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ORIGINAL CONTRIBUTION

Increased physical activity combined with more eating occasions is beneficial against dyslipidemias in children. The Healthy Growth Study

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Abstract
Purpose To identify lifestyle patterns associated with blood lipid levels in children.
Methods A representative sample of 2,043 schoolchildren (9–13 years) participated in a cross-sectional epidemiologic study conducted in 77 primary schools in four large regions in Greece. Dietary intakes, breakfast patterns and eating frequency, physical activity levels, sleep duration, anthropometric and physical examination data, biochemical indices and socioeconomic information (collected from parents) were assessed in all children. Principal component analysis was used to identify the lifestyle patterns.
Results A lifestyle pattern of more screen time, shorter sleep duration and higher sugar-sweetened beverage consumption was inversely associated with HDL cholesterol ($\beta = -0.077$; $P < 0.001$) and positively associated with total/HDL cholesterol ratio ($\beta = 0.049$; $P = 0.025$). Furthermore, a lifestyle pattern of more eating occasions and higher moderate-to-vigorous physical activity (MVPA) levels was inversely associated with total cholesterol ($\beta = -0.064$; $P = 0.006$), LDL (low-density lipoprotein) cholesterol ($\beta = -0.065$; $P = 0.004$) and total/HDL (high-density lipoprotein) cholesterol ratio ($\beta = -0.043$; $P = 0.049$) in multivariate models. Finally, children with MVPA levels and eating frequency higher than that corresponding to the second quartile of this lifestyle pattern (i.e., > 44.8 min of MVPA per day and > 4.7 meals per day) were 29.7, 32.6 and 43.1 % less likely of having abnormal levels of total cholesterol, LDL and total/HDL cholesterol ratio, respectively, according to the National Cholesterol Education Program (NCEP) cutoff points.
Conclusions A lifestyle pattern of more than approximately 45 min of MVPA and 5 eating occasions per day was significantly associated with reduced likelihood of dyslipidemias in schoolchildren (9–13 years).

Keywords Children · Serum lipids · Lifestyle patterns · Diet

This study is on behalf of the "Healthy Growth Study" group. The details of the "Healthy Growth Study" are given in the "Appendix."

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Introduction

Recent data have clearly demonstrated that serum lipid and lipoprotein levels can be measured above optimal values during early childhood [1]. Increased concentration of triglycerides, total and LDL (low-density lipoprotein) cholesterol and decreased levels of HDL (high-density lipoprotein) cholesterol in children are significantly associated with the development of arterial atherosclerotic lesions [2]. Likewise, there is a significant association of abnormal values of LDL, HDL, triglycerides and LDL/HDL ratio in childhood with intima media thickness (IMT), a marker of preclinical atherosclerosis in adulthood [3]. Accordingly, blood lipid levels in children and adolescents

are important indices of atherosclerosis and risk of cardiovascular diseases later in life [2].

The study of dietary patterns in nutritional epidemiology is common. Nutrients are rarely eaten in isolation, and nutrient-only investigations underestimate the possible interactions between multiple nutrients or between single foods and other dietary components [4]. Since food is usually consumed as meals with a variety of foods, simple consideration of nutrient intake ignores the complex, additive, synergistic or antagonistic effects of food combinations. Dietary patterns represent the types and amounts of foods consumed and focus on the entire diet rather than just a single food or nutrient [4].

Several epidemiologic studies in children and adults have produced important information on the possible relationship of common dietary patterns with blood lipid levels [5, 6]. These studies suggested that varying patterns of nutrition may affect measurable cardiovascular risk factors, including blood lipids, while other lifestyle factors, like physical activity and sleep duration, may also influence such relations. Physical activity in childhood has been inversely related to cardiovascular risk factors through several mechanisms, including an improved ratio between HDL and LDL concentration and decreased levels of triglycerides and diastolic blood pressure [7]. Short sleep duration in children 4–10 years was associated with increased health risks and specifically increased circulating LDL concentrations [8].

Therefore, the purpose of the current study was to examine not just dietary but lifestyle patterns associated with blood lipid levels in children, offering valuable information in the field and possibly in cardiovascular risk reduction strategies.

Methods

Sampling

The Healthy Growth Study was a large-scale cross-sectional epidemiologic study initiated in May 2007 and completed in June 2009. Approval to conduct the study was granted by the Greek Ministry of National Education and the Ethics Committee of Harokopio University of Athens and was conducted in accordance with the ethical standards specified in the 1964 Declaration of Helsinki. The study population comprised of schoolchildren 9–13 years, attending the 5th and 6th grades of primary schools located in municipalities within the counties of Attica, Aitoloakarnania, Thessaloniki and Iraklio. The sampling of schools was random, multistage and stratified by parents' educational level and total population of students attending schools within municipalities of these counties, as described in more detail elsewhere [6]. The study population was representative of the 9–13-year-old school children living in the four counties under study. Nonetheless, these counties are scattered throughout the Greek territory, covering the northern (i.e., Thessaloniki), central (i.e., Attica), western (i.e., Aitoloakarnania) and southern (i.e., Iraklio-Crete) parts of Greece. This combined with the random, multistage and stratified sampling procedures followed to recruit our sample is indicative of the representativeness of our population. The sampling procedure yielded 77 primary schools, representative of the total number of schools in the counties under study, which responded positively when they were invited to participate in the study. An extended letter explaining the aims of the study and a consent form for taking full measurements were provided to all parents or guardians having a child in these schools. Parents who agreed to the participation of their children in the study had to sign the consent form and provide their contact details. Signed parental consent forms were collected for 2,656 out of 4,145 children (response rate 64.1 %).

Dietary intake

Dietary intake data were obtained by trained dieticians and nutritionists via morning interviews with the children at the school site for two consecutive weekdays and 1 weekend day, using the 24-h recall technique. Specifically, all study participants were asked to describe the type and amount of foods and beverages consumed, during the previous day, provided that it was a usual day according to the participant's perception. To improve the accuracy of food description, standard household measures (cups, tablespoons, etc.) and food models were used to define amounts. At the end of each interview, the interviewers, who were dieticians rigorously trained to minimize the interviewer's effect, reviewed the collected data with the respondent in order to clarify entries, servings and possible forgotten foods. Food intake data were analyzed using the Nutritionist V diet analysis software (version 2.1, 1999, First Databank, San Bruno, California, USA), which was extensively amended to include traditional Greek recipes [9]. Furthermore, the database was updated with nutritional information of processed foods provided by independent research institutes, food companies and fast-food chains.

The food-grouping scheme was designed for all foods or entries (core and recipe) appearing in Nutritionist V. In total, 47 food groups were initially established, based on similar source characteristics and nutrient content. Composite food items, such as recipes, were analyzed and were assigned to food groups according to primary ingredients. A similar methodology for the extraction of food groups was previously reported in studies with a smaller sample

size, but with only one 24-h recall available [10]. Examples of foods included in the food groups were documented previously [11]. In total, 18 large food categories (whole-grain bread and cereals, refined bread and cereals, dairy products, vegetables, fruits, red meat, processed meat, eggs, potatoes, legumes, fish, other seafood, white meat, fat oils, fresh fruit juices, sugared beverages, namely regular soft beverages and sugared fruit juices, salty snacks and sweets) were created, and the relevant values were calculated as consumption in grams per day.

Breakfast and eating frequency patterns

Although there is no widely acceptable definition for breakfast, a more balanced breakfast should include as the first meal of the day choices from the following three food groups: milk and milk-derived products, cereals and fruits (fresh fruits or natural juices; no sugar) [12]. Using data from the 24-h recalls and on the basis of the aforementioned definition of breakfast, a categorical variable was developed to assess the adequacy of children's breakfast consumption. More specifically, the following categories were created: 0 = salty, sweet or other savory snacks, 1 = zero portions of dairy products, cereals and fruits, 2 = one portion of dairy products or cereals or fruits, 3 = one portion of dairy products and one portion of cereals or fruits and 4 = one or more portions of dairy products, cereals and fruits. Data from 24-h dietary recalls were also used to define eating frequency. More specifically, eating frequency was assessed by summing the number of snacks or meals in which any portion of food was consumed.

Physical activity levels

Physical activity during leisure time was assessed using a standardized activity interview, based on a questionnaire completed by the participants in the presence of a member of the research team. Further details regarding the reliability and validity of the questionnaire are provided elsewhere [13]. Respondents reported the time spent on various physical activities on 2 weekdays and 1 weekend day, most preferably Sunday. Reported activities were grouped by a member of the research team into moderate-to-vigorous physical activities (MVPA) (intensity higher than 4 metabolic equivalents, METs), including activities such as brisk walking, bicycling, gymnastics, dancing, basketball, soccer, athletics, tennis, swimming, jumping rope and general participation in active outdoors games. Given the age-group, MVPA was defined as continuous physical activities causing sweating and heavy breathing for periods longer than 15 min, but with occasional breaks in intensity, rather than the strict aerobic definition of 20 continuous minutes appropriate for adults. The other level of physical activity that was assessed in the present study was "Light" (i.e., intensity lower than 4 METs). Physical activity levels that have been proposed to positively affect cardiorespiratory function and to produce desirable health outcomes (i.e., better weight, glycemic, lipidemic control, etc.) are those higher than 4 METs, which corresponds to the level of moderate, vigorous and very vigorous physical activity [14, 15]. Finally, the average weekdays and weekend time (h/day) spent on sedentary activities and more specifically on TV/DVD/video watching, video games and recreational computer usage was characterized as screen time.

Anthropometry and physical examination

Participants underwent a physical examination by two trained members of the research team. The protocol and equipment used were the same in all schools. Weight was measured to the nearest 10 g using a Seca digital scale (Seca Alpha, Model 770, Hamburg, Germany). Students were weighed without shoes in the minimum clothing possible. Height was measured to the nearest 0.1 cm using a commercial stadiometer (Leicester Height Measure, Invicta Plastics, Oadby, UK) with the participant standing barefoot, keeping shoulders in a relaxed position, arms hanging freely and head in Frankfurt horizontal plane. Weight and height were used to calculate body mass index (BMI) using Quetelet's equation (weight (kg)/height $(m)^2$). The International Obesity Task Force (IOTF) cutoff points [16, 17] were used to categorize participants as "underweight," "normal weight," "overweight" or "obese." Waist circumference (WC) was measured to the nearest 0.1 cm with the use of a non-elastic tape (Hoechstmass, Germany) with the student standing at the end of a gentle expiration. The measuring tape was placed around the trunk, at the level of umbilicus midway, between the lower rib margin and the iliac crest. Furthermore, one well-trained and experienced female pediatrician in each prefecture determined pubertal maturation (Tanner stage) after thorough visual inspection of breast development in girls and genital development in boys [18].

Biochemical indices

Blood samples were obtained for biochemical and hematological screening tests between 08.30 and 10.30 after a 12-h overnight fast. Reminders were distributed the previous day to both parents and children to ensure compliance with fasting. Professional staff performed venipuncture to obtain a maximum of 23 ml blood. Part of the blood was collected in test tubes with no added anticoagulant, where it was allowed to clot for approximately

2 h, as this was designated for serum separation. Clotted blood was centrifuged at 3,000 rpm for 15 min, and the collected serum was divided into aliquots and stored at −80 °C. All serum samples were transported in dry ice to the Laboratory of Nutrition and Clinical Dietetics at Harokopio University, where central storage of backup samples at −80 °C took place.

Total cholesterol (TC), HDL and triglycerides (TG) were determined in duplicate using commercially available enzymatic colorimetric assays (Roche Diagnostics SA, Vasilia, Switzerland) on an automated analyzer (Roche/Hitachi Modular). LDL was calculated by the Friedewald equation [19]. The LDL/HDL and the TC/HDL ratios were also estimated. The National Cholesterol Education Program (NCEP) cutoff points for blood lipids were used to define dyslipidemias [20].

Socioeconomic, perinatal and other information collected from the parents

Data on the socioeconomic background of the families having at least one child participating in the study were collected from the parents (most preferably from the mother) during scheduled face-to-face interviews at school. For those parents not able to attend the meetings (approximately 5 % of the total sample), data were collected via telephone interviews. All interviews were conducted by research team members who were rigorously trained to minimize interviewer's effect by using a standardized questionnaire. The socioeconomic data collected from parents included parental educational level (years of education), home square meters (m^2), number of family members living at home, number of cars owned by the family and home property. All aforementioned data were grouped, scored and combined for the development of a Socio-Economic Status index (SES index) as follows: Scores 0, 1, 2 and 3 were assigned to <9, 9–12, 12–16 and >16 years of maternal and paternal education, respectively; scores 0, 1, 2 and 3 were assigned to <20, 20–25, 25–30 and >30 home m^2 per family member, respectively; scores 0, 1, 2 and 3 were assigned to 0, 1, 2 and ≥3 cars owned by the family, respectively; and scores 0 and 1 were assigned to rented or owned home, respectively. The total score of the SES index was obtained by summing the scores to each index component. The values of the total SES index score were ranging between 0 and 13, with higher values indicating higher SES of the family. The self-reported weight and height of the mother and the father were also recorded.

Pediatric records were used to obtain data about children's birth weight. Parents were also asked to report the time their children usually go to bed at night and wake up in the morning on weekdays and weekend days, respectively. This information was used for the calculation of children's night sleep duration.

Statistical analysis

Lifestyle component derivation

Principal component analysis (PCA) was used to identify lifestyle patterns. The Kaiser–Meyer–Olkin (KMO) criterion was applied, and it was equal to 0.6. The orthogonal rotation (varimax option) was used to derive optimal non-correlated components (lifestyle patterns). To decide the number of components to retain, Kaiser criterion (eigenvalues > 1) was used. Factor loadings represent the correlations of each habit with the lifestyle pattern score. Higher absolute values of factor loadings indicate that the habit predictor contributes most to the construction of this particular component. The lifestyle components (patterns) were named according to the factor loadings of those behaviors correlated most with the component (factor loadings > |0.4|). When a behavior had a factor loading value >|0.4| in more than one component, then this behavior was used to name the component for which it had the higher factor loading value.

Descriptive and other statistical analyses

Categorical variables were presented as frequencies. Associations between categorical variables were examined by using the chi-square test and the two-sample z test for proportions whenever appropriate. Multiple linear regression analysis was used to evaluate the associations between blood lipids and lifestyle patterns derived from PCA, which are treated as continuous variables. More specifically, 3 different models were applied: model 1, unadjusted; model 2, adjusted for sex and Tanner stage; and model 3, adjusted for sex, Tanner stage, waist circumference, mean parental BMI, SES index score and birth weight. The results from the linear regression models are presented as standardized beta coefficients, and the level of significance was defined at $P < 0.05$. Moreover, participants' lifestyle pattern scores were categorized into quartiles, so that for each lifestyle component, quartile 4 consisted of persons whose dietary and physical activity behaviors were most adherent to that particular pattern. Based on the statistical significant associations provided by the linear regression models, logistic regression analysis was performed to evaluate the association between the quartiles of each lifestyle component and the probability of dyslipidemias. The results of logistic regression models were presented as odds ratios (OR) and 95 % confidence interval (CI). Data were analyzed using Statistical Package for Social Sciences software (version 13.0, 2004, SPSS Inc, Chicago, IL).

Results

Full socioeconomic, demographic, parental BMI, perinatal, clinical, biochemical and dietary data, as well as information on the time spent on physical and sedentary activities and on sleep duration, were collected for 2,043 children (50.2 % females and 49.8 % males). Regarding the descriptive characteristics of the study sample, Table 1 illustrates that the total prevalence of overweight and obesity was 29.7 and 11.3 %, respectively, with a significantly higher prevalence of obesity in boys than in girls (13.3 vs. 9.3 %, $P < 0.05$). Furthermore, Table 1 shows that the prevalence of hypertriglyceridemia (i.e., TG > 100 mg/dL) was significantly higher in girls than in boys.

Table 2 summarizes the loadings of the factors retained from the PCA. The value of the KMO criterion indicates that the lifestyle variables entered in the analysis were strongly intercorrelated and that PCA could be correctly used for assessing "healthy" or "unhealthy" lifestyle patterns. The PCA analysis indicated five lifestyle components explaining 52.7 % of the total variance with regard to the examined variables. These components were characterized as follows: higher dairy consumption and more adequate breakfast (component 1); higher consumption of high fiber foods (component 2); more screen time, shorter sleep duration and higher consumption of sugared beverages (component 3); more time spent on MVPA and more eating occasions (component 4); higher red meat and lower fish consumption (component 5).

Table 1 Prevalence of overweight, obesity and dyslipidemias[†] in preadolescents

	Boys (n = 1,018) %	Girls (n = 1,025) %	Total (n = 2,043) %
Weight status			
Normal weight	56.0	62.1	59.0
Overweight	30.7	28.6	29.7
Obese	13.3*	9.3	11.3
Total cholesterol (mg/dL)			
<170[a]	55.7	57.9	56.8
170–199[b]	30.7	29.2	30.0
≥200[c]	13.6	13.0	13.3
HDL cholesterol (mg/dL)			
≥35[a]	96.6	95.6	96.1
<35[d]	3.4	4.4	3.9
LDL cholesterol (mg/dL)			
<110[a]	71.5*	75.5	73.5
110–129[b]	18.1	16.0	17.0
≥130[c]	10.4	8.5	9.4
Triglycerides (mg/dL)			
<75[a]	80.3*	72.8	76.5
75–99[b]	13.0*	16.7	14.8
≥100[c]	6.8*	10.5	8.7

* $P < 0.05$ for differences between sexes based on the chi-square test and the two-sample z test for proportions

[†] Adapted from NCEP guidelines for children and adolescents [20]. Letter superscripts indicate categorization of children based on the proposed thresholds of serum lipids, that is, [a] acceptable levels; [b] borderline-high levels; [c] high levels, [d] low levels

Table 3 presents the associations derived from the three different models of the linear regression analyses between serum lipids (i.e., TC, HDL, LDL, TG), total/HDL cholesterol and HDL/LDL cholesterol ratio with each one of the five lifestyle components. Total cholesterol, LDL cholesterol and total/HDL cholesterol ratio were found to be inversely associated with lifestyle component 4 ($\beta = -0.064$, $P = 0.006$; $\beta = -0.065$, $P = 0.004$; and $\beta = -0.043$; $P = 0.049$, respectively), even after adjusting for several potential confounding factors in model 3. Furthermore, lifestyle component 3 was inversely associated with HDL cholesterol ($\beta = -0.077$; $P < 0.001$) and positively associated with total/HDL cholesterol ratio ($\beta = 0.049$; $P = 0.025$). Several other significant associations between certain lifestyle components and serum lipids were also observed in models 1 and 2; however, these became statistically insignificant after controlling for several potential confounding factors in model 3.

The logistic regression analysis summarized in Table 4 showed that children at the second quartile of lifestyle component 4 were 29.7, 32.6 and 43.1 % less likely of having total cholesterol levels > 170 mg/dL, LDL cholesterol levels > 110 mg/dL and total/HDL cholesterol ratio > 4.5 compared to children at the first quartile, after controlling for several potential confounding factors. Furthermore, children at the second quartile of lifestyle component 4 were found to spend more time on MVPA and to have more eating occasions than children in the first quartile (i.e., 44.8 ± 28.6 vs. 20.0 ± 22.6 min/day and 4.7 ± 0.7 vs. 4.1 ± 0.8 eating occasions/day). Similar findings were also observed in children at the third and fourth quartiles.

Discussion

During the past decades, the prevalence of dyslipidemia, a major cardiovascular risk factor, has been rapidly increasing in Greek children [21], while dyslipidemia in childhood tracks into adulthood and represents an increasing risk of cardiovascular diseases later in life [3]. Accordingly, several studies need to identify factors that may influence abnormal lipid profile in children, specifically lifestyle factors including diet, physical activity and sleep pattern.

Table 2 Factor loadings derived from principal component analysis regarding certain behaviors in preadolescents

Predictors (behaviors)	Component				
	1	2	3	4	5
Dairy consumption[a]	0.793[g]	0.154	−0.113	0.099	0.002
Fruit consumption	0.051	0.620[g]	−0.006	0.149	0.000
Vegetable consumption	−0.050	0.739[g]	0.113	−0.037	0.038
Whole-grain food consumption[b]	0.108	0.501[g]	−0.223	−0.090	−0.110
Red meat consumption	0.014	−0.055	0.066	0.116	0.719[g]
Fish consumption	−0.013	−0.011	0.069	0.114	−0.753[g]
Sugared beverages consumption[c]	−0.171	−0.154	0.560[g]	0.362	0.114
Adequate breakfast consumption	0.760[g]	−0.088	−0.016	−0.130	0.034
Eating frequency	0.446[g]	0.352	0.123	0.476[g]	−0.035
Screen time[d]	0.040	−0.062	0.680[g]	−0.214	−0.018
Time spent on physical activity[e]	−0.039	−0.004	−0.124	0.822[g]	−0.001
Sleep duration[f]	0.033	−0.072	−0.580[g]	0.008	0.033
Explained variance (%)	12.1	11.4	10.2	9.7	9.3

[a] Consumption of milk, yogurt and cheese (g/day)

[b] Consumption of whole-grain bread and cereals (g/day)

[c] Consumption of regular soft beverages and sugared fruit juices (g/day)

[d] Time spent on watching TV/DVD/video and/or using games consoles/computer for fun (h/day)

[e] Time spent on moderate-to-vigorous physical activity (min/day)

[f] Night sleep duration on weekdays (h/day)

[g] Habit with the highest factor loading (>|0,4|) within the component

The results of the present study in terms of anthropometric and biochemical indices, concerning prevalence of overweight and obesity, shown in Table 1, are in accordance with the proportional results from previous studies in Greek children [22–24], introducing obesity levels around 8–12 % and overweight levels around 25–30 %. Additionally, our data related to the prevalence of dyslipidemia are consistent with similar studies conducted in Greece [21] and the United States [25]. However, gender-specific prevalence of hypertriglyceridemia has been rarely investigated in children and adolescents and has produced inconsistent results [26, 27], precluding comments on our findings for higher prevalence in girls.

Statistical analysis revealed predominantly five lifestyle components. Out of these, only component 3 comprising a greater screen time, shorter sleep duration and higher consumption of sugared beverages and component 4 comprising more time spent on MVPA and more eating occasions were significantly associated with blood lipid profile after adjustment for sex, Tanner stage, waist circumference, parental BMI, SES index and birth weight.

Consumption of low-nutrient, energy-dense foods, including sugared sweetened beverages, is very popular among children and adolescents, especially those who eat most of their meals away from home [28]. Indeed, beverages containing sugar were related to cardiovascular risk factors in childhood, both independently and through development of obesity leading to dyslipidemia [29]. In adulthood, increased soft drink intake was associated with risk of metabolic syndrome, while daily consumption of more than 1 soft drink/day was associated with reduced HDL cholesterol levels and increased triglycerides [30]. Likewise, decreased sleep duration and increased time spent on watching TV and playing video games for fun were associated, respectively, negatively or positively with blood lipid levels. Thus, in adolescent girls, short sleep duration is correlated with risk of hypercholesterolemia in adulthood [31], while obese children having short sleep duration seem to have significant association with elevated levels of LDL [8]. Additionally, screen time at the age of 7–13 years also seems as an important behavior affecting lipid profile [32]. In adolescents, screen time is dose dependent correlated with the risk of developing metabolic syndrome and subsequently, among others, of introducing elevated TG and decreased HDL levels [33]. Therefore, our results concerning strong association of sleep duration, screen time and sugar beverages consumption with blood lipids in children are in accordance with previous relevant studies, indicating predominant lifestyle patterns possibly capable of modifying lipid profile in children.

Component 4 was also inversely associated with TC, LDL and total/HDL cholesterol ratio. Physical activity is a matter of great importance, as it seems that there is a remarkable decline in its levels between the ages of

Table 3 Single (model 1) and multiple (models 2 and 3) linear regression analysis models, exploring the association of lifestyle components with serum lipids levels ($n = 2{,}043$)

Components	Model 1 (unadjusted)		Model 2		Model 3	
	β	P value	β	P value	β	P value
Total cholesterol (mg/dL)						
Lifestyle component 1	−0.001	0.955	−0.004	0.855	−0.006	0.779
Lifestyle component 2	0.048	0.029	0.047	0.034	0.040	0.080
Lifestyle component 3	−0.052	0.019	−0.047	0.036	−0.038	0.098
Lifestyle component 4	−0.062	0.005	−0.069	0.002	−0.064	0.006
Lifestyle component 5	0.025	0.262	0.023	0.312	0.015	0.521
HDL cholesterol (mg/dL)						
Lifestyle component 1	0.052	0.018	0.050	0.025	0.024	0.264
Lifestyle component 2	0.056	0.012	0.054	0.015	0.029	0.187
Lifestyle component 3	−0.104	<0.001	−0.097	<0.001	−0.077	<0.001
Lifestyle component 4	0.007	0.744	0.004	0.857	−0.007	0.756
Lifestyle component 5	0.017	0.445	0.009	0.678	0.013	0.538
LDL cholesterol (mg/dL)						
Lifestyle component 1	−0.025	0.258	−0.028	0.210	−0.022	0.343
Lifestyle component 2	0.030	0.174	0.030	0.183	0.031	0.169
Lifestyle component 3	−0.015	0.448	−0.013	0.558	−0.009	0.695
Lifestyle component 4	−0.063	0.004	−0.074	0.001	−0.065	0.004
Lifestyle component 5	0.024	0.282	0.022	0.326	0.012	0.592
Triglycerides (mg/dL)						
Lifestyle component 1	−0.027	0.228	−0.023	0.304	0.003	0.895
Lifestyle component 2	−0.021	0.334	−0.022	0.311	<0.001	0.983
Lifestyle component 3	0.061	0.006	0.056	0.012	0.032	0.136
Lifestyle component 4	−0.068	0.002	−0.041	0.067	−0.026	0.221
Lifestyle component 5	−0.020	0.368	−0.003	0.885	−0.012	0.587
Total/HDL cholesterol ratio						
Lifestyle component 1	−0.045	0.040	−0.046	0.038	−0.021	0.331
Lifestyle component 2	−0.015	0.512	−0.014	0.526	0.003	0.887
Lifestyle component 3	0.066	0.003	0.061	0.006	0.049	0.025
Lifestyle component 4	−0.058	0.009	−0.059	0.010	−0.043	0.049
Lifestyle component 5	−0.004	0.858	0.002	0.938	−0.010	0.648
HDL/LDL cholesterol ratio						
Lifestyle component 1	0.024	0.275	0.022	0.315	0.012	0.605
Lifestyle component 2	0.015	0.501	0.014	0.530	−0.002	0.929
Lifestyle component 3	−0.026	0.246	−0.023	0.305	−0.019	0.395
Lifestyle component 4	0.047	0.034	0.049	0.031	0.040	0.077
Lifestyle component 5	−0.007	0.746	−0.012	0.597	−0.006	0.774

β Standardized beta coefficient

Model 1, unadjusted; Model 2, adjusted for Tanner stage and sex; Model 3, adjusted for sex, Tanner stage, waist circumference, parental BMI, SES index and birth weight

Lifestyle component 1: higher dairy consumption and more adequate breakfast

Lifestyle component 2: higher consumption of high fiber food (i.e., fruits, vegetables and whole-grain food)

Lifestyle component 3: more screen time, shorter sleep duration and higher consumption of sugared beverages

Lifestyle component 4: more time spent on physical activity and more eating occasions

Lifestyle component 5: higher red meat consumption and lower fish consumption

Table 4 Mean levels of behaviors comprising lifestyle components 3 and 4 and likelihood of dyslipidemias across quartiles of lifestyle components 3 and 4

Behaviors of component 3	Quartiles of lifestyle component 3 (more screen time, shorter sleep duration and higher consumption of sugared beverages)				
	1st quartile Mean ± SD	2nd quartile Mean ± SD	3rd quartile Mean ± SD	4th quartile Mean ± SD	*P* value
Screen time (h/day)	1.35 ± 0.69	1.85 ± 0.83	2.42 ± 0.97	3.61 ± 1.68	<0.001
Sleep duration (h/day)	9.42 ± 0.58	8.98 ± 0.52	8.73 ± 0.55	8.35 ± 0.73	<0.001
Sugared beverages consumption (g/day)	21.9 ± 57.8	44.2 ± 82.7	82.5 ± 117.1	221.9 ± 222.4	<0.001*
Serum lipids		OR (95 % CI)	OR (95 % CI)	OR (95 % CI)	
HDL cholesterol < 35 mg/dl[†]	1.00	0.650 (0.309–1.368)	1.025 (0.530–1.983)	1.480 (0.802–2.731)	
Total/HDL cholesterol > 4.5[†]	1.00	1.056 (0.563–1.979)	1.229 (0.671–2.252)	0.929 (0.490–1.764)	
Behaviors of component 4	**Quartiles of lifestyle component 4 (more time spent on physical activity and more eating occasions)**				
	1st quartile Mean ± SD	2nd quartile Mean ± SD	3rd quartile Mean ± SD	4th quartile Mean ± SD	*P* value
Time spent on MVPA (min/day)	20.0 ± 22.6	44.8 ± 28.6	72.4 ± 37.7	142.0 ± 67.7	<0.001*
Meal frequency (no. of meals/day)	4.10 ± 0.84	4.70 ± 0.74	5.05 ± 0.75	5.22 ± 0.72	<0.001
Serum lipids		OR (95 % CI)	OR (95 % CI)	OR (95 % CI)	
Total cholesterol > 170 mg/dl[†]	1.00	0.703 (0.554–0.909)	0.705 (0.544–0.914)	0.619 (0.476–0.805)	
LDL cholesterol > 110 mg/dl[†]	1.00	0.674 (0.507–0.896)	0.695 (0.522–0.925)	0.667 (0.499–0.890)	
Total/HDL cholesterol > 4.5	1.00	0.569 (0.320–0.931)	0.308 (0.153–0.620)	0.603 (0.342–0.980)	

OR odds ratio, *CI* confidence interval

Adjusted for sex, Tanner stage, waist circumference, parental BMI, SES index and birth weight

* *P* value derived from the nonparametric Kruskal–Wallis test

[†] The cutoff values were adapted from NCEP guidelines for children and adolescents [20]

4–13 years in both sexes [34]. Additionally, findings from several epidemiologic studies have shown significant associations between higher physical activity levels and cardiovascular disease risk factors in children, mainly through an improved ratio of HDL/LDL cholesterol, attributed to increased lipoprotein lipase activity [7]. Alongside, eating frequency is a very popular dietary pattern investigated in many recent relevant studies [6]. However, most data concern adults rather than children and adolescents. In adulthood, increasing number of eating occasions from 3 to 6 or more for several weeks leads to a reduction in total and LDL cholesterol levels, possibly due to a relevant reduction in endogenous cholesterol synthesis or the enhancement of the reverse cholesterol transport pathway [35]. Additionally, a nibbling meal pattern caused significant decreases in total, LDL cholesterol and triglycerides compared to gorging [36]. In another recent study in diabetic children, skipping meals was correlated with higher levels of LDL cholesterol compared with having a steady number of meals daily [37].

Our results further amplify these findings as children being in the highest quartile of eating frequency and time spent on MVPA were less likely to have an abnormal lipid profile, implying a possible dose-dependent correlation. Recent recommendations for children and adolescents concerning physical activity (60 min/day) [38] and eating occasions (at least 4/day) [39] focus on general health and prevention of overweight and obesity, which is a possible explanation for the slight diversification of our findings (45 min/day of physical activity and 5 eating occasions/day) that concentrate more on dyslipidemias.

Components 1 and 2 were also associated with blood lipids after adjustment for only sex and Tanner stage, but the associations lost their significance when adjusted for several confounders. These findings were well described previously, as it was reported that dietary patterns characterized by high fiber consumption were inversely correlated with abnormal lipid profile in children and adults [5, 6], while skipping breakfast was associated with elevated levels of total and LDL cholesterol in both children and adults [40].

The current study has both strengths and limitations. The Healthy Growth Study was a large-scale epidemiological study conducted in a representative sample of children from four prefectures within the wider region of Greece. Furthermore, the adjustment for specific confounders resulted in the extraction of more accurate results. However, as the study has a cross-sectional design, it is not

suitable for interpreting causal relationships. Finally, although all statistical conditions for performing PCA analysis were kept in the current study, still the use of variables measured in different scales should be considered as another limitation.

The importance of healthy food choices in the prevention of cardiovascular diseases is now unequivocal. As we continue to explore the roles of nutrients and other compounds in our foods and their complex interrelations in influencing cardiovascular risk, it is now of great importance to identify lifestyle patterns that might also relate to future risk of developing cardiovascular diseases. In the present study, a pattern consisting of more than approximately 45 min of MVPA and 5 eating occasions per day was found to be significantly associated with reduced likelihood of dyslipidemias in schoolchildren (9–13 years). This pattern can be physiologically explained and employed in practical public health advice and individual counseling for reducing the future risk of cardiovascular diseases later in life.

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Conflict of interest None of the authors has any conflict of interest to declare.

Appendix

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