

Prebiotic supplementation improves appetite control in children with overweight and obesity: a randomized controlled trial^{1–3}

Megan P Hume,⁴ Alissa C Nicolucci,⁴ and Raylene A Reimer^{4,5*}

⁴Faculty of Kinesiology and ⁵Department of Biochemistry and Molecular Biology, Cumming School of Medicine, University of Calgary, Calgary, Alberta, Canada

ABSTRACT

Background: Prebiotics have been shown to improve satiety in adults with overweight and obesity; however, studies in children are limited.

Objective: We examined the effects of prebiotic supplementation on appetite control and energy intake in children with overweight and obesity.

Design: This study was a randomized, double-blind, placebo-controlled trial. Forty-two boys and girls, ages 7–12 y, with a body mass index (BMI) of ≥ 85 th percentile were randomly assigned to 8 g oligofructose-enriched inulin/d or placebo (maltodextrin) for 16 wk. Objective measures of appetite included energy intake at an ad libitum breakfast buffet, 3-d food records, and fasting satiety hormone concentrations. Subjective appetite ratings were obtained from visual analog scales before and after the breakfast. Children's Eating Behavior Questionnaires were also completed by caregivers.

Results: Compared with placebo, prebiotic intake resulted in significantly higher feelings of fullness ($P = 0.04$) and lower prospective food consumption ($P = 0.03$) at the breakfast buffet at 16 wk compared with baseline. Compared with placebo, prebiotic supplementation significantly reduced energy intake at the week 16 breakfast buffet in 11- and 12-y-olds ($P = 0.04$) but not in 7- to 10-y-olds. Fasting adiponectin ($P = 0.04$) and ghrelin ($P = 0.03$) increased at 16 wk with the prebiotic compared with placebo. In intent-to-treat analysis, there was a trend for prebiotic supplementation to reduce BMI z score to a greater extent than placebo (-3.4% ; $P = 0.09$) and a significant -3.8% reduction in per-protocol analysis ($P = 0.043$).

Conclusions: Independent of other lifestyle changes, prebiotic supplementation in children with overweight and obesity improved subjective appetite ratings. This translated into reduced energy intake in a breakfast buffet in older but not in younger children. This simple dietary change has the potential to help with appetite regulation in children with obesity. This trial was registered at clinicaltrials.gov as NCT02125955. *Am J Clin Nutr* doi: 10.3945/ajcn.116.140947.

Keywords: pediatric, obesity, appetite, prebiotic, dietary fiber, oligofructose-enriched inulin

INTRODUCTION

More than one-third of US and Canadian children are overweight or obese (1, 2). The current obesogenic environment, which includes

readily available, highly palatable foods that are low in dietary fiber, is one factor contributing to the increase in childhood obesity (3, 4). Despite the increase in childhood obesity throughout the world, body composition varies greatly within populations, even within families who share similar environments and lifestyles (4). Intrapopulation variability in adiposity suggests that there are individual-level traits that may increase a child's risk of developing obesity. Appetitive traits, including low satiety responsiveness and high food responsiveness, may be important individual-level traits that cause a child to overeat or to eat in the absence of hunger, thereby contributing to chronic energy imbalance and weight gain (4, 5).

Adolescent obesity is predictive of adulthood obesity and achieving a healthy body weight and lifestyle in childhood must therefore be a leading priority for strategies aimed at reducing obesity. Typical nutrition-based treatments for the management of adulthood obesity often include restrictive diets that reduce energy intake and/or dramatically alter macronutrient intake; however, these approaches are less effective in children and may promote weight gain via binge eating, increased desire for restricted foods, and heightened consumption of "snacks" throughout the day (6–8). Alternatively, focusing on the addition of certain foods and nutrients that are known to reduce the risk of obesity, such as dietary fiber, represents a potentially more suitable management option in the pediatric population (9–11). Importantly, more comprehensive multidisciplinary and family-based interventions that include dietary changes have shown success in reducing BMI z scores in children (12).

Increasing dietary fiber intake by using a specific type of fiber called prebiotics may stimulate satiety hormones and enhance

¹ Supported by grants from the BMO Financial Group, Alberta Children's Hospital Foundation, Alberta Children's Hospital Research Institute, and the Canadian Institutes of Health Research (MOP115076-1). The oligofructose-enriched inulin (Synergy1) was provided by Beneo (Mannheim, Germany).

² The funding agencies had no role in the design of the study or preparation of the manuscript and had no influence on the data collection, analysis, or interpretation or manuscript publication.

³ Supplemental Methods are available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at <http://ajcn.nutrition.org>.

*To whom correspondence should be addressed. E-mail: reimer@ucalgary.ca.

Received June 28, 2016. Accepted for publication January 26, 2017.

doi: 10.3945/ajcn.116.140947.

appetite control, thereby helping to manage body weight (13, 14). Health benefits of prebiotics are most often attributed to their ability to stimulate the growth and activity of health-promoting bacteria in the gut while simultaneously improving appetite and postprandial glucose and insulin concentrations (10–21 g prebiotic/d) (15, 16). Prebiotics have been shown to decrease food intake and reduce body fat in overweight and obese adults (13). To date, to our knowledge, only 2 published studies have examined the effects of prebiotics on body weight in children: a study in which the prebiotic was combined with increased calcium intake and the primary outcome was change in bone mineral density in healthy normal-weight adolescents (17) and a trial by Liber and Szajewska (18) in which the primary outcome was body weight changes in children with overweight and obesity after 12 wk of prebiotic intake. However, beyond a self-reported 3-d food record in the study by Liber and Szajewska (18), there is a lack of knowledge on the effects of prebiotics on appetite regulation in children. Therefore, our objective was to assess the effects of 16 wk of prebiotic supplementation on subjective and objective measures of appetite control as well as BMI *z* score in children with overweight and obesity.

METHODS

Informed consent

This project received ethical approval from the Conjoint Health Research Ethics Board of the University of Calgary (ethics ID: REB13-0975), and the study was registered at clinicaltrials.gov (NCT02125955).

Participants

A total of 42 male and female participants with overweight or obesity (BMI \geq 85th percentile) between the ages of 7 and 12 y were voluntarily recruited in 2014–2015 from the city of Calgary, Canada, to participate in this randomized, double-blind, placebo-controlled trial. This study was part of a larger clinical trial that assessed multiple outcomes (19). The primary outcome of this particular study was the effect of prebiotics on appetite (serum satiety hormones, appetite ratings, and food intake) and, secondarily, BMI *z* score. Based on the outcomes of the entire trial, sample size was calculated for percentage body fat and BMI *z* score on the basis of data from studies that examined the effects of lifestyle intervention (diet and exercise) in children with overweight and obesity. Specifically, with an estimated reduction in BMI *z* score of 0.26 and an SD of 0.22 based on a power of 0.8 to detect a significant difference ($P = 0.05$, 2-sided), a minimum of 24 participants ($n = 12/\text{group}$) were needed (20). With an estimated difference in percentage body fat of 6% and an SD of 6.26 based on a power of 0.8 to detect a significant difference ($P = 0.05$, 2-sided), a minimum of 36 participants ($n = 18/\text{group}$) were needed (21); 8 additional participants (4/group) were added to account for dropouts. We were also adequately powered to detect differences in serum ghrelin on the basis of the diet and exercise interventions of Kelishadi et al. (22).

Participants were randomly assigned to the prebiotic or placebo group by using a computer-generated randomization list that was formulated to stratify by age, sex, and BMI percentile. The

randomization sequence was generated by an investigator not involved in recruiting participants, and a research assistant was responsible for product distribution to ensure the correct product was provided to the groups. Randomization sequences were not revealed to the study staff. Participants and study staff were blinded to the treatments, which were provided to the participants in identical individual-serving foil packets. Overweight and obese boys and girls, ages 7–12 y, with BMIs \geq 85th percentile for their age and sex, were eligible to participate in this study. Given that puberty has been shown to affect fat mass, digestion, and energy storage and expenditure, pubertal stage was assessed before study participation (23, 24). Participants were required to be at Tanner development stage ≤ 3 , as determined by a pediatrician from the Alberta Children's Hospital during a brief confidential physical examination after confirmation of the caregivers' consent. Girls were required to be premenarchal. Participants were excluded if they had used prebiotic or probiotic supplements or had been administered antibiotics within 3 mo of entering the study; had type 1 or type 2 diabetes, cardiovascular health conditions, or liver diseases; had previous gastrointestinal surgery; were taking drugs that influenced appetite, weight, and/or metabolism, including herbal supplements; were following a diet designed for weight loss at the time of study commencement; or had undergone a weight change of >3 kg within 3 mo of study commencement. Last, participants who were unable to provide caregiver consent were excluded from the study.

Dietary intervention

Participants were randomly assigned to consume either 8 g prebiotic/d (oligofructose-enriched inulin; Synergy 1; Beneo) or an equicaloric dose of a 3.3-g maltodextrin placebo/d (Agenamalt 20.222; Agrana) for 16 wk. Maltodextrin was selected as a placebo due to its physical and chemical properties, which gave it a similar taste and appearance, and its successful use as a placebo in previous prebiotic supplementation trial in adults with overweight and obesity (13) and a similar study examining the impact of oligofructose supplementation on satiety in normal-weight adults (25). The prebiotic fiber and placebo were supplied to the participants in individualized identical foil packets. The prebiotic packet contained the entire dose of 8 g oligofructose-enriched inulin and the placebo packet contained the entire dose of 3.3 g maltodextrin. Therefore, participants in both groups were required to consume 1 packet/d. Both groups were instructed to add the powder from 1 packet to 250 mL water and consume the entire mixture ~ 15 –30 min before their dinner meal. Participants were provided with a reusable water bottle on their initial test day to allow for consistent measurement throughout the study and across the study groups. The dose of prebiotic and placebo was increased over a period of 2 wk to allow for adaptation to the fiber and to minimize gastrointestinal symptoms (flatulence and bloating). For the first 2 wk, each participant was instructed to mix 1 packet into 250 mL water but only drink half of the mixture and therefore consume half of the daily dose. For the remaining 14 wk, participants were asked to consume the entire water bottle containing the complete daily dose of prebiotic fiber or maltodextrin. Participants were asked to return all used (empty) and unused (full) packets to assess for compliance.

Maintenance of dietary habits and physical activity

The goal of this study was to analyze the effects of the prebiotic supplementation independent of any other lifestyle changes such as diet or exercise. Thus, participants purchased and prepared their own meals throughout the study with no influence from the research team. Individuals were instructed to eat until comfortably full and maintain their usual level of physical activity. Caregivers recorded their child's physical activity by using the Godin's Leisure-Time Exercise Questionnaire at baseline and at 8 and 16 wk (26).

Food intake

Food and beverage intakes were assessed with the use of 3-d weighed food records. Caregivers were provided with a food scale and instructed to weigh and record all foods and beverages consumed by their child for 2 weekdays and 1 weekend day at baseline and at 8 and 16 wk. This information was analyzed with the use of the Food Works software program (The Nutrition Company).

Ad libitum breakfast buffet

Participants' energy intake was determined at an ad libitum breakfast buffet that took place at baseline and again at the end of the study (week 16). Participants were offered breakfast foods in reasonable surplus amounts to eliminate researcher-imposed limits to voluntary food intake. The energy content of food choices was predetermined. Participants were advised to eat until comfortably full. After participants consumed all they wished to, research staff measured the total weight of the remaining food not consumed and used this information to calculate energy intake.

Blood sampling

A registered nurse from the Alberta Children's Hospital collected ~8 mL fasted blood from each participant at baseline and at the end of the study (week 16). To obtain the serum for satiety hormone analysis, blood samples were collected in cooled microfuge tubes containing Diprotin A (0.034 mg/mL blood; MP Biomedicals), Sigma protease inhibitor (1 mg/mL blood; Sigma-Aldrich), and Pefabloc (1 mg/mL blood; Roche). Serum was stored at -80°C until analyzed for gut hormones, adipokines, and insulin.

Serum analysis: gut hormones, adipokines, and insulin

Serum samples were analyzed in duplicate for gut hormones involved in appetite control, including active glucagon-like peptide 1 (GLP-1),⁶ total peptide tyrosine tyrosine (PYY), active ghrelin, and total glucose-dependent insulinotropic polypeptide (GIP) along with leptin and insulin with the use of the Milliplex Map Human Metabolic Hormone Magnetic Bead Panel–Metabolic Multiplex Assay (Millipore; see **Supplemental Methods** for details). Serum was also analyzed for the adipokines, adiponectin, and resistin by using the Milliplex Map Human Adipokine

Magnetic Bead Panel 1–Endocrine Multiplex Assay kit. Eve Technologies Corporation performed the plate analysis with the use of Luminex 200 instrumentation and software.

Visual analog scale

Subjective sensations of appetite were determined by using a 100-mm visual analog scale (VAS) at baseline and at the end of study immediately before and after the ad libitum breakfast buffet according to our previous work (27). The “Before Breakfast” and “After Breakfast” VAS consisted of 5 and 6 questions, respectively. Questions range from “How hungry do you feel?” to “How full do you feel?” and were anchored by “I am not hungry at all” or “Not at all full” and “I have never been more hungry” or “totally full.”

Children's Eating Behavior Questionnaire

Subjective appetite and eating behaviors were also assessed by using the Children's Eating Behavior Questionnaire (CEBQ) (28), which was completed at baseline and at 8 and 16 wk. Caregivers were asked to complete the questionnaire with their child's input. This 35-item questionnaire was designed to assess eating behavior styles that are related to obesity risk and is divided into 8 subscales: Satiety Responsiveness, Food Responsiveness, Enjoyment of Food, Emotional Overeating, Emotional Undereating, Desire to Drink, Slowness in Eating, and Food Fussiness.

BMI z score

BMI z score, also known as the BMI SD score, is a measure of relative weight and height adjusted for age and sex applied to a reference standard. These scores are considered to be more appropriate for determining longitudinal changes in body weight and adiposity while also being a superior measure for comparing between-group mean values (29). Thus, BMI z score was used to assess body weight changes in participants. The Children's Hospital of Philadelphia Pediatric z score calculator, which uses CDC growth charts, was used to calculate BMI z score (<http://stokes.chop.edu/web/zscore/>).

Statistical analyses

Data are presented as means \pm SEMs. The primary statistical analyses were performed on an intent-to-treat (ITT) basis, regardless of compliance, in which missing values were imputed via the means of the last observation carried forward. Data were analyzed with SPSS 20.0 software for Windows (IBM Corporation). Differences between groups at baseline were determined by using independent *t* tests for continuous variables and chi-square tests for categorical variables. Changes from baseline in subjective and objective measures of appetite were determined by subtracting initial values from the final values, except in the case of 3-d dietary food records and CEBQ scores. Analysis of change in ad libitum breakfast buffet energy intake, gut hormones, adipokines, insulin, and VAS score was carried out by using ANCOVA, with age and sex included as covariates. CEBQs and 3-d weighed food records were analyzed by using repeated-measures ANCOVA, with age and sex included as covariates. Baseline scores were also used as a covariate when

⁶ Abbreviations used: CEBQ, Children's Eating Behavior Questionnaire; FFAR, free-fatty acid receptor; GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide 1; ITT, intent-to-treat; PYY, peptide tyrosine tyrosine; SCFA, short-chain fatty acid; VAS, visual analog scale.

they were determined to be significantly different between groups and were incorporated into the model. Change in BMI z score from baseline was calculated by subtracting the initial values from the final values and analyzed with the use of ANCOVA, with age and sex included as covariates. Both ITT and per-protocol analyses of children who completed the study were completed for BMI z score. Physical activity was analyzed by using repeated-measures ANOVA.

RESULTS

Participants

Forty-two participants provided signed consent to participate in the study and were randomly assigned to either the prebiotic or placebo group (**Figure 1**). A total of 38 participants completed the study, 20 in the prebiotic group (10 girls and 10 boys) and 18 in the placebo group (7 girls and 11 boys). There were no significant differences between groups at baseline in terms of sex, height, weight, and BMI z score (**Table 1**). The retention rate was 90%. One of the participants did not attend baseline testing and did not participate in the study. Three additional participants

dropped out (2 from the prebiotic group and 1 from the placebo group) over the course of the study. Reasons for withdrawing from the study included personal matters (time commitment issues) and others not specified. When an informal interview was conducted at the end of the study to assess blinding, 50% and 72% of the prebiotic and placebo participants, respectively, correctly guessed their group assignment.

Food intake

On the basis of the weighed 3-d food records, both the prebiotic and placebo groups reported lower energy intake (kilocalories per day) at 8 and 16 wk with a significant effect of time ($P = 0.047$) but not treatment ($P = 0.12$) or time \times treatment interaction ($P = 0.92$) (**Table 2**). Repeated-measures ANCOVA, with sex and age as covariates, indicated that there were no significant time \times treatment interaction effects for macro- and micronutrient intakes ($P > 0.05$ for all), but there was a main effect of treatment for protein ($P = 0.048$), calcium ($P = 0.032$), and sodium ($P = 0.042$), which was reflected in higher baseline intake in placebo compared with prebiotic participants.

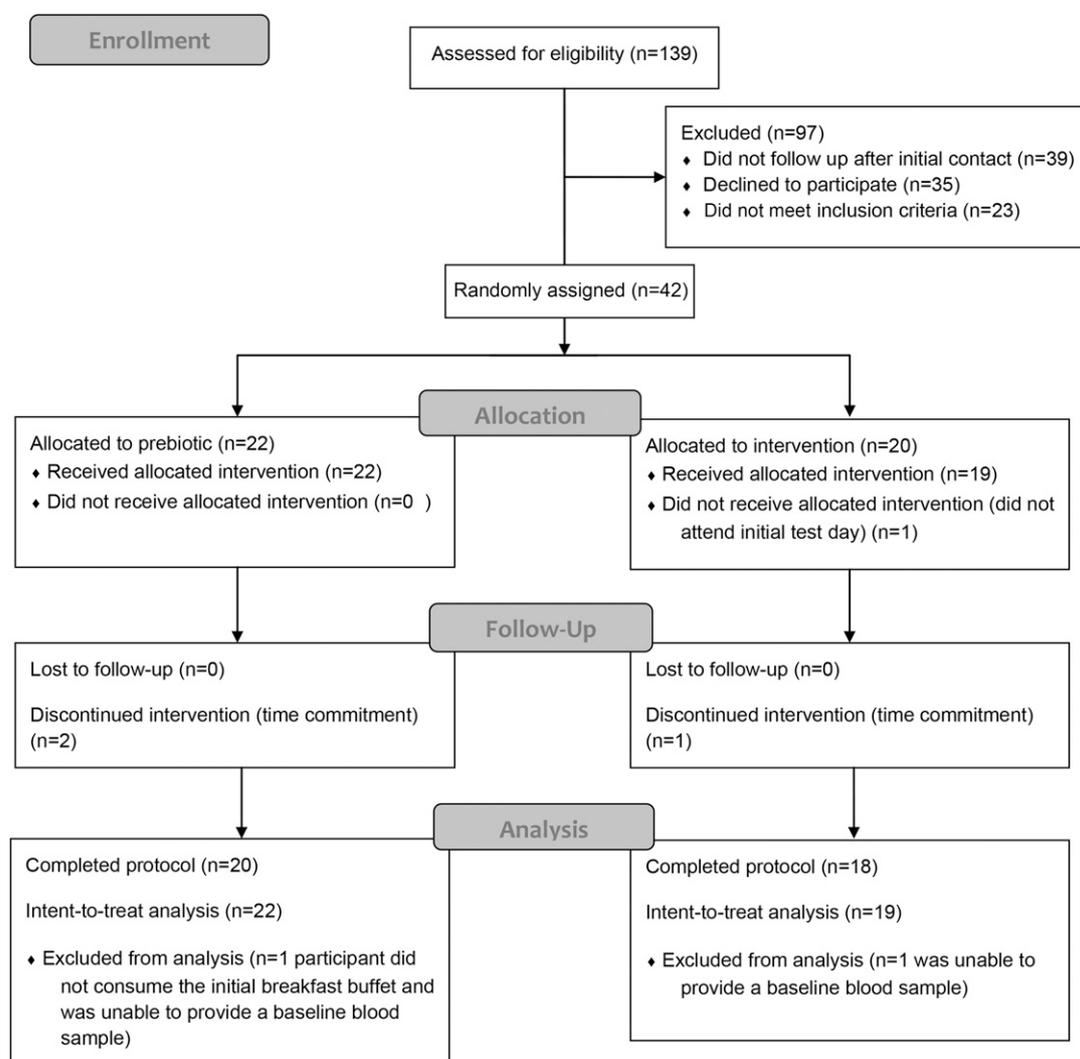


FIGURE 1 Study participant flow diagram showing participant recruitment and withdrawals from the prebiotic and placebo groups.

TABLE 1
Baseline characteristics of participants according to treatment group¹

	Prebiotic group	Placebo group	<i>P</i>
Participants (F/M), <i>n/n</i>	10/12	8/12	0.72
Age, y	10.4 ± 1.6	10.2 ± 1.6	0.72
Height, cm	148.1 ± 2.4	147.1 ± 2.8	0.78
Body weight, kg	58.5 ± 3.1	59.6 ± 4.5	0.84
BMI <i>z</i> score	2.03 ± 0.07	2.04 ± 0.10	0.94

¹ Values are means ± SEMs unless otherwise indicated. *n* = 22 and *n* = 20 in the prebiotic and placebo groups, respectively; 1 participant did not attend the initial test day so the researcher-measured values are for *n* = 19 for placebo. Baseline data were analyzed by using independent *t* tests for continuous variables and chi-square tests for categorical variables.

Ad libitum breakfast buffet

There was no significant effect of treatment on energy consumed at the ad libitum breakfast buffet when all ages were included in the analysis (all ages, 7–12 y old; *n* = 40; *P* = 0.18) (Table 3). There was, however, a significant effect of treatment × age (*P* = 0.017) on energy intake at the breakfast buffet; therefore, participants were stratified into a younger (7–10 y; *n* = 22) age group and an older (11–12 y; *n* = 18) age group. Prebiotic intake significantly reduced energy intake in older participants at the final breakfast buffet compared with placebo (*P* = 0.04). Energy intake in the prebiotic group was reduced by ~113 kcal, whereas energy intake increased by 137 kcal in the placebo group. In the younger participants, energy intake increased in both groups at the final breakfast buffet compared with baseline. The magnitude of increased energy intake in the placebo group (188 kcal) was higher than in the prebiotic group (60 kcal), but this was not significantly different (*P* > 0.05).

Gut hormones, insulin, and adipokines

There was a significant increase in fasting ghrelin with prebiotic supplementation compared with placebo (*P* = 0.04) (Table 4). The prebiotic group’s ghrelin concentrations increased by nearly 28% from baseline, whereas the placebo group showed an increase of ~8% from the start of the study. There were no significant differences in changes in fasting GIP, GLP-1, PYY, and insulin concentrations from baseline to the final test days (*P* > 0.05).

There was a significant change from baseline for adiponectin (*P* = 0.005), with the prebiotic group showing increased concentrations and the placebo group showing a decrease over the 16-wk intervention. There were no differences in fasting resistin or leptin between groups.

Subjective ratings of appetite: VAS

Differences in subjective appetite ratings were measured by using VASs before and after an ad libitum breakfast buffet at baseline and at the end of the study. “Pre” refers to appetite ratings before breakfast meal consumption and “post” refers to appetite ratings after breakfast meal consumption.

From week 0 to week 16, the prebiotic reduced the amount of food that participants thought they could consume at the breakfast buffet (*P* = 0.03), which is shown in Figure 2A as prospective food consumption before breakfast. The lower ratings of prospective

TABLE 2
Energy and nutrient intakes at 16 wk obtained via weighed 3-d food records in participants receiving a prebiotic or placebo¹

Group	Baseline	Midpoint	Final
Energy, kcal/d			
Prebiotic	2100 ± 118	1887 ± 139 ^Y	1650 ± 103 ^Y
Placebo	2234 ± 136	1925 ± 160 ^Y	1756 ± 119 ^Y
Protein, g/d			
Prebiotic	87.1 ± 6.9*	77.0 ± 6.7	79.0 ± 6.0
Placebo	103.3 ± 7.2	87.5 ± 7.0	77.2 ± 6.2
Carbohydrates, g/d			
Prebiotic	229.9 ± 21.1	218.4 ± 25.5	220.4 ± 17.9
Placebo	298.4 ± 21.1	272.8 ± 25.5	237.4 ± 17.9
Dietary fiber, g/d			
Prebiotic	18.8 ± 1.5	39.4 ± 6.1	40.8 ± 4.8
Placebo	18.7 ± 1.7	33.4 ± 5.9	36.7 ± 5.7
Sugars, g/d			
Prebiotic	105.8 ± 11.3	98.8 ± 10.6	93.9 ± 9.3
Placebo	112.2 ± 13.0	103.4 ± 12.2	104.4 ± 10.7
Fat, g/d			
Prebiotic	79.4 ± 6.7	27.8 ± 4.5	29.1 ± 5.3
Placebo	81.1 ± 7.7	32.3 ± 5.2	32.8 ± 6.1
Saturated fat, g/d			
Prebiotic	25.1 ± 2.8	23.1 ± 2.2	19.2 ± 1.4
Placebo	29.3 ± 3.1	20.0 ± 2.4	18.1 ± 1.6
Cholesterol, mg/d			
Prebiotic	303.6 ± 37.7	234.0 ± 28.7	234.0 ± 24.5
Placebo	270.2 ± 43.1	247.7 ± 32.9	198.4 ± 28.1
Calcium, mg/d			
Prebiotic	916.0 ± 118.8**	854.6 ± 119.6	731.1 ± 51.7
Placebo	1256.5 ± 146.6	1040.0 ± 83.6	1126.6 ± 185.7
Iron, mg/d			
Prebiotic	17.3 ± 1.5	13.2 ± 1.1	12.6 ± 1.2
Placebo	19.0 ± 1.6	15.7 ± 1.1	15.8 ± 1.2
Sodium, mg/d			
Prebiotic	3226.0 ± 188.1 [†]	2621.6 ± 258.3	2667.5 ± 242.3
Placebo	4004.0 ± 418.1	3310.5 ± 281.0	3244.7 ± 263.6
Vitamin D, μg/d			
Prebiotic	1.5 ± 0.2	0.6 ± 0.2	0.8 ± 0.2
Placebo	0.7 ± 0.3	0.7 ± 0.2	0.6 ± 0.2

¹ Values are means ± SEMs. *n* = 21 and *n* = 19 in the prebiotic and placebo groups, respectively. Intent-to-treat analysis with sex and age as covariates was used (ANCOVA): *treatment (*P* = 0.048), treatment × time (*P* = 0.630); **treatment (*P* = 0.032), treatment × time (*P* = 0.538); [†]treatment (*P* = 0.042), treatment × time (*P* = 0.811); ^Ysignificant effect of time (*P* = 0.047) compared with baseline.

food consumption seen after consumption of the final breakfast meal did not differ between groups (*P* > 0.05) (Figure 2B). There was no difference in fullness before breakfast (Figure 2C), but participants reported feeling significantly “more full” after their breakfast meal at week 16 in the prebiotic compared with the placebo group than after their breakfast meal consumed at week 0 (*P* = 0.04) (Figure 2D). The ratings of pre- and post-breakfast buffet hunger did not differ between the prebiotic and placebo groups (*P* > 0.05) (Figure 2E, F).

CEBQ

The subscales of Enjoyment of Food, Emotional Overeating, Desire to Drink, Emotional Undereating, Food Responsiveness, Slowness in Eating, and Food Fussiness were similar between groups at baseline and at 16 wk (Table 5). In

TABLE 3Energy intake at the ad libitum breakfast buffet in participants receiving a prebiotic or placebo¹

Group	Baseline, kcal	Final, kcal	Change in energy intake from baseline (ANCOVA adjusted), kcal
All subjects			
Prebiotic (<i>n</i> = 21)	473.9 ± 55.1	486.7 ± 61.1	12.4 ± 58.7
Placebo (<i>n</i> = 19)	499.8 ± 77.2	586.9 ± 72.4	131.6 ± 63.7
Age 7–10 y			
Prebiotic (<i>n</i> = 12)	453.7 ± 53.6	536.7 ± 73.9	59.5 ± 72.0
Placebo (<i>n</i> = 10)	607.3 ± 65.2	735.6 ± 57.4	188.2 ± 86.3
Age 11–12 y			
Prebiotic (<i>n</i> = 9)	498.5 ± 107.2	401.0 ± 98.4	−113.2 ± 72.7*
Placebo (<i>n</i> = 9)	282.8 ± 50.6	401.6 ± 112.3	136.6 ± 77.4

¹ Values are means ± SEMs. Intent-to-treat analysis with sex and age as covariates was used. A significant treatment × age effect ($P = 0.017$) justified analysis according to 2 age groups. *Different from placebo, $P < 0.05$.

contrast, there was a significant increase in the Satiety Responsiveness subscale in both groups over time (effect of time, $P = 0.007$). Specifically, caregivers reported significant increases in Satiety Responsiveness from baseline and mid-point to the end of the study ($P = 0.001$ and $P = 0.04$, respectively).

BMI z score

In ITT analysis, there was a trend ($P = 0.09$) for the prebiotic to reduce the BMI z score by 3.4% (-0.066 ± 0.026) compared with a 0.49% (-0.009 ± 0.019) reduction for the placebo. In per-protocol analysis, the 3.8% (-0.078 ± 0.027) reduction by the prebiotic was significant compared with the 0.35% (-0.007 ± 0.020) reduction with the placebo (per-protocol analysis, $P = 0.043$).

Physical activity

According to the Godin's Leisure-Time Exercise Questionnaire, there was no significant change in exercise frequency and duration between the 2 groups ($P > 0.05$).

Compliance and tolerance

We observed a mean return rate of 87% ± 0.03% and 91% ± 0.03% of total allotted packets for the prebiotic and placebo groups, respectively. Two participants who dropped out forgot to bring their packets to their 4-wk anthropometrics appointment and a short time later withdrew from the study entirely. Therefore, compliance data were collected from 39 participants. Compliance was calculated by using an equation in which the total number of returned empty packets was divided by the expected number of packets (i.e., the total number of packets that would have been consumed if participants were 100% compliant with the treatment). Independent *t* tests indicated that there were no significant differences in compliance with the powder between groups ($P = 0.30$).

With regard to gastrointestinal side effects, 70% and 61% of participants in the prebiotic and placebo groups, respectively, reported no changes in flatulence and bloating during the study. Similar numbers of participants in the prebiotic (25%) and placebo (28%) groups experienced a mild increase in flatulence

and bloating. The remaining 5% and 11% of participants in the prebiotic and placebo groups, respectively, reported a moderate increase in flatulence and bloating. None of the participants belonging to either group reported a severe increase in flatulence and bloating. Finally, 61% of participants indicated that the powder was very acceptable in terms of consuming it on a day-to-day basis, whereas the remaining 39% rated the powder as moderately acceptable.

TABLE 4Gut hormones, insulin, and adipokines in participants receiving a prebiotic or placebo¹

Group	Baseline (week 0)	Final (week 16)	Change from baseline (ANCOVA adjusted)
GIP, pg/mL			
Prebiotic	41.9 ± 6.8	51.0 ± 5.8	9.0 ± 7.3
Placebo	49.1 ± 6.8	55.7 ± 36.5	6.8 ± 7.5
Ghrelin, pg/mL			
Prebiotic	90.8 ± 12.4	125.4 ± 14.1	34.5 ± 8.6*
Placebo	86.6 ± 10.8	93.8 ± 11.4	7.4 ± 9.3
Insulin, pg/mL			
Prebiotic	1034.8 ± 128.1	1124.0 ± 131.5	81.2 ± 87.2
Placebo	1323.8 ± 243.3	1412.2 ± 226.5	97.5 ± 92.2
GLP-1, pg/mL			
Prebiotic	36.1 ± 6.2	42.2 ± 6.6	6.0 ± 6.2
Placebo	31.7 ± 3.7	43.2 ± 6.8	11.7 ± 7.0
PYY, pg/mL			
Prebiotic	209.3 ± 27.2	220.2 ± 28.2	10.9 ± 19.8
Placebo	158.9 ± 32.7	154.0 ± 28.9	−4.9 ± 10.7
Adiponectin, μg/mL			
Prebiotic	8.32 ± 0.51	9.81 ± 1.05	1.49 ± 0.61*
Placebo	8.27 ± 1.75	6.62 ± 1.16	−1.65 ± 0.65
Resistin, ng/mL			
Prebiotic	4.5 ± 1.0	4.8 ± 1.1	0.31 ± 1.21
Placebo	8.3 ± 1.9	9.3 ± 1.9	0.94 ± 1.21
Leptin, ng/mL			
Prebiotic	23.4 ± 2.9	24.4 ± 28.2	1.0 ± 19.1
Placebo	30.0 ± 4.9	32.1 ± 52.1	2.1 ± 2.1

¹ Values are means ± SEMs. $n = 21$ and $n = 18$ in the prebiotic and placebo groups, respectively. Intent-to-treat analysis with sex and age as covariates was used (ANCOVA). *Different from placebo, $P < 0.05$. GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide 1; PYY, peptide tyrosine tyrosine.

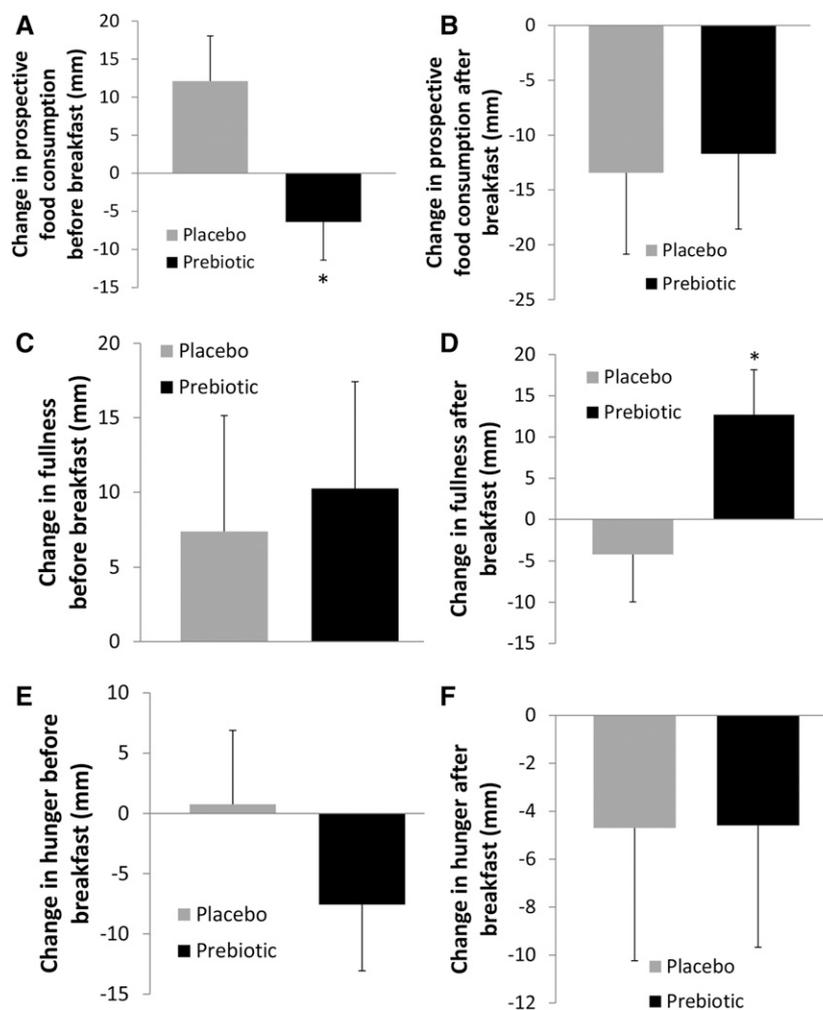


FIGURE 2 Change (from baseline to 16 wk) in prospective food consumption (A and B) and in fullness (C and D) and hunger (E and F) ratings taken before and after a breakfast buffet in participants who received a prebiotic ($n = 21$) or placebo ($n = 19$). Values are means \pm SEMs. *Different from placebo, $P < 0.05$ (ANCOVA, including the covariates of sex and age; intent-to-treat analysis).

DISCUSSION

Studies in rodents and adult humans have shown that prebiotics, chiefly oligofructose and inulin, result in enhanced satiety, reduced energy intake, and weight loss (13, 25, 30–32); however, studies in the obese pediatric population are limited. To the best of our knowledge, this is the first randomized, double-blind, placebo-controlled trial to specifically examine appetite control in children with overweight and obesity in response to long-term prebiotic intake. Our findings suggest that prebiotics may help improve appetite control in the context of pediatric obesity.

Improvements in subjective appetite included lower prospective food consumption and increased fullness after 16 wk of oligofructose-enriched inulin. Cani et al. (25) showed that healthy adults who consumed oligofructose for 2 wk reported increased fullness and reduced prospective food consumption after the dinner meal but not after breakfast, as was seen in our children. These meal-related differences may be attributed to the timing of prebiotic intake given that participants in Cani et al. (25) consumed 4 g with breakfast and 4 g with dinner, whereas our children consumed the full 8-g dose just before their dinner meal. Thus, the beneficial effects of prebiotics on appetite may not be acutely evident but rather may contribute to a state of prolonged satiety, wherein the

prebiotic effect leads to delayed initiation of subsequent meals and reduced subsequent meal size. This phenomenon of delayed action has been described by others as the second-meal effect. Specifically, whole grain-rye foods, when consumed at an evening meal, significantly reduced energy intake at a lunch meal the following day and this may have been due to mechanisms related to colonic fermentation (33). Inulin, when used as a fat-replacer in a breakfast sausage, was also shown to reduce total energy intake at the end of a test day in healthy men, despite no apparent effect on satiety during breakfast itself (34). Complete colonic fermentation of the inulin may be followed by a late postabsorptive satiety trigger (34).

In contrast to VAS scores, CEBQ results changed in a similar manner between groups, which suggests a possible “training effect” among caregivers. Formally known as the Hawthorne effect (35), participants’ awareness that their child’s eating behavior is being observed can lead to reported changes in eating behavior that align with researchers’ expectations or desires (36). This effect has been noted in other studies that examined behavior in a similar demographic (36).

Past studies have shown that prebiotic intake can lead to reduced energy intake in healthy and overweight and obese adults (13, 25, 37). Similar to these findings, overweight and obese

TABLE 5
CEBQ scores in participants receiving a prebiotic or placebo¹

Subscale item and group	Baseline ² (week 0)	Midpoint (week 8)	Final (week 16)
Enjoyment of Food			
Prebiotic	4.21 ± 0.12	4.72 ± 0.73	3.63 ± 0.13
Placebo	4.40 ± 0.14	4.39 ± 0.86	4.16 ± 0.15
Emotional Overeating			
Prebiotic	2.84 ± 0.17	2.90 ± 0.19	2.64 ± 0.19
Placebo	3.27 ± 0.18	2.95 ± 0.21	2.86 ± 0.21
Satiety Responsiveness			
Prebiotic	2.44 ± 0.00	2.59 ± 0.11	2.78 ± 0.09*
Placebo	2.44 ± 0.00	2.52 ± 0.13	2.76 ± 0.10*
Slowness in Eating			
Prebiotic	2.37 ± 0.15	2.30 ± 0.13	2.60 ± 0.11
Placebo	2.07 ± 0.17	2.22 ± 0.15	2.60 ± 0.12
Desire to Drink			
Prebiotic	2.85 ± 0.18	2.83 ± 0.17	2.68 ± 0.15
Placebo	3.00 ± 0.21	2.84 ± 0.20	2.82 ± 0.18
Food Fussiness			
Prebiotic	2.85 ± 0.17	2.96 ± 0.19	2.96 ± 0.07
Placebo	2.66 ± 0.20	2.52 ± 0.22	2.87 ± 0.09
Emotional Undereating			
Prebiotic	2.71 ± 0.12	2.63 ± 0.13	2.55 ± 0.15
Placebo	2.76 ± 0.14	2.68 ± 0.15	2.57 ± 0.18
Food Responsiveness			
Prebiotic	3.65 ± 0.00	3.32 ± 0.14	3.25 ± 0.13
Placebo	3.65 ± 0.00	3.40 ± 0.17	3.17 ± 0.16

¹ Values are means ± SEMs. $n = 22$ and $n = 19$ in the prebiotic and placebo groups, respectively. Intent-to-treat analysis was used with repeated-measures ANCOVA [all baseline, midpoint, and final CEBQ scale comparisons (covariates = sex and age) and baseline Satiety Responsiveness and Food Responsiveness for Satiety Responsiveness and Food Responsiveness analysis, respectively]. *Significant effect of time ($P = 0.007$) with both groups increasing Satiety Responsiveness from baseline to final (time × treatment, $P = 0.230$). CEBQ, Children's Eating Behavior Questionnaire.

²Independent t test was used.

children in the prebiotic group consumed less energy than those in the placebo group at the final ad libitum breakfast buffet, but this was only significant in the older children: the 11- and 12-year-olds in the prebiotic group reduced meal energy intake by 113 kcal compared with an increase of 137 kcal in the placebo group. A reduction in this objective measure of energy intake could be attributed to the beneficial changes in prospective food consumption and fullness experienced. The physiologic mechanisms by which prebiotics improve satiety and regulate appetite are likely mediated in part by hormones that control appetite, such as GLP-1, PYY, ghrelin, and leptin (13, 31, 38). Short-chain fatty acids (SCFAs), metabolites of gut microbial fermentation of prebiotics, bind to specific receptors [free-fatty acid receptor (FFAR) 2] on colonic L-cells and trigger the secretion of GLP-1 and PYY (39). Our results that showed increased fasting ghrelin and no changes in GLP-1 and PYY are surprising in light of studies in adults that showed increased GLP-1 and PYY and reduced ghrelin after prebiotic intake (13, 38). The observed increase in fasting ghrelin does align with the concept that diet-induced weight loss and caloric restriction tend to increase ghrelin in obese subjects as a defense mechanism (40, 41). However, our findings are limited to the fasted state and are in fact consistent with previous studies that showed no differences in fasting concentrations of GLP-1 and PYY in adults who consumed 21 g oligofructose/d for 12 wk (13). The significant reductions in ghrelin and increases in PYY that occurred in the

adult study were seen during a meal tolerance test, which takes into account the responsiveness of these hormones to nutrient stimulus. A full postprandial survey of gut hormones in obese children would be needed to provide a comprehensive understanding of the role of these hormones in appetite control in this population. In addition to stimulating satiety hormones, studies in mice also showed that the SCFA acetate may suppress appetite by crossing the blood-brain barrier and acting directly on central homeostatic mechanisms, therefore potentially triggering a reduction in food and energy intake (42). Other mechanisms by which the gut microbiota may influence host appetite control have recently been reviewed by Fetissov (43).

In the current study, we found that oligofructose-enriched inulin significantly increased fasting adiponectin. A cross-sectional study in nondiabetic women showed that increased fiber intake and a reduced starch-to-fiber ratio were positively associated with adiponectin (44). Higher fiber intake could increase adiponectin due to increased SCFA production, given that physiologic concentrations of SCFAs can activate FFAR3 on adipocytes in mice and stimulate the release of another adipocyte-derived hormone, leptin (45). However, such studies have yet to show a positive effect of SCFAs on adiponectin secretion. For instance, Freeland and Wolever (46) examined whether the route of administration (intravenous compared with rectal infusion) of the SCFA acetate would affect the secretion of multiple gut hormones and adipokines, including adiponectin in 6 hyperinsulinemic women. There

was no effect of acetate, regardless of route of administration, on adiponectin (46). In addition to this particular study, there are few studies that looked at the role of SCFAs on adiponectin release, and therefore the mechanism by which prebiotics affect adiponectin is not known and may in fact be SCFA-independent.

Although ITT analysis did not show a significant reduction in BMI z score in our participants ($P = 0.09$), per-protocol analysis of participants who completed the study was significant ($P = 0.043$). The reduction in BMI z score of 0.078 over 4 mo is comparable to other, more intensive interventions, such as a nutrition education plus physical activity intervention in obese children in which a reduction of 0.13 in BMI z score occurred at 6 mo and a final reduction of 0.18 in BMI z score at 1 y was observed (47). It remains to be seen if extending the duration of our prebiotic intervention would continue to reduce BMI z score over time.

The strengths of this study include a sufficiently large sample size of both sexes across a fairly wide age range and the examination of a variety of subjective and objective measures of appetite. The limitations of using parent-reported 3-d food records to measure energy intake are recognized (48), and our initial training session attempted to minimize reporting error. Fasting blood samples precluded the examination of postprandial effects on satiety hormones, and energy intake during the buffet was only significantly different in separate age group analysis. Participants in our study were primarily white and of middle to high socioeconomic status, thus limiting generalizability to more diverse populations. Last, future studies should include children with comorbid conditions, because our participants were otherwise healthy overweight and obese children.

In conclusion, the results of this dietary intervention study highlight the potential of prebiotic supplementation in the management of pediatric overweight and obesity, with significant improvements in sensations of appetite and marked reductions in energy intake in children 11–12 y of age. Additional studies are required to examine postprandial satiety hormone concentrations after a test meal to develop a better understanding of how prebiotics induce physiologic effects on appetite regulation in overweight and obese children.

The authors' responsibilities were as follows—MPH and ACN: conducted the research; MPH: analyzed the data and wrote the manuscript; RAR: had final responsibility for the final content; and all authors: designed the research and read and approved the final manuscript. RAR previously held a research grant from Beneo for work unrelated to the current study. The remaining authors reported no conflicts of interest related to the study.

REFERENCES

- Ogden CL, Carroll MD, Kit BK, Flegal KM. Prevalence of childhood and adult obesity in the United States, 2011–2012. *JAMA* 2014;311:806–14.
- Roberts KC, Shields M, de Groh M, Aziz A, Gilbert JA. Overweight and obesity in children and adolescents: results from the 2009 to 2011 Canadian Health Measures Survey. *Health Rep* 2012;23:37–41.
- Belsky DW. Appetite for prevention: genetics and developmental epidemiology join forces in obesity research. *JAMA Pediatr* 2014;168:309–11.
- Carnell S, Wardle J. Appetite and adiposity in children: evidence for a behavioral susceptibility theory of obesity. *Am J Clin Nutr* 2008;88:22–9.
- van Jaarsveld CH, Boniface D, Llewellyn CH, Wardle J. Appetite and growth: a longitudinal sibling analysis. *JAMA Pediatr* 2014;168:345–50.
- Field AE, Austin SB, Taylor CB, Malspeis S, Rosner B, Rockett HR, Gillman MW, Colditz GA. Relation between dieting and weight change among preadolescents and adolescents. *Pediatrics* 2003;112:900–6.
- Fisher JO, Birch LL. Restricting access to foods and children's eating. *Appetite* 1999;32:405–19.
- Fisher JO, Birch LL. Restricting access to palatable foods affects children's behavioral response, food selection, and intake. *Am J Clin Nutr* 1999;69:1264–72.
- Huang JY, Qi SJ. Childhood obesity and food intake. *World J Pediatr* 2015;11:101–7.
- Kimm SY. The role of dietary fiber in the development and treatment of childhood obesity. *Pediatrics* 1995;96:1010–4.
- Van Itallie TB. Dietary fiber and obesity. *Am J Clin Nutr* 1978;31(10, Suppl):S43–52.
- Hampel S, Odar Stough C, Poppert Cordt K, Best C, Blackburn K, Drever Gillete ML. Effectiveness of a hospital-based multidisciplinary pediatric weight management program: two-year outcomes of PHIT kids. *Child Obes* 2016;12:20–5.
- Parnell JA, Reimer RA. Weight loss during oligofructose supplementation is associated with decreased ghrelin and increased peptide YY in overweight and obese adults. *Am J Clin Nutr* 2009;89:1751–9.
- Parnell JA, Raman M, Rioux KP, Reimer RA. The potential role of prebiotic fibre for treatment and management of non-alcoholic fatty liver disease and associated obesity and insulin resistance. *Liver Int* 2012;32:701–11.
- Kellow NJ, Coughlan MT, Reid CM. Metabolic benefits of dietary prebiotics in human subjects: a systematic review of randomised controlled trials. *Br J Nutr* 2014;111:1147–61.
- Parnell JA, Reimer RA. Prebiotic fibres dose-dependently increase satiety hormones and alter Bacteroidetes and Firmicutes in lean and obese JCR:LA-cp rats. *Br J Nutr* 2012;107:601–13.
- Abrams SA, Griffin IJ, Hawthorne KM, Ellis KJ. Effect of prebiotic supplementation and calcium intake on body mass index. *J Pediatr* 2007;151:293–8.
- Liber A, Szajewska H. Effect of oligofructose supplementation on body weight in overweight and obese children: a randomised, double-blind, placebo-controlled trial. *Br J Nutr* 2014;112:2068–74.
- Nicolucci AC, Hume MP, Reimer RA. Effect of prebiotic-induced changes in gut microbiota on adiposity in obese and overweight children. *FASEB J* April 2015;29:276.6.
- Reinehr T, Schaefer A, Winkel K, Finne E, Toschke AM, Kolip P. An effective lifestyle intervention in overweight children: findings from a randomized controlled trial on "Obeldicks light". *Clin Nutr* 2010;29:331–6.
- Savoie M, Shaw M, Dziura J, Tamborlane WV, Rose P, Guandalini C, Goldberg-Gell R, Burgert TS, Cali AM, Weiss R, et al. Effects of a weight management program on body composition and metabolic parameters in overweight children: a randomized controlled trial. *JAMA* 2007;297:2697–704.
- Kelishadi R, Hashemipour M, Mohammadifard N, Alikhassy H, Adeli K. Short- and long-term relationships of serum ghrelin with changes in body composition and the metabolic syndrome in pre-pubescent obese children following two different weight loss programmes. *Clin Endocrinol (Oxf)* 2008;69:721–9.
- Molnár D, Schutz Y. The effect of obesity, age, puberty and gender on resting metabolic rate in children and adolescents. *Eur J Pediatr* 1997;156:376–81.
- Novak LP. Changes in total body water during adolescent growth. *Hum Biol* 1989;61:407–14.
- Cani PD, Joly E, Horsmans Y, Delzenne NM. Oligofructose promotes satiety in healthy humans: a pilot study. *Eur J Clin Nutr* 2006;60:567–72.
- Godin G, Shepard RJ. Godin Leisure-Time Exercise Questionnaire. *Med Sci Sports Exerc* 1997;29 Suppl:S36–8.
- Lambert JE, Parnell JA, Han J, Sturzenegger T, Paul HA, Vogel HJ, Reimer RA. Evaluation of yellow pea fibre supplementation on weight loss and the gut microbiota: a randomized controlled trial. *BMC Gastroenterol* 2014;14:69.
- Wardle J, Guthrie CA, Sanderson S, Rapoport L. Development of the Children's Eating Behaviour Questionnaire. *J Child Psychol Psychiatry* 2001;42:963–70.
- Must A, Anderson SE. Body mass index in children and adolescents: considerations for population-based applications. *Int J Obes (Lond)* 2006;30:590–4.

30. Cani PD, Dewever C, Delzenne NM. Inulin-type fructans modulate gastrointestinal peptides involved in appetite regulation (glucagon-like peptide-1 and ghrelin) in rats. *Br J Nutr* 2004;92:521–6.
31. Delzenne NM, Cani PD, Daubioul C, Neyrinck AM. Impact of inulin and oligofructose on gastrointestinal peptides. *Br J Nutr* 2005;93 Suppl 1:S157–61.
32. Bomhof MR, Saha DC, Reid DT, Paul HA, Reimer RA. Combined effects of oligofructose and *Bifidobacterium animalis* on gut microbiota and glycemia in obese rats. *Obesity (Silver Spring)* 2014;22:763–71.
33. Ibrügger S, Vigsnaes LK, Blennow A, Skufflic D, Raben A, Lauritzen L, Kristensen M. Second meal effect on appetite and fermentation of wholegrain rye foods. *Appetite* 2014;80:248–56.
34. Archer BJ, Johnson SK, Devereux HM, Baxter AL. Effect of fat replacement by inulin or lupin-kernel fibre on sausage patty acceptability, post-meal perceptions of satiety and food intake in men. *Br J Nutr* 2004;91:591–9.
35. McCambridge J, Witton J, Elbourne DR. Systematic review of the Hawthorne effect: new concepts are needed to study research participation effects. *J Clin Epidemiol* 2014;67:267–77.
36. Adolphus K, Lawton CL, Dye L. The effects of breakfast on behavior and academic performance in children and adolescents. *Front Hum Neurosci* 2013;7:425.
37. Verhoef SP, Meyer D, Westterterp KR. Effects of oligofructose on appetite profile, glucagon-like peptide 1 and peptide YY3-36 concentrations and energy intake. *Br J Nutr* 2011;106:1757–62.
38. Cani PD, Lecourt E, Dewulf EM, Sohet FM, Pachikian BD, Naslain D, De Backer F, Neyrinck AM, Delzenne NM. Gut microbiota fermentation of prebiotics increases satietogenic and incretin gut peptide production with consequences for appetite sensation and glucose response after a meal. *Am J Clin Nutr* 2009;90:1236–43.
39. Chambers ES, Morrison DJ, Frost G. Control of appetite and energy intake by SCFA: what are the potential underlying mechanisms? *Proc Nutr Soc* 2015;74:328–36.
40. Arrigo T, Gitto E, Ferrau V, Munafo C, Alibrandi A, Marseglia GL, Salpietro A, Miraglia Del Giudice M, Leonardi S, Ciprandi G, et al. Effect of weight reduction on leptin, total ghrelin and obestatin concentrations in prepubertal children. *J Biol Regul Homeost Agents* 2012;26(1, Suppl):S95–103.
41. Larder R, O’Rahilly S. Shedding pounds after going under the knife: guts over glory—why diets fail. *Nat Med* 2012;18:666–7.
42. Frost G, Sleeth ML, Sahuri-Arisoylu M, Lizarbe B, Cerdan S, Brody L, Anastasovska J, Ghourab S, Hankir M, Zhang S, et al. The short-chain fatty acid acetate reduces appetite via a central homeostatic mechanism. *Nat Commun* 2014;5:3611.
43. Fetissov SO. Role of the gut microbiota in host appetite control: bacterial growth to animal feeding behaviour. *Nat Rev Endocrinol* 2017;13:11–25.
44. Al Essa HB, Ley SH, Rosner B, Malik VS, Willett WC, Hu FB. High fiber and low starch intakes are associated with circulating intermediate biomarkers of type 2 diabetes among women. *J Nutr* 2016;146:306–17.
45. Xiong Y, Miyamoto N, Shibata K, Valasek MA, Motoike T, Kedziarski RM, Yanagisawa M. Short-chain fatty acids stimulate leptin production in adipocytes through the G protein-coupled receptor GPR41. *Proc Natl Acad Sci USA* 2004;101:1045–50.
46. Freeland KR, Wolever TM. Acute effects of intravenous and rectal acetate on glucagon-like peptide-1, peptide YY, ghrelin, adiponectin and tumour necrosis factor-alpha. *Br J Nutr* 2010;103:460–6.
47. Pedrosa C, Oliveira BM, Albuquerque I, Simoes-Pereira C, Vaz-de-Almeida MD, Correia F. Metabolic syndrome, adipokines and ghrelin in overweight and obese schoolchildren: results of a 1-year lifestyle intervention programme. *Eur J Pediatr* 2011;170:483–92.
48. Bingham SA. Limitations of the various methods for collecting dietary intake data. *Ann Nutr Metab* 1991;35:117–27.