


## ORIGINAL ARTICLE

# Effects of a high-protein intake on metabolic targets for weight loss in children with obesity: a randomized trial

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## Summary

### Objective

The objective of this research is to study effects of a 4-week high-protein (HP) diet on energy intake, resting energy expenditure (REE), protein turnover and body composition in children with obesity.

### Methods

In this randomized placebo-controlled single-blind crossover study, children with obesity ( $n = 14$ ; mean age: 10.1 years  $\pm$  1.2 standard deviation; body mass index–standard deviation score [BMI–SDS]: 2.8  $\pm$  0.5) received an *ad libitum* HP (+50 g protein per day) or normal-protein (NP) diet for 4 weeks with a washout period of  $\geq 2$  weeks. Energy intake, REE, protein turnover, weight, BMI–SDS and body composition were measured.

### Results

No differences were found in energy intake or REE between HP and NP. There was an increased urea production and phenylalanine hydroxylation after HP compared with NP ( $p < 0.05$ ). There was an increased rise in fat-free mass after HP compared with NP ( $\Delta$ HP: 0.8  $\pm$  0.8 kg vs.  $\Delta$ NP: 0.1  $\pm$  0.6 kg,  $p < 0.05$ ). BMI and BMI–SDS increased during the study (BMI–SDS start: 2.8  $\pm$  0.5, end: 2.9  $\pm$  0.5,  $p < 0.05$ ) without a difference between groups.

### Conclusions

A 4-week HP diet with *ad libitum* food intake did not affect energy intake and energy expenditure in children with obesity. BMI increased, although that could be partly explained by an increase in fat-free mass.

**Keywords:** Body composition, dietary protein, energy expenditure, protein turnover.

## Introduction

The prevalence of childhood overweight and obesity is high. Currently, about 25–30% of the children in Europe and the USA are overweight and 5–15% are obese (1–3). Children with obesity are likely to become overweight or obese adults, with an increased risk of type 2 diabetes mellitus, cardiovascular disease and certain types of cancer (4–6). Already among children with obesity, there is a rising prevalence of the metabolic syndrome (7,8). Thus, effective strategies for weight loss and weight maintenance in children are needed.

In adults, randomized controlled trials with diet interventions using a high-protein (HP) intake have shown increased weight loss and improved weight maintenance after weight loss (9). Several metabolic targets have been shown to be affected by an HP diet: (1) suppressed appetite and reduced energy intake (10,11), (2) increased energy expenditure (12–15) and (3) preservation of fat-free mass (FFM) (16,17). The mechanism via which an HP diet affects metabolic targets like energy expenditure and preservation of FFM may be an increased protein and amino acid metabolism (10,18). The body has no storage capacity to cope with HP intake and therefore

has to metabolize it immediately. Gluconeogenesis has a major contribution to increased energy expenditure at an HP diet (19); however, the extent to which processes of protein metabolism, e.g. protein synthesis and protein oxidation, are actually affected by a relatively HP intake is not clear yet (13,20). Stable isotope techniques allow assessment of protein synthesis and breakdown as well as gluconeogenesis (21), and these will add to the understanding of what metabolic processes are affected by an HP diet.

With regard to children with overweight or obesity, some studies have focused on the effects an HP intake on weight loss and weight maintenance. In a large European study with 827 children, it was shown that an HP diet in combination with a low glycaemic index was protective against obesity (22). Other studies have shown less effects of an HP intake on body weight in children with overweight or obesity (23–25). Thus, the results of studies in children with obesity are inconclusive. However, before a long-term study focusing on body weight can be conducted, first, a short-term pilot study is needed to study which metabolic targets are affected by an HP intake in children with obesity. To the best of our knowledge, there are no studies that have assessed the effect of an HP diet on metabolic targets in children with obesity using different types of high-quality and well-validated techniques, like indirect calorimetry and stable isotope techniques. In the present study, the objective was to study the effects of a 4-week HP intake on energy intake, energy expenditure, protein turnover and body composition, i.e. fat mass and FFM, in children with obesity. The aim was to increase protein intake with approximately  $50 \text{ g d}^{-1}$  to reach an HP intake of  $\geq 20\%$  of energy, which generally is regarded as HP intake (9). The HP intake was applied via two HP supplements daily, in addition to *ad libitum* food intake. It was hypothesized that the HP supplements increased satiety, resulting in a lower *ad libitum* food intake from the regular diet. Altogether, it was hypothesized that, similarly to what has been shown in adults, an HP intake may have beneficial effects on energy intake, energy expenditure and body composition for children with obesity and may contribute to obtaining a healthy body weight.

## Methods

### Subjects

This study was conducted in two academic hospitals in the Netherlands: the Erasmus MC–Sophia Children's Hospital in Rotterdam and the VU University Medical Center in Amsterdam. Inclusion criteria were obesity (body mass index–standard deviation score [BMI-SDS]  $> 2.0$ ),

prepubertal according to the classification of Tanner, aged between 8 and 12 years and motivation to participate in the study of both the child and his or her parents. Exclusion criteria were obesity caused by a somatic treatable disorder or use of systemic steroids. The paediatrician checked the inclusion and exclusion criteria, and eligible children and their parents were subsequently informed about the study by the researcher. Motivation to participate in the study was assessed by the paediatrician based on his or her experiences with the family. The children and parents had visited the paediatrician and other disciplines in the hospital in the preceding months on several occasions because of the obesity the child suffered. The study was presented to the child and the parents as a trial studying potential beneficial effects of an HP intake, which in the end could be helpful for treatment and prevention strategies for children with obesity. Informed consent was obtained from the parents of all participants. The study was approved by the Medical Ethics Committee of the Erasmus MC. Participants were recruited from January 2011 to June 2014; the children were enrolled in the study one by one, and study measurements took place from May 2011 to September 2014. In general, the children attended school during the study; however, for some of the children, part of the study took part during a school holiday.

### Study design

The study had a randomized placebo-controlled single-blind crossover design with 4 weeks of an HP diet and 4 weeks of a normal-protein (NP) diet separated by a washout period of at least 2 weeks. For practical scheduling reasons, the washout period could last more than 2 weeks. One group of subjects received the diets in the order HP–NP (group 1), and the other group of subjects received the diets in the order NP–HP (group 2). The allocation sequence was generated using computerized randomization.

On day 0 and day 28 of both intervention periods (NP0, NP28, HP0 and HP28), the children came to the research unit. They underwent a series of measurements in the fasted state including anthropometrics, body composition, resting energy expenditure (REE), appetite and measurement of protein turnover using stable isotope techniques. Measurements took place at similar times on each test day, with  $t = 0$  min referring to baseline blood sampling and  $t = 360$  min to collection of the final blood samples. Other measurements took place between these time points.

The children kept a daily compliance diary during the intervention periods, and the children and their parents were in contact with the researchers by e-mail and/or

telephone once per 3 or 4 d. In the compliance diary, the children reported whether they consumed both supplements. The compliance diaries were discussed during the e-mails/phone calls and on day 28 of both intervention periods. This was to support compliance and to obtain an impression on the level of supplement intake. No quantitative data on compliance to the supplements were collected or analysed.

## Diets

In both HP and NP, the children consumed their *ad libitum* habitual diets and two supplements per day.

The protein supplement was offered as protein powder (Protifar Plus<sup>®</sup>, Nutricia, The Netherlands), which was processed into a milkshake using (fruit) yoghurt or chocolate milk. The children were provided with a list of recipes in order to prepare a milkshake with a taste the child preferred most. Depending on the recipe, the HP milkshakes contained between 179 and 189 kcal and 24.9–27.7 g protein, 5.6–17.3 g carbohydrates and 0.8–4.4 g fat per milkshake. When the children were on the NP diet, they consumed two iso-energetic milkshakes containing a carbohydrate and fat powder (Duocal<sup>®</sup>, Nutricia, The Netherlands) that was combined with fruit juice or fruit sauce and processed into a milkshake. Again, the children were provided with a list of recipes. Depending on the recipe, the NP milkshakes contained between 177 and 182 kcal and 0.2–1.3 g protein, 32.5–34.8 g carbohydrates and 4.5–4.8 g fat per milkshake. The milkshakes were consumed at the start of the breakfast and at the start of the dinner. The children were instructed to first consume the entire milkshake and after that to continue with the rest of normal breakfast or dinner, respectively, and consume as much or little from the meal as they preferred (i.e. *ad libitum*). In the washout period, the children ate their habitual diet *ad libitum* and did not receive supplements. The powder supplements were weighted into two daily portions by one of the researchers, while the children and their caregivers were blinded to the treatment.

## Measurements

### *Energy intake and appetite*

The children kept a 3-d food diary on three consecutive days (two weekdays and one weekend day) in week 3 of both intervention periods. On day 28 of both intervention periods, the children were interviewed by a trained researcher in order to cross-check the food diaries. The parents assisted their children with keeping the diary and with the interview. The results of the interview were

analysed for energy and macronutrient intake using the computerized nutritional tables of the Erasmus MC. The reported energy intake was compared with the measured REE, and a value of <1.3 was considered as underreporting and excluded from further analysis (26).

Appetite, i.e. hunger, fullness, desire to eat and prospective consumption, was rated on 100-mm visual analogue scales, anchored with 'not at all' and 'extremely' on each test day at time point  $t = 150$  min.

### *Energy expenditure (indirect calorimetry)*

The REE and respiratory quotient were calculated from the measurements of O<sub>2</sub> consumption and CO<sub>2</sub> production for 20 min using a ventilated hood system (Datex Division Instrumentarium, Finland). The formula of Brouwer (27) was used to calculate energy expenditure; urinary nitrogen excretion was neglected in the calculation.

### *Body weight and body composition*

Body weight and height were measured, and subsequently, BMI (in kg m<sup>-2</sup>) was calculated. BMI-SDS was calculated with the software programme Growth Analyser version 3 (Dutch Growth Foundation, Rotterdam, The Netherlands; www.growthanalyser.org). Body fat and FFM were measured using dual-energy X-ray absorptiometry (DEXA). Each scan of each child was made on the same machine (Lunar Prodigy, GE Healthcare at the Erasmus MC and Hologic Discovery at the VU University Medical Center) by a trained professional. The fat mass index (FMI) and the fat-free mass index (FFMI, in kg m<sup>-2</sup>) were calculated.

### *Stable isotope techniques: protein turnover and glucose production*

In order to calculate the amount of stable isotopes to be infused, the FFM was estimated using the formula of Deurenberg *et al.* (28) estimating that total body water was 73% of FFM.

On days 0 and 28 of both intervention periods, a Venflon catheter (Becton Dickinson, Franklin Lakes, NJ, USA) was placed in a superficial dorsal vein of the hand for blood sampling and another Venflon catheter was placed in a superficial vein of the other arm for intravenous infusion. A 6-h-primed continuous infusion of a mixture of stable isotopes (obtained from Cambridge Isotopes, Andover, MA, USA) was started after baseline blood sampling ( $t = 0$ ). [ring-<sup>2</sup>H<sub>5</sub>]Phenylalanine (98% enriched; prime: 5 μmol kg<sup>-1</sup> FFM; continuous: 3 μmol kg<sup>-1</sup> FFM h<sup>-1</sup>), [3,3-<sup>2</sup>H<sub>2</sub>]tyrosine (98% enriched; prime: 2 μmol kg<sup>-1</sup> FFM; continuous: 2 μmol kg<sup>-1</sup> FFM

$\text{h}^{-1}$ ) and [ring- $^2\text{H}_4$ ]tyrosine (98% enriched; prime:  $0.7 \mu\text{mol kg}^{-1}$  FFM) were used to measure protein oxidation and synthesis, and [ $^{15}\text{N}_2$ ]urea (98% enriched; prime:  $40 \mu\text{mol kg}^{-1}$  FFM; continuous:  $4 \mu\text{mol kg}^{-1}$  FFM  $\text{h}^{-1}$ ) was used for measurement of urea production. [6,6- $^2\text{H}_2$ ] Glucose (99% enriched; prime:  $22 \mu\text{mol kg}^{-1}$  FFM; continuous:  $20 \mu\text{mol kg}^{-1}$  FFM  $\text{h}^{-1}$ ) and oral  $^2\text{H}_2\text{O}$  (99.9% enriched, Eurisotop, Saint-Aubin Cedex, France;  $5 \text{ g kg}^{-1}$  body water) were used to measure endogenous glucose production and gluconeogenesis. The  $^2\text{H}_2\text{O}$  was consumed hourly between  $-60$  and  $180$  min. At  $240$ ,  $300$  and  $360$  min, blood samples were taken to measure enrichment of [ $^2\text{H}_5$ ]phenylalanine, [ $^2\text{H}_2$ ]tyrosine, [ring- $^2\text{H}_4$ ]tyrosine, [ $^{15}\text{N}_2$ ]urea, [ $^2\text{H}_2$ ]glucose, [6,6- $^2\text{H}_2$ ]glucose and plasma  $^2\text{H}_2\text{O}$  enrichment.

### Stable isotope techniques: gas chromatography, mass spectrometry and calculations

Blood samples were centrifuged for  $10$  min at  $3,500g$ , and plasma was stored at  $-80^\circ\text{C}$  until further analysis.

Plasma isotopic enrichments of [ring- $^2\text{H}_5$ ]phenylalanine, [3,3- $^2\text{H}_2$ ]tyrosine and [ring- $^2\text{H}_4$ ]tyrosine were determined by gas chromatography–mass spectrometry (GC-MS) (Agilent 5975C GC-MS, Agilent Technologies, Amstelveen, The Netherlands) after using the *N*-ethoxycarbonyl ethyl ester derivative according to a modified method of Husek (29).

Plasma urea enrichment was determined by GC-MS (Agilent 5975C GC-MS, Agilent Technologies) as described by Vlaardingerbroek *et al.* (30).

Enrichments of glucose labelled with  $^2\text{H}$  were measured by GC-MS (Agilent 5975C, Agilent Technologies, Wilmington, DE, USA) using the penta-acetate derivative as previously described (31). Plasma isotopic enrichment of [6,6- $^2\text{H}_2$ ]glucose (M+2) was determined by monitoring fragment ions at a mass-to-charge ratio (*m/z*) of  $169$  and  $171$ . Selective ion monitoring of *m/z*  $170/169$  was performed to determine the M+1 enrichment of deuterium in the circulating glucose carbons (C-1, 3, 4, 5, 6, 6) (31).

Isotopic enrichment of deuterium in plasma body water was determined by isotope ratio mass spectrometry (DeltaPlus XP IRMS, Thermo Fisher, Bremen, Germany).

Whole body protein kinetics, urea production and endogenous glucose production were calculated by conventional isotope dilution equations using a model during steady-state enrichment (21,32). Phenylalanine hydroxylation (rate of phenylalanine conversion into tyrosine) was calculated as described by Marchini *et al.* (33,34). Fractional gluconeogenesis was calculated using the average deuterium enrichment method (31).

### Statistical analysis

The Shapiro–Wilk normality test was used to determine whether data were normally distributed. Data are presented as mean  $\pm$  standard deviation unless otherwise indicated. For the variables energy intake, macronutrient intake, appetite, REE, respiratory quotient, protein turnover, endogenous glucose production, body weight and body composition comparisons between delta HP and delta NP, i.e.  $\Delta\text{HP} = \text{HP28} - \text{HP0}$  and  $\Delta\text{NP} = \text{NP28} - \text{NP0}$ , and between HP28 and NP28 were made using a paired *t*-test if normally distributed or a Wilcoxon signed rank test if not normally distributed. A *p*-value  $< 0.05$  was considered as statistically significant. Statistical procedures were performed using SPSS version 20 (IBM SPSS Statistics, Chicago, IL, USA).

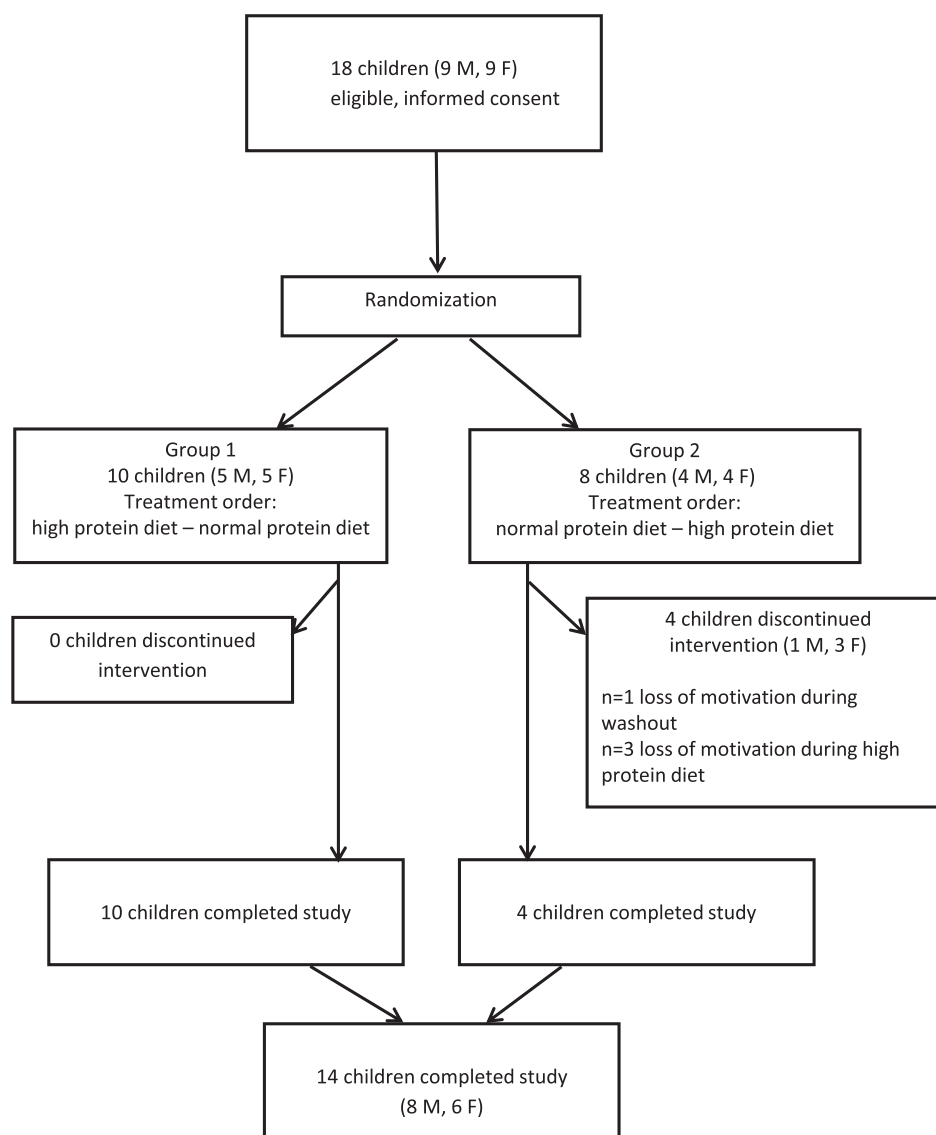
## Results

### Subjects

The flow chart of this study is presented in Figure 1. Eighteen healthy children (nine boys and nine girls) with obesity were randomized to participate in the study; baseline characteristics are described in Table 1. Four subjects dropped out of the study leaving 14 subjects who completed the study: eight boys and six girls with a mean age at start of the study of  $10.1 \pm 1.2$  years, body weight of  $60.06 \pm 13.42$  kg, height of  $1.45 \pm 0.09$  m, BMI of  $28.4 \pm 4.2$   $\text{kg m}^{-2}$  and BMI-SDS of  $2.8 \pm 0.5$ . Eleven children participated at the Erasmus MC–Sophia Children's Hospital, and three participated at the VU University Medical Center. There were no significant differences in age, sex or BMI-SDS at baseline between those who did and did not complete the study. The reason for not completing the study was loss of motivation to comply with the study requirements, i.e. to consume two milkshakes per day and to undergo the assessments, during the washout period ( $n = 1$ ) and loss of motivation during HP ( $n = 3$ ); all were subjects in group 2. There were no significant differences at baseline between subjects in group 1 and subjects in group 2. The mean washout period was  $5 \pm 2$  weeks, with a minimum of 3 weeks and a maximum of 9 weeks.

### Energy and macronutrient intake, compliance and appetite

Energy intake was considered as underreported in three children and left out of analysis in two cases during HP and two during NP. The energy and macronutrient intake is presented in Table 2. Protein intake was  $21\% \pm 3\%$  of



**Figure 1** Flow chart of the randomized placebo-controlled single-blind crossover study on the effects of a 4-week high-protein intake in children with obesity. M, male; F, female.

energy during HP, indicating a successful intervention to reach an HP intake.

There was no quantitative data collection or analysis from the compliance diaries. In general, the children indicated that they consumed the milkshakes entirely and that on a few occasions they forgot to take the milkshakes. All in all the compliance diaries showed a good compliance to milkshakes without major differences between HP and NP.

There were no differences in hunger, fullness, desire to eat and prospective consumption as measured with 100-mm visual analogue scales between HP and NP (Table 3).

#### Resting energy expenditure and respiratory quotient

The REE was  $1,575 \pm 264$  and  $1,574 \pm 247$  kcal/24 h at HP0 and HP28, respectively, and  $1,595 \pm 239$  and  $1,590 \pm 256$  kcal/24 h at NP0 and NP28, respectively. There were no differences in REE between HP and NP.

The respiratory quotient was  $0.80 \pm 0.07$  and  $0.79 \pm 0.07$  at HP0 and HP28, respectively, and  $0.81 \pm 0.05$  and  $0.80 \pm 0.06$  at NP0 and NP28, respectively. There were no differences in respiratory quotient between HP and NP.



**Table 1** Baseline characteristics of subjects enrolled in a randomized placebo-controlled single-blind crossover study with a 4-week high-protein intake ( $n = 18$ )

	Group 1 ( $n = 10$ )	Group 2 ( $n = 8$ )
M/F	5/5	4/4
Age (year)	10.0 ± 1.1	10.2 ± 1.5
Body weight (kg)	57.34 ± 6.58	59.17 ± 17.73
Height (m)	1.44 ± 0.09	1.45 ± 0.10
BMI ( $\text{kg m}^{-2}$ )	27.7 ± 3.4	27.7 ± 4.8
BMI-SDS	2.7 ± 0.4	2.7 ± 0.6

Treatment order: high-protein diet–normal-protein diet (group 1); normal-protein diet–high-protein diet (group 2).  
BMI, body mass index; F, female; M, male.

**Table 2** Energy and macronutrient intake during a randomized placebo-controlled single-blind crossover study with a 4-week high-protein intake in children with obesity ( $n = 12$ )

	Normal-protein diet	High-protein diet
Energy ( $\text{kcal d}^{-1}$ )	2,156 ± 252	2,311 ± 292
Protein ( $\text{g d}^{-1}$ )	72 ± 24	120 ± 15**
Protein (En%)	13 ± 3	21 ± 3**
Fat ( $\text{g d}^{-1}$ )	81 ± 15	78 ± 17
Fat (En%)	34 ± 3	30 ± 5*
Carbohydrate ( $\text{g d}^{-1}$ )	283 ± 32	283 ± 48
Carbohydrate (En%)	53 ± 5	49 ± 5

\*Significant difference between high-protein diet and normal-protein diet,  $p < 0.05$  paired  $t$ -test.

\*\*Significant difference between high-protein diet and normal-protein diet,  $p < 0.01$  paired  $t$ -test.

**Table 3** Appetite ratings in the fasted state measured with 100-mm visual analogue scales during a randomized placebo-controlled single-blind crossover study with a 4-week high-protein intake in children with obesity ( $n = 14$ )

	Normal-protein diet		High-protein diet	
	Day 0	Day 28	Day 0	Day 28
Hunger (mm)	57 ± 27	68 ± 25	50 ± 28	61 ± 37
Fullness (mm)	21 ± 24	17 ± 19	17 ± 19	13 ± 16
Desire to eat (mm)	53 ± 34	63 ± 31	52 ± 30	52 ± 35
Prospective consumption (mm)	52 ± 21	59 ± 28	57 ± 27	60 ± 32

No significant differences between NP0 and NP28 and between HP0 and HP28.

### Protein turnover and endogenous glucose production

Protein turnover and endogenous glucose production were measured in a subgroup of  $n = 8$  children from the

Erasmus MC–Sophia Children's Hospital who had data available for all four study visits. There were no significant differences in age, BMI, BMI-SDS or body composition between this subgroup and the total study population. There were no significant differences between HP0 and NP0. Ra urea, Ra tyrosine and the phenylalanine hydroxylation were increased at HP28 compared with NP28 (Table 4). In addition, the phenylalanine balance was more negative at HP28 compared with NP28. There were no differences in endogenous glucose production or fractional gluconeogenesis between HP and NP (Table 4).

### Body weight and body composition

When analysed together, regardless of the type of diet, body weight and BMI increased significantly during participation in the study, which had an average duration of  $13 \pm 1$  weeks. At the start of the study, body weight was  $60.06 \pm 13.42$  kg, and it was  $62.82 \pm 14.24$  kg at the end of the study ( $p < 0.001$ ; BMI start:  $28.4 \pm 4.2$   $\text{kg m}^{-2}$ , end:  $29.3 \pm 4.5$   $\text{kg m}^{-2}$ ,  $p < 0.01$ ). This resulted in a significant increase in BMI-SDS (start:  $2.8 \pm 0.5$ , end:  $2.9 \pm 0.5$ ,  $p < 0.05$ ). The increase in body weight and BMI was significantly larger in the second intervention period compared with the first intervention period (body weight  $\Delta$ 1st intervention period:  $0.86 \pm 0.95$  kg vs.  $\Delta$ 2nd intervention period:  $1.5 \pm 0.67$  kg,  $p < 0.05$ ; BMI  $\Delta$ 1st intervention period:  $0.2 \pm 0.6$   $\text{kg m}^{-2}$  vs.  $\Delta$ 2nd intervention period:  $0.6 \pm 0.4$   $\text{kg m}^{-2}$ ,  $p < 0.05$ ).

When analysed for HP and NP separately, body weight and BMI increased significantly during HP and during NP (Table 5). There were no significant differences in body weight, BMI or BMI-SDS between HP and NP (Table 5).

For practical reasons, DEXA scans were not available in several occasions. Therefore, only data of the children with DEXA scans on all four occasions ( $n = 10$ ) were analysed. Both during HP and during NP, fat mass and FMI increased significantly (Table 5). In addition, during HP, FFM and FFMI increased significantly (Table 5), and delta FFM and delta FFMI were increased during HP compared with NP (Table 5).

### Discussion

This study showed that a diet intervention with a 4-week HP intake did not affect *ad libitum* energy intake, appetite or REE in children with obesity. Body weight, BMI and BMI-SDS as well as the fat mass and FMI of the children increased significantly during the study period. However, during the HP diet, the FFM and the FFMI also increased, which may partly explain the increase in BMI during the HP diet. FFM, of which muscle mass is the major

**Table 4** Protein turnover and endogenous glucose production during a randomized placebo-controlled single-blind crossover study with a 4-week high-protein intake in children with obesity ( $n = 8$ )

	Normal-protein diet			High-protein diet		
	Day 0	Day 28	Delta	Day 0	Day 28	Delta
Ra urea ( $\mu\text{mol kg}^{-1} \text{h}^{-1}$ )	304 ± 46	284 ± 73	-20.2 ± 65.5	319 ± 96	377 ± 62*	57.8 ± 86.9
Ra phenylalanine ( $\mu\text{mol kg}^{-1} \text{h}^{-1}$ )	54.4 ± 12	44.7 ± 13	-9.7 ± 15.8	55.1 ± 14	51.7 ± 8.3	-3.3 ± 15.9
Ra tyrosine ( $\mu\text{mol kg}^{-1} \text{h}^{-1}$ )	46.1 ± 12	37.7 ± 15	-8.4 ± 11.4	45.7 ± 11	45.9 ± 9.9*	0.2 ± 10.5
Phenylalanine hydroxylation ( $\mu\text{mol kg}^{-1} \text{h}^{-1}$ )	13 ± 4.8	9.4 ± 4.9	-3.6 ± 5.7	13 ± 4.9	12.3 ± 3.5*	-0.6 ± 6
Non-hydroxylation phenylalanine disposal ( $\mu\text{mol kg}^{-1} \text{h}^{-1}$ )	41.4 ± 7.4	35.3 ± 9.5	-6.1 ± 10.8	42.1 ± 9.8	39.4 ± 6.5	-2.7 ± 10.4
Phenylalanine balance ( $\mu\text{mol kg}^{-1} \text{h}^{-1}$ )	-13 ± 4.8	-9.4 ± 4.9	3.6 ± 5.7	-13 ± 4.9	-12.3 ± 3.5*	0.6 ± 6
Endogenous glucose production ( $\text{mg kg}^{-1} \text{min}^{-1}$ )	3.2 ± 0.3	2.9 ± 0.4	-0.3 ± 0.5	3.2 ± 0.3	3.1 ± 0.3	-0.1 ± 0.3
Fractional gluconeogenesis (%)	66 ± 14	69 ± 14	3 ± 10.0	63 ± 12	67 ± 6	4 ± 8

Delta: day 28 minus day 0.

No significant differences between NP0 and NP28 and between HP0 and HP28.

\*Significant difference between day 28 high-protein diet and day 28 normal-protein diet,  $p < 0.05$  paired  $t$ -test.

**Table 5** Body weight and body composition during a randomized placebo-controlled single-blind crossover study with a 4-week high-protein intake in children with obesity ( $n = 14$ )

	Normal-protein diet			High-protein diet		
	Day 0	Day 28	Delta	Day 0	Day 28	Delta
Body weight (kg)	61.04 ± 13.30	62.13 ± 13.43***	1.1 ± 0.5	60.37 ± 13.99	61.60 ± 14.31**	1.23 ± 1.13
Body mass index ( $\text{kg m}^{-2}$ )	28.7 ± 4.2	29.0 ± 4.2**	0.3 ± 0.3	28.4 ± 4.5	28.9 ± 4.6*	0.4 ± 0.6
BMI-SDS	2.8 ± 0.5	2.9 ± 0.5	0.0 ± 0.1	2.8 ± 0.6	2.8 ± 0.5	0.1 ± 0.1
Body fat (%)	46.4 ± 4.3	47.3 ± 4.4	0.8 ± 1.2	46.3 ± 4.1	46.5 ± 4.5	0.2 ± 1.1
Body fat (kg)	29.6 ± 8.9	30.6 ± 8.8*	1.0 ± 0.9	29.1 ± 8.9	30.1 ± 9.2*	0.9 ± 0.9
Fat-free mass (kg)	33.4 ± 6.7	33.5 ± 6.9	0.1 ± 0.6	33.1 ± 7.5	33.9 ± 7.6*	0.8 ± 0.8*
Fat mass index ( $\text{kg m}^{-2}$ )	14.0 ± 3.2	14.4 ± 3.3*	0.4 ± 0.4	13.8 ± 3.3	14.1 ± 3.4*	0.4 ± 0.5
Fat-free mass index ( $\text{kg m}^{-2}$ )	15.8 ± 1.6	15.7 ± 1.7	-0.1 ± 0.4	15.7 ± 1.9	16.0 ± 2.0*	0.3 ± 0.4*

Delta: day 28 minus day 0.

Body fat, fat-free mass, fat mass index and fat-free mass index were analysed in  $n = 10$  children.

\*\*\* $p < 0.001$  paired  $t$ -test/Wilcoxon signed rank test between day 28 and day 0 within a diet or between delta high-protein diet and delta normal-protein diet.

\*\* $p < 0.01$  paired  $t$ -test/Wilcoxon signed rank test between day 28 and day 0 within a diet or between delta high-protein diet and delta normal-protein diet.

\* $p < 0.05$  paired  $t$ -test/Wilcoxon signed rank test between day 28 and day 0 within a diet or between delta high-protein diet and delta normal-protein diet.

component, determines energy expenditure to a much greater extent than fat mass does. In the long term, an increase in muscle mass may have a significant effect on energy expenditure (35). The sparing of FFM with an HP diet during weight loss or weight maintenance after weight loss has been shown before (16,17,36); the present study shows that an HP diet increases FFM during weight gain. The small but significant increase in FFM after the HP diet may in the long term result in increased energy expenditure and less metabolic disturbances. However, the present study showed no such a benefit in the short term. The overall weight gain during the study

period can be explained by a higher energy intake as compared with the energy needs, which is likely to be at least partly explained by the absence of compensation of energy intake from normal food intake.

Several studies in adults did observe effects of an HP diet on metabolic targets (10–17,19,37). The amount of protein the children consumed in the present study in the HP diet (21 En%) was similar to most of the studies in adults. The lack of beneficial effects on appetite, energy intake or energy expenditure may partly be explained by differences in timing in comparison with previous studies. In the present study, appetite and

energy expenditure were measured in the fasted state after 4 weeks on the diet whereas in other studies, it was measured immediately after an HP meal (10) or continuously while on an HP diet for 24–36 h (12–15). Although considered to be very relevant, in the present study, it was chosen not to add questionnaires to complete at home, given that the burden for the participants was already high.

Protein metabolism was measured using stable isotope techniques to study which processes were affected by an HP intake and could be affecting metabolic targets like energy expenditure and body composition. The results show increased protein oxidation after the HP diet reflected by an increased urea production and phenylalanine hydroxylation. Although not statistically significant, the non-hydroxylation phenylalanine disposal was also slightly increased after the HP diet. However, the phenylalanine breakdown, i.e. the rate of appearance of phenylalanine, was increased to a larger extent resulting in a more negative phenylalanine balance, indicating a higher protein breakdown than protein synthesis. These results are in line with those of Pasiakos *et al.* who also showed increased oxidation and a decreased net balance with increased protein intake in overweight adults (38). The increase in FFM during the HP diet suggests a net increase in protein synthesis, which is in contrast with the more negative phenylalanine balance. In our design, we were not able to measure protein turnover in the postprandial state. Therefore, we do not know whether the increased protein intake may have resulted in a higher protein synthesis during those periods. It can only be speculated that the increase in FFM that was observed during the HP diet can be explained by an increased protein synthesis in the postprandial phase.

In the present study, no effects of the HP diet on appetite or energy intake were observed. Studies that did show a satiating effect of an HP intake have predominantly been performed in adults. However, Lomenick *et al.* have shown a short-term satiating effect of an HP intake in children (39). In two longer-term studies with HP diets in children with obesity, there was no effect on appetite sensations (23,24). The potential satiating effect of HP effect was not strong enough under *ad libitum* conditions, i.e. when children are free to choose what, when and how much to eat. Eating behaviour is determined by several internal and external cues, including taste preferences, emotions and social factors (40). Apparently, the effect of an HP diet was not as strong as compared with all these other signals and the subjects stayed in a positive energy balance during the study.

One of the limitations of the study is that the number of subjects is relatively low. Unfortunately, the study stopped before the planned number of 40 subjects was

reached because of severe difficulty recruiting participants. For the participants of the study, the burden was high because of the frequent visits to the research unit and regular blood sampling on those study visits. From the 18 children that were included in the study, four (22%) dropped out before the end of the study. Dropout is a major concern in studies in children with obesity, and dropout rates much higher than those in the present study have been reported (41). All dropouts occurred during the second half of the intervention, and all were on the HP diet in this period. It cannot be ruled out that the HP diet itself affected the motivation and willingness to comply with the study requirements. However, there was no indication that compliance to the HP supplements decreased during the study period. During recruitment, a lot of effort was put in including only those children and parents who were highly motivated. Nevertheless, it seems that during the study period, the motivation to comply with the study requirements decreased. The study did not aim to induce body-weight loss, but it was expected that in such a highly motivated population, the results would show a stabilization of BMI-SDS during the study period. The fact that this did not happen shows how difficult body-weight management is for children with obesity. It may be speculated that the effect of the diet that the children would experience was not as big as the participants had hoped for and that this resulted in a loss of motivation. The attention that was paid to inclusion of only those children and parents who were highly motivated may have resulted in a study population that is not fully representative of the total population. This should be taken into account when interpreting the results for a broader audience. The number of subjects in the study should also be seen in the light of the study design and type of measurement. It was a crossover study where the children were their own control, thereby reducing the effect of confounding. In addition, for measurements using stable isotope techniques, studies with eight subjects are not uncommon (21). A major strength of the study is the use of different types of high-quality and well-validated techniques, like indirect calorimetry, DEXA scanning and stable isotope techniques to measure different outcomes relating to energy and protein metabolism. These are costly and relatively invasive techniques for children, adding to the uniqueness of this study.

This study was designed to study the mechanistic effects of an HP intake in children with obesity. It was hypothesized that the HP supplements increased satiety, resulting in a lower *ad libitum* food intake from the regular diet. However, the results show that this was not the case. The results show a small beneficial effect on FFM; however, no effects on energy intake or energy



expenditure were observed. Moreover, the BMI-SDS and fat mass of the children significantly increased during the study period, which indicates that the obesity became worse during the study period. It needs further study whether an HP intake may be supportive for children with obesity in reducing hunger feelings and supporting FFM when on a diet with a reduced energy content for weight loss. In conclusion, a 4-week HP diet with *ad libitum* food intake did not affect energy intake and energy expenditure in children with obesity. BMI increased, although that could be partly explained by an increase in FFM.

## Conflict of Interest Statement

The authors declare no conflicts of interest.

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This study was registered at the Netherlands National Trial Register, [www.trialregister.nl](http://www.trialregister.nl), as NTR2372.

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