Open Journal of
Pediatrics & Neonatal Care

Research Article

High Sensitivity C - Reactive Protein Levels are Associated with High Energy Intake, Processed Foods, Total Fat and Saturated Fats Intake in Children -

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Submitted: 15 December 2016; **Approved:** 23 February 2017; **Published:** 28 February 2017

Citation this article: Boscaini C, Artifon M, Pellanda LC. High Sensitivity C – Reactive Protein Levels are Associated with High Energy Intake, Processed Foods, Total Fat and Saturated Fats Intake in Children. Open J Pediatr Neonatal Care. 2017;2(1): 017-030.

ABSTRACT

Objective: Food habits may be associated with inflammation, but there is little information about processed and ultra-processed foods in children. Thus, our aim was to investigate the relationship between processed and ultra-processed foods, energy intake, total fat and saturated fats intake and high sensitivity C - reactive protein levels (hs - CRP) in children.

Design: A cross-sectional study with a population-based cluster sample.

Setting: A southern Brazilian city.

Subjects: A total of 602 children aged 5-13 years were included. Food consumption was evaluated through food frequency questionnaire and two 24-h recall questionnaires. Nutritional status was assessed by body mass index, waist circumference, and skin folds. Blood samples were collected to measure hs - CRP, Total Cholesterol (TC) and fractions, triglycerides, insulin, and glucose.

Results: hs - CRP values above 0.1 mg/dl were observed in 16.9%. Processed foods, energy intake, fat intake and saturated fat intake were associated with elevated values of hs - CRP, (PR: 2.46 - 95%IC: 1.20 - 5.04). (PR: 1.09 - 95% IC: 1.03 - 1.15), (PR: 1.14 - 95% IC: 1.04 - 1.25), (PR: 1.19 - 95% IC: 1.04 - 1.37), respectively. Body mass index, waist circumference, skin folds, insulin and high-density lipoprotein were increased in school children with hs - CRP levels above 0.1 mg/dl ($p < 0.001$).

Conclusions: Body mass index, waist circumference, skin folds, insulin and high-density lipoprotein were associated with high sensitivity C - reactive protein levels.

Keywords: Child; C - reactive protein; Diet

INTRODUCTION

Recently, inflammation has been understood to be a key pathogenic mechanism in the initiation and progression of Cardiovascular Disease (CVD) [1] and great attention has been given to inflammatory markers for their ability to predict CVD risk [2]. Serum C - Reactive Protein (CRP) levels were a long-term predictor of risk of cardiovascular and non- cardiovascular mortality independent of known risk factors [3].

Relevant data assessing the relationship of diet on CRP levels in children are scarce [4]. A study demonstrated that both total fat intake and percentage of energy from fat were positively associated with CRP levels in children [5]. Another study [6] observed an inverse link between CRP and dietary intake of fruit, folate, and vitamin C (nutrients that are found in abundance in fruits and vegetables) among adolescents. Frequent consumption of “inflammatory” foods is positively related to CRP levels in children [7].

Epidemiological studies examining the dietary intake of children have shown increases in total energy consumption, inadequate intake of nutrients such as fiber, and excessive consumption of other nutrients such as lipids, saturated fatty acids and refined sugar [8-10], in addition to low consumption of fruits and vegetables [11]. Increased consumption of high energy density foods, such as snack food, sweets, and soft drinks have also been observed among children [12].

The perfect diet plan to reduce or prevent chronic low-grade inflammation is still unclear. Research is needed to examine the impact of different dietary components on inflammation in children. Thus, the aim of this study was to investigate the relationship between high-sensitivity C - reactive protein (hs - CRP) and energy intake, processed foods, total fat and saturated fats intake in school children.

METHODS

Design and location of the study

This population-based cross-sectional study was conducted in municipal and state public schools of Garibaldi, in the Rio Grande do Sul (RS). Garibaldi is located in the Northeast mountainous region – the Serra Gaúcha, 110 km distant from the capital city Porto Alegre. It has currently 30,165 inhabitants, and a total of 21 municipal and state elementary schools, with 1,464 students enrolled between 5 to 10 years old. Data was collected between 2011 and 2012 in a representative sample of the city´s schools.

Population

Considering the outcome of this study, the sample size was determined according to the study by Lazarou, et al. [7], which identified an inadequate eating pattern and hs - CRP values above 0.10 mg/ dl in 31.3% of children. The sample size was determined as 330 school children, considering a sampling error of 5% and 95% confidence level. The project was approved by the Research Ethics Committee of Institute of Cardiology of Rio Grande do Sul. Children using drugs that could interfere with the results of the study, with acute inflammatory processes or whose parents refused to sign an informed consent were not included in the study.

Dietary data

The data collection team included a nutritionist and trained Nutrition students. A Food Frequency Questionnaire (FFQ) for children over 5 years of age, prepared by the Food and Nutrition Surveillance System [13] and intended to identify food practices leading to obesity risk, was used for assessing eating habits. The mother or caregiver familiar with the routine eating schedule of the child answered the questions. The questionnaire assessed how often the child consumed foods or drinks related to a healthy diet (for example, daily consumption of beans, fruits, and vegetables) or to a poor diet (for example, frequent consumption of fried foods and sweets) in the last 7 days. All individuals responded to a 24 hour-recall (24 hr) and, in randomly selected children representing 50% of the sample, a second 24 hr was applied through telephone interviews.

A photographic model of homemade measurements, which allowed the respondent to determine which utensil(s) were usually used in daily life and portions consumed of certain foods [14], was used to facilitate the registration of the quantity of food eaten. In addition, a set of household utensils consisting of 14 items (5 spoons, 1 ladle, 1 skimmer, 3 dishes, 2 glasses, 2 cups) was shown to the school children or responsible/caregiver for the indication of measurements used daily. School children were instructed to indicate as accurately as possible the quantities of food consumed, using the following terms: leveled, shallow or full, and small, medium or large units. These approaches minimize memory bias, standardize measures and diminish the errors of estimating portion. Energy and nutrients contents were calculated using the Diet twin software. All values of nutritional composition of foods mentioned in the food diaries were included in the original database software, with basis on the Brazilian Table of Food Composition – TACO [15], the Food Composition Table - IBGE [16] and, in the case of industrialized foods specified by brand, on the values given on the label.

The participants were trained to standardization in data collection and received a standardized form for the 24 hr. A manual with the standardization of homemade measures was prepared, to assist in the process of calculating the 24 hr.

Anthropometric parameters

The anthropometric measurements were repeated three times, non-consecutively, using the average of the values for analysis. The school child was barefoot and wearing light clothes (shorts for boys and shorts and t-shirts for girls). Weight and height were measured using, respectively, a digital scale (Techline®) with a variation of 100 g and a stadiometer with the accuracy of 0.1 cm, set in a smooth wall without a footer and square. The school children stood in vertical position, with feet parallel and with the heels, shoulders, and buttocks touching the wall.

The Body Mass Index (BMI), determined as weight in kilograms divided by height in meters squared, was used to assess the nutritional status, based on the BMI-for-age standards determined by the World Health Organization (WHO) and values ≥ + 1 was considered as overweight [17].

For measurement of the Waist Circumference (WC), the school children were placed in standing position, with the abdomen relaxed and with the arms along the body. The measuring tape was positioned around the natural waistline, in the narrower region between the thorax and the pelvis, at the midpoint between the last rib and the iliac crest, with a firm but not compressive force. The measurement was made at the time of expiration. The WC was classified according to the percentile distribution, suggested by the International Diabetes Federation (IDF) [18].

Body composition was assessed through the sum of the tricipital and subscapular skin folds.

Daily hours of TV/video game/computer

The mean number of hours spent by the school child watching Television (TV), playing video games and/or computer in the previous week was used as a marker of physical activity. This activity was dichotomized based on international recommendations [19] into < 2 hours per day (hrs/ day) or > 2 hrs/ day.

High sensitivity C - reactive protein measurements

Blood samples were collected between 8 am and 11.30 am, following an overnight fast. To control for plasma volume shifts, venous blood was sampled after the participants had assumed a seated position for at least 30 minutes. Blood samples were obtained from an antecubital vein and collected in an Ethylene Diamine Tetra-acetic Acid (EDTA) vacutainer tube. Serum was isolated by centrifugation at 3500 rpm for 10 minutes. Samples were aliquoted and frozen at 270uC within two hours of collection. The analysis was undertaken within five months of collection. Hs-CRP concentration was measured using a latex-enhanced immunoturbidity - metric assay. The lower detection limit reported for the assay was 0.01 mg/ dl. The laboratory analytical variance for the measurement of hs-CRP was 5.5%.

The cut-off for hs - CRP was 0.1 mg/ dL, which was the lowest cut-off value which has been proposed to be associated with an elevated risk for cardiovascular disease due to chronic low-grade inflammation in children [2].

Statistical analysis

Generalized Linear Models analysis, using Poisson logistic was used to test the association between dietary practices with the likelihood of having hs-CRP levels > 0.1 mg/ dL, adjusting for potential confounders of BMI, gender, age, insulin and High Density Lipoprotein (HDL-cholesterol), after checking collinearity between anthropometric variables, using the Variance Inflation Factor (VIF). The best-adjusted model between all using anthropometric variables was verified by the Akaike's information criteria. All reported p-values are based on two-sided tests, and a significance level was set to 0.05. SPSS 21.0 software (Statistical Package for Social Sciences, Chicago, IL, USA) was used for all statistical calculations.

RESULTS

A total of 612 school children were evaluated. Among them, 602 presented complete data considered in this study, 599 presented complete information concerning the food frequency questionnaire, and 576 completed the 24 hR recall. Among school children assessed, 51.5% were male. Mean age was 8.6 years (± 1.46). The level of hs - CRP was higher than 0.1 mg/dl in 16.9% of the participants (n = 102).

The anthropometric markers assessed, BMI, WC, and skin folds, were statistically increased in children with hs-CRP above 0.1 mg/ dl ($p < 0.001$). Inverse behavior was observed for HDL-cholesterol levels ($p < 0.001$). No statistically significant differences were observed for the other biochemical parameters evaluated (Table 1).

Hs-CRP levels above 0.1 mg/ dl were more frequent among girls (PR: 1.71 - 95% CI: 1.18 - 2.47). Increased values of hs-CRP were around three-fold more probable among overweight children (PR: 2.96 - 95% CI: 2.05-4.28), over four times higher among school children with WC above the 90th percentile (PR: 4.27 - 95% CI: 2.42 - 7.54) (Table 2).

The food frequency analysis after control of confounding variables showed that school children consuming processed foods were around 2.5 more likely to have increased levels of hs - CRP (PR: 2.46 - 95% IC: 1.20 - 5.04) (Table 2).

The total energy intake was higher in school children with high hs - CRP, and each 300 calories more per day in food added up 9% to the likelihood of the occurrence of the outcome under study (PR: 1.09 - 95% IC: 1.03-1.15). Analysis of the role of diet macronutrients showed that an increase of 20 g/day of fat in the diet increases in 14% the likelihood for the elevation of the hs - CRP (PR: 1.14 - 95% IC: 1.04 - 1.25). The evaluation of the type of fat showed that a daily increased of 10 g of saturated fat results in a 19% increase in the likelihood of high hs - CRP levels (PR: 1.19 - 95% IC: 1.04 - 1.37). No significant relationships were observed among the outcome under study and the other macronutrients, others types of fat, dietary cholesterol and dietary fiber (Table 3).

Table 1: Association between gender, anthropometric, biochemical markers, TV/videogame/computer hours/day and high sensitivity C - reactive protein in school children, RS, Brazil (n = 602), 2014.

	Total		**hs - CRP < = 0.10 mg/ dl**		**hs - CRP > 0.10 mg/ dl**		***p* value**
	n	%	n	%	n	%	
Gender							
Male	297	48.5	271	87.4	39	12.6	
Female							
Age							
5-9 years							0.003
10-13 years	315	51.5	229	78.4	63	21.6	
BMI (kg/ m²)							
< = +1 SD	385	62.9	341	90.2	37	9.8	< 0.001
> + 1 SD	227	37.1	159	71.0	65	29.0	
WC							
< = P 90	545	89.8	461	85.7	77	14.3	< 0.001
> P 90	62	10.2	35	58.3	25	41.7	
TV/videogame/ computer hours/day							
< 2 hours/day	76	12.4	61	81.3	14	18.7	0.64
> = 2 hours/day	529	86.4	435	83.5	86	16.5	
	mean	**SD**	**mean**	**SD**	**mean**	**SD**	
Age (years)	8.6	1.4	8.6	1.4	8.6	1.4	0.66
Skin folds (mm)[a]	16.0[b]	12.0 - 24.1[c]	18.5	9.6	28.0	16.7	< 0.001[d]
Total Cholesterol (mg/dl)	166.2	26.9	165.8	26.7	168.0	26.7	0.44
LDL – Cholesterol (mg/dl)	96.6	24.4	95.9	24.3	99.8	24.5	0.15
HDL – Cholesterol (mg/dl)	50.1	10.5	50.6	10.5	47.6	10.2	< 0.001
Tryglicerides (mg/dl)[a]	90.0	78.0 - 108.0	97.1	31.8	102.4	38.8	0.14[d]
Glucose (mg/dl)	82.9	7.7	83.0	7.2	82.4	9.7	0.44
Insulin (μ UI/dl)[a]	4.9	3.2 - 7.1	5.6	4.5	7.8	9.8	0.04[d]

[a]Non-parametric; [b]Median; [c]P25 - P75 - percentile 25 and 75; [d]Non-parametric test - Mann - Whitney U Test.
hs - CRP: high sensitivity C - reactive protein; SD: standard deviation; BMI: Body Mass Index; WC: Waist Circumference; P 90: percentile 90; HDL: High Density Lipoprotein; LDL: Low Density Lipoprotein

Table 2: Association between specific foods (measured by Food Frequency Questionnaire) and high sensitivity C - reactive protein in school children, RS, Brazil (n = 602), 2014.

	hs - CRP > 0.10 mg/dl		**hs - CRP > 0.10 mg/dl Adjusted**	
Food Frequency Questionnaire	**n (%)**	**PR (95% IC)**	**PR (95% IC)**	***p* value**
Processed foods				
Yes	7 (6.9)	1	1	0.013*
No	95 (19.1)	2.76 (1.32 - 5.76)	2.46 (1.20 - 5.04)	
Raw salad				
Intake	79 (18.1)	1	------	0.24
Not intake	23 (14.1)	0.77 (0.50 - 1.19)	------	
Fresh fruit or fruit salad				
Intake	96 (17.1)	1	------	0.77
Not intake	6 (15.4)	0.89 (0.42 - 1.91)	------	
Beans				
Intake	87 (18.2)	1	------	0.14
Not intake	15 (12.5)	0.68 (0.41 - 0.14)	------	
Fries, French fries and savory packet				
Not intake	17 (14.9)	1	------	0.50
Intake	85 (17.5)	0.85 (0.52 - 1.37)	------	
Soda				
Intake	13 (22.8)	1	------	0.22
Not intake	89 (16.4)	1.38 (0.83 - 2.32)	------	

hs - CRP: High Sensitivity C - Reactive Protein; PR: Prevalence Ratio; CI: Confidential Interval.
Poisson regression: adjusted for BMI, HDL - cholesterol, insulin, gender and age.

Table 3: Association between, energy intake, diet macronutrients, total fiber and high sensitivity C - reactive protein in school children, RS, Brazil (n = 602), 2014.

	hs - CRP ≤ 0.10 mg/ dl		hs - CRP > 0.10 mg/ dl		*p* Value	PR IC (95%)	PR adjusted IC (95%)
	MD	P25 - 75	MD	P25 - 75			
Energy intake (EI)	1865.3	1489.4 - 2287.3	1962.9	1638.3 - 2501.6	0.01 0.02*	1.1 (1.01 - 1.17)	1.09 (1.03-1.15)
Breakfast (EI)	241.86	153.8 - 361.2	256.8	156.5 - 372.6	0.68	1.0 (0.99 - 1.00)	---------
Morning snack (EI)	169.01	74.7 - 284.2	149.3	83.9 - 309.7	0.17	1.0 (1.00 - 1.00)	---------
Lunch(EI)	533.13	363.1 - 746.3	536.9	410.6 - 780.2	0.07	1.0 (1.00 - 1.00)	---------
Afternoon snack (EI)	540.8	377.0 - 729.9	533.2	374.5 - 850.1	0.72	1.0 (0.99 - 1.00)	---------
Dinner (EI)	379.9	243.6 - 548.7	400.4	292.1 - 586.3	0.17	1.0 (1.00 - 1.00)	---------
Night snack (EI)	199.7	108.1 - 375.6	243.0	136.9 - 487.1	0.33	1.0 (1.00 - 1.00)	---------
Macronutrients							
Protein (g/day)	65.4	49.7 - 89.8	71.9	56.5 - 91.3	0.19	1.0 (0.99 - 1.00)	---------
Carbohydrate (g/day)	252.6	201.6 - 326.4	275.1	208.1 - 352.9	0.12	1.0 (1.00 - 1.00)	---------
Fat (g/day)	58.4	40.9 - 74.7	60.1	46.9 - 83.7	0.008 0.005*	1.2 (1.03 - 1.28)	1.14 (1.04 - 1.25)
Other dietary components							
Saturated Fat (g/day)	17.05	12.3 - 23.9	18.7	12.8 - 26.1	0.01 0.009*	1.2 (1.04 - 1.38)	1.19 (1.04 - 1.37)
Cholesterol (mg/day)	177.2	118.9 - 271.6	180.7	127.0 - 289.5	0.19	1.0 (1.00 - 1.00)	---------
Total Fiber (g/day)	11.5	7.6 - 16.3	11.5	8.8 - 17.1	0.93	0.9 (0.98 - 1.01)	---------

EI: Energy Intake; MD: Median; P25-75: Percentile 25 and 75; hs - CRP: High Sensitivity C - Reactive Protein; PR: Prevalence Ratio; CI: Confidential Interval.
**p* Value from Poisson regression; **Poisson regression adjusted for: BMI, HDL - cholesterol, insulin, gender and age.

DISCUSSION

The mains findings of this paper are that processed foods, high energy intake, total fat and saturated fat intake are linked to hs - CRP concentrations in a sample of school children from Brazil. Furthermore, it was found that overall and central obesity contributes to the increased hs-CRP levels. This is the first study among Brazilian school children aged up to 5 years, to examine the relationship between obesity and diet to hs - CRP levels. Although causality cannot be inferred from a cross-sectional study, the current data are consistent with the existing literature in population studies among children of other ethnicities regarding the obesity and diet-inflammation hypothesis.

A number of diseases, particularly chronic diseases of later life, are thought to be connected to chronic-low grade inflammation. Recently described diets are characterized by the removal of large groups of food or nutrients, such as wheat, corn, soy, milk, eggs, meat, and certain vegetables. These foods are considered pro-inflammatory, and are associated with increased inflammation, obesity and chronic diseases such as diabetes and hypertension. Their removal or at least the decrease in consumption of these may be beneficial in the recovery process of the inflammatory state [20]. The results of the present study showed a statistically significant association between hs - CRP and dietary intake. The consumption of processed foods was significantly associated with higher values of hs - CRP. A significant relationship was also observed between increased likelihood of inflammation and higher energy intake, so that an increase of 300 calories/ day in diet resulted in a 9% increase in inflammation likelihood. A 14% increase in this risk was related to each extra 20 g of total fat ingested daily, and the likelihood almost doubled (19%) when the intake of saturated fat increased in 10 g/ day. This relationship was observed even after statistical adjustment for confounding variables, such as BMI, HDL-cholesterol, insulin, age and gender.

Both the type and amount of dietary fat can impact plasma and tissue fatty acid composition and modify inflammation [21].

Poor diet is often cited as the cause of the obesity epidemic. Americans and their children are eating away from home more, eating fewer meals and having more snacks, and substituting sweets, pizzas, and snack foods for balanced meals [22]. Consumption of these “empty calorie” foods displaces healthier nutrient-dense options such as fruits, vegetables, whole grains, and calcium-rich foods. School-aged children exceed fat and saturated fat recommendations, and only 17% meet fruit intake recommendations [23]. In Brazil, a study showed only 30.2% of adolescents eats fruits five times a week or more, and that 21.3% of the studied population does not eat fruits even once a week. For fruits and vegetables, frequencies were 34.8% and 19.7%, respectively [24]. Around 69.9% of Brazilian school children were shown to consume beans, which have a beneficial effect on health [25], five or more times a week, while 6.6% of these populations do not consume beans even once a week [24].

No statistically significant differences were observed between the consumption of beans, fruits, vegetables or legumes and hs - CRP. In a study with children over 13 years of age, Lazarou, et al. [7] found no statistically significant association between quality of diet and inflammation, but observed a strong association between overall and central obesity and higher concentrations of hs - CRP. Weight reduction after successful lifestyle intervention, combined hypocaloric diet, and moderate physical activity, results in improvements of blood inflammatory markers in obese children and adolescents [26]. Effects of lifestyle modification with high-fiber, low-fat diet in a 2-week residential program showed significant reductions in all serum lipids (except HDL cholesterol) and CRP [27].

In children, physical fitness is negatively associated with metabolic risk factors and inflammatory markers such as CRP. Adiposity is positively related to serum CRP in prepubescent children, independent of the effects of fitness or physical activity. Physical activity is inversely associated with CRP levels and habitual physical activity and adiposity as focal points for the design of interventions attempting to reduce chronic systemic inflammation in children [28]. The level of physical activity was not measured in the present study, but the number of daily hours that the children remained in front of the TV, video game and/or computer was used as a marker to assess sedentarily. No association was observed between this variable and the outcome results, but there is a large body of evidence from all study designs, which suggests that decreasing any type of sedentary time is associated with lower health risk in youth aged 5-17 years. In particular, the evidence suggests that daily TV viewing in excess of 2 hours/ day is associated with reduced physical and psychosocial health, and that lowering sedentary time leads to reductions in BMI [29].

The role of chronic low-grade inflammation as a possible link between obesity and its metabolic and cardiovascular sequelae has been convincingly showed during the last years [30]. This study shows that BMI, WC and skin folds have significantly increased markers of inflammatory status. These results suggest that the association between excess adiposity during childhood and adolescence and increased inflammation and impaired endothelial function may promote the activation of mechanisms linked to the initiation of the atherosclerotic process.

Our data showed a strong association between skin folds and hs - CRP in school children. This correlation was similar to other studies where plasma hs - CRP levels were positively associated with adiposity [31-33].

Higher hs - CRP values are associated with cardiovascular risk factors, like lower HDL-cholesterol levels in this population of school children. The previous study already suggested that high CRP levels in children are associated with cardiovascular risk factors including HDL - cholesterol [34,35-39]. The finding is of interest because the low-grade inflammation has been found to predict the development of CVD [40] and it is generally accepted that traditional CVD risk factors such as obesity and hypercholesterolemia and have pediatric origins [34].

The new food guide for the Brazilian population highlighted the daily exposure to various strategies that are used by food industries to disseminate their products. It stands out that more than two-thirds of food commercials aired on television refer to ultra-processed products [41]. The guide proposes actions to promote an adequate and healthy diet, seeking to reverse the trend of increasing obesity and other chronic diseases related to food [42].

The Pan American Health Organization (PAHO) published Nutrient Profile Model PAHO with new definitions for the classification of processed and ultra-processed food, according to regional criteria for acceptable amounts of critical nutrients such as salt, sugar, and Tran's fats in the form of a nutrient profile model. Thus, countries can determine what kind of food can be sold or served in schools. Among the recommended measures, is the restriction on child advertising of unhealthy foods? The initiative reflects on the alarming prevalence of obesity in the Americas - the largest in the world [33].

Our study was limited by its cross-sectional nature. The causality of the associations could not be ascertained because of the cross-sectional study design. However, the results suggest that the development of obesity in school children may already be associated with modifications of the metabolic pathways important for the growth, development, and regulation of the cardiovascular system.

Dietary macronutrient intakes were examined with blood levels and hs - CRP, instead of food groups, which could better assess dietary quality. However, previous studies support analyses of dietary macronutrient intakes with blood lipid levels [43]. Moreover, dietary assessment is particularly challenging in children, due to challenges of memory, estimation in portion sizes, and misreporting [44]. All self-reported dietary assessment methods (eg: 24 hour - recalls and FFQs) tend to underreport energy and nutrient intake [45]. In our study, the FFQ was chosen because of its use in other similar populations, ease of implementation in a school-based setting, and relatively low cost. Inaccurately reported intake has important implications in studies that deal with assessing the role of diet in childhood obesity and related health risk factors.

In summary, BMI, WC, skin folds, insulin and high-density lipoprotein was associated with hs - CRP, which may predispose to an increased risk of atherosclerotic damage later in life. It is suggested that processed foods have inflammatory action, as well as inducing an increase in the energy intake, total fat, and saturated fat. Further studies are required to assess the direction of the association and to clarify the mechanistic connections among excess adiposity, food consumption, and inflammation, and whether these biomarkers could have a clinically significant role in the prediction of cardiovascular risk later in life.

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