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Higher Protein Diets Consumed Ad Libitum Improve Cardiovascular Risk Markers in Children of Overweight Parents from Eight European Countries[1–4]

Camilla T. Damsgaard,[5]* Angeliki Papadaki,[6] Signe M. Jensen,[5] Christian Ritz,[5] Stine-Mathilde Dalskov,[5] Petr Hlavaty,[7] Wim H. M. Saris,[8] J. Alfredo Martinez,[9] Teodora Handjieva-Darlenska,[10] Malene R. Andersen,[11] Steen Stender,[11] Thomas M. Larsen,[5] Arne Astrup,[5] Christian Mølgaard,[5] and Kim F. Michaelsen[5] on behalf of Diogenes

[5]Department of Nutrition, Exercise and Sports, Faculty of Science, University of Copenhagen, Copenhagen, Denmark; [6]Department of Social Medicine, Preventive Medicine and Nutrition Clinic, Heraklion, Crete, Greece; [7]Obesity Management Centre, Institute of Endocrinology, Prague, Czech Republic; [8]Department of Human Biology, Nutrition and Toxicology Research Institute Maastricht, Maastricht University, Maastricht, The Netherlands; [9]Department of Physiology and Nutrition, University of Navarra, CIBERobn, Pamplona, Spain; [10]Department of Pharmacology and Toxicology, Faculty of Medicine, Medical University, Sofia, Bulgaria; and [11]Department of Clinical Biochemistry, Copenhagen University Hospital, Gentofte, Denmark

Abstract

Dietary strategies to improve early cardiovascular markers in overweight children are needed. We investigated the effect of dietary protein and glycemic index (GI) on cardiovascular markers and metabolic syndrome (MetS) scores in 5- to 18-y-old children of overweight/obese parents from 8 European centers. Families were randomized to 1 of 5 diets consumed ad libitum: high protein (HP) or low protein (LP) combined with high GI (HGI) or low GI (LGI), or a control diet. At 6 centers, families received dietary instruction (instruction centers); at 2 centers, free foods were also provided (supermarket centers). Diet, anthropometry, blood pressure, and serum cardiovascular markers (lipid profile, glucose regulation, and inflammation) were measured in 253 children at baseline, 1 mo, and/or 6 mo. Protein intake was higher in the HP groups (19.9 ± 1.3% energy) than in the LP groups at 6 mo (16.8 ± 1.2% energy) ($P = 0.001$). The GI was 4.0 points lower (95% CI: 2.1, 6.1) in the LGI compared with the HGI groups ($P < 0.001$). In the supermarket centers, the HP and LP groups differed more in protein intake than did the groups in the instruction centers ($P = 0.009$), indicating better compliance. The HP diets evoked a 2.7-cm (95% CI: 0.9, 5.1) smaller waist circumference and a 0.25-mmol/L (95% CI: 0.09, 0.41) lower serum LDL cholesterol compared with the LP diets at 6 mo ($P < 0.007$). In a separate supermarket center analysis, the HP compared with LP diets reduced waist circumference ($P = 0.004$), blood pressure ($P < 0.01$), serum insulin ($P = 0.013$), and homeostasis model of assessment-insulin resistance ($P = 0.016$). In the instruction centers, the HP compared with the LP diets reduced LDL cholesterol ($P = 0.004$). No consistent effect of GI was seen and the MetS scores were not affected. In conclusion, increased protein intake improved cardiovascular markers in high-risk children, particularly in those undergoing most intensive intervention. J. Nutr. 143: 810–817, 2013.

Introduction

Children of obese parents are more than twice as likely as children of normal-weight parents to become obese (1). Overweight and obesity in childhood carry over into adulthood and have been associated with coronary events and mortality later in life (2,3). In adults, metabolic syndrome (MetS)[12] is defined as a cluster of cardiovascular disease (CVD) risk markers, including abdominal obesity, dyslipidemia, glucose intolerance, and hypertension (4). Overweight and obese children also show these metabolic changes (5), and blood pressure levels and cholesterol

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* To whom correspondence should be addressed. Email: ctd@life.ku.dk.

[3] This trial was registered at clinicaltrials.gov as NCT00390637.

[4] Supplemental Table 1 is 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://jn.nutrition.org.

[12] Abbreviations used: CRP, C-reactive protein; CVD, cardiovascular disease; Diogenes, Diet, Obesity and Genes; GI, glycemic index; HGI, high glycemic index; HOMA-IR, homeostasis model of assessment-insulin resistance; HP, high protein; LGI, low glycemic index; LP, low protein; MAP, mean arterial blood pressure; MetS, metabolic syndrome.

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concentrations have been shown to track from childhood into adulthood (6). There is a need to develop dietary strategies to improve early CVD risk markers, particularly among overweight and obese children.

In adults, high protein intake (7,8) and low-glycemic index (GI) diets (9,10) have been shown to reduce body weight, blood pressure, TG, and C-reactive protein (CRP) and to improve glucose regulation and insulin sensitivity. In children, protein intake has been associated with lower blood pressure (11,12). However, evidence from randomized controlled trials on the specific effects of GI and protein intake on early CVD markers in children is sparse. In the Diet, Obesity and Genes (Diogenes) study, we previously found an increase in body fat percentage in children assigned to a low protein (LP) intake and high-GI (HGI) diet, whereas the percentage of overweight/obese children decreased among those assigned to a high protein intake and a low-GI diet (13). Whether these dietary changes also affect metabolic and CVD markers in children remains to be explored.

The NHANES III 1988–1994 showed that the prevalence of MetS was 4% in 12- to 19-y-old adolescents (14), this number had increased to 6% in the NHANES 1999–2000 (15). However, the use of a single, fixed definition of MetS is problematic in children. Blood pressure, lipid concentrations, and anthropometric variables change with age and pubertal development (4) and various cutoffs for these variables have been used in children and adolescents (16). When continuous variables are reduced to dichotomous ones, information is lost and we do not know which cutoffs in children appropriately indicate increased disease risk. Also, in most pediatric populations, the prevalence of MetS will be low and therefore the statistical power to detect associations with dietary exposures will be limited (16). MetS scores that incorporate a number of metabolic risk markers into one continuous age- and sex-adjusted value for each child are therefore highly relevant when assessing the effect of diet on metabolic risk in children.

The aim of this paper was to examine the effect of diets with a high or low protein content and high or low GI on early CVD markers (waist circumference, blood pressure, lipid profile, glucose regulation, and CRP) and 2 continuous MetS scores in 5- to 18-y-old children of overweight and obese parents. The paper is based on secondary and exploratory outcomes from the Diogenes study, a pan-European randomized trial investigating the ability of dietary protein and GI to prevent weight gain (17).

Materials and Methods

Study design. The children included in this study participated together with their families in the Diogenes study (17). Families were enrolled from November 2005 to April 2007 at 8 European centers: Maastricht (The Netherlands), Copenhagen (Denmark), Cambridge (United Kingdom), Heraklion (Greece), Potsdam (Germany), Pamplona (Spain), Sofia (Bulgaria), and Prague (The Czech Republic). The study was conducted according to the Declaration of Helsinki, and the local ethical committees in the respective countries approved all procedures involving human participants. Written informed consent was obtained from all custody holders of the child and from the child itself when considered mature enough to understand the procedure.

A thorough description of the study design and procedures was provided by Larsen et al. (17). After screening, eligible adults underwent an 8-wk, low-calorie diet period; during this period, the enrolled children received no intervention. During the last weeks of the adult low-calorie diet, children attended a baseline clinical examination visit at which anthropometry was measured, blood samples were collected, and 3-d weighted dietary records were provided (**Fig. 1**). Families in which at least one parent lost ≥8% of body weight during the low-calorie diet

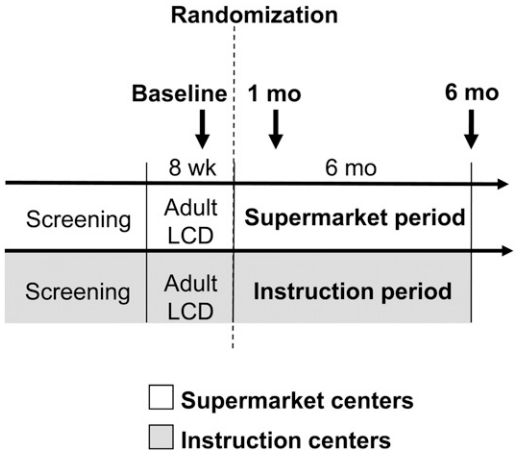

FIGURE 1 The design of the dietary intervention for the children in the supermarket centers and instruction centers. LCD, low calorie diet.

were randomized to 1 of 5 diets consumed ad libitum with regard to energy: low protein (LP)/low GI (LGI); LP/high GI (HGI); high protein (HP)/LGI; HP/HGI; or a control diet, which followed current national dietary guidelines and had a medium protein content and no GI specifications (13,17). Eligible families were allocated to diet groups by a simple block randomization procedure with stratification according to center, the number of eligible parents in each family, and the number of parents with a BMI >34 kg/m^2 in each family. After randomization, free foods were provided to the participants from laboratory supermarkets in the Danish and Dutch centers for 6 mo in addition to dietary instructions. In the remaining 6 centers, dietary instructions were provided for 6 mo without provision of free foods. Apart from the baseline visit, children attended a second clinical examination visit 1 mo into their randomized diet (1 mo) and a third visit at the end of the dietary period (6 mo). The same measurements were performed at all 3 visits.

Participants. Eligible families were healthy, with at least one overweight (BMI ≥27 kg/m^2) parent younger than 65 y and at least one child aged 5–18 y independent of weight status. Details on recruitment and adult exclusion criteria were provided in a previous paper (17). Children were excluded if they: *1*) were on special diets; *2*) had food intolerances; *3*) had systemic infections or chronic diseases; *4*) used medication that might affect the study outcomes; or *5*) reported drug or alcohol abuse, defined as regularly drinking >21 alcoholic units/wk (males) or >14 alcoholic units/wk (females). The 253 children included in the study population for this paper were aged 5–18 y, were randomized to 1 of the 5 diets, had a recorded randomization date, and had recorded values for MetS markers (waist circumference, blood pressure, serum glucose, insulin, and lipid profile) at baseline, 1 mo, and/or 6 mo.

Dietary instruction and recording. At randomization, trained dieticians gave detailed instructions on the ad libitum diets to the families, as previously described in detail (18). A points-based system was chosen as the primary tool to attain the desired proportion of dietary protein and carbohydrate in the prescribed diets to facilitate ad libitum eating and give flexibility in terms of the types of foods included at different eating occasions. The target protein intake was 10–15% energy in the LP groups and 23–28% energy in the HP groups, which was within the acceptable range (10–30% energy) set by the Food and Nutrition Board, U.S. Institute of Medicine for 4- to 18-y olds (19). Children in the LGI and HGI groups were advised to choose the foods with a low and high GI, respectively, within a food group. The aim was a 15-point GI difference between the LGI and HGI groups [for assignment of GI values to foods, see (20)]. The target fat content of all diets was 25–30% energy. The control group did not receive advice regarding the macronutrient composition or GI of their diet. All dietary groups received healthy eating advice positioned within the context of national healthy eating recommendations and including lowering total and saturated fat intake,

emphasizing a minimum intake of fruit and vegetables per day, consuming 2 portions/wk of fish, and restricting sugary foods/drinks and alcohol. Children were encouraged to participate in the instruction sessions for the adults; otherwise, parents were told to help their children follow their randomized diets. Moreover, during the intervention, children were requested to attend 6 counseling sessions together with their parents. At the counseling sessions, dieticians gave intensive guidance on weight control and reinforced the diet composition instruction messages through advice on food choice and behavior modification (18).

Dietary adherence was measured by dietary recording. All families were provided with weighing scales and were told to weigh and record their intake of all foods and liquids for 3 consecutive days (2 weekdays and 1 weekend day) (13). Parents were instructed to help their children with the recording when appropriate. The dietary records were checked for completeness on return to clarify any uncertainties and were analyzed as described in previous papers (17,20).

Clinical examinations. Standard operating procedures were drawn up for all investigations performed to ensure standardization across centers. The children had been fasting for 4 h, except for 350–500 mL water, prior to the clinical examination visits. Height and body weight were measured by standard procedures as described in an earlier paper (13). Gender- and age-specific Z-scores for height and BMI were calculated by using WHO AnthroPlus software (21,22). Weight status (overweight and obesity) was determined based on age- and sex-specific cutoffs defined to correspond to a BMI of 25 and 30 kg/m^2 at age 18 y, as according to Cole et al. (23). Waist circumference was measured to the nearest 0.5 cm with a tape measure in the vertical plane midway between the lower rib and the iliac crest. The mean of 2 subsequent measurements was calculated. Systolic and diastolic blood pressure was measured 3 times with an automatic device after at least 5 min rest in supine position. The mean value of the last 2 measurements was used. Mean arterial blood pressure (MAP) was calculated as: (2× diastolic pressure + systolic pressure)/3.

Blood collection and analysis. Blood was drawn after a 10-min rest from the antecubital vein into vacutainers containing clot activator and gel for serum separation. After 10–30 min coagulation at room temperature, samples were centrifuged at 2500 × *g* for 15 min at room temperature. Within 30 min of centrifugation, serum was transferred to cryo vials and stored at −80°C until analysis. Serum total cholesterol, HDL cholesterol, and TG concentrations were quantified by enzyme immunoassays (Ortho-Clinical Diagnostics, Johnson & Johnson) for the Vitros 5.1 FS analyzer. LDL and VLDL cholesterol concentrations were calculated using Friedewald's equation (24). Serum glucose was measured by a colorimetric assay (Ortho-Clinical Diagnostics) for the Vitros 950 analyzer and serum insulin was measured by an immunoassay (Siemens Healthcare Diagnostics) for the ADVIA Centaur XP. Serum CRP was quantified by a high sensitivity immunoassay (hsCRP, Ortho-Clinical Diagnostics) for the Vitros 5.1 FS analyzer with a detection limit of 0.1 mg/L. Forty-six children had one or more CRP values below the detection limit; these values were defined as 0.05 mg/L. Nine children (3.6%) had one or more CRP values >10 mg/L; these were taken as indications of acute inflammation and the CRP values were excluded from the dataset. Intra- and inter-assay CV for the analyses were: 1.2 and 1.8% (total cholesterol); 1.5 and 1.9% (HDL cholesterol); 0.8 and 1.5% (TG); 1.4 and 2.1% (glucose); 2.8 and 5.9% (insulin); and 2.9 and 1.8% (hsCRP). Homeostasis model of assessment-insulin resistance (HOMA-IR) was calculated as fasting glucose (mmol/L) × fasting insulin (mIU/L)/22.5.

MetS prevalence and scores. Prevalence of MetS among the 10- to 18-y olds was determined according to the pediatric criteria defined by the International Diabetes Federation (25), which include cutoffs for waist circumference, systolic or diastolic blood pressure, plasma glucose, TG, and HDL cholesterol. A British reference material was used for waist circumference percentiles (26). According to the International Diabetes Federation, MetS cannot be reliably diagnosed in children <10 y of age and therefore the prevalence was not determined in these children (25).

Two different continuous pediatric MetS scores were considered; MetS score 1 was based on the sum of age- and gender-adjusted Z-scores of the 5 variables, waist circumference, HOMA-IR, TG, MAP, and HDL cholesterol, as suggested for pediatric populations by Eisenmann (16). MetS score 2 was calculated as a weighted mean of the 5 variables, where the weights were based on the first principal component in a principal component analysis (27). All 5 variables were adjusted for gender and age before entering the scores. The reference values for standardization were based on all children with a baseline value for all 5 MetS markers.

Statistical analyses. Age-adjusted gender differences in baseline characteristics were compared using ANCOVA with robust estimation of SEs. Chi-square tests were used for comparing the gender distribution and frequencies of normal-weight, overweight, and obese children. Differences in MetS prevalence between visits were assessed using Fisher's exact test. Included children and those not included were compared using unpaired *t* tests and chi-square tests.

Dietary differences at 1 and 6 mo were evaluated using ANCOVA, including protein (control, low, or high), GI (control, low, or high), and baseline as fixed effects and country as random effect. Second, to evaluate whether or not adherence with regard to protein intake and dietary GI differed between the 2 center types at 6 mo, the initial ANCOVA was extended by protein-center type and GI-center type interactions. If these terms were significant, protein intake and dietary GI at 6 mo were evaluated separately for the 2 center types. Outcomes were transformed if necessary to meet model requirements.

The effect of protein (control, LP, and HP) and GI (control, LGI, and HGI) on the development of the CVD risk markers and MetS scores during the intervention (from baseline through 1 to 6 mo) was evaluated using linear mixed models, including random effects for individual participants, families, and countries to account for repeated sampling at each level. The linear mixed models allowed the effects of protein, GI, and their interaction to vary with time (since randomization) and time squared. Likelihood ratio tests were used to assess whether changes during the intervention depended on protein, GI, or both. Outcomes were transformed if necessary to meet model requirements and in this case, approximate CIs for effect sizes on the original scales were based on a Taylor expansion. If dietary adherence differed between the supermarket and instruction centers, secondary analyses were performed in the 2 center types separately using linear mixed models as described above. However, for the secondary analyses, family and center random effects were not included due to the reduced sample size.

Statistical analyses were performed using STATA 12.0 (StataCorp). Numbers in the text are shown as mean ± SEM unless otherwise stated. Significance was established at $P < 0.05$. In post hoc analyses of the cardiovascular outcomes, a Bonferroni corrected significance level was applied ($P < 0.0167$) to account for multiple comparisons.

Results

A total of 1139 children were registered for screening and 817 of these attended a baseline visit and were randomly assigned to the 5 diets together with their parents. Of these, the 253 children (31%) in whom the MetS markers were measured at baseline and at least at one of the other examination visits were included in the present study after removal of one outlier in MAP. Compared with those registered for screening but not included in the present study, the included children were slightly older (mean ± SD) (12.4 ± 3.4 y vs. 11.6 ± 4.0 y) and had a different country distribution (6/25/5/12/13/21/5/14% vs. 20/15/14/13/7/13/8/9% from The Netherlands/Denmark/United Kingdom/Greece/Germany/Spain/Bulgaria/The Czech Republic) ($P < 0.0001$). However, they did not differ with regard to gender distribution or proportion of overweight or obese children. Comparable differences were seen between the 253 included children and the 564 children who were measured at baseline and randomized to the 5 diets but not included in the present study (data not shown). Of the 253 children included, 204 children provided

data at 1 mo, 178 children provided data at 6 mo, and 129 children provided data at baseline, 1 mo, and 6 mo.

The included girls had lower age-adjusted waist circumference, serum glucose, and systolic blood pressure and higher serum total cholesterol compared with the boys at baseline (**Table 1**). The prevalence of MetS among the 10- to 18-y olds (n = 201) was 4.8 and 4.2% among the girls and boys, respectively. These numbers did not change during the intervention (P = 0.84).

The 5 dietary groups had a comparable age and sex distribution: the median (IQR) age was 13.6 (10.6–15.9) y, 13.7 (11.6–16.0) y, 12.0 (9.4–14.4) y, 13.0 (9.9–15.9) y, and 13.1 (10.7–15.5) y and the proportion of girls was 46, 46, 57, 64, and 43% in the control, LP/LGI, LP/HGI, HP/LGI, and HP/HGI groups, respectively.

At baseline, 1 mo, and 6 mo, 194, 153, and 121 children (77, 61, and 48% of the total study population, respectively) had dietary recordings (**Supplemental Table 1**). According to these, none of the groups met their target intakes of 10–15% energy and 23–28% energy protein in the LP and HP groups, respectively, or the 15-point target GI difference between the LGI and HGI groups during the intervention. The HP groups had a 3.1% energy (95% CI: 1.4, 5.2) (P = 0.001) higher protein intake and a 4.4% energy (95% CI: 1.0, 8.1) (P = 0.012) lower carbohydrate intake than the LP groups at 6 mo. The HP groups also had higher protein intake compared with control (P = 0.002) at 6 mo. The LGI groups had a 4.0-point (95% CI: 2.1, 6.1) lower dietary GI compared with the HGI groups at 6 mo (P < 0.001) and lower GI compared with control (P = 0.008) (Supplemental Table 1). Secondary analyses showed that with regard to protein intake, the HP and LP groups differed more in the supermarket centers (23.7 ± 1.4% energy in the HP groups vs. 16.9 ± 1.3% energy in the LP groups) (P = 0.001) than in the instruction centers (18.6 ± 1.3% energy in the HP groups vs. 17.6 ± 1.3% energy in the LP groups) (P = 0.31) at 6 mo (P = 0.009 for difference between center types). The effect of the GI intervention on dietary GI did not differ between the center types at 6 mo.

TABLE 1 Baseline characteristics of the included children[1]

	Girls	Boys	P value[2]
n	128	125	
Age, *y*	13.2 (10.6–16.2)	12.8 (10.3–15.2)	0.23
Height-for-age Z-score	0.75 (0.28–1.40)	0.77 (0.07–1.59)	0.92
BMI-for-age Z-score	1.12 (0.34–1.92)	1.52 (0.59–2.27)	0.06
Normal weight, *n (%)*	66 (52)	60 (48)	0.57
Overweight, *n (%)*	43 (34)	39 (31)	0.68
Obese, *n (%)*	19 (15)	26 (21)	0.22
Waist circumference, *cm*	73.9 (65.7–81.7)	76.5 (66.0–85.8)	0.01
Systolic blood pressure, *mm Hg*	108 9101–117)	113 (105–121)	<0.001
Diastolic blood pressure, *mm Hg*	63 (57–70)	61 (56–68)	0.40
MAP, *mm Hg*	78 (72–84)	78 (73–85)	0.26
Serum glucose, *mmol/L*	4.6 (4.3–4.8)	4.7 (4.5–5.0)	0.001
Serum insulin, *pmol/L*	83 (56–111)	65 (40–102)	0.06
HOMA-IR	2.4 (1.5–3.2)	2.0 (1.2–3.2)	0.25
Serum total cholesterol, *mmol/L*	4.2 (3.9–4.6)	4.1 (3.5–4.6)	0.02
Serum LDL cholesterol, *mmol/L*	2.35 (2.08–2.73)	2.39 (1.97–2.67)	0.39
Serum HDL cholesterol, *mmol/L*	1.37 (1.17–1.60)	1.30 (1.12–1.50)	0.08
Serum TG, *mmol/L*	0.81 (0.63–1.09)	0.72 (0.54–0.96)	0.09
Serum CRP,[3] *mg/L*	1.03 (0.39–2.43)	0.97 (0.46–2.36)	0.79

[1] Values are unadjusted medians (IQRs) or *n* (%). CRP, C-reactive protein; HOMA-IR, homeostasis model of assessment-insulin resistance; MAP, mean arterial blood pressure.
[2] P value adjusted for age in ANCOVA with robust SEs.
[3] n = 100 (girls) and n = 98 (boys) for serum CRP.

The HP diets resulted in a reduction in waist circumference, serum total cholesterol, and serum LDL cholesterol compared with the LP diets during the intervention but only the effects on waist circumference and serum LDL cholesterol remained significant after Bonferroni correction. Changes in serum TG and CRP during the intervention were greater in children who consumed the LGI compared with the HGI diets, but not after Bonferroni correction (**Table 2**). The estimated differences between the HP and LP groups at 6 mo were 2.7 cm (95% CI: 0.9, 5.1) (P = 0.006) in waist circumference and 0.25 mmol/L (95% CI: 0 0.09, 0.41) (P = 0.002) in serum LDL cholesterol. No effects of protein or GI were seen on body weight or BMI Z-scores during the intervention (data not shown). There were no interactions between protein and GI with regard to the development of the outcomes during the intervention (Table 2).

Because dietary adherence based on the dietary recordings differed between the supermarket and instruction centers, secondary analyses were performed in the 2 center types separately. In the children in the supermarket centers, waist circumference (P = 0.004), diastolic blood pressure (P = 0.007), MAP (P = 0.005), serum insulin (P = 0.013), and HOMA-IR (P = 0.016) were reduced with the HP compared with the LP diets and serum insulin and homeostasis model of assessment were reduced with the LGI compared with the HGI diets during the intervention (P = 0.04). In the children in the instruction centers, the HP diets reduced serum LDL cholesterol (P = 0.004) compared with the LP diets and the LGI diets increased serum CRP and TG (P = 0.047) compared with the HGI diets (P = 0.022). However, only the effects of protein on waist circumference, diastolic blood pressure, MAP, serum insulin, HOMA-IR, and serum LDL cholesterol remained significant after Bonferroni correction in these separate center analyses. The estimated reductions with the HP compared with the LP diets in the supermarket centers at 6 mo were 3.1 cm (95% CI: 1.0, 5.3) (P = 0.004) in waist circumference, 1.0 mm Hg (95% CI: 0.3, 1.7) (P = 0.007) in diastolic blood pressure, 6.5 mm Hg (95% CI: 1.5, 15.0) (P = 0.016) in MAP, 6 pmol/L (95% CI: 2, 13) (P = 0.014) in serum insulin, and 0.8 points (95% CI: 0.2, 1.7) (P = 0.016) in HOMA-IR. In the instruction centers, the serum LDL cholesterol reduction with the HP compared with the LP diets was estimated at 1.7 mmol/L (95% CI: 0.7, 3.3) (P = 0.003) at 6 mo. BMI-for-age Z-scores were reduced in the HP groups compared with the LP groups (P = 0.023) and with the control (P = 0.015) in the supermarket centers only. However, only the difference between the HP groups and control group remained significant after correction. No effects of protein or GI were seen on body weight during the intervention in the 2 center types separately.

Discussion

This substudy of the Diogenes trial is apparently the first large-scale, randomized, controlled trial to assess the effects of dietary protein and GI on CVD risk markers in overweight children. We found that an ad libitum diet with a modestly increased protein content reduced waist circumference and LDL cholesterol despite no apparent reduction in body weight or BMI Z-scores. Moreover, our secondary analyses also showed improvements in blood pressure and glucose regulation with HP diets in those children who were exposed to the intensive supermarket intervention and who reported a higher adherence to the diets. These findings are supported by those of a recent meta-analysis of

TABLE 2 Cardiovascular risk markers and MetS scores in children of overweight parents who consumed 1 of 5 diets ad libitum for 6 mo[1]

Variable by diet group	Baseline	1 mo	6 mo	Protein × GI	*P* value protein	GI
Children, *n*						
Control	56	43	35			
LP/LGI	55	47	38			
LP/HGI	44	37	26			
HP/LGI	45	34	37			
HP/HGI	53	43	42			
Waist circumference, *cm*				0.32	0.02[2]	0.88
Control	75.8 (67.5–87.6)	76.0 (64.3–86.5)	77.8 (69.6–86.8)			
LP/LGI	75.0 (66.0–81.6)	73.0 (64.8–83.3)	73.5 (66.0–84.2)			
LP/HGI	74.6 (67.6–84.4)	75.3 (68.0–85.8)	74.0 (69.0–80.0)			
HP/LGI	70.0 (63.5–81.5)	70.7 (62.4–78.9)	68.0 (61.0–76.4)			
HP/HGI	77.5 (68.8–88.8)	76.5 (64.8–86.9)	76.8 (70.9–83.3)			
MAP, *mm Hg*				0.66	0.66	0.20
Control	79 (73–85)	80 (72–86)	80 (68–86)			
LP/LGI	77 (73–84)	79 (67–82)	76 (70–80)			
LP/HGI	76 (72–83)	77 (72–80)	75 (68–83)			
HP/LGI	79 (75–85)	77 (71–81)	74 (69–81)			
HP/HGI	78 (73–84)	76 (72–82)	77 (71–82)			
Serum glucose, *mmol/L*				0.90	0.98	0.63
Control	4.8 (4.5–5.1)	4.8 (4.6–5.0)	4.8 (4.5–5.2)			
LP/LGI	4.7 (4.4–4.9)	4.7 (4.4–4.9)	4.7 (4.5–5.0)			
LP/HGI	4.6 (4.3–4.8)	4.7 (4.5–5.1)	4.6 (4.3–5.1)			
HP/LGI	4.6 (4.4–4.8)	4.6 (4.3–4.7)	4.5 (4.2–4.8)			
HP/HGI	4.6 (4.4–4.8)	4.7 (4.4–4.9)	4.6 (4.5–4.9)			
Serum insulin, *pmol/L*				0.28	0.63	0.51
Control	77 (50–113)	74 (42–108)	77 (50–127)			
LP/LGI	76 (45–100)	66 (37–88)	71 (47–114)			
LP/HGI	65 (41–112)	87 (41–121)	84 (50–104)			
HP/LGI	81 (47–106)	63 (40–96)	49 (40–78)			
HP/HGI	72 (51–104)	72 (41–98)	71 (43–93)			
HOMA-IR				0.30	0.66	0.46
Control	2.4 (1.5–3.3)	2.2 (1.3–3.3)	2.5 (1.6–3.9)			
LP/LGI	2.2 (1.4–3.4)	2.0 (1.2–2.7)	2.1 (1.4–3.3)			
LP/HGI	1.8 (1.2–3.4)	2.8 (1.2–3.8)	2.5 (1.5–3.1)			
HP/LGI	2.3 (1.3–3.3)	1.8 (1.2–2.9)	1.4 (1.2–2.2)			
HP/HGI	2.1 (1.5–2.9)	2.1 (1.3–2.8)	2.1 (1.3–2.8)			
Serum total cholesterol, *mmol/L*				0.22	0.02[3]	0.58
Control	4.1 (3.8–4.6)	4.3 (3.8–4.6)	4.1 (3.6–4.8)			
LP/LGI	4.3 (3.9–4.6)	4.2 (3.6–4.5)	4.3 (3.9–4.7)			
LP/HGI	4.0 (3.8–4.5)	4.1 (3.7–4.4)	4.3 (3.8–4.5)			
HP/LGI	4.3 (3.7–4.8)	4.1 (3.7–4.5)	4.1 (3.7–4.5)			
HP/HGI	4.1 (3.4–4.3)	4.0 (3.3–4.3)	4.0 (3.7–4.2)			
Serum LDL cholesterol, *mmol/L*				0.17	0.002[4]	0.62
Control	2.40 (1.96–2.67)	2.46 (1.98–2.79)	2.32 (1.83–2.73)			
LP/LGI	2.40 (2.10–2.77)	2.22 (1.90–2.56)	2.37 (2.09–2.70)			
LP/HGI	2.33 (2.05–2.65)	2.27 (2.04–2.57)	2.50 (2.15–2.90)			
HP/LGI	2.41 (2.10–2.78)	2.40 (1.98–2.80)	2.32 (1.95–2.59)			
HP/HGI	2.29 (2.00–2.53)	2.24 (1.78–2.62)	2.31 (1.94–2.52)			
Serum HDL cholesterol, *mmol/L*				0.63	0.83	0.84
Control	1.32 (1.11–1.60)	1.33 (1.11–1.67)	1.37 (1.08–1.64)			
LP/LGI	1.45 (1.21–1.68)	1.41 (1.15–1.71)	1.40 (1.23–1.66)			
LP/HGI	1.26 (1.15–1.48)	1.30 (1.05–1.51)	1.27 (1.15–1.50)			
HP/LGI	1.39 (1.20–1.58)	1.27 (1.14–1.44)	1.38 (1.25–1.57)			
HP/HGI	1.29 (1.01–1.48)	1.24 (1.01–1.47)	1.21 (1.10–1.47)			

(Continued)

TABLE 2 *Continued*

Variable by diet group	Baseline	1 mo	6 mo	Protein × GI	*P* value protein	GI
Serum TG, *mmol/L*				0.48	0.59	0.05[5]
Control	0.91 (0.58–1.15)	0.75 (0.57–1.13)	0.84 (0.64–1.24)			
LP/LGI	0.69 (0.54–0.98)	0.76 (0.51–1.09)	0.78 (0.64–1.00)			
LP/HGI	0.77 (0.56–1.14)	0.77 (0.62–1.12)	0.69 (0.62–1.00)			
HP/LGI	0.75 (0.59–0.98)	0.71 (0.59–0.95)	0.80 (0.59–1.01)			
HP/HGI	0.82 (0.57–1.00)	0.77 (0.53–1.10)	0.77 (0.51–1.03)			
Serum CRP, *mg/L*				0.52	0.65	0.02[6]
Control	1.29 (1.10–1.57)	1.27 (1.10–1.54)	1.22 (1.04–1.50)			
LP/LGI	1.44 (1.21–1.68)	1.46 (1.24–1.68)	1.46 (1.21–1.72)			
LP/HGI	1.26 (1.15–1.48)	1.25 (1.15–1.48)	1.18 (1.14–1.46)			
HP/LGI	1.39 (1.28–1.58)	1.37 (1.28–1.56)	1.40 (1.25–1.59)			
HP/HGI	1.29 (1.00–1.48)	1.25 (1.00–1.48)	1.27 (1.00–1.47)			
MetS score 1				0.76	0.99	0.26
Control	0.02 (−1.33–1.24)	−0.21 (−1.17–1.09)	−0.43 (−1.18–1.12)			
LP/LGI	0.06 (−1.18–0.96)	−0.13 (−1.74–0.45)	−0.39 (−1.52–0.57)			
LP/HGI	−0.17 (−1.10–1.00)	−0.06 (−1.21–1.03)	−0.16 (−1.13–0.42)			
HP/LGI	0.45 (−0.61–1.07)	0.51 (−0.74–1.37)	−0.15 (−1.63–0.77)			
HP/HGI	0.15 (−0.70–1.29)	−0.10 (−0.77–1.48)	0.51 (−0.88–0.97)			
MetS score 2				0.38	0.99	0.43
Control	0.06 (−1.19–1.16)	−0.05 (−1.13–1.33)	−0.38 (−1.30–1.46)			
LP/LGI	0.34 (−0.82–1.41)	0.66 (−0.52–1.71)	0.57 (−0.54–1.75)			
LP/HGI	0.06 (−1.22–0.87)	−0.26 (−1.06–0.44)	0.24 (−0.84–1.12)			
HP/LGI	0.21 (−0.27–1.30)	0.42 (−0.43–1.35)	0.84 (−0.38–1.56)			
HP/HGI	−0.12 (−1.17–0.73)	0.31 (−0.64–0.95)	0.32 (−0.61–1.21)			

[1] Values are unadjusted median (IQR). *P* values are shown for the protein-GI interaction and for the effects of protein (control, LP, HP) and GI (control, LGI, HGI) on the development of the outcomes modeled over time during the intervention based on linear mixed models adjusted for age, gender, family, country, participant, baseline values, and time since randomization. CRP, C-reactive protein; GI, glycemic index; HGI, high glycemic index; HOMA-IR, homeostasis model of assessment-insulin resistance; HP, high protein; LGI, low glycemic index; LP, low protein; MAP, mean arterial blood pressure; MetS, metabolic syndrome.
[2] HP < LP, $P = 0.016$.
[3] HP < LP, $P = 0.018$; not significant after Bonferroni correction.
[4] HP < LP, $P = 0.002$.
[5] LGI > HGI, $P = 0.044$; not significant after Bonferroni correction.
[6] LGI > HGI, $P = 0.024$; not significant after Bonferroni correction.

randomized controlled trials in mainly overweight and obese adults showing reductions in BMI, waist circumference, blood pressure, insulin, TG, and increased HDL cholesterol with HP compared with LP diets (28). The evidence from studies in children is sparse. In a pilot study among 6- to 12-y-old overweight children, a low-carbohydrate diet reduced total cholesterol and TG as well as body weight (29). However, whether the carbohydrate reduction caused an increase in dietary protein, fat, or both was not monitored. In cross-sectional studies among children, negative associations between protein intake and blood pressure have been observed (11,30). A comparable association for blood pressure was seen in 9-y-old Australians, but only among the girls (12). In Swiss children, total dietary protein was positively associated with fasting insulin and negatively associated with QUICKI, an index of insulin sensitivity, but not with blood pressure (31). In the adults from the Diogenes study, CRP concentrations decreased with the LP compared with HP diets during the 6-mo weight maintenance period following the 8-wk, low-calorie diet (9). No differences in blood pressure or serum lipid profile were seen despite a 0.9-kg lower weight regain in the HP compared with the LP groups. In the adults, a higher insulin response with the LP/HGI compared with the control diet was also seen (32), whereas we found no interactions between protein and GI in the children, possibly because relatively small differences in dietary GI were evoked in the present study. It is possible that children respond differently or are more sensitive to dietary protein manipulations than adults. The cardio-protective effects in the present study were seen with modest differences in protein intake (~3% energy), whereas the mean difference in the mentioned meta-analysis by Santesso et al. (28) was ~9% energy. Also, although the mean protein intake of ~24% energy in the HP groups in the supermarket centers in the present study was within the acceptable range for children >4 y of age set by the U.S. Institute of Medicine (19), it was higher than the 10–20% energy recommended in the Nordic countries (33). In infants, high protein intake has been shown to promote weight gain and may increase the risk of later obesity (34).

To our surprise, serum TG and CRP tended to increase in the children with the LGI diets. This is in contrast to the findings in the adults from the Diogenes trial, in whom CRP concentrations were improved by low-GI diets (9). Moreover, in the supermarket centers separately, low dietary GI tended to improve glucose regulation in the present study. However, the clinical importance of low-GI diets is uncertain. Recent meta-analyses of prospective cohort studies showed modest improvements in CVD risk with low-GI diets, but mainly in women (35,36). A meta-analysis of dietary intervention trials showed improved blood glucose and insulin sensitivity but no clear effect on TG with lower GI (10). Few studies have investigated the effects of GI on CVD risk markers in children. In a small study in overweight and obese

11-y olds, replacement of high-GI foods with low-GI foods for 6 wk reduced body fat percentage and the waist:hip ratio and tended to reduce HOMA-IR, HDL cholesterol, and TG (37). In obese, insulin-resistant adolescents, insulin and HOMA-IR decreased more with a low-GI diet than a control diet (38). In contrast to the present study, both diets were calorie restricted and reduced body weight, but the low-GI diet led to greater reductions in waist circumference. In 11- to 25-y-old participants, Slyper et al. (39) found negative associations between HDL cholesterol and glycemic load, which reflects both the GI of dietary carbohydrate and the amount of carbohydrate ingested. Mechanistically, we may expect serum TG to be reduced by lowering the GI, because higher postprandial glucose and insulin concentrations can act directly on the liver to slow the clearance of TG-rich chylomicron particles (40). However, considering the relatively low accuracy with which the GI of foods can be determined, the reductions in GI were small (~4 points) in the present study and may even have been over-reported. Moreover, our secondary analyses showed that the CRP and TG increases were only found in the instruction centers, in which the families were less adherent to the diets, and the effect disappeared after Bonferroni correction.

In the present study, the dietary intervention did not affect the 2 MetS scores, probably because the main effects were seen on variables that were not included in the scores. However, together with the reductions in waist circumference, improvements in these cardiovascular markers are still thought to be important for the prevention of CVD. The prevalence of the actual MetS in pediatric populations increases with the degree of overweight but also varies considerably with the MetS definition used. As mentioned above, we used the International Diabetes Federation Criteria, by which 4–5% of the children in the present study could be diagnosed with MetS. These numbers are comparable with the 4.4% reported in a national representative sample of American 12- to 17-y olds from the NHANES 1999–2004 using the same criteria (41). In population-based samples of 10- to 15-y olds from Denmark, Estonia, and Portugal, the prevalence of MetS was 0.2–1.4% using the IDF criteria (42). Much higher prevalences (11–32%) of MetS were found among European samples of obese children using various definitions for diagnosis, as reviewed by Tailor et al. (43).

Due to overweight and obese parents, the children were at high risk of obesity and associated comorbidities in adulthood and are therefore a highly relevant target group for prevention. Although the dietary intervention was primarily targeted at the parents, the dietary records showed that the children's diet also changed in the desired direction, at least in the supermarket centers. The dietary targets for protein and GI were not met in most children; however, this is not unexpected with a free-living, family-based intervention primarily aimed at the parents. Nevertheless, the results indicate that even modest changes in protein intakes can improve CVD risk markers in children. Due to the limited number of children who gave blood samples, only about one-half of the pediatric study population that was assessed for weight loss earlier (13) was included in the present paper and an intention-to-treat analysis was not applicable. Although the prevalence of overweight and obesity did not differ between included children and those not included, the study population may belong to a select group of children of motivated and health-concerned parents. However, the data set is unique, because we have been able to repeatedly collect blood samples from healthy children from 8 countries. Puberty is known to affect the CVD risk markers as well as fat accumulation and distribution, and therefore it would have been desirable to be able to control for pubertal stage. However, during the planning of the study, such measurements were judged to be culturally inappropriate in some centers and logistically unfeasible.

In conclusion, this pan-European multicenter study showed that a dietary intervention that increased protein intake improved CVD risk markers in children of overweight and obese parents, particularly among those undergoing the most intensive intervention. In contrast, small reductions in dietary GI had no consistent effect on early CVD risk markers. This study is an important contribution to our knowledge of evidence-based strategies to prevent obesity-related diseases in childhood. The potential effects of dietary GI on early CVD risk markers and inflammation should be explored further.

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