Longitudinal associations of diet quality with serum biomarkers of lipid and amino acid metabolism from childhood to adolescence: the PANIC study

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Abstract

Studies on longitudinal associations between diet quality and lipid and amino acid metabolism in children and adolescents are limited. We studied associations between diet quality and serum markers of lipid and amino acid metabolism in the Physical Activity and Nutrition in Children (PANIC) study. These analyses included 403 children aged 6–9 years at baseline, 360 re-examined two years later at age 9–11 years, and 219 eight years later at age 15–17 years. Food intake was recorded over four days, and diet quality assessed using the Finnish Children Healthy Eating Index (FCHEI). Fasting serum fatty acids, amino acids, apolipoproteins, and lipoprotein particle sizes were analyzed via nuclear magnetic resonance spectroscopy. Linear mixed-effects models, adjusted for sex, age, body fat percentage, pubertal stage, and physical activity, were used to analyze the associations. Better diet quality was linked to increased serum polyunsaturated fatty acids and reduced saturated and monounsaturated fatty acids, alanine and very low-density lipoprotein (VLDL) particle size. Consuming more vegetables, fruits, berries, vegetable oils and margarine with at least 60% fat, fish, and whole grains associated with higher serum polyunsaturated fatty acids and lower saturated fatty acids and smaller VLDL particles. Conversely, consuming higher-fat dairy products and sugary products associated with higher saturated and monounsaturated fatty acids, branched-chain and aromatic amino acids, and larger VLDL particles. A diet rich in fruits, vegetables, unsaturated fats, and fiber, with reduced processed meat and sugar consumption, promotes favorable metabolic changes relevant to cardiometabolic health.

Short title: Diet and metabolites from childhood to youth

Keywords: Diet quality, food consumption, metabolites, children, adolescents

Abbreviations: AAA; aromatic amino acids; ApoA1, apolipoprotein A1; ApoB, apolipoprotein B; BCAA, branch-chained amino acids; DHA, docosahexaenoic acid; FA, fatty acids; FCHEI, Finnish Children Healthy Eating Index; FDR, false discovery rate; HDL, high-density lipoprotein; LA, linoleic acid; LDL, low-density lipoprotein; MUFA, monounsaturated fatty acids; NCD, Non-communicable disease; NMR, nuclear magnetic resonance spectroscopy; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; VLDL, very-low-density lipoprotein.

Introduction

The burden of non-communicable diseases (NCDs), such as obesity, type 2 diabetes, and cardiovascular diseases, has increased globally [1,2]. Although NCDs exhibit their clinical manifestations in adulthood, the pathophysiological processes leading to these diseases can begin at a very young age [3–6]. Lower diet quality has been recognized as an underlying risk factor for NCDs [7,8]. Furthermore, several serum metabolites, small molecules resulting from metabolic processes in the body, have been associated with both lower diet quality and an increased risk of NCDs in adults [9]. For instance, higher serum concentrations of branched-chain amino acids (BCAAs) and saturated fatty acids (SFAs) as well as larger very-low density lipoprotein (VLDL) particles have been linked to higher blood pressure, insulin resistance, and cardiovascular diseases in cross-sectional studies in adults [9,10]. Moreover, higher serum levels of some diet-associated metabolites, such as BCAAs, have been related to risk factors for NCDs already in children [11]. These findings raise curiosity in the role of blood metabolites as mediating factors between diet and NCDs and whether these effects are seen already in childhood.

Nutrition and metabolomics research in children and adolescents has largely focused on the associations of dietary factors with blood fatty acids [12–18]. Interestingly, we previously observed that better diet quality, characterized by a higher intake of dietary sources of polyunsaturated fatty acids (PUFAs) and fiber and a lower intake of sugary products, was reflected in a more unsaturated serum fatty acid profile, a larger VLDL particle size, and lower serum levels of alanine, histidine, and glycine in children [19]. Our results suggest that diet quality might influence blood levels of various metabolites related to cardiometabolic health [9,10], thus also underlining the importance of studying a wide range of blood metabolites rather than focusing on only a limited number of biomarkers to provide a more comprehensive view. Additionally, in Finnish children and adolescents, the consumption of vegetables, fruit, and berries is low and the consumption of dietary SFAs and sucrose is over the recommendations of the Finnish dietary guidelines [20–22]. These results also highlight the importance of studying the association between diet quality and metabolites in a population sample of Finnish children and adolescents. However, only few reports on the longitudinal associations of dietary factors with circulating metabolites of amino acid and lipid metabolism in children and adolescents have been published [23,24]. The results of these reports, which were based on the same Finnish dietary intervention study, showed that better adherence to Nordic nutritional recommendations was reflected in higher serum levels of

PUFAs, lower serum levels of SFAs, and a larger low-density lipoprotein (LDL) particle size. This existing, yet scarce, evidence emphasizes the need for further longitudinal studies examining the associations of diet with blood metabolites from childhood to adolescence.

Given the limited evidence on the longitudinal associations of dietary factors with blood metabolites in youth, we investigated the associations of diet quality with serum metabolites in a general population of children followed up for eight years until adolescence. We hypothesized that good diet quality is associated with serum levels of metabolites considered beneficial for cardiometabolic health, such as higher PUFAs and lower BCAAs.

Methods

Study design and participants

These analyses on the longitudinal associations of diet quality with serum biomarkers of lipid and amino acid metabolism from childhood to adolescence are based on the baseline, 2-year and 8-year data of the Physical Activity and Nutrition in Children (PANIC) study, which is an 8-year physical activity and diet intervention and long-term follow-up study in a population sample of children from the city of Kuopio, Finland. The PANIC study protocol was approved by the Research Ethics Committee of the Hospital District of Northern Savo in 2006 (Statement 69/2006) and in 2015 (Statement 422/2015). The parents or caregivers gave their written informed consent, and the children provided their assent to participation. The PANIC study has been carried out in accordance with the principles of the Declaration of Helsinki as revised in 2008. The trial was registered at ClinicalTrials.gov (NCT01803776). Examinations were performed in the PANIC study facilities at the University of Eastern Finland.

Altogether, 736 children aged 6–9 years were invited to participate in the baseline examinations of the PANIC study between 2007 and 2009 (Figure 1). Of all the children invited, 512 (70%) attended. The participants did not differ in sex, age, or body mass index standard deviation score from all children who started the first grade in 2007–2009 based on data from the standard school health examinations performed for all Finnish children before the first grade. Eight children were excluded at baseline because of disability or withdrawal from the study. The final study sample thus included 504 children at baseline.

Overall, 440 children aged 9-11 years (87% of those participating in baseline examinations) attended the 2-year examinations, and 277 adolescents aged 15-17 years (55% of those

participating in baseline examinations) attended the 8-year examinations. Adolescents, who participated in the 8-year examinations, did not differ from those who did not attend these examinations in terms of baseline age, BMI-SDS, sex distribution, or allocation to study groups [25]. The present study sample consisted of 403 children at baseline, 360 children at two years, and 219 adolescents at eight years with complete data on variables used in the statistical analyses.

Data on the basic characteristics were available for 403 children (194 girls, 209 boys), except linoleic acid, for which data from 382 children were available at baseline, and lipoprotein subclasses for which data from 401 children were available. Data for 360 children (171 girls, 189 boys) were available at two years and for 219 adolescents (108 females, 111 males) at eight years, except body fat percentage, for which data for 346 children were available at two years and data for 217 adolescents were available at eight years.

Assessment of diet

At the baseline and 2-year examinations, food consumption and energy intake were assessed by food records filled out by the parents or caregivers on four predefined consecutive days, including either two weekdays and two weekend days for 99.5 % of the participants or three weekdays and one weekend day for 0.5 % of the participants [26]. At the 8-year examinations, food records were filled out by the adolescents themselves. The dietary data collection was conducted after blood sampling to ensure that participants had time to receive detailed guidance on how to accurately complete their dietary records. A clinical nutritionist checked the returned food records together with the children and their parents at the baseline and 2-year examinations and with the adolescents at the 8-year examinations and filled in any missing information. Food consumption (g/d) was calculated from the food records by using the Micro Nutrica® dietary analysis software, version 2.5 (The Social Insurance Institution of Finland), which uses Finnish and international data on the nutrient compositions of foods [27] and was regularly updated by a clinical nutritionist of the PANIC study.

We computed the Finnish Children Healthy Eating Index (FCHEI) [28] to assess overall diet quality. FCHEI consists of five food categories: 1) vegetables, fruit, and berries, 2) higher-fat vegetable oils and vegetable-oil-based margarine (≥60% fat), 3) low-fat (<1%) milk, 4) fish, and 5) foods with high sugar content. The consumption of these foods was divided by energy intake and categorized to deciles. Deciles were scored, a higher decile getting a higher score, apart from sugary products that were inversely scored. The sum of scores from the five

categories was calculated, with a minimum of 0 indicating lowest possible diet quality and a maximum of 50 indicating best possible diet quality.

In addition to the FCHEI food categories, we assessed separately the consumption of high-fat (≥1%) milk, high-fat (≥1%) sour milk products, lower-fat vegetable oils and vegetable-oil-based margarine (<60% fat), red meat, sausages, high-fiber (≥5%) grain products, and low-fiber (<5%) grain products. These foods also reflect diet quality and were chosen for the present study based on the dietary factors used in our previous studies [19] and on existing studies examining the associations of dietary factors, such as the consumption of low-fat or high-fat dairy products [17], meat [29], and dietary fatty acids [30,31], with blood metabolites in children.

Biochemical analyses

Venous blood samples were collected after a 12-hour fast, centrifuged, and stored at a temperature of -75 °C until biochemical analyses. The blood samples were collected at baseline, two years, and eight years. Serum concentrations of total fatty acids (FAs), SFAs, monounsaturated fatty acids (MUFAs), PUFAs, omega-3-FAs, omega-6-FAs, docosahexaenoic acid (DHA), linoleic acid (LA), alanine, glutamine, glycine, histidine, isoleucine, leucine, valine, total BCAAs (including isoleucine, leucine, and valine), aromatic amino acids (AAAs, including phenylalanine and tyrosine), apolipoprotein B (apoB), and apolipoprotein A1 (apoA1) as well as the sizes of VLDL, LDL, and high-density lipoprotein (HDL) particles were measured using high-throughput nuclear magnetic resonance (NMR) spectroscopy metabolomics analysis (Nightingale Health Ltd, Kuopio Finland) [32]. We also assessed the degree of serum FA unsaturation (presence and amount of double bonds in carbon chains of FAs), the percentage of SFAs from total FAs (SFA%), the percentage of MUFAs from total FAs (MUFA%), the percentage of PUFAs from total FAs (PUFA%), the percentage of omega-3-FAs from total FAs (omega-3-FA%), the percentage of omega-6-FAs from total FAs (omega-6-FA%), the ratio of omega-6-FAs to omega-3-FAs (omega-6 FA/omega-3 FA), the percentage of DHA from total FAs (DHA%), the percentage of LA from total FAs (LA%), and the ratio of apoB to apoA1 (apoB/apoA1).

Other assessments

All the following assessments were done at baseline, two years, and eight years. Body weight was measured after a 12-hour fast, emptied bladder, and standing in light underwear using a weight scale integrated into a calibrated InBody® 720 bioelectrical impedance device

(Biospace, Seoul, South Korea) to an accuracy of 0.1 kg. Body weight was measured twice, and the mean of these values was used. Body height was measured standing in the Frankfurt plane without shoes using a wall-mounted stadiometer to an accuracy of 0.1 cm. Height was measured three times, and the mean of the nearest two values was used. Body mass index was calculated by dividing weight (kg) by height (m) squared and body mass index standard deviation score (BMI-SDS) was calculated. Body fat percentage was assessed by a dual energy X-ray absorptiometry method with a Lunar® Dual-energy X-ray absorptiometry device (GE Medical Systems, Madison, WI, USA). A research physician assessed pubertal status according to breast development for girls (scored M 1–5) and according to testicular volume measured by an orchidometer for boys (scored G 1–5) using the staging method described by Tanner [33,34].

Physical activity was objectively measured using a combined heart rate and movement sensor (Actiheart®, CamNtech Ltd., Papworth, UK), attached to the chest with two standard ECG electrodes. Participants were instructed to wear the device continuously for at least four days (2 weekdays, 2 weekend days), including sleep and water-based activities, without altering their usual behavior. Heart rate data were individually calibrated using results from a maximal cycle ergometer exercise test. Moderate-to-vigorous physical activity was defined as activity exceeding an intensity of four metabolic equivalents (METs).

Statistical methods

Basic characteristics of the children at baseline were analyzed using the SPSS Statistics software, version 27.0 (IBM Corporation, IBM SPSS Statistics for Windows, Armonk, NY, USA). The associations of changes in the indicators of diet quality with changes in serum metabolite concentrations over eight years were analyzed by linear mixed-effects models using the R Statistical Software, version 4.1.3 (2022). These models were adjusted for sex, age, body fat percentage, pubertal status and moderate-to-vigorous physical activity. To assess the sensitivity of our findings to the choice of random effects structure, we compared models with random intercepts only to models with random slopes and intercepts. A random subject-specific intercept was used in the models. The skewness of the distributions of the outcome variables was tested. For variables with skewed distributions, including DHA, DHA%, LA%, omega-6 FA/omega-3 FA, total BCAAs, isoleucine, and leucine, logarithmic transformations were made, and the linear mixed-effect analyses were conducted with the logarithmic values. Associations with p-values < 0.05 were considered statistically

significant. A Benjamini–Hochberg false-discovery-rate (FDR) corrected p-value < 0.2 was used to control for possible false positive results caused by multiple comparisons. The original sample size calculations of the PANIC study were based on the primary outcomes of the intervention, specifically fasting insulin and homeostatic model assessment of insulin resistance in children, as described earlier [19,35]. In short, to estimate the required sample size, we aimed to detect a difference of at least 0.30 standard deviations between the intervention group (60% of participants) and the control group (40%), with 80% power and a significance level of 0.05 (two-sided test). Accounting for a potential 20% loss due to dropouts or missing data, we determined that the study would need at least 275 children in the intervention group and 183 in the control group.

Results

Basic characteristics, diet quality, and serum metabolites

At baseline, boys were taller, had lower body fat percentage and higher energy intake, had higher PUFA%, omega-6 FA%, LA%, apoA1, lower total FA, SFA, MUFA, MUFA%, glutamine, apoB and apoB/apoA1 and consumed more red meat and sausages than girls (Tables 1 and 2). At baseline, there were no differences in average diameter of lipoprotein sizes but boys had higher concentrations of medium LDL and HDL particles and lower concentrations of medium and small VLDL particles and large and small LDL particles. At two years, boys had lower body fat percentage, higher energy intake, consumed more high-fat milk and red meat than girls. Boys also had lower alanine, glutamine, and tyrosine levels, smaller LDL particles, and a higher concentration of medium and small HDL particles compared to girls. At eight years, boys were taller, weighed more, and had a lower body fat percentage and higher energy intake. Their diet quality was lower, they consumed less vegetables, fruit, and berries as well as high-fat sour milk products and consumed more low-fat milk, red meat, sausages, and sugary products compared to girls. Boys also had lower concentrations of total FA, SFA, PUFA, omega-3 FA, omega-6 FA, DHA, LA, and omega-6%, DHA%, LA%, and a lower degree of FA unsaturation. Boys had smaller HDL particle size, lower concentrations large and medium HDL particles, and lower serum apoA1 concentrations. Conversely, boys had higher MUFA%, omega-6/omega-3 ratio, glutamine, BCAAs (including isoleucine, leucine and valine separately), phenylalanine, and tyrosine

concentrations. They also had larger VLDL particle size, higher concentrations of large VLDL particles, and higher apoB/apoA1 ratio.

Associations of changes in diet quality with changes in serum fatty acids over eight years

Increased FCHEI was associated with increased omega-3-FAs, degree of FA unsaturation, PUFA%, omega-3-FA%, omega-6-FA% and LA% over eight years after adjustment for sex, age, body fat percentage, pubertal status, and moderate-to-vigorous physical activity (Tables 3 and 4). Increased FCHEI was associated with decreased concentrations of SFAs, MUFAs, SFA%, MUFA%, and omega-6-FA/omega-3-FA. Increased consumption of vegetables, fruit, and berries was associated with increased PUFAs, omega-3-FAs, omega-3-FA%, and degree of FA unsaturation but with decreased SFA% and omega-6 FA/omega-3 FA. Increased consumption of higher-fat vegetable oils and vegetable-oil-based margarine (≥60% fat) was associated with an increased degree of FA unsaturation, PUFA%, omega-6-FA% and decreased SFA%. Increased consumption of lower-fat vegetable-oil-based margarine (<60% fat) was associated with increased MUFA% and decreased omega-6-FA%. Increased consumption of low-fat milk was associated with a higher degree of FA unsaturation. A higher consumption of high-fat (≥1%) milk was associated with increased SFAs, SFA% and decreased PUFA% and omega-6 FA%. Increased consumption of fish was associated with increased omega-3 FA and omega-3-FA% but with decreased omega-6-FA/omega-3-FA. Increased red meat consumption was associated with a higher degree of FA unsaturation. Increased sausage consumption was related to increased SFA% and omega-6-FA/omega-3-FA and decreased PUFA% and omega-3-FA%. Increased consumption of high-fiber grain products was related to increased degree of FA unsaturation, PUFA%, omega-3-FA%, and omega-6-FA% but decreased SFA%, MUFA%, and omega-6-FA/omega-3-FA. Increased consumption of sugary products was associated with increased SFA%, MUFA%, and omega-6-FA/omega-3-FA and decreased omega-3-FA, degree of FA unsaturation, PUFA%, omega-3-FA%, and LA%. The associations of vegetable, fruit, and berry consumption with SFA%, consumption of higher-fat vegetable oils and vegetable-oil-based margarine (≥60% fat) with degree of FA unsaturation, low-fat milk consumption with degree of FA unsaturation, fish consumption with omega-3-FA and omega-3-FA%, red meat consumption with degree of FA unsaturation, sausage consumption with SFA%, PUFA%, omega-3-FA% and omega-6-FA/omega-3-FA and sugary product consumption with omega-6 FA/omega-3 FA did not remain statistically significant after FDR correction.

Associations of changes in diet quality with changes in serum amino acids over eight years

Increased FCHEI was associated with decreased alanine over eight years after adjustment for sex, age, body fat percentage, pubertal status and physical activity (Table 5). Increased consumption of vegetables, fruit, and berries was associated with decreased alanine, glycine and isoleucine. Increased consumption of lower-fat vegetable oils and vegetable-oil-based margarine (<60% fat) was related to increased alanine. Increased consumption of low-fat (<1%) milk was related to increased leucine and phenylalanine but decreased alanine. Increased consumption of high-fat (≥1%) milk was related to increased total BCAAs, isoleucine, leucine, valine, phenylalanine, and tyrosine but decreased glycine. Increased consumption of high-fat (≥1%) sour milk products was associated with higher total BCAAs and valine. Increased red meat consumption was associated with increased histidine but decreased isoleucine. All these longitudinal associations, except the association between lower-fat vegetable oils and vegetable-oil-based margarine (<60% fat) with alanine, low-fat (<1%) milk and alanine, leucine and phenylalanine, high-fat (≥1%) milk with glycine, high-fat (≥1%) sour milk products and total BCAA and valine, and the association between red meat consumption with histidine and isoleucine remained statistically significant after FDR correction.

Associations of changes in diet quality with changes in serum lipoprotein particle sizes and serum apolipoproteins over eight years over eight years

Increased FCHEI was associated with decreased VLDL particle size and lower concentration of large VLDL particles after adjustment for sex, age, body fat percentage, pubertal status and physical activity (Table 6 and 7). Increased consumption of vegetables, fruit and berries was associated with higher concentrations of medium and small VLDL particles and large and small LDL particles. Increased consumptions of lower-fat vegetable oils and vegetable-oil-based margarine (<60% fat) was associated with increased VLDL particle size and higher concentration of large VLDL particles, decreased LDL particle size and lower concentration of large HDL particles. Increased consumption of sugary products was associated with increased VLDL particle size and higher concentration of large and small VLDL particles and a smaller HDL particle size and lower concentration of large HDL particles. The associations of lower-fat vegetable oils and vegetable-oil-based margarine (<60% fat) with VLDL particle

size and lower concentrations of large HDL particles, consumption of sugar products with the concentration of medium VLDL did not remain statistically significant after FDR correction.

No associations were found for diet quality and food consumption with apolipoproteins over eight years (Table 8).

Discussion

We observed that improved diet quality, as indicated by increased consumptions of vegetables, fruit, and berries, higher-fat vegetable oils and vegetable-oil-based margarine (≥60% fat), fish, and high-fiber grain products and a decreased consumption of sugary products were longitudinally associated with increased serum PUFAs and decreased serum MUFAs and SFAs from childhood to adolescence. Increased consumptions of dairy products and sausages were also longitudinally related to increased serum BCAAs and AAAs. Improved overall diet quality was related to decreased serum VLDL particle size and lower concentration of large VLDL particles. Conversely, worsened diet quality, as indicated by increased consumptions of lower-fat vegetable-oils and vegetable-oil-based margarines (<60% fat) and sugary products, was longitudinally associated with increased VLDL size. Additionally, increased consumptions of lower-fat vegetable-oils and vegetable-oil-based margarines (<60% fat) were associated with decreased LDL size.

Diet quality and serum fatty acids

Increased FCHEI was related to increased serum PUFAs and decreased serum SFAs and MUFAs from childhood to adolescence. These results align with the findings of our previous cross-sectional study, in which better diet quality was related to higher serum PUFAs measured with NMR, and our 2-year intervention study, in which the intervention group showed an increased plasma proportion of PUFAs in cholesteryl esters and phospholipids measured with gas chromatography [19,36]. Also supporting our observations, another Finnish intervention study showed that better adherence to a dietary intervention based on nutritional recommendations was associated with higher serum PUFAs and lower serum SFAs from childhood to adulthood [37]. In the present study, increased consumptions of vegetables, fruit, and berries and high-fiber grain products were associated with increased serum PUFAs, such as omega-3-FAs, and decreased serum proportion of SFA to total FA from childhood to adolescence. A higher consumption of vegetables, fruit and berries might be an indicator of better overall diet quality, which then is reflected in a more favorable serum fatty acid profile. It is also possible that the participants who ate more vegetables, fruit, and berries were more

health conscious and thus ate more foods containing lots of PUFAs and had an improved serum fatty acid profile than other participants. Additionally, high-fiber grain products are important sources of PUFAs in our study population [26]. We observed that an increased consumption of direct dietary sources of PUFAs, such as higher-fat vegetable oils and vegetable-oil-based margarine (≥60% fat) and fish, was reflected as an increased serum PUFA%, serum degree of FA unsaturation and decreased serum SFA%. Similar results from cross-sectional studies in children have been described earlier [31,38]. In the present study, an increased sugary product consumption was reflected as decreased serum PUFAs and increased serum SFAs. This might be related to the increased intake of SFAs since sugary products such as ice cream and chocolate are major dietary sources of SFAs in our study population of children [26].

Higher circulating levels of PUFAs have been associated with a lower risk of many cardiometabolic disturbances, such as insulin resistance and elevated blood pressure, and are thought to be beneficial for cardiometabolic health due to their anti-inflammatory properties in adults [10,39,40]. Moreover, blood omega-3-FAs have been inversely associated with cardiometabolic risk factors, such as elevated blood pressure, since childhood [41]. Thus, our results suggest that improved diet quality, characterized by an increased consumption of unsaturated fat products and fiber as well as a reduced consumption of sugary products, has beneficial effects on FA metabolism from childhood to adolescence.

Diet quality and serum amino acids

Increased FCHEI was associated with decreased serum alanine from childhood to adolescence, the results being consistent with our previous cross-sectional findings [19]. Interestingly, in the present longitudinal study, some indicators of impaired diet quality were associated with increased serum BCAAs, associations which were not seen in our earlier cross-sectional study. Increased consumptions of low-fat and high-fat milk were reflected in increased serum BCAAs and AAAs, whereas an increased consumption of vegetables, fruit, and berries was associated with decreased serum BCAAs and AAAs. Our longitudinal findings are similar with those of a Danish intervention study in children [29], in which an increased protein intake as meat or dairy was reflected in increased serum BCAAs in the intervention and control groups. In another intervention study among infants, complementary feeding of a protein rich diet with meat or dairy products increased serum BCAA levels [42]. Moreover, in a Finnish dietary intervention study aiming towards dietary choices based on

nutritional recommendations, serum BCAA levels were lower among boys in the intervention group than among boys in the control group, although the difference was statistically nonsignificant [23]. These observations together suggest that diet quality, particularly diets high in protein, predominantly derived from dairy and meat sources, and a lower consumption of vegetables, fruit, and berries affect amino acid metabolism and may lead to higher blood concentrations of BCAAs and AAAs. Our results also indicated that these associations are independent of physical activity. These findings may be clinically important as higher circulating BCAAs have been associated with a higher risk of insulin resistance in children, adolescents, and adults [9,11,43]. Higher circulating levels of some AAAs, such as phenylalanine, have also been linked to adverse cardiovascular outcomes in adults [9,44] and insulin resistance in children [11].

Diet quality and serum lipoprotein particle sizes

Improved overall diet quality was associated with decreased serum VLDL particle size from childhood to adolescence, whereas impaired diet quality and increased consumptions of lower-fat vegetable oils and vegetable-oil-based margarine (<60% fat) and sugary products were associated with increased serum VLDL particle size and higher concentration of large VLDL particles. These longitudinal findings are consistent with the results of our previous cross-sectional study [19]. However, in dietary intervention studies, no such effect of diet affecting circulating VLDL particle size in children has been observed [37,45]. Nevertheless, in adults, better diet quality has been associated with smaller circulating VLDL particle size [46], thus supporting our present results. A plausible mechanism for the observed associations of an increased consumption of high-fat and high-sugar foods with increased VLDL particle size could be increased liver adiposity due to the increased dietary intake of SFAs and fructose [19]. Liver adiposity has been linked to the secretion of larger VLDL particles [47], which in turn are associated with the formation of smaller, more dense LDL particles that are atherogenic due to their fibrinolytic, oxidative, and inflammatory properties [48]. In fact, smaller LDL particle size has been associated with adverse cardiovascular health outcomes, such as coronary artery disease and myocardial infarction, in adults [49,50]. Therefore, larger VLDL particles might contribute to the development of cardiovascular diseases which underlines the biological significance of diet quality and the possible public health relevance of our findings.

In the present study, increased consumption of sugary products was associated with decreased serum HDL particle size and a lower concentration of large HDL particles from childhood to adolescence. Some studies have observed that smaller circulating HDL particles are associated with a higher risk of cardiovascular diseases, such as coronary heart disease, in adults [51]. This is possibly due to the higher susceptibility of smaller HDL particles to degradation compared to larger HDL particles [52,53].We also observed that an increased consumption of lower-fat vegetable oils and vegetable-oil-based margarine (<60% fat) was associated with decreased serum LDL particle size, which is unfavorable for cardiovascular health due to the atherogenic nature of small LDL particles [48–50]. In adults, better diet quality has been associated with a lower quantity of circulating small LDL and HDL particles [46,54], while studies examining the associations of dietary factors with lipoprotein particle sizes in children are scarce. Of single dietary factors, a higher PUFA intake has been associated with larger circulating HDL and LDL particle sizes in adults [55,56]. Thus, the present findings concerning the associations of increased consumptions of sugary products and lower-fat vegetable oils with decreased serum HDL and LDL sizes might be due to a higher intake of SFAs and a lower intake of PUFAs. This is because sugary products are often sources of SFAs, and lower-fat vegetable-oil-based fats are lower in PUFA content [26]. Considering the previously observed associations of lipoprotein particle sizes with cardiometabolic health [48–53], our present results underline the importance of good diet quality in improving lipoprotein metabolism and preventing cardiometabolic diseases since childhood.

We also observed a direct association of vegetable, fruit, and berry consumption with the concentration of small LDL particles. It is possible that fructose intake from this food group is affects serum lipoprotein profile as fructose intake has been observed to influence the concentration of small LDL particles in children [57,58] However, due to the complexity of human metabolism, other possible factors could explain the observed findings.

Diet quality and serum apolipoproteins

We did not find any associations of diet quality with serum apolipoproteins from childhood to adolescence. We previously observed a cross-sectional association between a higher consumption of vegetables, fruit, and berries with higher serum apoB/apoaA1 [19], but no such association was found in the present longitudinal study.

Study strengths and limitations

We examined the longitudinal associations of diet quality with serum metabolites in a relatively large population sample of children followed for eight years until adolescence using data on all these variables from three time points. Another strength of our study is that most participants of this population-based sample of children followed up until adolescence have not been exposed to possible confounding factors, such as alcohol consumption, smoking, chronic diseases, and medications. Additionally, we were able to control for sex, age, body fat percentage, pubertal status and physical activity, which were assessed at all three time points. Food consumption was assessed comprehensively by 4-day dietary records reviewed by clinical nutritionists, thus improving the accuracy and reliability of the dietary data from the records. Overall diet quality was assessed using FCHEI, which has been validated in Finnish children [28]. To assess serum biomarkers, we used high-throughput NMR spectroscopy analysis which is a robust technique with multiple advantages in metabolomic research [59]. We also acknowledge the limitations of this study. First, the assessment of diet is always prone to misreporting [60]. Diet was reported by the parents or caregivers of the children at the baseline and 2-year examinations but by the adolescents themselves at the 8-year examinations, which might result in differences in reporting. For example, adolescents' perceptions of their body image can lead to the misreporting of their diet [61]. Additionally, FCHEI has been validated in Finnish children but not adolescents. Due to the complexity of eating behavior and human metabolism, it was not possible to consider all possible confounding factors in the statistical analyses. Another limitation of this study is the loss of 50% of the original sample over the eight years. While those who participated in the 8-year examinations did not differ from non-participants in terms of baseline age, BMI-SDS, sex distribution, or study group allocation, this level of attrition still reduces statistical power and may limit the overall generalizability of the findings. However, linear mixed-effects models used in our study for the analyses are especially suitable for analyzing longitudinal datasets containing unbalanced data as the mixed-effect models assume that the data are missing at random. Finally, we studied Finnish children and adolescents, and the results are therefore not generalizable to all population groups. Thus, the interpretation of the present results should be done carefully.

Conclusions

We observed longitudinal associations of overall diet quality and single indicators of diet quality with serum metabolites in a general population of children followed up until adolescence. Better diet quality was associated with a serum metabolite profile characterized by higher PUFAs, lower SFAs, BCAAs, and AAAs as well as a smaller VLDL particle size, independent of body fat percentage and pubertal status. The findings of this study are particularly relevant given the existing dietary challenges within Finnish children and adolescents [20–22], among whom a low consumption of vegetables, fruit, and berries and a higher consumption of dietary SFA and sugary products than recommended remains a concern. Thus, addressing these issues is fundamental for improving metabolic health in children and adolescents and building a base for precision nutrition [62]. These results suggest that healthy dietary choices since childhood modify the serum metabolite profile towards a more favorable direction for cardiometabolic health moving on to adolescence.

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Conflict of Interest

The authors have no competing interests to declare that are relevant to the content of this article.

Authorship

TAL, US, and AME designed the study and received funding for it. US, SS, TS, AME, and TAL collected the data. SL, AME, EAH, and TAL formulated the research question. SH and SL conducted statistical analyses. SL, SH, EAH, US, SS, TS, AME, and TAL interpreted the findings. SL drafted the manuscript. SH, EAH, US, SS, TS, AME, and TAL critically reviewed the manuscript and approved its final version.

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Table 1. Basic characteristics and dietary factors of the study participants at baseline, at the 2-year examinations and at the 8-year examinations.

	Baseline			2 years			8 years		
	Girls (194)	Boys (209)	p-value	Girls (171)	Boys (189)	p-value	Girls (108)	Boys (111)	p-value
	Mean (SD) / *Median (IQR)	Mean (SD)/ *Median (IQR)		Mean (SD)/ *Median (IQR)	Mean (SD) / *Median (IQR)		Mean (SD) / *Median (IQR)	Mean (SD) / *Median (IQR)	
Age (years)	7.62 (0.39)	7.64 (0.38)	0.67	9.7 (0.44)	9.8 (0.44)	0.42	15.8 (0.38)	15.9 (0.45)	0.14
Height (cm)	128 (5.7)	130 (5.3)	**0.007**	140 (6.6)	141 (6.3)	0.09	166 (5.9)	176 (7.4)	< **0.001**
Weight (cm)	26.7 (5.2)	27.0 (4.5)	0.50	33.8 (7.4)	35.1 (7.6)	0.10	58.0 (8.9)	65.9 (15.8)	< **0.001**
BMI (kg/m^2)	16.2 (2.2)	16.0 (1.9)	0.49	17.1 (2.6)	17.5 (2.8)	0.19	21.1 (2.8)	21.1 (4.2)	0.99
BMI SDS	-0.2 (1.1)	-0.2 (1.1)	0.47	-0.13 (1.0)	-0.09 (1.1)	0.74	0.08 (0.85)	-0.11 (1.1)	0.16
Body fat percentage[†]	22.5 (7.7)	16.9 (7.5)	< **0.001**	25.4 (8.5)	21.8 (9.9)	<**0.001**	28.9 (6.8)	17.1 (9.2)	< **0.001**
Energy intake (kcal/d)	1550 (284)	1724 (306)	< **0.001**	1577 (296)	1806 (352)	<**0.001**	1617 (436)	2054 (558)	< **0.001**
Finnish Children Healthy Eating Index score	23.6 (6.5)	22.9 (7.2)	0.27	24.5 (7.3)	23.8 (7.5)	0.38	24.0 (7.0)	22.1 (7.1)	**0.04**
Vegetables, fruit, and berries (g/d)	216 (110)	206 (118)	0.10	205 (111)	210 (119)	0.73	270 (154)	192 (132)	<**0.001**

Higher-fat vegetable oils and vegetable-oil-based margarine (≥60% fat)	10.7 (8.6)	11.6 (9.6)	0.64	*13.7 (17.1)	*14.7 (23.6)	0.32	*11.8 (13.1)	*13.2 (18.4)	0.5
Lower-fat vegetable oils and vegetable-oil-based margarine (<60% fat)	*0 (5.0)	*0 (5.9)	0.18	*0 (0)	*0 (0)	0.68	*0 (0)	*0 (0)	0.34
Low-fat (<1%) milk (g/d)	*350 (492)	*426 (544)	0.08	*400 (469)	*449 (446)	0.2	*200 (425)	*375 (529)	**<0.001**
High-fat (≥1%) milk (g/d)	*92.4 (227)	*99.6 (250)	0.55	*44.5 (123)	*74.1 (161)	**0.03**	*19.7 (83.5)	*30.9 (103)	0.06
High-fat (≥1%) sour milk products (g/d)	*70.4 (88.4)	*62.5 (112.5)	0.43	*50 (100)	*50.0 (125.0)	0.50	*10.6 (75.0)	0 (100)	**0.02**
Fish (g/d)	4.9 (22.0)	*8.23 (28.1)	0.13	*11 (28)	*7.5 (28.8)	0.82	*5.0 (28.5)	*15.1 (38.4)	0.17
Red meat (g/d)	51.3 (26.3)	60.8 (33.3)	**0.007**	53.4 (31.9)	68.6 (40.3)	**< 0.001**	*39.8 (49.1)	*81.9 (80.8)	**<0.001**
Sausages (g/d)	*12.1 (26.9)	*18.0 (26.2)	**0.001**	*11.8 (30.8)	*16.3 (33.1)	0.22	*1.63 (17.4)	*20.0 (55.0)	**<0.001**

High-fiber (≥5%) grain products (g/d)	60.9 (36.4)	65.6 (42.0)	0.34	70.6 (43.6)	79.4 (46.9)	0.07	*71.4 (69.9)	*78.1 (95.5)	0.23
Low-fiber (<5%) grain products (g/d)	108 (45.4)	119 (59.7)	0.10	99.6 (53.0)	114 (77.7)	0.05	112 (76.9)	124 (63.8)	0.22
Sugary products (g/d)	176 (129)	203 (144)	0.08	*146 (174)	*189 (192)	0.16	*104 (158)	*158 (275)	**0.003**

*For variables with skewed distributions, medians and interquartile ranges are presented.

†For body fat percentage at two years, data from 346 children and at eight years, data from 217 adolescents were available.

Table 2. Serum metabolites of the study participants at baseline, at the 2-year examinations and at the 8-year examinations.

	Baseline			2 years			8 years		
	Girls (194)	Boys (209)	p-value	Girls (171)	Boys (189)	p-value	Girls (108)	Boys (111)	p-value
	Mean (SD)	Mean (SD)		Mean (SD)	Mean (SD)		Mean (SD)	Mean (SD)	
Fatty acids									
Total FA (mmol/l)	11.3 (1.14)	11.1 (1.08)	**0.04**	10.2 (1.30)	10.3 (1.36)	0.47	10.0 (1.54)	9.44 (1.36)	**0.003**
SFA (mmol/l)	3.79 (0.41)	3.71 (0.41)	**0.04**	3.37 (0.47)	3.41 (0.50)	0.40	3.31 (0.53)	3.12 (0.49)	**0.006**
MUFA (mmol/l)	2.67 (0.38)	2.57 (0.37)	**0.007**	2.29 (0.39)	2.31 (0.43)	0.73	2.32 (0.46)	2.23 (0.49)	0.21
PUFA (mmol/l)	4.84 (0.47)	4.80 (0.43)	0.34	4.55 (0.51)	4.59 (0.52)	0.42	4.40 (0.62)	4.08 (0.46)	< **0.001**
Omega-3 FA (mmol/l)	0.43 (0.11)	0.423 (0.11)	0.79	0.38 (0.11)	0.38 (0.11)	0.90	0.35 (0.13)	0.30 (0.09)	< **0.001**
Omega-6 FA (mmol/l)	4.41 (0.39)	4.37 (0.36)	0.28	4.17 (0.44)	4.22 (0.45)	0.37	4.04 (0.52)	3.78 (0.40)	< **0.001**
DHA (mmol/l)	0.37 (0.19)	0.35 (0.15)	0.18	0.23 (0.04)	0.23 (0.04)	0.70	0.22 (0.05)	0.19 (0.03)	< **0.001**
LA (mmol/l)*	3.50 (0.52)	3.50 (0.45)	0.96	3.54 (0.44)	3.56 (0.45)	0.64	3.36 (0.50)	3.10 (0.41)	< **0.001**
Degree of unsaturation	1.25 (0.08)	1.25 (0.08)	0.49	1.32 (0.05)	1.33 (0.05)	0.87	1.31 (0.05)	1.28 (0.05)	< **0.001**
SFA/total FA (%)	33.6 (1.10)	33.5 (1.15)	0.35	33.0 (1.14)	33.04 (1.04)	0.42	33.1 (1.20)	33.1 (1.38)	0.92
MUFA/total FA (%)	23.6 (1.52)	23.1 (1.52)	**0.008**	22.4 (1.46)	22.3 (1.76)	0.56	22.99 (1.52)	23.48 (1.96)	**0.04**

PUFA/total FA (%)	42.9 (2.13)	43.4 (2.25)	**0.02**	44.67 (1.78)	44.68 (2.10)	0.97	43.9 (1.91)	43.4 (2.46)	0.09
Omega-3 FA/total FA (%)	3.75 (0.79)	3.81 (0.83)	0.45	3.64 (0.86)	3.63 (0.86)	0.97	3.46 (0.83)	3.11 (0.73)	0.60
Omega-6 FA/total FA (%)	39.1 (1.87)	39.6 (1.96)	**0.02**	41.02 (1.78)	41.05 (2.11)	0.88	40.48 (1.87)	40.33 (2.42)	**0.003**
Omega-6 FA/ Omega-3 FA				11.9 (3.07)	12.03 (3.28)	0.70	12.38 (3.15)	13.66 (3.27)	**0.003**
DHA/total FA (%)	1.63 (0.12)	1.37 (0.09)	0.35	2.23 (0.33)	2.23 (0.33)	0.86	2.15 (0.32)	1.99 (0.33)	< **0.001**
LA/total FA (%)	30.9 (3.43)	31.6 (2.52)	**0.03**	34.70 (1.90)	34.59 (2.04)	0.60	33.58 (2.03)	33.0 (2.26)	**0.04**
Amino acids									
Alanine (µmol/l)	293 (53.2)	285 (58.7)	0.15	305 (57.8)	292 (60.6)	**0.04**	364 (69.3)	357 (67.0)	0.45
Glutamine (µmol/l)	686 (63.8)	672 (58.2)	**0.02**	707 (52.9)	683 (57.2)	< **0.001**	694 (74.2)	728 (62.7)	< **0.001**
Glycine (µmol/l)	267 (40.2)	270 (49.0)	0.54	279 (51.5)	277 (58.6)	0.77	288 (59.4)	282 (44.9)	0.40
Histidine (µmol/l))	92.2 (7.89)	91.2 (7.48)	0.20	91.3 (7.39)	90.7 (8.05)	0.41	95.2 (9.74)	96.0 (9.51)	0.55
Total BCAA (µmol/l)	391 (54.8)	385 (55.6)	0.27	387 (54.0)	383 (50.8)	0.42	404 (57.4)	477 (96.7)	<**0.001**
Isoleucine (µmol/l)	52.1 (11.1)	50.7 (9.74)	0.17	50.0 (8.86)	49.0 (8.98)	0.33	55.5 (10.2)	70.0 (20.0)	<**0.001**
Leucine (µmol/l)	111 (17.0)	110 (17.4)	0.43	112 (16.7)	112 (16.1)	0.87	119 (17.5)	146 (36.5)	<**0.001**

Valine (µmol/l)	227 (30.2)	224 (31.0)	0.27	225 (31.0)	222 (29.3)	0.30	229 (33.1)	263 (43.4)	**<0.001**
Phenylalanine (µmol/l)	65.2 (6.85)	64.2 (7.05)	0.34	63.0 (7.64)	62.0 (6.77)	0.21	68.7 (9.1)	71.8 (10.2)	**0.02**
Tyrosine (µmol/l)	63.4 (9.33)	61.7 (10.2)	0.09	63.6 (9.48)	60.9 (11.6)	**0.007**	65.6 (10.1)	67.6 (15.2)	0.25
Average diameter of lipoprotein particles									
VLDL (nm)	37.5 (0.89)	37.4 (0.90)	0.18	37.5 (0.82)	37.6 (0.99)	0.26	37.8 (0.84)	38.3 (1.1)	**< 0.001**
LDL (nm)	23.7 (0.06)	23.7 (0.06)	0.57	23.9 (0.07)	23.9 (0.07)	**0.04**	23.9 (0.07)	23.9 (0.06)	0.23
HDL (nm)	9.73 (0.15)	9.75 (0.13)	0.22	9.71 (0.16)	9.70 (0.16)	0.66	9.74 (0.15)	9.60 (0.15)	**< 0.001**
Lipoprotein subclasses[†]									
Large VLDL	6.55×10^{-6} (4.22×10^{-6})	5.93×10^{-6} (3.70×10^{-6})	0.11	4.91×10^{-6} (2.72×10^{-6})	5.03×10^{-6} (3.23×10^{-6})	0.71	5.80×10^{-6} (2.63×10^{-6})	6.82×10^{-6} (3.56×10^{-6})	**0.02**
Medium VLDL	3.40×10^{-5} (9.80×10^{-6})	3.12×10^{-5} (8.85×10^{-6})	**<0.001**	2.32×10^{-5} (7.59×10^{-6})	2.31×10^{-5} (7.71×10^{-6})	0.86	2.31×10^{-5} (7.88×10^{-6})	24.3×10^{-5} (7.60×10^{-6})	0.27
Small VLDL	3.60×10^{-5} (1.07×10^{-5})	3.32×10^{-5} (9.49×10^{-6})	**0.006**	2.68×10^{-5} (8.18×10^{-6})	2.62×10^{-5} (8.64×10^{-6})	0.55	2.82×10^{-5} (8.44×10^{-6})	2.89×10^{-5} (7.78×10^{-6})	0.50
Large LDL	6.43×10^{-4} (1.32×10^{-4})	6.07×10^{-4} (1.16×10^{-4})	**0.003**	6.45×10^{-4} (1.25×10^{-4})	6.34×10^{-4} (1.20×10^{-4})	0.40	6.0×10^{-4} (1.31×10^{-4})	6.07×10^{-4} (1.13×10^{-4})	0.85
Medium LDL	6.06×10^{-3} (9.47×10^{-4})	6.31×10^{-3} (9.12×10^{-4})	**0.003**	2.41×10^{-4} (4.86×10^{-5})	2.43×10^{-4} (4.77×10^{-5})	0.72	2.25×10^{-4} (4.97×10^{-5})	2.34×10^{-4} (5.17×10^{-5})	0.16

Small LDL	1.93 x 10^{-4} (3.04 x 10^{-5})	1.83 x 10^{-4} (2.66 x 10^{-5})	< **0.001**	1.48 x 10^{-4} (2.43 x 10^{-5})	1.47 x 10^{-4} (2.24 x 10^{-5})	0.79	1.37 x 10^{-4} (2.43 x 10^{-5})	1.39 x 10^{-4} (2.28 x 10^{-5})	0.57
Large HDL	2.66 x 10^{-3} (9.07 x 10^{-4})	2.76 x 10^{-3} (7.81 x 10^{-4})	0.23	1.74 x 10^{-3} (6.02 x 10^{-4})	1.77 x 10^{-3} (6.68 x 10^{-4})	0.56	1.79 x 10^{-3} (5.58 x 10^{-4})	1.24 x 10^{-3} (4.60 x 10^{-4})	< **0.001**
Medium HDL	6.06 x 10^{-3} (9.47 x 10^{-4})	6.31 x 10^{-3} (9.12 x 10^{-4})	**0.007**	4.16 x 10^{-3} (6.22 x 10^{-4})	4.37 x 10^{-3} (7.36 x 10^{-4})	**0.004**	4.14 x 10^{-3} (6.61 x 10^{-4})	3.54 x 10^{-3} (5.25 x 10^{-4})	< **0.001**
Small HDL	1.46 x 10^{-2} (1.29 x 10^{-3})	1.48 x 10^{-2} (1.37 x 10^{-3})	0.18	9.98 x 10^{-3} (9.12 x 10^{-4})	10.4 x 10^{-2} (9.61 x 10^{-4})	< **0.001**	9.65 x 10^{-3} (9.77 x 10^{-4})	9.49 x 10^{-3} (8.38 x 10^{-4})	0.20
Apolipoproteins									
Apolipoprotein B (g/l)	0.81 (0.16)	0.76 (0.14)	**0.002**	0.71 (0.13)	0.70 (0.13)	0.55	0.67 (0.14)	0.67 (0.12)	0.98
Apolipoprotein A1 (g/l)	1.45 (0.19)	1.50 (0.18)	**0.02**	1.53 (0.17)	1.58 (0.20)	< **0.01**	1.52 (0.18)	1.36 (0.14)	< **0.001**
Apolipoprotein B/Apolipoprotein	0.56 (0.13)	0.51 (0.10)	< **0.001**	0.47 (0.10)	0.45 (0.10)	0.08	0.45 (0.10)	0.50 (0.11)	<**0.001**

BCAAs, branched-chain amino acids; DHA, docosahexaenoic acid; FAs, fatty acids; HDL, high-density lipoprotein; LA, linoleic acid; LDL, low-density lipoprotein; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids; SFAs, saturated fatty acids; VLDL, very low-density lipoprotein.

*For linoleic acid data from 382 children were available at baseline.

†For lipoprotein subclasses, data from 401 children were available at baseline.

Table 3. Longitudinal associations of diet quality with serum fatty acids over eight years*

	Total FAs (mmol/l)	SFAs (mmol/l)	MUFAs (mmol/l)	PUFAs (mmol/l)	Omega-3 FAs (mmol/l)	Omega-6 FAs (mmol/l)	DHA (mmol/l)	LA (mmol/l)	Degree of FA unsaturation
Finnish Children Healthy Eating Index	-0.007 (0.27)	-0.005 **(0.02)**†	-0.0042 **(0.04)**†	0.0017 (0.48)	0.0018 **(<0.001)**†	-0.0001 (0.94)	0.0010 (0.45)	0.0033 (0.26)	0.0011 **(0.002)**†
Vegetables, fruit, and berries (g/d)	0.0006 (0.13)	0.0001 (0.45)	0.0001 (0.29)	0.0003 **(0.02)**†	0.0001 **(<0.001)**†	0.0002 (0.09)	0.0001 (0.15)	0.0003 (0.06)	6.17×10^{-5} **(0.002)**†
Higher-fat vegetable oils and vegetable-oil-based margarine (≥60% fat)	-0.003 (0.40)	-0.0022 (0.09)	-0.0014 (0.21)	0.0004 (0.77)	-3.34×10^{-5} (0.92)	0.0005 (0.70)	-0.0001 (0.89)	5.08×10^{-5} (0.98)	**0.0004 (0.03)**
Lower-fat vegetable oils and vegetable-oil-based margarine (<60% fat)	0.007 (0.25)	0.0026 (0.27)	0.004 (0.002)	0.0005 (0.84)	0.0007 (0.22)	-0.0002 (0.94)	-0.0004 (0.74)	0.0004 (0.89)	-0.0004 (0.17)
Low-fat (<1%) milk (g/d)	-2.48×10^{-5} (0.87)	-2.73×10^{-5} (0.63)	-1.53×10^{-5} (0.76)	1.04×10^{-5} (0.86)	1.24×10^{-5} (0.36)	-2.53×10^{-6} (0.96)	-1.88×10^{-5} (0.56)	1.40×10^{-6} (0.84)	1.63×10^{-5} **(0.05)**
High-fat (≥1%) milk (g/d)	0.0002 (0.29)	0.0002 **(0.03)**†	5.28×10^{-5} (0.45)	6.3×10^{-6} (0.94)	-5.26×10^{-6} (0.79)	1.11×10^{-5} (0.88)	-1.75×10^{-5} (0.70)	-9.78×10^{-5} (0.33)	-2.02×10^{-5} (0.08)
High-fat (≥1%) sour milk	-0.0004	-9.84×10^{-5}	$-9.29 \times 10^{-}$	-0.0002	$-3.83 \times 10^{-}$	-0.0002	6.47×10^{-5}	-0.0003	$-2.40 \times 10^{-}$

products (g/d)	(0.47)	(0.64)	^{5} (0.62)	(0.33)	^{5} (0.46)	(0.34)	(0.60)	(0.21)	^{5} (0.45)
Fish (g/d)	-0.0007 (0.67)	-0.0005 (0.16)	-0.0001 (0.82)	-0.0001 (0.85)	0.0003 **(0.04)**	-0.0005 (0.45)	0.0003 (0.53)	0.0001 (0.89)	8.10×10^{-5} (0.43)
Red meat (g/d)	0.0004 (0.72)	3.90×10^{-5} (0.93)	-0.0002 (0.65)	0.0005 (0.27)	0.0002 (0.08)	0.0003 (0.41)	0.0003 (0.23)	0.0002 (0.79)	0.0001 **(0.03)**
Sausages (g/d)	0.0013 (0.41)	0.0008 (0.18)	0.0007 (0.21)	-8.24×10^{-5} (0.89)	-0.0002 (0.15)	0.0001 (0.80)	-0.0002 (0.54)	-0.0006 (0.43)	-3.33×10^{-5} (0.70)
High-fiber (≥5%) grain products (g/d)	-0.0012 (0.18)	-0.0006 (0.05)	-0.0006 (0.06)	-6.13×10^{-5} (0.86)	0.0001 (0.10)	-0.0002 (0.53)	4.69×10^{-5} (0.81)	0.0004 (0.85)	0.0001 **(0.01)**†
Low-fiber (<5%) grain products (g/d)	0.0002 (0.80)	0.0001 (0.70)	4.5×10^{-5} (0.85)	6.42×10^{-6} (0.98)	-8.13×10^{-5} (0.20)	8.53×10^{-5} (0.72)	-0.0001 (0.35)	-8.79×10^{-5} (0.80)	-5.39×10^{-5} (0.17)
Sugary products (g/d)	0.0002 (0.46)	0.0002 (0.16)	0.0002 (0.09)	-9.37×10^{-5} (0.42)	-7.79×10^{-5} **(0.004)**†	-1.31×10^{-5} (0.90)	6.22×10^{-6} (0.92)	-0.0003 (0.07)	-3.00×10^{-5} **(0.07)**†

FAs, fatty acids; SFAs, saturated fatty acids; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids; DHA, docosahexaenoic acid; LA, linoleic acid.

*Data are unstandardized regression coefficients from linear mixed-effects models adjusted for sex, age, body fat percentage, pubertal status and physical activity. P-values are reported in parentheses. Statistically significant associations are indicated by bolded P-values.

†Association remained statistically significant after Benjamini-Hochberg false-discovery-rate correction for multiple testing.

Table 4. Longitudinal associations of diet quality with serum fatty acid ratios over eight years*

	SFA/total FA (%)	MUFA/total FA (%)	PUFA/total FA (%)	Omega-3 FA/ total FA (%)	Omega-6 FA/ total FA (%)	Omega-6 FA/ Omega-3 FA	DHA/total FA (%)	LA/total FA (%)
Finnish Children Healthy Eating Index	-0.027 **(<0.001)**†	-0.022 **(0.006)**†	0.050 **(<0.001)**†	0.020 **(<0.001)**†	0.030 **(0.003)**†	-0.0053 **(<0.001)**†	0.0020 (0.1)	0.0035 **(0.009)**†
Vegetables, fruit, and berries (g/d)	-0.001 **(0.02)**	-2.69×10^{-5} (0.96)	0.0009 (0.18)	0.0010 **(<0.001)**†	-0.0002 (0.78)	-0.0003 **(<0.001)**†	6.16×10^{-5} (0.41)	7.43×10^{-5} (0.36)
Higher-fat vegetable oils and vegetable-oil-based margarine (≥60% fat)	-0.013 **(<0.001)**†	-0.0072 (0.13)	0.020 **(0.002)**†	0.0007 (0.76)	0.020 **(0.001)**†	-0.0001 (0.88)	0.0002 (0.74)	0.0011 (0.16)
Lower-fat vegetable oils and vegetable-oil-based margarine (<60% fat)	-0.001 (0.84)	0.022 **(0.007)**†	-0.022 (0.06)	0.0040 (0.36)	-0.026 **(0.01)**†	-0.0009 (0.51)	-0.0012 (0.35)	-0.0006 (0.68)
Low-fat (<1%) milk (g/d)	-0.0002 (0.24)	-0.0001 (0.48)	0.0003 (0.23)	0.0001 (0.27)	0.0002 (0.44)	-7.87×10^{-6} (0.81)	3.24×10^{-5} (0.28)	3.18×10^{-5} (0.32)
High-fat (≥1%) milk (g/d)	0.0009 **(<0.001)**†	2.53×10^{-6} (0.99)	-0.0009 **(0.02)**†	-0.0001 (0.42)	-0.0008 **(0.03)**†	-1.54×10^{-5} (0.74)	-3.81×10^{-5} (0.37)	-3.49×10^{-5} (0.44)
High-fat (≥1%) sour milk products (g/d)	0.0005 (0.36)	-0.0002 (0.77)	-0.0003 (0.81)	-0.0002 (0.59)	-7.06×10^{-5} (0.94)	6.15×10^{-5} (0.62)	0.0001 (0.36)	4.89×10^{-6} (0.97)
Fish (g/d)	-0.0029	0.0003	0.0027	0.0039	-0.0014	-0.0011	0.0003	3.20×10^{-5}

	(0.10)	(0.92)	(0.42)	**(0.003)**	(0.65)	**(0.004)**†	(0.38)	(0.94)
Red meat (g/d)	-0.001	-0.0025	0.0035	0.0013	0.0023	-0.0003	0.0003	-0.0001
	(0.36)	(0.11)	(0.09)	(0.10)	(0.24)	(0.28)	(0.24)	(0.68)
Sausages (g/d)	0.003	0.0037	-0.0067	-0.0022	-0.046	0.0007	-0.0004	-0.0006
	(0.04)	(0.08)	**(0.02)**	**(0.04)**	(0.08)	**(0.04)**	(0.19)	(0.08)
High-fiber (≥5%) grain products	-0.002	-0.0030	0.0054	0.0018	0.0034	-0.0005	0.0002	0.0003
(g/d)	**(0.006)**†	**(0.01)**†	**(<0.001)**†	**(0.004)**†	**(0.02)**†	**(0.02)**†	(0.21)	(0.16)
Low-fiber (<5%) grain products	0.0004	0.0002	-0.0006	-0.0008	0.0002	0.0002	-0.0002	-8.4 x 10-^{5}
(g/d)	(0.51)	(0.87)	(0.65)	(0.13)	(0.86)	(0.21)	(0.29)	(0.60)
Sugary products (g/d)	0.0008	0.0010	-0.0017	-0.0008	-0.0010	0.0002	-3.43 x 10^{-5}	-0.0002
	(0.007)†	**(0.01)**†	**(0.001)**†	**(<0.001)**†	(0.05)	**(0.002)**	(0.57)	**(<0.001)**†

SFAs, saturated fatty acids; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids; DHA, docosahexaenoic acid; FAs, fatty acids; LA, linoleic acid

*Data are unstandardized regression coefficients from linear mixed-effects models adjusted for sex, age, body fat percentage, pubertal status and physical activity. P-values are reported in parentheses. Statistically significant associations are indicated by bolded P-values.

†Associations remained statistically significant after Benjamini-Hochberg false-discovery-rate correction for multiple testing.

Table 5. Longitudinal associations of diet quality with serum amino acids over eight years*

	Alanine (µmol/l)	Glutamine (µmol/l)	Glycine (µmol/l)	Histidine (µmol/l)	Total BCAAs (µmol/l)	Isoleucine (µmol/l)	Leucine (µmol/l)	Valine (µmol/l)	Phenylalanine (µmol/l)	Tyrosine (µmol/l)
Finnish Children Healthy Eating Index	-0.0008 **(0.008)**†	-0.0002 (0.55)	-0.0004 (0.09)	-6.80×10^{-5} (0.10)	-0.0001 (0.89)	-0.0009 (0.35)	0.0002 (0.85)	-6.01×10^{-5} (0.72)	8.36×10^{-6} (0.83)	2.36×10^{-5} (0.64)
Vegetables, fruit, and berries (g/d)	-3.96×10^{-5} **(0.03)**†	-2.74×10^{-5} (0.13)	-2.83×10^{-5} **(0.04)**†	-1.49×10^{-6} (0.54)	-6.11×10^{-5} (0.17)	-0.0001 **(0.03)**†	-4.32×10^{-5} (0.37)	-1.29×10^{-5} (0.20)	-5.78×10^{-7} (0.80)	-1.46×10^{-6} (0.63)
Higher-fat vegetable oils and vegetable-oil-based margarine (≥60% fat)	-0.0001 (0.55)	0.0001 (0.51)	0.0001 (0.33)	-5.09×10^{-6} (0.83)	-0.0002 (0.62)	-0.0008 (0.20)	3.55×10^{-5} (0.94)	-7.58×10^{-5} (0.44)	-1.64×10^{-5} (0.47)	7.81×10^{-6} (0.79)
Lower-fat vegetable oils and vegetable-oil-based margarine (<60% fat)	0.0007 **(0.03)**	-0.0001 (0.71)	0.0001 (0.60)	4.28×10^{-5} (0.31)	-0.0002 (0.83)	-0.0001 (0.91)	-0.0004 (0.60)	-1.52×10^{-6} (0.99)	3.91×10^{-5} (0.32)	1.39×10^{-5} (0.79)
Low-fat (<1%) milk (g/d)	-1.51×10^{-5} **(0.04)**	-2.76×10^{-6} (0.71)	-6.79×10^{-6} (0.24)	7.64×10^{-7} (0.44)	2.62×10^{-5} (0.14)	-5.52×10^{-6} (0.82)	3.78×10^{-5} **(0.05)**	4.92×10^{-6} (0.22)	2.31×10^{-6} **(0.01)**	1.78×10^{-6} (0.15)
High-fat (≥1%) milk (g/d)	3.59×10^{-6} (0.73)	-9.24×10^{-6} (0.38)	-2.32×10^{-5} **(0.004)**	1.68×10^{-6} (0.23)	9.45×10^{-5} **(<0.001)**†	9.38×10^{-5} **(0.005)**†	7.64×10^{-5} **(0.005)**†	2.53×10^{-5} **(<0.001)**†	3.18×10^{-6} **(0.02)**†	4.37×10^{-6} **(0.01)**†
High-fat (≥1%) sour milk products	-3.22×10^{-5}	-1.38×10^{-5}	-2.21×10^{-5}	-9.89×10^{-9}	0.0001 **(0.05)**	0.0001 (0.20)	0.0001 (0.13)	3.53×10^{-5} **(0.02)**	5.45×10^{-6} (0.13)	6.57×10^{-6}

(g/d)	(0.26)	(0.63)	(0.30)	(1.00)						(0.17)
Fish (g/d)	-4.54 x 10^{-5}	-3.97 x 10^{-5}	-1.25 x 10^{-5}	-1.40 x 10^{-5}	-0.0002	-0.0002	-0.0003	-4.66 x 10^{-5}	2.36 x 10^{-6}	-5.08 x 10^{-6}
	(0.62)	(0.67)	(0.85)	(0.26)	(0.33)	(0.54)	(0.16)	(0.36)	(0.84)	(0.74)
Red meat (g/d)	7.58 x 10^{-6}	5.94 x 10^{-5}	-3.85 x 10^{-5}	1.87 x 10^{-5}	-8.73 x 10^{-6}	-0.0004	-0.0001	-1.72 x 10^{-7}	-3.73 x 10^{-6}	5.90 x 10^{-6}
	(0.90)	(0.30)	(0.36)	**(0.02)**	(0.54)	**(0.03)**	(0.48)	(1.00)	(0.62)	(0.55)
Sausages (g/d)	8.80 x 10^{-5}	-9.52 x 10^{-6}	1.12 x 10^{-5}	4.88 x 10^{-6}	0.0002	0.0002	0.0002	5.48 x 10^{-5}	8.86 x 10^{-6}	1.30 x 10^{-5}
	(0.27)	(0.90)	(0.85)	(0.65)	(0.31)	(0.44)	(0.47)	(0.21)	(0.38)	(0.33)
High-fiber (≥5%) grain products (g/d)	-3.38 x 10^{-5}	1.42 x 10^{-5}	-1.60 x 10^{-5}	4.50 x 10^{-6}	-6.99 x 10^{-5}	-0.0002	-5.64 x 10^{-5}	-1.28 x 10^{-5}	-4.40 x 10^{-6}	3.53 x 10^{-6}
	(0.45)	(0.75)	(0.62)	(0.45)	(0.51)	(0.26)	(0.63)	(0.60)	(0.43)	(0.63)
Low-fiber (<5%) grain products (g/d)	-2.28 x 10^{-5}	5.66 x 10^{-5}	-1.85 x 10^{-5}	3.84 x 10^{-6}	0.0001	8.58 x 10^{-5}	0.0001	2.66 x 10^{-5}	2.22 x 10^{-6}	1.04 x 10^{-5}
	(0.52)	(0.11)	(0.48)	(0.42)	(0.23)	(0.46)	(0.17)	(0.17)	(0.62)	(0.08)
Sugary products (g/d)	1.37 x 10^{-5}	5.56 x 10^{-6}	1.17 x 10^{-5}	2.17 x 10^{-6}	-4.39 x 10^{-5}	-1.74 x 10^{-5}	-5.14 x 10^{-5}	1.04 x 10^{-5}	-2.02 x 10^{-6}	-2.21 x 10^{-6}
	(0.35)	(0.71)	(0.29)	(0.28)	(0.22)	(0.72)	(0.19)	(0.20)	(0.28)	(0.37)

BCAAs, branched-chain amino acids.

*Data are unstandardized regression coefficients from linear mixed effects model adjusted for age, sex, body far percentage, pubertal status and physical activity. P-values are reported in parentheses. Statistically significant associations are indicated by bolded P-values.

†Associations remained statistically significant after Benjamini-Hochberg false-discovery-rate correction for multiple testing.

Table 6. Longitudinal associations of diet quality with lipoprotein particle size over eight years*

	VLDL diameter (nm)	LDL diameter (nm)	HDL diameter (nm)
Finnish Healthy Eating Index	-0.012	0.0008	0.0011
	(0.008)†	(0.06)	(0.10)
Vegetables, fruit, and berries (g/d)	0.0004	2.01×10^{-5}	-5.44×10^{-5}
	(0.13)	(0.42)	(0.18)
Higher-fat vegetable oils and vegetable-oil-based margarine (≥60% fat)	-0.0039	0.0002	0.0004
	(0.13)	(0.44)	(0.31)
Lower-fat vegetable oils and vegetable-oil-based margarine (<60% fat)	0.0098	-0.0011	-0.0009
	(0.03)	**(0.009)**†	(0.21)
Low-fat (<1%) milk (g/d)	-3.82×10^{-5}	5.83×10^{-6}	-1.34×10^{-6}
	(0.73)	(0.56)	(0.94)
High-fat (≥1%) milk (g/d)	1.25×10^{-5}	6.32×10^{-6}	-1.80×10^{-5}
	(0.94)	(0.66)	(0.45)
High-fat (≥1%) sour milk products (g/d)	-0.0004	3.46×10^{-6}	3.37×10^{-5}
	(0.39)	(0.93)	(0.59)
Fish (g/d)	-0.0003	0.0001	3.44×10^{-5}
	(0.81)	(0.42)	(0.86)
Red meat (g/d)	0.0007	4.95×10^{-5}	-0.0002

	(0.40)	(0.53)	(0.14)
Sausages (g/d)	0.0021	2.58×10^{-5}	4.53×10^{-5}
	(0.07)	(0.81)	(0.79)
High-fiber (≥5%) grain products (g/d)	-0.0009	9.46×10^{-6}	6.82×10^{-5}
	(0.17)	(0.88)	(0.48)
Low-fiber (<5%) grain products (g/d)	0.0009	-6.02×10^{-5}	-3.72×10^{-5}
	(0.09)	(0.21)	(0.63)
Sugary products (g/d)	0.0006	-3.66×10^{-6}	-7.10×10^{-5}
	(0.001)†	(0.07)	**(0.03)**†

VLDL, very low-density lipoprotein; LDL, low-density lipoprotein; HDL, high-density lipoprotein.

*Data are unstandardized regression coefficients from linear mixed-effects models adjusted for sex, age, body fat percentage, pubertal status and physical activity. P-values are reported in parentheses. Statistically significant associations are indicated by bolded P-values.

†Associations remained statistically significant after Benjamini-Hochberg false-discovery-rate correction for multiple testing.

Table 7. Longitudinal associations of diet quality and dietary factors with lipoprotein subclasses over 8 years*

	Large VLDL (mmol/l)	Medium VLDL (mmol/l)	Small VLDL (mmol/l)	Large LDL (mmol/l)	Medium LDL (mmol/l)	Small LDL (mmol/l)	Large HDL (mmol/l)	Medium HDL (mmol/l)	Small HDL (mmol/l)
Finnish Children Healthy Eating Index	-4.58×10^{-8} **(0.01)**†	-6.31×10^{-8} (0.13)	-7.43×10^{-8} (0.11)	-1.82×10^{-7} (0.75)	-4.11×10^{-7} (0.11)	-1.16×10^{-7} (0.35)	3.88×10^{-6} (0.26)	-3.78×10^{-6} (0.37)	-1.16×10^{-7} (0.35)
Vegetables, fruit, and berries (g/d)	1.68×10^{-9} (0.12)	5.70×10^{-9} **(0.02)**†	6.50×10^{-9} **(0.02)**†	6.78×10^{-8} **(0.05)**†	2.86×10^{-8} (0.06)	1.67×10^{-8} **(0.02)**†	-2.23×10^{-7} (0.27)	-2.00×10^{-7} (0.42)	1.98×10^{-7} (0.63)
Higher-fat vegetable oils and vegetable-oil-based margarine (≥60% fat)	-1.48×10^{-8} (0.16)	-2.28×10^{-8} (0.34)	-2.05×10^{-8} (0.44)	-2.93×10^{-7} (0.38)	1.45×10^{-7} (0.32)	-6.58×10^{-8} (0.35)	1.45×10^{-6} (0.46)	8.16×10^{-7} (0.73)	-6.58×10^{-8} (0.35)
Lower-fat vegetable oils and vegetable-oil-based margarine (<60% fat)	4.47×10^{-8} **(0.02)**†	-1.91×10^{-10} (0.85)	7.33×10^{-8} (0.12)	-1.04×10^{-6} (0.08)	-1.47×10^{-7} (0.58)	-1.19×10^{-7} (0.35)	-7.24×10^{-6} **(0.04)**	-5.94×10^{-6} (0.16)	-1.19×10^{-7} (0.35)
Low-fat (<1%) milk (g/d)	-1.28×10^{-10} (0.77)	-1.91×10^{-10} (0.85)	-3.71×10^{-10} (0.74)	-1.27×10^{-9} (0.93)	-3.27×10^{-9} (0.61)	-4.97×10^{-10} (0.87)	-6.00×10^{-8} (0.48)	-1.55×10^{-7} (0.13)	-4.97×10^{-10} (0.87)
High-fat (≥1%) milk (g/d)	-1.98×10^{-10} (0.75)	1.50×10^{-9} (0.30)	1.35×10^{-9} (0.40)	3.28×10^{-8} (0.11)	1.42×10^{-8} (0.11)	6.05×10^{-9} (0.17)	4.56×10^{-8} (0.70)	2.77×10^{-7} (0.06)	6.05×10^{-9} (0.17)
High-fat (≥1%) sour milk products	-2.52×10^{-10}	-1.04×10^{-9}	-1.42×10^{-9}	1.78×10^{-9} (0.97)	-6.83×10^{-9}	1.38×10^{-9} (0.90)	-8.92×10^{-8}	-3.61×10^{-7}	1.38×10^{-9} (0.90)

(g/d)	(0.88)	(0.79)	(0.74)		(0.77)		(0.78)	(0.35)	
Fish (g/d)	2.37×10^{-9} (0.67)	-1.03×10^{-9} (0.93)	7.50×10^{-9} (0.58)	-7.45×10^{-8} (0.66)	-8.58×10^{-8} (0.25)	-3.30×10^{-8} (0.36)	-8.68×10^{-7} (0.39)	-2.16×10^{-6} (0.08)	-3.30×10^{-8} (0.36)
Red meat (g/d)	1.49×10^{-9} (0.67)	1.01×10^{-8} (0.18)	1.19×10^{-8} (0.16)	7.66×10^{-8} (0.47)	4.53×10^{-8} (0.33)	1.40×10^{-8} (0.53)	-1.08×10^{-6} (0.08)	7.91×10^{-7} (0.29)	1.40×10^{-8} (0.53)
Sausages (g/d)	7.18×10^{-9} (0.13)	1.02×10^{-8} (0.33)	1.08×10^{-8} (0.36)	1.31×10^{-7} (0.37)	2.86×10^{-8} (0.66)	2.84×10^{-8} (0.36)	7.91×10^{-7} (0.36)	6.83×10^{-7} (0.51)	2.84×10^{-8} (0.36)
High-fiber (≥5%) grain products (g/d)	-3.33×10^{-9} (0.21)	-6.05×10^{-9} (0.30)	-5.67×10^{-9} (0.39)	-9.25×10^{-8} (0.26)	-4.40×10^{-8} (0.23)	-1.67×10^{-8} (0.34)	-1.32×10^{-7} (0.78)	-5.00×10^{-7} (0.39)	-1.67×10^{-8} (0.34)
Low-fiber (<5%) grain products (g/d)	1.95×10^{-9} (0.35)	3.38×10^{-9} (0.47)	2.40×10^{-9} (0.65)	-7.00×10^{-9} (0.92)	1.49×10^{-8} (0.61)	6.80×10^{-9} (0.63)	-2.42×10^{-7} (0.53)	-5.98×10^{-8} (0.90)	6.80×10^{-9} (0.63)
Sugary products (g/d)	2.84×10^{-9} **(0.001)**†	4.07×10^{-9} **(0.04)**	5.09×10^{-9} **(0.02)**†	-5.38×10^{-9} (0.85)	1.76×10^{-8} (0.15)	4.61×10^{-9} (0.44)	-3.59×10^{-7} **(0.03)**†	-1.65×10^{-7} (0.41)	4.61×10^{-9} (0.44)

*Data are unstandardized regression coefficients from linear mixed-effects models adjusted for sex, age, body fat percentage, pubertal status and physical activity. P-values are reported in parentheses. Statistically significant associations are indicated by bolded P-values.

†Associations remained statistically significant after Benjamini-Hochberg false-discovery-rate correction for multiple testing.

Table 8. Longitudinal associations of diet quality and dietary factors with apolipoproteins over 8 years*.

	Apolipoprotein B (g/l)	Apolipoprotein A1 (g/l)	Apolipoprotein B/Apolipoprotein A1
Finnish Children Healthy Eating Index	-0.0005	-0.0005	-0.0003
	(0.40)	(0.61)	(0.50)
Vegetables, fruit and berries (g/d)	7.22×10^{-5}	-4.39×10^{-5}	5.61×10^{-5}
	(0.06)	(0.40)	(0.05)
Higher-fat vegetable oils and vegetable-oil-based margarine (≥60% fat)	-0.0004	0.0006	-0.0005
	(0.30)	(0.27)	(0.09)
Lower-fat vegetable oils and vegetable-oil-based margarine (<60% fat)	-0.0009	-0.0017	5.92×10^{-5}
	(0.18)	(0.05)	(0.91)
Low-fat (<1%) milk (g/d)	-3.50×10^{-6}	-1.87×10^{-5}	4.08×10^{-6}
	(0.83)	(0.39)	(0.74)
High-fat (≥1%) milk (g/d)	3.84×10^{-5}	4.50×10^{-5}	1.17×10^{-5}
	(0.09)	(0.14)	(0.50)
High-fat (≥1%) sour milk products (g/d)	-2.94×10^{-6}	-5.05×10^{-5}	5.42×10^{-6}
	(0.96)	(0.53)	(0.90)
Fish (g/d)	-9.97×10^{-5}	-0.0004	5.22×10^{-5}
	(0.59)	(0.15)	(0.71)
Red meat (g/d)	0.0001	-7.62×10^{-5}	0.0001

	(0.37)	(0.64)	(0.23)
Sausages (g/d)	0.0002	0.0001	8.41×10^{-5}
	(0.34)	(0.59)	(0.49)
High-fiber (≥5%) grain products (g/d)	-9.31×10^{-5}	-8.57×10^{-5}	-4.36×10^{-5}
	(0.30)	(0.49)	(0.52)
Low-fiber (<5%) grain products (g/d)	2.93×10^{-6}	1.24×10^{-6}	4.10×10^{-6}
	(0.97)	(0.99)	(0.94)
Sugary products (g/d)	1.03×10^{-5}	-4.51×10^{-5}	2.71×10^{-5}
	(0.74)	(0.28)	(0.24)

*Data are unstandardized regression coefficients from linear mixed-effects models adjusted for sex, age, body fat percentage, pubertal status and physical activity. P-values are reported in parentheses. Statistically significant associations are indicated by bolded P-values.

†Associations remained statistically significant after Benjamini-Hochberg false-discovery-rate correction for multiple testing.

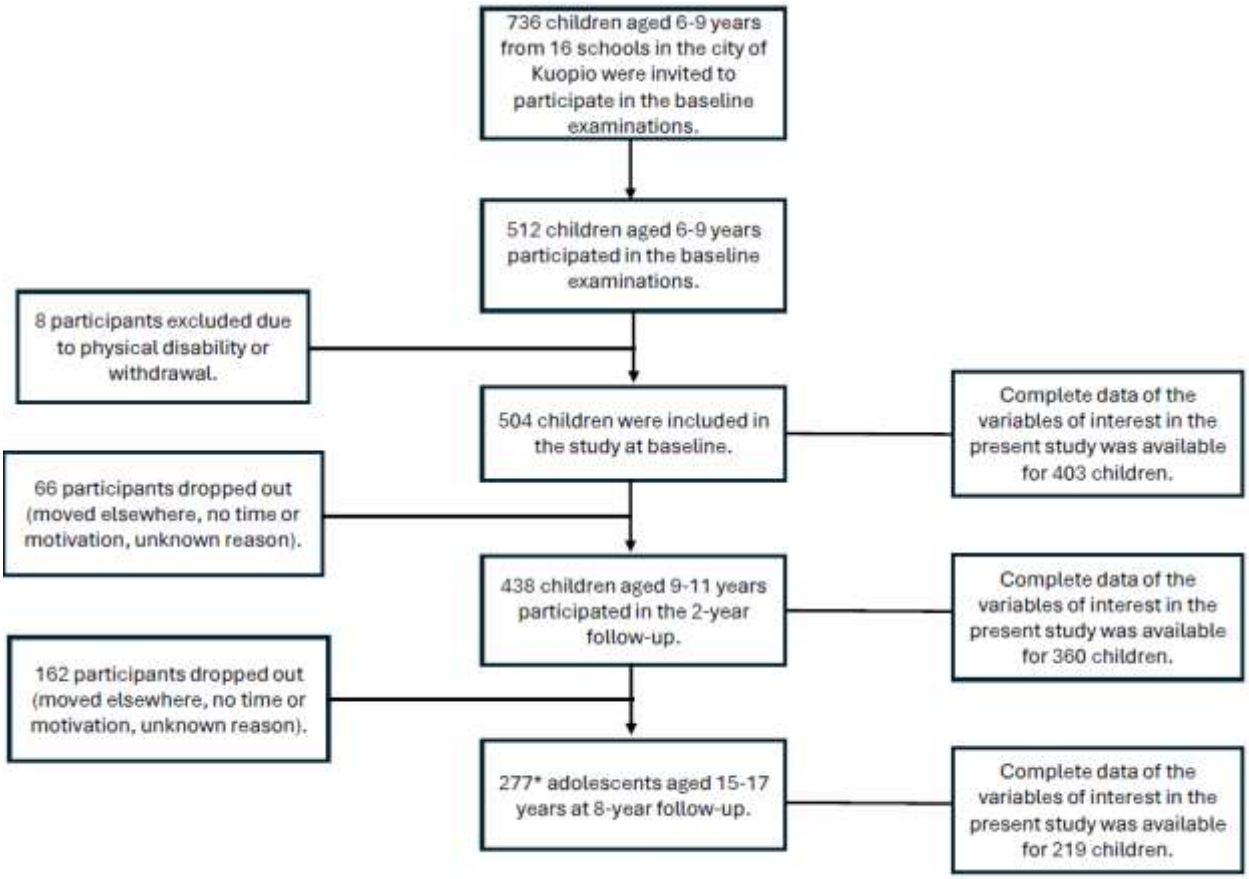

Figure 1: Flowchart of baseline, 2-year, and 8-year examinations in the Physical Activity and Nutrition in Children (PANIC) study. *One of the participants did not attend the 2-year examinations but attended the 8-year examinations.