the association between macronutrient composition and prospective changes in obesity-related variables, overcoming the cross-sectional design limitations from previous studies. In this study, the dietary protein (%) remained stable from baseline to follow-up, which provides the basis for prospective analyses. The collection of dietary information and anthropometrics was conducted independently and prospectively by trained personnel and standardized procedures, which minimized information bias. In addition, using the information from the comprehensive questionnaire surveys of parents and children, we were able to adjust for a wide range of important confounders, including children's demographics, lifestyle, and maternal education level. Importantly, this study explored proportions-based nutritional geometry in analyzing the relationship of macronutrient balance to the prospective risk of obesity in preschool children. Nutritional geometry has major strengths in that it provides a graphical visualization of the effects of nutritional mixtures and allows the individual and interactive effects of nutrients to be explored and disentangled, which are not captured by conventional regression methodologies [31].

This study has several limitations. Firstly, this study had a relatively small sample size, especially for body composition measurements, as only a subset of children was measured, which may have affected the precision of the effect estimates. However, since we found robust evidence for protein prioritization in this sample of preschool children and prospective evidence for the PLH based on the inverse association of dietary protein dilution with WHtR and HHtR increases, we believe lack of power was not a substantial issue in the present study. Secondly, as in other diet assessment studies, reporting bias may be a problem. However, this bias appears to be subtler when focused on protein intake rather than carbohydrate or fat intake. From previous studies, it has been shown that the underreporting of fat and carbohydrate-rich foods is much more substantial compared with reporting protein-rich foods and is exacerbated especially by those with increasing overweight and obesity or among those with low education [46–49]. Thirdly, although we have adjusted for several potential confounders, as with any observational study, the risk of unmeasured or residual confounding might still be a concern. Lastly, this study

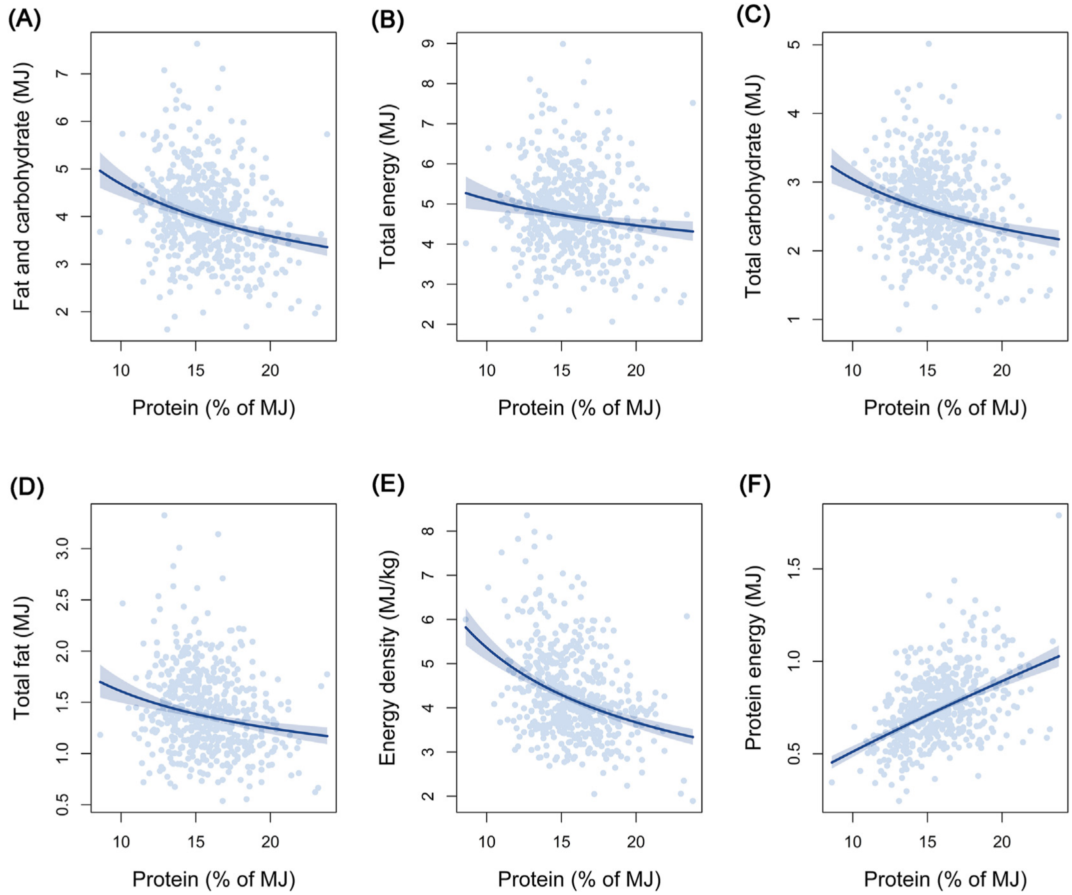

Fig. 4. Power functions between proportional protein intake and energy intake from different dietary components. Energy proportion from protein (% of MJ) was inversely associated with intakes of **(A)** combined fat and carbohydrate, **(B)** total energy, **(C)** total carbohydrate, **(D)** total fat, **(E)** energy density, and directly associated with **(F)** protein energy (all p-value <0.05). Each model was fully adjusted for sex, age, physical activity, maternal education level, and fibre intake. MJ, megajoule.

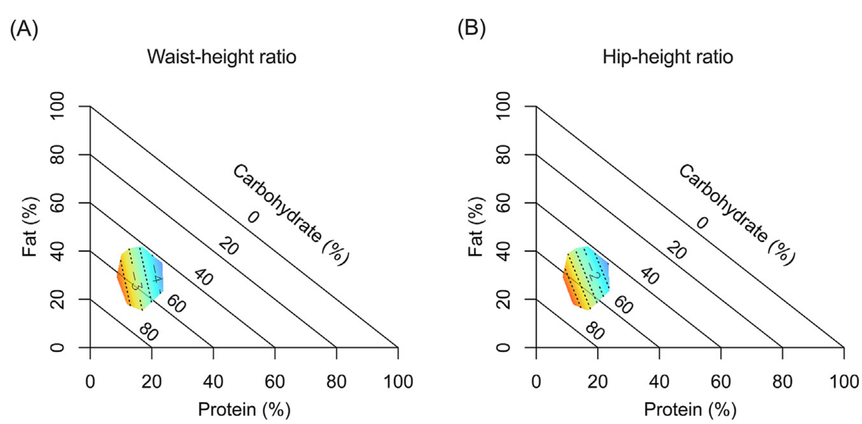

Fig. 5. Right-angle mixture triangle for dietary macronutrient distribution and changes of waist- and hip-height ratio. Response surfaces show the predicted **(A)** waist- and **(B)** hip-height ratio changes superimposed onto a composition triangle where cool colours represent the lowest values and warm colours represent the highest values. All points on the triangle represent 100% of dietary energy, being the sum of protein (x-axis) + fat (y-axis) + carbohydrate. Carbohydrate is shown as diagonal lines with higher intake closer to the origin. The response surface has been trimmed to display predictions for values observed in the dataset. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

was conducted in a population sample of healthy-weight children who were all predisposed to obesity, and the results may not generalize to all healthy-weight children. Thus, more research expanding to larger cohorts is needed to identify the optimal dietary protein and macronutrient composition on the risk of obesity to optimize dietary recommendations for children.

5. Conclusion

In this study in preschool children, protein intake was the most tightly regulated macronutrient, and energy intake was an inverse function of dietary protein concentration, indicating the evidence for protein leverage. Increases in WHtR and HHtR were principally associated with the dietary protein dilution, supporting the PLH. These findings highlight the importance of protein in children's diets, which seems to have significant implications for childhood obesity risk and overall health.

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Author contributions

The authors' responsibilities were as follows—HZ and BLH: were responsible for the study concept and design; SCL, NJO, and BLH: performed the data and project management; HZ and AMS: performed the data cleaning and analysis; HZ, AMS, CS, SJS, DR, and BLH: interpreted the data; HZ and BLH: drafted the manuscript; and all authors: read and approved the final manuscript and agree to be accountable for all aspects of the work.

Data share statement

Data described in the manuscript will be made available upon request pending application and approval.

Conflict of interest

All authors declared no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clnu.2023.09.025.

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