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Influence of the Mediterranean diet on carotid intima–media thickness in hypercholesterolaemic children: A 12-month intervention study

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Lipid profile

Abstract *Background and aims:* The Mediterranean diet has been recognised as having a protective role on the cardiovascular system due to its low lipid and high antioxidant content. Lipid profile and oxidant status represent two important risk factors related to endothelial dysfunction, even at early stages of cardiovascular diseases. The aim of the study was to evaluate the influence of a 12-month Mediterranean diet on the variation of lipid profile and carotid intima–media thickness (cIMT) in pre-pubertal hypercholesterolaemic children.
Methods and results: We performed a cross-sectional study comparing lipid profile and cIMT in a group of 68 pre-pubertal children (36 with hypercholesterolaemia and 32 controls). In addition, in the hypercholesterolaemic children a 12-month intervention programme with a Mediterranean diet was started to evaluate the variation of lipid profile and cIMT. At baseline, hypercholesterolaemic children showed a significantly higher cIMT (both right and left carotid artery) compared to controls (both $p < 0.05$). After 12 months of diet intervention, a significant reduction of total cholesterol, LDL-cholesterol and cIMT was documented (all $p < 0.05$). Furthermore, at the end of follow-up, delta body mass index-Standard Deviation score and delta LDL-cholesterol were significantly and independently related to the changes of cIMT (both $p < 0.05$).

Abbreviations: AHA, American Heart Association; BMI, body mass index; cIMT, carotid intima–media thickness; DBP, diastolic blood pressure; HDL-C, HDL-cholesterol; HOMA-IR, Homeostasis Model Assessment of Insulin Resistance; IR, insulin resistance; LDL-C, LDL-cholesterol; PUFAs, plant-derived n-3 polyunsaturated fatty acids; SDS, Standard Deviation score; SBP, systolic blood pressure; TC, total cholesterol; TG, triglycerides.

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Conclusion: The Mediterranean diet represents a valid approach in the treatment of hypercholesterolaemia even during childhood.

Introduction

Hypercholesterolaemia is a key component of the development and progression of angiopathy. In fact, total cholesterol (TC) and LDL-cholesterol (LDL-C) seem to be the major determinants of the atherosclerotic process [1]. The chronic exposure to increased lipid levels induces anatomic arterial changes even in childhood [2], and autopsy studies showed the presence of fatty streaks in the aortas of 3-year-old children [3].

Over the last years, high-resolution B-mode ultrasound measurement of the carotid intima—media thickness (cIMT) has been developed as a reliable and non-invasive method to predict the development of atherosclerosis. It has to be acknowledged that to date no normal values of cIMT for age and sex in healthy children have been established yet. Nonetheless, data in children with familial hypercholesterolaemia [4], hypertension [5], type 1 diabetes [6], and severe obesity [7] have clearly shown that high values of cIMT are a strong predictor of development of atherosclerosis later on in life. These data suggest that early therapeutic approaches during childhood aimed to positively modify these vascular findings could play a major role in reducing the risk of cardiovascular diseases in adulthood. Although lifestyle changes, characterised by nutritional approach and physical activity, represent the main strategy for the prevention of cardiovascular diseases in the paediatric age group, the data are still controversial. In fact, an interventional study in a group of obese children documented a decreased cIMT after a weight loss programme [8], while another study showed a reduction of the oxidative and pro-inflammatory state after a 6-week diet without changes in terms of cIMT [9].

Among the nutritional approaches, the Mediterranean diet has been recognised as having a protective role on the cardiovascular system [10,11]. The Lyon Diet Heart Study, a randomised secondary prevention trial, reported a relevant decrease of the recurrence rate in survivors of myocardial infarction following the Mediterranean diet [12]. This dietary pattern represents one of the healthiest diets characterised by low intake of total and saturated fats and increased intake of marine or plant-derived n-3 polyunsaturated fatty acids (PUFAs), fresh fruits and vegetables, legumes, high-fibre cereals, antioxidants, vegetable proteins and B vitamins [13]. However, up to now only few studies demonstrated the beneficial effects of the Mediterranean diet in youths [14—16].

The aim of this study was to investigate whether the Mediterranean diet could influence the lipid profile in prepubertal children with hypercholesterolaemia during a 12-month interventional period. Furthermore, we evaluated the potential association between changes in cIMT and the main metabolic and anthropometric variables after the Mediterranean dietary intervention.

Methods

A total of 36 pre-pubertal hypercholesterolaemic children were recruited from the Department of Pediatrics, University of Chieti, Italy. Hypercholesterolaemia was defined according to TC or LDL-C level above the 75th percentile for age and sex found on two distinct occasions [17]. Children with familial lipid disorders or family history for dyslipidaemia were excluded. All subjects were otherwise in good health and were not affected by other diseases (renal dysfunctions, endocrine disorders, hereditary diseases and systemic inflammation) or were taking any medication. None of the children was involved in regular and programmed physical activity. As control group, we recruited 32 healthy normocholesterolaemic pre-pubertal children admitted to the Department of Paediatrics for minor diseases. A physical examination was performed including anthropometric parameters (height, height-Standard Deviation score (SDS), weight, body mass index (BMI), BMI-SDS) and bio-impedance to determine fat mass. Based on BMI-SDS, children with values between -2 and 2 were defined as normal-weight, whereas children with values >2 SD were defined as obese. The pubertal stage was defined based on the Tanner criteria (all children had pre-pubertal characteristics corresponding to stage 1 both at baseline and after 12 months of diet). Furthermore, basal blood pressure was measured.

Fasting blood samples were obtained to evaluate lipids (TC, triglycerides (TG), LDL-C and HDL-cholesterol (HDL-C)), and glucose and insulin for the assessment of insulin resistance (IR). cIMT was measured by high-resolution B-mode ultrasound.

Hypercholesterolaemic children were encouraged to follow a Mediterranean diet for 12 months. During this period, all subjects had a follow-up visit after 6 months to obtain anthropometric parameters and lipid profile. At the end of the intervention, anthropometric parameters, metabolic parameters and cIMT were obtained.

This study was approved by Ethical Committee of University of Chieti. Written informed consent was obtained from parents and oral consent from children.

Anthropometric measurements

Body weight was determined to the nearest 0.1 kg and height was measured in triplicate with the Harpenden stadiometer to the nearest 0.1 cm. BMI and BMI-SDS for age and sex were calculated [18].

Mediterranean dietary intervention

At the start of the study, a 3-day dietary recording was performed by one physician and two nutritionists to

examine nutrient intakes of hypercholesterolaemic subjects. The recording demonstrated an unbalanced diet of a mean of 1672 calories day^{-1} (1285–2059 calories day^{-1}) distributed as follows: 49% carbohydrates, 36% lipids and 15% proteins. Lipids were distributed as follows: 20–25% unsaturated fats and more than 10% saturated fats; a daily cholesterol intake of 221 ± 97 mg was recorded. All children had low vegetable and fruit intake. Therefore, they were instructed to follow a traditional Mediterranean diet based on a food pattern typical of southern Italy in the early 1960s [13]. In detail, both parents and children received individual nutritional counselling to describe the main principles and health benefits of the Mediterranean diet and to furnish details regarding food preparation. The daily calories' intake was above 1605 calories (1397–1811 calories day^{-1}). The dietary goals were to obtain <30% of total energy from fats (10–15% monounsaturated fats, 10% PUFAs and <10% saturated fats), 12–14% from proteins and >55% from carbohydrates. The recommended daily cholesterol intake was <200 mg (mean 145 ± 27 mg day^{-1}). A daily intake of fruits, fibres and vegetables rich in antioxidants and vitamins was recommended, and a daily intake of 200 ml of milk or yogurt was suggested. Every diet was elaborated according to a 1-week scheme that included food relative weight, calories and possible substitutes distributed as follows: low-fat meat, fish rich in omega-3 fatty acids, beans, low-fat cheese, cold cuts without fat and eggs (once a week). We suggested using extra-virgin olive oil, which contains a high amount of monounsaturated fatty acids, moderate quantity of saturated fatty acids, low amount of PUFAs and, of note, high amount of micronutrients with antioxidant properties [19,20].

In the entire study population a strict follow-up programme was organised and a monthly telephone call was made to improve the compliance to the diet and to avoid dropout. Furthermore, each patient was invited to report with parental help a 3-day dietary recording twice a month. A short questionnaire aimed at maintaining parental involvement and at obtaining further details regarding the food and drink consumed was included in the diary. An expert nutritionist reviewed the diet programme with the child and a parent every 3 months and encouraged compliance. If needed, further details were sought about the food and drink recorded, such as portion sizes and preparation methods. Adherence to the Mediterranean diet was measured using the Mediterranean Diet Quality Index (KIDMED) questionnaire; all children showed an index score >8 reflecting good adherence [21].

Laboratory procedures

Biochemical analysis

Serum TC, HDL-C and TG were determined by a calorimetric enzymatic method. LDL-C was calculated according to the Friedewald formula (LDL-C = TC − HDL-C − (TG/5)). Apolipoprotein A1 (Apo A1) and Apo B were measured by rate nephelometry.

Plasma glucose was determined using the glucose oxidase method and plasma insulin measured with a two-site immunoenzymometric assay (AIA-PACK IRI; Tosoh, Tokyo, Japan). The limit of detection was 0.5 μU ml^{-1} with interassay coefficients of variation <7% for quality control. For determination of IR we used the Homeostasis Model Assessment of IR (HOMA-IR) calculated as follows: (fasting insulin (μU ml^{-1}) × fasting glucose (mmol l^{-1}))/22.5.

Instrumental procedures

Blood pressure

Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured twice in the right arm after 10 min rest in a supine position using a calibrated sphygmomanometer and averaged.

Bioelectrical impedance analysis

Impedance signal (*Z*, in ohms), resistance (*R*, in ohms) and reactance (*X*c, in ohms) were estimated to calculate fat mass. Measurements were conducted with the same single-frequency, phase-sensitive, impedance analyser (STA/BIA, Akern System Srl, Florence, Italy) and analysed by the Bodygram system [22,23].

Carotid ultrasonography

High-resolution B-mode ultrasonography of the right and left carotid arteries was performed with a linear 14-mHz Philips Sonos transducer. Children were examined in the supine position with the head turned slightly to the left and then to the right. The common, internal and external carotid arteries were identified by combined B-mode and colour-Doppler ultrasound examinations. A careful search was performed to obtain an optimal visualisation of the vessel wall, demonstrating the typical double lines representing the intima–media layer. cIMT was defined as the distance between the leading edge interface of the far wall and the leading edge of the median adventitia interface of the far wall, as previously described [24]. The ultrasound protocol requires the visualisation of the near and far wall of the right and left common carotid and internal carotid arteries and bifurcation in three different projections: anterior, lateral and posterior, for a total of 12 carotid segments per patient; these determinations were averaged (mean cIMT). All procedures were performed according to recent recommendations proposed by the American Heart Association (AHA) [25]. For quality control assessment, 10% of the patients were re-examined and these repeated measurements gave a coefficient of variation of 3%. All measurements were obtained by the same ultrasonographer who was blinded to any individual characteristic of the subjects.

Statistical analysis

Values are expressed as means and SD for normally distributed data or medians and interquartile range for non-normally distributed data (TG, insulin and HOMA-IR). Variables have been evaluated by independent-samples *t*-test at baseline and by repeated-measures analysis of variance (ANOVA) during the intervention period with *post-hoc* Bonferroni analysis. Non-normally distributed data were log transformed before analysis. Differences in sex variable were analysed by Fisher's exact test. At baseline, to

evaluate a possible relationship between variables of interest (BMI-SDS, HOMA-IR, lipids, Apo A1 and Apo B) and cIMT, including the mean value of right and left carotid artery (mean cIMT), the Pearson correlation analysis was performed. At the end of the study, to investigate the joint independent effect of obesity and lipid profile on the reduction of cIMT, a multiple stepwise linear regression analysis was performed in hypercholesterolaemic children. In the model we used the delta mean of cIMT as the dependent variable and delta BMI-SDS and delta LDL-C as independent variables. A p value of <0.05 was considered statistically significant. Analysis was performed using the computer program Statistical Package for the Social Sciences (SPSS), version 16.0 software for Windows.

Power calculation

For the primary 'end' point, change in cIMT between baseline and 1-year treatment, assuming a difference of 0.02 mm with a SD of 0.04 [26], and an alpha level of 0.05, a sample size of 36 would provide a power of 83%. The same sample size would give a power of 91% to detect a difference in LDL-C of 15.5 mg dl^{-1} between baseline and 1-year treatment with a SD of 0.7 [26].

Results

Baseline characteristics of the study cohort

The baseline characteristics of hypercholesterolaemic and control children are reported in Table 1. The children were similar for age, sex, weight and height, while height-SDS was lower in hypercholesterolaemic children than in controls ($p = 0.001$). Furthermore, BMI-SDS and fat mass were higher in hypercholesterolaemic children than in the control group (both $p < 0.001$). No difference was documented in SBP and DBP.

Hypercholesterolaemic children showed higher TC and LDL-C than controls (both $p < 0.001$), while no difference was documented in HDL-C and TG. Furthermore, Apo B was higher in hypercholesterolaemic children than in controls ($p < 0.001$), whereas the Apo A1/Apo B ratio was lower in hypercholesterolaemic subjects than in the control group ($p = 0.001$). No difference was documented in Apo A1.

Table 1 Baseline clinical and biochemical characteristics of hypercholesterolaemic and control children.

	Hypercholesterolaemic children ($n = 36$)	Control children ($n = 32$)	p[a]
Anthropometric parameters			
Age	7.5 ± 2.3	6.9 ± 1.8	NS
Sex	18M/18F	15M/17F	NS[b]
Pubertal stage	1	1	
Height (cm)	123.3 ± 15.7	127.5 ± 16.0	NS
Height-SDS	0.16 ± 1.69	1.69 ± 1.71	0.001
Weight (kg)	32.3 ± 14.1	27.8 ± 7.3	NS
BMI-SDS	2.88 ± 2.88	0.73 ± 1.29	<0.001
Fat mass (%)	27.7 ± 6.3	20.2 ± 3.9	<0.001
SBP (mmHg)	101 ± 8	98 ± 9	NS
DBP (mmHg)	61 ± 8	63 ± 7	NS
Lipid profile			
Total cholesterol (mg/dl)	260.6 ± 51.2	162.5 ± 16.1	<0.001
HDL-cholesterol (mg/dl)	53.0 ± 15.6	53.8 ± 12.5	NS
LDL-cholesterol (mg/dl)	182.0 ± 53.8	94.3 ± 13.5	<0.001
TG (mg/dl)[c]	84 [47–127]	61 [51–80]	NS
Apo A1 (mg/dl)	155.4 ± 28.5	155.6 ± 17.3	NS
Apo B (mg/dl)	136.5 ± 42.9	70.2 ± 21.3	<0.001
Apo A1/Apo B	1.28 ± 0.60	2.11 ± 0.36	0.001
Insulin resistance			
Glucose (mg/dl)	89.4 ± 7.4	85.0 ± 7.3	0.01
Insulin (μU/mL)[c]	7.4 [5.4–11]	3.5 [2.8–4.9]	<0.001
HOMA-IR[c]	1.54 [1.15–2.58]	0.67 [0.62–1.07]	<0.001
cIMT			
Right cIMT (mm)	0.36 ± 0.05	0.29 ± 0.11	0.002
Left cIMT (mm)	0.39 ± 0.07	0.31 ± 0.04	<0.001
Mean cIMT (mm)	0.37 ± 0.04	0.29 ± 0.06	<0.001

Data are means ± SD or medians (interquartile range). Significant values for $p < 0.05$. NS = Non significant value.
M = male; F = female; SDS = SD score; BMI = body mass index; SBP = systolic blood pressure; DBP = diastolic blood pressure; TG = triglycerides; HOMA-IR = Homeostasis Model Assessment of Insulin Resistance; cIMT = carotid intima–media thickness.
[a] Independent-samples t test.
[b] Fisher's test.
[c] Values are log transformed.

Glucose and insulin were higher in hypercholesterolaemic children than in controls (both $p < 0.05$). In addition, hypercholesterolaemic children showed higher HOMA-IR values than the control group ($p < 0.001$).

Both right and left cIMT and mean cIMT measurements were higher (all $p < 0.05$) in hypercholesterolaemic children than in controls.

Characteristics of hypercholesterolaemic children during the intervention period (Tables 2 and 3)

All children with hypercholesterolaemia followed the Mediterranean diet and completed the study.

All children grew regularly, as shown by a significant higher height at final assessment ($p < 0.001$). Hypercholesterolaemic children showed a significant decrease in BMI-SDS ($p < 0.05$) and fat mass ($p = 0.001$) at final assessment.

After 12 months, hypercholesterolaemic children showed a significant reduction in TC and LDL-C (both $p < 0.001$) and a significant increase in HDL-C ($p < 0.05$), whereas no change was documented in TG. A significant increase in Apo A1 and in the Apo A1/Apo B ratio was detected after 12 months (both $p < 0.05$), whereas no difference was documented in Apo B.

Moreover, glucose and HOMA-IR decreased between baseline and 12 months (both $p < 0.05$).

Finally, after 12 months of diet both right and left cIMT as well as mean cIMT were significantly reduced (all $p < 0.05$).

In order to avoid any possible influence of obesity on lipids and cIMT, a sub-analysis of metabolic variables was performed in normal-weight and obese hypercholesterolaemic children (Table 4).

Table 3 Insulin resistance and carotid intima–media thickness of hypercholesterolaemic children at baseline and after 12 months.

	Baseline ($n = 36$)	After 12 months ($n = 36$)	p[a]
Insulin resistance			
Glucose (mg/dl)	89.4 ± 7.4	84.1 ± 11.3	0.02
Insulin (μU/mL)[b]	7.4 [5.4–11]	7.5 [5.6–10.5]	NS
HOMA-IR[b]	1.54 [1.15–2.58]	1.50 [1.00–1.85]	0.02
cIMT			
Right cIMT (mm)	0.36 ± 0.05	0.32 ± 0.04	0.002
Left cIMT (mm)	0.39 ± 0.07	0.33 ± 0.03	<0.001
Mean cIMT (mm)	0.37 ± 0.04	0.32 ± 0.03	<0.001

Data are means ± SD or medians (interquartile range). Significant values for $p < 0.05$. NS = Non significant value.
HOMA-IR = Homeostasis Model Assessment of Insulin Resistance; cIMT = carotid intima–media thickness.
[a] Repeated-measures ANOVA.
[b] Values are log transformed.

Correlations and linear regression analysis

In order to detect any association between cIMT and main anthropometric and metabolic parameters, a Pearson correlation was performed. cIMT directly correlated with BMI-

Table 2 Clinical and biochemical characteristics of hypercholesterolaemic children during the intervention period.

	Baseline ($n = 36$)	After 6 months ($n = 36$)	After 12 months ($n = 36$)	p[a]
Anthropometric parameters				
Age (years)	7.5 ± 2.3	8.1 ± 2.3	8.7 ± 2.3	<0.001
Pubertal stage	1	1	1	
Height (cm)	123.3 ± 15.7	127.6 ± 15.2	131.7 ± 14.7	<0.001
Height-SDS	0.16 ± 1.69	0.26 ± 1.41	0.38 ± 1.42	NS
Weight (kg)	32.3 ± 14.1	34.4 ± 14.2	36.5 ± 14.7	<0.001
BMI-SDS	2.88 ± 2.88	2.81 ± 2.39	2.68 ± 2.56	0.04
Fat mass (%)	27.7 ± 6.3	26.3 ± 6.2	26.0 ± 6.2	0.001
SBP (mmHg)	101 ± 8	102 ± 8	104 ± 7	0.02
DBP (mmHg)	61 ± 8	61 ± 10	62 ± 13	NS
Lipid profile				
Total cholesterol (mg/dl)	260.6 ± 51.2	237.2 ± 49.5	235.1 ± 48.0	<0.001
HDL-cholesterol (mg/dl)	53.0 ± 15.6	56.7 ± 16.1	59.7 ± 18.5	0.04
LDL-cholesterol (mg/dl)	182.0 ± 53.8	159.2 ± 47.9	160.3 ± 52.2	<0.001
TG (mg/dl)[b]	84 [47–127]	73 [60–94]	72 [53–104]	NS
Apo A1 (mg/dl)	155.4 ± 28.5	162.0 ± 26.7	171.6 ± 32.5	<0.001
Apo B (mg/dl)	136.5 ± 42.9	127.0 ± 28.3	117.5 ± 40.9	NS
Apo A1/Apo B	1.28 ± 0.60	1.30 ± 0.62	1.53 ± 0.68	0.02

Data are means ± SD or medians (interquartile range). Significant values for $p < 0.05$. NS = Non significant value.
SDS = SD score; BMI = body mass index; SBP = systolic blood pressure; DBP = diastolic blood pressure; TG = triglycerides.
[a] Repeated-measures ANOVA.
[b] Values are log transformed.

Table 4 Biochemical characteristics of obese and normal-weight hypercholesterolaemic children during the intervention period.

	Obese hypercholesterolaemic children ($n = 20$)			Normal-weight hypercholesterolaemic children ($n = 16$)		
	Baseline	After 12 months	p[a]	Baseline	After 12 months	p[a]
Lipid profile						
Total cholesterol (mg/dl)	267.4 ± 51.6	242.9 ± 45.9	<0.001	252.2 ± 50.9	225.4 ± 50.4	<0.001
HDL-cholesterol (mg/dl)	53.6 ± 19.6	55.1 ± 13.8	NS	52.3 ± 9.1	65.6 ± 22.2	NS
LDL-cholesterol (mg/dl)	186.9 ± 57.8	167.8 ± 47.2	<0.001	175.9 ± 49.6	150.9 ± 58.0	0.003
TG (mg/dl)[b]	112.0 [78—164]	96.5 [75—120]	NS	47.5 [44.5—95.5]	57.5 [46—68.7]	NS
Apo A1 (mg/dl)	158.1 ± 33.9	158.3 ± 25.3	NS	152.1 ± 20.6	185.0 ± 34.4	0.002
Apo B (mg/dl)	149.6 ± 47.2	122.5 ± 23.0	0.01	120.2 ± 30.9	112.5 ± 54.0	NS
Apo A1/Apo B	1.24 ± 0.74	1.30 ± 0.53	0.01	1.33 ± 0.37	1.75 ± 0.76	NS
Insulin resistance						
Insulin (μU/mL)[b]	8.5 [5.4—11]	8.0 [7.4—11.8]	NS	5.9 [4.5—11.3]	5.8 [5.4—8]	NS
HOMA-IR[b]	1.88 [1.1—2.5]	1.73 [1.4—2.1]	0.02	1.27 [1.0—2.6]	1.37 [0.9—1.7]	NS
cIMT						
Right cIMT (mm)	0.36 ± 0.05	0.33 ± 0.04	NS	0.36 ± 0.04	0.31 ± 0.03	0.002
Left cIMT (mm)	0.39 ± 0.07	0.33 ± 0.04	0.02	0.39 ± 0.07	0.32 ± 0.02	<0.001
Mean cIMT (mm)	0.37 ± 0.05	0.33 ± 0.04	0.02	0.38 ± 0.03	0.32 ± 0.02	<0.001

Data are means ± SD or medians (interquartile range). Significant values for $p < 0.05$. NS = Non significant value.
TG = triglycerides; HOMA-IR = Homeostasis Model Assessment of Insulin Resistance; cIMT = carotid intima—media thickness.
[a] Repeated-measures ANOVA.
[b] Values are log transformed.

SDS ($p = 0.009$, $\beta = 0.33$), LDL-C ($p < 0.001$, $\beta = 0.55$) and HOMA-IR ($p < 0.001$, $\beta = 0.56$). No associations were found between cIMT and other variables of interest.

A linear regression analysis was performed to evaluate the potential influence of changes in BMI-SDS and LDL-C on the delta mean of cIMT during the longitudinal intervention study. In hypercholesterolaemic children delta BMI-SDS and delta LDL-C were significantly and independently related to the delta mean of cIMT (Table 5).

Furthermore, to avoid the effect of obesity, a regression analysis was performed in normal-weight and obese hypercholesterolaemic children separately. In normal-weight children, delta LDL-C ($p = 0.03$, $\beta = 0.496$) was significantly correlated with the delta mean of cIMT, while no association was found between delta BMI-SDS and the delta mean of cIMT. In obese hypercholesterolaemic children a significant and independent association was found between delta mean of cIMT and delta BMI-SDS ($p = 0.04$, $\beta = 0.272$) and delta LDL-C ($p = 0.007$, $\beta = 0.352$).

Table 5 Multiple stepwise linear regression: correlation between delta mean carotid intima—media thickness (mm) and other main parameters within obese ($n = 20$) and normal-weight ($n = 16$) hypercholesterolaemic children after diet intervention.

	β coefficient	p
Delta BMI-SDS	0.748	0.003
Delta LDL-cholesterol (mg/dl)	0.496	0.03

Significant values for $p < 0.05$.
SDS = SD score; BMI = body mass index.

Discussion

To the best of our knowledge, in this study we demonstrated for the first time that a 12-month Mediterranean diet was able to improve the lipid profile, which in turn was associated with a significant reduction of cIMT values in a group of hypercholesterolaemic pre-pubertal children.

Dyslipidaemias are disorders of lipoprotein metabolism that lead to an excess of TC, LDL-C or TG and/or to a deficiency of HDL-C. It has clearly been demonstrated that these disorders have negative effects on the risk of cardiovascular diseases [27].

In our study, cIMT was significantly higher in children with hypercholesterolaemia compared with control subjects, even if the difference between these two groups was not extremely wide. These results are in agreement with previous studies showing strong evidence for significantly higher cIMT in children with dyslipidaemia compared with healthy populations, suggesting that unfavourable vascular changes occur early in life [3,4].

The American Academy of Paediatrics guidelines recommend an early diagnosis and treatment of children affected by lipid abnormalities to reduce the risk of heart diseases in this age group [27]. In this respect, the AHA developed practical recommendations for management of high-risk paediatric populations, including therapeutic lifestyle changes with dietetic guidelines: total fat <30% of calories, saturated fat <10% of calories, cholesterol <300 mg day^{-1}, avoidance of trans fats and adequate calories for growth [28]. Similarly to these approaches, the Mediterranean diet represents a nutritional model characterised by low amounts of saturated fat and high levels of monounsaturated fat and antioxidants. This diet has been demonstrated to improve the lipid profile with beneficial

effects on the cardiovascular system during adolescence [14—16]. Of note, in a recent study the lipid profile improved after a structured dietician training to a Mediterranean-style diet in children and adolescents with type 1 diabetes [29].

According to these studies, we found that a 12-month Mediterranean diet was able to reduce TC and LDL-C levels by approximately 10%. Although this reduction could represent a small change in lipids levels, the children included in our study represent a pure and low-risk population. In fact, in our study cohort relevant confounding factors (diabetes, familial lipid disorders, smoking and alcohol) were excluded as it is well known that they strongly influence changes in cIMT in childhood and adulthood. Therefore, in the absence of these confounding factors, it could be supposed that relatively small changes in lipid profile could be still able to induce significant changes in cIMT in pre-pubertal children.

Hypercholesterolaemia can induce early structural changes of the arterial wall leading to an increased cIMT. A direct correlation between cIMT and the atherosclerotic process has been found even during childhood [3,30]. Wiegman et al. [31] reported that a 2-year statin therapy was able to induce a significant regression of carotid atherosclerosis in children with familial hypercholesterolaemia. However, lifestyle and dietary intervention represent the first approaches for management of newly diagnosed children with dyslipidaemia. Interestingly, in our study after a 12-month Mediterranean diet we found a significant reduction of cIMT, documenting the effectiveness of this diet in positively influencing cIMT in hypercholesterolaemic children.

Of note, in our study population IR was significantly reduced after 12 months of Mediterranean diet. This result is consistent with recent findings in adults. In fact, a previous study in IR adults has shown that the Mediterranean diet is able to improve insulin sensitivity [32], with an inverse association between the overall score of adherence to the Mediterranean diet and high HOMA-IR [33]. Interestingly, a recent study has reported that maternal diets with low Mediterranean-diet adherence score are associated with high IR markers at birth [34]. These important results have been supposed to be related to the effect of the Mediterranean diet on glucose metabolism.

In addition to the effects on reduction of cIMT and improvement of lipid profile, we documented in hypercholesterolaemic children a significant reduction of BMI-SDS. Furthermore, at the end of the intervention period, we found an independent association between the variation of lipid profile, BMI-SDS and cIMT. Therefore, to avoid the influence of obesity, we performed a sub-analysis comparing obese to normal-weight hypercholesterolaemic children. Of note, obese children demonstrated that both lipid profile and BMI-SDS influenced the reduction of cIMT, whereas in normal-weight children we could not find any association between BMI-SDS and cIMT. These findings suggest that not only the reduction of BMI-SDS but also especially the improvement of lipid profile represent useful tools in the prevention of cardiovascular risk in pre-pubertal age. It has to be noted that hypercholesterolaemic children grew regularly, confirming that the Mediterranean diet is safe and does not interfere with linear growth [35].

Although it might be argued that a major limitation of the present study is related to evaluation of cIMT by high-resolution B-mode ultrasonography [36], at the moment this technique represents the most feasible, noninvasive and reliable method for the paediatric age group. On the other hand, as cIMT can be influenced by puberty [37] or can represent a non-atherosclerotic adaptive response to changes in shear and tensile stress, it has to be acknowledged that the selection of pre-pubertal children significantly reduced the influence of confounding factors. Another limitation of this study could be represented by the possible influence of daily physical activity on the obtained results. In fact, although an important inclusion criterion was that none of the children was involved in regular and programmed physical activity, we were not able to avoid the influence of the physical activity performed during school time. However, it has to be acknowledged that the school exercise programme included only 2 h a week, therefore having a minimal influence on the study results.

A further limitation of our study is the lack of a control group of hypercholesterolaemic children taking a non-Mediterranean diet in order to exclude whether the metabolic and vascular changes were just related to the adherence to a well-structured diet or to the Mediterranean diet.

In conclusion, our study demonstrates that the Mediterranean diet represents an effective nutritional intervention for the reduction of cardiovascular risk even in very young hypercholesterolaemic children. Therefore, paediatricians should be highly encouraged to adopt and promote this dietary approach to prevent cardiovascular diseases later in life.

Conflict of interest

None.

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