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Associations between *a priori*-defined dietary patterns and longitudinal changes in bone mineral density in adolescents

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Abstract

Objective: To quantify short- and long-term associations between dietary patterns defined *a priori* and bone mineral density (BMD) during adolescence.
Design: Dietary patterns were defined at 13 years old using a Mediterranean diet (MD) quality index, the Dietary Approaches to Stop Hypertension (DASH) diet index and the Oslo Health Study (OHS) dietary index. Linear regression coefficients were used to estimate associations between dietary patterns and forearm BMD at 13 and 17 years, measured by dual-energy X-ray absorptiometry.
Setting: Public and private schools of Porto, Portugal.
Subjects: The EPITeen cohort comprising 1180 adolescents born in 1990, recruited at schools during the 2003/2004 school year and re-evaluated in 2007/2008.
Results: In girls, at 13 years, mean BMD (g/cm^2) in the first and third tertiles was 0·369 and 0·368 for the MD pattern, 0·368 and 0·369 for the DASH diet, and 0·370 and 0·363 for the OHS index. In boys, mean BMD (g/cm^2) in the first and third tertiles was 0·338 and 0·347 for the MD pattern, 0·342 and 0·346 for the DASH diet, and 0·344 and 0·342 for the OHS index. None of these differences were significant. Mean BMD at 17 years and prospective variation were also not significantly different between tertiles of adherence to each score. However, a trend of increased BMD at 13 years with greater adherence to the MD pattern was observed in boys (adjusted coefficient = 0·248; 95 % CI 0·052, 0·444).
Conclusions: The selected dietary patterns may not capture truly important dietary differences in determining BMD or diet may not be, beyond nutrient adequacy, a limiting determinant of BMD.

Keywords
Dietary patterns
Bone density
Cohort study
Adolescence

Osteoporotic fractures are a major cause of morbidity and mortality whose burden is expected to increase in the future[1,2]. Research has been focused on understanding the mechanisms of age-related bone loss, but growing evidence emphasizes the influence of early-life factors in the attainment of adult bone mass[3,4]. It has been suggested that high peak bone mass protects against fragility fractures later in life[3–5]. Diet is known to modulate achievement of the full genetic potential for skeletal mass during adolescence[4,6] but research has typically examined the effects on bone mineral density (BMD) of single nutrients or foods (e.g. Ca, P, vitamin D, vitamin K and protein) or food groups such as dairy products, fruit and vegetables[7–9]. However, meals consist of a variety of foods with complex combinations of nutrients that are likely to be interactive and many foods and nutrients are highly correlated, making it difficult to separate their effects. Effects of single nutrients may be too subtle to detect with the available measurement instruments, but combined effects as expressed in a dietary pattern may be sufficiently large.

Dietary patterns analysis is a more comprehensive approach to dietary exposure assessment and to examine its relationship with bone health[10,11]. Dietary choices in epidemiological studies may be assessed through compliance with dietary patterns defined *a priori* based on presumed health effects[12]. Indices to measure adherence to the Mediterranean diet (MD) have been used to explore the risk of obesity, CVD and cancer[13]. Although some components of this pattern have been associated with bone quality in children[14], the overall effect of adherence to this type of diet on bone accrual remains unknown.

The Dietary Approaches to Stop Hypertension (DASH) diet is a Ca-rich diet that emphasizes fruits, vegetables and low-fat dairy products[15–17]. Although originally designed for blood pressure reduction, several aspects of this pattern may benefit bone such as the rich Ca, K and Mg contents[18].

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MD and DASH do not specifically assess foods with a possible negative effect on bone quality. Such information is of major interest and can be obtained with the Oslo Health Study (OHS) dietary index built on prevailing knowledge about foods that potentially influence bone health. The OHS is computed as the ratio between soft drinks and fruit and vegetables consumption and has been directly associated with metabolic syndrome and inversely associated with BMD[19,20].

In addition to being a critical period in the acquisition of bone mass[5], adolescence is also a period of social and psychological influences on food choices[21].

To the best of our knowledge, there are no studies examining the association between *a priori*-defined dietary patterns and BMD in adolescents. By using prospective data from a population-based cohort, our objective was to quantify the associations between forearm BMD in early and late adolescence and adherence to *a priori*-defined dietary patterns in early adolescence, using the Mediterranean Diet Quality Index for children and adolescents (KIDMED), the DASH diet index and the OHS dietary index.

Experimental methods

The EPITeen cohort was assembled during the 2003/2004 school year, when all public and private schools in Porto that provided teaching to adolescents born in 1990 were approached. Nineteen private and all public schools (79·2 % *v.* 100 %, $P=0·04$) agreed to participate in the study. Executive boards were asked to provide contact information for each student's family. Parents received written explanation of the purpose and the study and meetings were arranged in each school in order to explain research aims and procedures. We identified 2787 eligible adolescent students, of whom 2160 (1044 boys) agreed to participate and provided information for at least part of the protocol (77·5 % participation at the individual level). Similar participation proportions were obtained in public and private schools. The follow-up evaluation of the cohort took place in the 2007/2008 school year and 1716 participants were successfully re-evaluated (79·4 %). Sampling procedures and detailed methods have been described elsewhere[22]. The study was approved by the Ethics Committee of the University Hospital of São João, Porto and policies and procedures were developed to guarantee data confidentiality and protection. Written informed consent was obtained from both parents and adolescents.

Physical examination

In both evaluations, forearm BMD (g/cm^2) was measured at the distal radius of the non-dominant forearm by dual-energy X-ray absorptiometry (DXA) using a Lunar® PIXI device (GE Medical Systems, Madison, WI, USA). In cases of reported previous fracture of the non-dominant arm, the dominant arm was assessed. Anthropometry was obtained while the student stood barefoot in light indoor clothing. Weight was measured using a digital scale to the nearest tenth of a kilogram (Tanita Corporation, Tokyo, Japan) and height was measured in centimetres, to the nearest tenth, using a portable stadiometer (Seca Deutschland, Hamburg, Germany); BMI was calculated.

Home questionnaires variables

At 13 years of age, dietary intake was evaluated using an FFQ designed according to Willett *et al.*[23] and adapted for the Portuguese population and previously validated in Porto adults[24,25]. The FFQ was adapted for adolescents through the addition of foods more frequently eaten by this age group[26,27]. The FFQ comprised ninety-one food items and an open-ended section to add items not listed in the questionnaire but eaten at least once per week. Participants were asked to report, over the prior 12-month period, the frequency of consumption of each food in one of nine categories, ranging from 'never or less than once per month' to 'six or more times per day'. The FFQ did not include specific questions on portion size and the frequency reported was multiplied by a standard portion size to estimate the average daily intake. Seasonal variation of food consumption was considered by multiplying the frequency and portion of seasonal items by a seasonal factor of 0·25 (i.e. equivalent to consumption during a 3-month period). Food consumption was converted into energy and nutrient intakes with the software program Food Processor Plus (1997; ESHA Research, Salem, OR, USA) based on values from the US Department of Agriculture and which was updated with values for typical Portuguese foods[24].

Information on regular practice of physical activity at 13 years was quantified through the frequency spent in sport activities of at least twenty consecutive minutes beyond compulsory school activities. Adolescents were classified according to parental educational level measured as the number of successfully completed years of formal schooling of the parent with higher education. Since examination at school limited conditions to evaluate adolescents' pubertal development according to Tanner stages[28], age at menarche referred at 13 years of age was recorded as a pubertal development indicator for girls.

Data analysis

The degree of adherence to the MD pattern was measured using the Mediterranean Diet Quality Index for children and adolescents (KIDMED)[29]; adherence to the DASH diet was evaluated through a DASH diet index score proposed by Fung and colleagues[30]; and the total score of the OHS dietary index[20] was calculated.

The original KIDMED includes sixteen components. Items denoting a negative connotation with respect to the MD are assigned a value of −1, and those with a positive

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aspect are assigned +1. This index assesses the daily consumption of at least one serving of fruit and vegetables, rating higher when consumption is greater. Weekly consumption of at least two to three servings of nuts and fish and of more than one serving of pulses is evaluated. It also assesses the consumption of pasta or rice at least five times weekly; grains or cereals, daily for breakfast; dairy products, three servings daily; and olive oil for culinary use. Items denoting a negative connotation with respect to the MD include frequent intake of sweets, candy, commercially baked goods, pastries, fast foods, and non-consumption of breakfast[29]. The higher the KIDMED score, the more Mediterranean and favourable is the dietary pattern. We eliminated the items regarding breakfast since our dietary evaluation was based on an FFQ. Excellent agreement ($\kappa = 0{\cdot}86$) between the original KIDMED and the adapted version was found in a different sample of 117 adolescents aged 13 years.

We used a DASH score awarding points for high intake of fruit, vegetables, nuts, legumes, dairy products and whole grains according to quintile rankings (i.e. participants in the lowest quintile were assigned 1 point and those in the highest quintile were assigned 5 points). Regarding the intake of Na, red and processed meat, and sweetened beverages, participants in lower quintiles of intake scored higher[30]. Higher scores reflect higher compliance with DASH characteristics.

The OHS dietary index was constructed based upon the literature about foods that potentially influence Ca balance or acid load, and consequently bone health. It is a ratio between the intake of soft drinks (negative for bone health owing to its role in acid load increase and promotion of urinary loss of Ca) and the intake of fruit/berries, fruit juice and cooked and raw vegetables (positive for bone owing its role in promotion of Ca absorption and reduction of Ca loss)[20]. Thus, it is expected that higher scores on this index, reflecting unhealthier patterns, are negatively associated with BMD. Our index was based on the sum of intake frequency categories of three items (range 3–27): 'colas', 'ice tea soft drinks' and 'other soft drinks', divided by the sum of intake frequency categories of three items (range 3–27): 'vegetables' (fresh or cooked), 'vegetable soup' and 'fruit' (fresh, canned or juice). This adaptation was due to differences in the FFQ used.

Categorical and continuous variables were summarized as percentages and as means and standard deviations, respectively, by tertile of the final KIDMED, DASH diet index and OHS dietary index scores, separately for boys and girls. Differences in proportions were tested with the χ^2 test. One-way ANOVA and Kruskal–Wallis one-way ANOVA were used to compare continuous variables between independent samples.

Using adherence to the MD dietary pattern, to the DASH diet and to the OHS dietary index as the main exposures and forearm BMD as the outcome, associations were estimated cross-sectionally (KIDMED score_{13} *v.* BMD_{13}, DASH diet score_{13} *v.* BMD_{13} and OHS dietary index score_{13} *v.* BMD_{13}) and prospectively (KIDMED score_{13} *v.* ΔBMD, DASH diet score_{13} *v.* ΔBMD, OHS dietary index score_{13} *v.* ΔBMD, KIDMED score_{13} *v.* BMD_{17}, DASH diet score_{13} *v.* BMD_{17} and OHS dietary index score_{13} *v.* BMD_{17}), using linear regression coefficients and 95 % confidence intervals. BMD was used in mg/cm^2 to improve readability. In addition to crude estimates, linear regression coefficients were also adjusted for height and weight at 13 years and at 17 years old, total energy intake at 13 years old, parental educational level, regular practice of physical activity and mean Ca intake. In girls, coefficients were further adjusted to menarche age to address confounding by pubertal status.

Sample description

From a total of 2160 adolescents who participated in the first evaluation, 1264 (591 boys) were included in the cross-sectional analysis (complete information on forearm BMD, anthropometric variables, parental educational level, age at menarche and FFQ). Of those 1264 adolescents, 1075 (85·0 %) participated in the follow-up evaluation. The final sample in the longitudinal analyses included 1023 adolescents (474 boys) after exclusion of participants with missing information in the follow-up evaluation (Fig. 1).

When participants with and without missing information for any of the above-mentioned variables in the baseline evaluation were compared (896 *v.* 1264 participants, respectively), we found no differences in female proportion, in BMD and in BMI means at 13 years. However, adolescents with missing information had lower median (25th percentile, 75th percentile (P_{25}, P_{75})) parental educational level (9 (6, 12) years *v.* 11 (7, 16) years, respectively; $P < 0{\cdot}001$).

No significant differences in female proportion, in BMD and in BMI means at 13 years were found between participants with complete information in both baseline and follow-up evaluations and participants with missing information (1023 with complete information *v.* 896 with missing information in the baseline evaluation + 189 lost to follow-up + fifty-two with missing information in the follow-up evaluation). However, adolescents with complete information had higher median (P_{25}, P_{75}) parental educational level (12 (8, 16) years *v.* 9 (6, 12) years, $P < 0{\cdot}001$).

Results

Mean forearm BMD increased from 0·360 (SD 0·057) g/cm^2 at 13 years to 0·435 (SD 0·052) g/cm^2 at 17 years in girls and from 0·342 (SD 0·050) to 0·452 (SD 0·075) g/cm^2 in boys.

There were no statistically significant differences in mean KIDMED and DASH diet scores by gender (KIDMED score: 5·2 (SD 2·0) for boys, 5·1 (SD 2·1) for girls,

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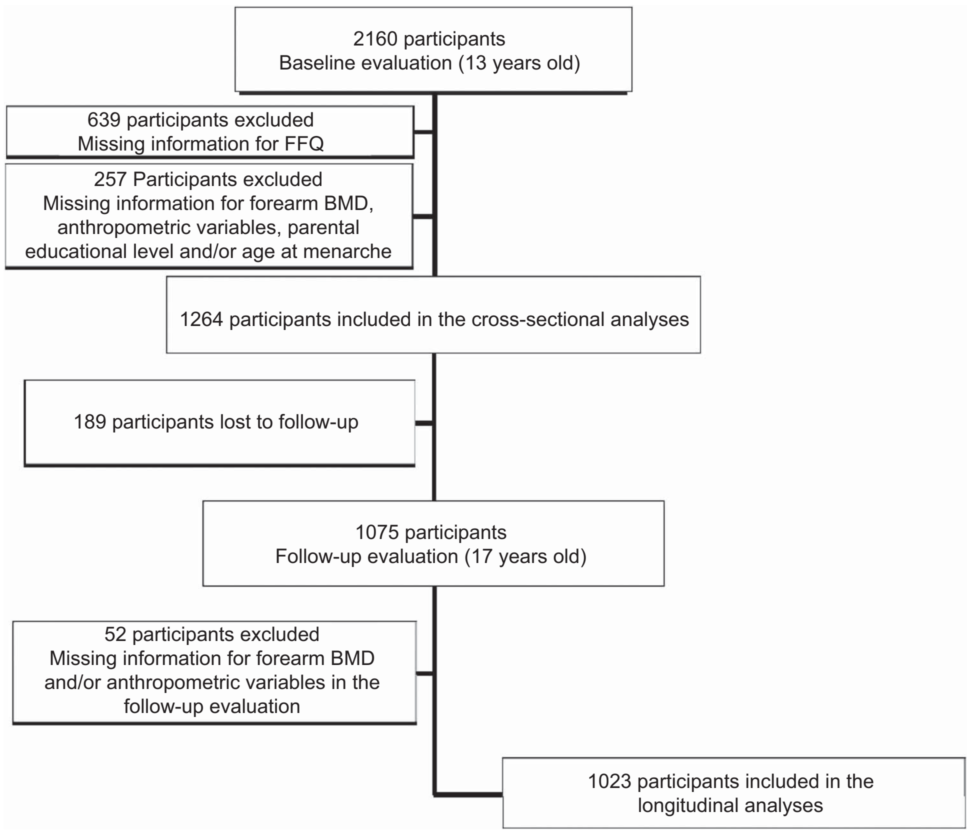

Fig. 1 Flowchart of the included and excluded participants in the cross-sectional and longitudinal analyses (BMD, bone mineral density)

$P=0{\cdot}410$; DASH diet score: 23·7 (SD 4·5) for boys, 23·7 (SD 4·5) for girls, $P=0{\cdot}908$). The mean OHS dietary index score was significantly higher in boys than in girls (1·1 (SD 0·6) *v.* 1·0 (SD 0·5), respectively, $P=0{\cdot}005$).

Average values of anthropometric, sociodemographic and behavioural characteristics in the baseline evaluation, stratified according to tertile of each *a priori* dietary pattern, are presented in Table 1 for girls and boys. Proportional distributions are presented for categorical variables.

We observed that adolescent girls and boys with healthier dietary patterns, i.e. whose dietary patterns were more similar to the MD pattern, to the DASH diet and who had lower scores in the OHS dietary index, were significantly more likely to have parents with higher education (all $P<0{\cdot}001$).

For both sexes, adolescents with higher adherence to the MD pattern, with higher adherence to the DASH diet and with lower OHS dietary index scores tended to practise physical activity more frequently. Significantly higher frequency of physical activity was observed in girls when adherence to the MD pattern ($P=0{\cdot}008$) and the DASH diet ($P=0{\cdot}032$) was higher and in boys when OHS dietary index score was lower ($P=0{\cdot}038$).

Although adolescents in the highest tertile of adherence to the MD pattern, as compared with those in the first and second tertiles of adherence, had significantly higher energy intake ($P<0{\cdot}001$), these adolescents had no statistically significant differences in BMI in the baseline evaluation. We also found no meaningful differences between tertiles of adherence to the MD pattern with regard to mean BMI at 17 years in the follow-up evaluation (data not shown).

As in the baseline evaluation (Table 1), we also found no significant differences in mean follow-up BMI by tertile of adherence to the DASH diet and of OHS dietary index score among males and by tertile of OHS dietary index score among females (data not shown). Although significant differences in girls' mean follow-up BMI by tertile of adherence to the DASH diet have been observed (first tertile: 21·7 (SD 3·4); second tertile: 22·6 (SD 3·5); third tertile: 21·9 (SD 3·2); $P=0{\cdot}027$), a dose–response relationship was not present.

Table 2 shows the average baseline and follow-up BMD for each of the *a priori* dietary patterns in tertiles. We observed no statistically significant differences in mean BMD, measured in the baseline and in the follow-up evaluations, or in BMD change by tertile of adherence to the *a priori*-defined dietary patterns.

Table 3 summarizes the cross-sectional and prospective associations between adherence to the MD pattern, to the DASH diet and to the OHS dietary index and BMD in girls and boys, by presenting crude and adjusted linear

Table 1 Description of average BMI, regular physical activity, parental educational level and total energy intake at 13 years old (baseline evaluation, 2003/2004) according to tertile of adherence to the Mediterranean diet (MD) pattern, to the Dietary Approaches to Stop Hypertension (DASH) diet and to the Oslo Health Study (OHS) dietary index among Portuguese girls and boys, EPITeen cohort

Girls (13 years old)

	MD pattern							DASH diet							OHS dietary index						
	1st tertile (*n* 247)		2nd tertile (*n* 232)		3rd tertile (*n* 194)			1st tertile (*n* 272)		2nd tertile (*n* 204)		3rd tertile (*n* 197)			3rd tertile (*n* 226)		2nd tertile (*n* 224)		1st tertile (*n* 223)		
	Mean or *n*	SD or %	Mean or *n*	SD or %	Mean or *n*	SD or %	*P*	Mean or *n*	SD or %	Mean or *n*	SD or %	Mean or *n*	SD or %	*P*	Mean or *n*	SD or %	Mean or *n*	SD or %	Mean or *n*	SD or %	*P*
Median score (P_{25}, P_{75})	3·0	2·0, 4·0	5·0	5·0, 6·0	7·0	7·0, 8·0		20·0	18·0, 21·0	24·0	23·0, 25·0	29·0	28·0, 30·0		1·4	1·2, 1·8	0·9	0·8, 1·0	0·6	0·4, 0·7	
BMI (kg/m^2)	21·3	3·6	21·2	3·6	21·0	3·4	0·566	21·0	3·6	21·6	3·9	20·9	3·3	0·131	21·1	3·7	21·1	3·7	21·3	3·4	0·773
Weight (kg)	53·3	10·4	52·8	10·2	53·5	10·0	0·767	52·4	10·3	54·1	10·8	53·2	9·4	0·193	52·2	10·4	53·6	10·7	53·7	9·5	0·243
Height (cm)	158·5	5·9	158·6	6·0	159·9	5·7	0·007	157·5	6·5	158·2	6·3	159·4	5·9	0·004	157·0	6·3	159·2	6·8	158·6	5·6	<0·001
Parental educational level (*n*, %)																					
≤6 years	80	32·4	59	25·4	26	13·4		87	32·0	49	24·0	29	14·7		71	31·4	53	23·7	41	18·4	
7–9 years	64	25·9	50	21·6	29	15·0		64	23·5	39	19·1	40	20·3		52	23·0	54	24·1	37	16·6	
10–12 years	67	27·1	58	25·0	54	27·8		70	27·7	59	28·9	50	25·4		68	30·1	52	23·2	59	26·5	
>12 years	36	14·6	65	28·0	85	43·8	<0·001	51	18·8	57	27·9	78	39·6	<0·001	35	15·5	65	29·0	86	38·6	<0·001
Physical activity (*n*, %)																					
Never	73	29·6	64	27·6	34	17·5		83	30·5	52	25·5	36	18·3		64	28·3	57	25·4	50	22·4	
≤1 time/week	74	30·0	66	28·4	45	23·2		74	27·2	63	30·9	48	24·4		61	27·0	68	30·4	56	25·1	
2–3 times/week	68	27·5	72	31·0	73	37·6		77	28·3	59	28·9	77	39·1		69	30·5	76	33·9	68	30·5	
>3 times/week	27	10·9	27	11·6	39	20·1		32	11·8	27	13·2	34	17·3		27	12·0	21	9·4	45	20·2	
Missing information	5	2·0	3	1·3	3	1·6	0·008	6	2·2	3	1·5	2	1·0	0·032	5	2·2	2	0·9	4	1·8	0·063
Total energy intake (kJ/d)	9460	3376	10 280	3121	11 347	2611	<0·001	10 280	3255	10 276	3481	10 309	2694	0·994	10 648	3540	10 251	3155	9958	2741	0·059
Total energy intake (kcal/d)	2261	807	2457	746	2712	624	<0·001	2457	778	2456	832	2464	644	0·994	2545	846	2450	754	2380	655	0·059
Ca intake (mg/d)	956	431	1113	397	1316	399	<0·001	990	436	1107	411	1291	396	<0·001	1067	456	1084	431	1191	407	0·005
Ca intake (mg/4184 kJ (1000 kcal))	433	160	465	140	491	124	<0·001	407	137	463	134	534	135	<0·001	430	155	446	128	508	139	<0·001

Boys (13 years old)

	MD pattern							DASH diet							OHS dietary index						
	1st tertile (*n* 219)		2nd tertile (*n* 186)		3rd tertile (*n* 186)			1st tertile (*n* 238)		2nd tertile (*n* 192)		3rd tertile (*n* 161)			3rd tertile (*n* 196)		2nd tertile (*n* 199)		1st tertile (*n* 196)		
	Mean or *n*	SD or %	Mean or *n*	SD or %	Mean or *n*	SD or %	*P*	Mean or *n*	SD or %	Mean or *n*	SD or %	Mean or *n*	SD or %	*P*	Mean or *n*	SD or %	Mean or *n*	SD or %	Mean or *n*	SD or %	*P*
Median score (P_{25}, P_{75})	3·0	2·0, 4·0	6·0	5·0, 6·0	7·0	7·0, 8·0		20·0	18·0, 21·0	25·0	23·0, 26·0	29·0	27·0, 30·0		1·6	1.4, 1·9	1·0	0·9, 1·1	0·6	0·5, 0·7	
BMI (kg/m^2)	21·0	3·6	20·7	3·7	20·6	3·3	0·619	20·8	3·5	20·7	3·7	20·8	3·3	0·945	21·1	3·4	20·5	3·6	20·7	3·6	0·254
Weight (kg)	55·5	11·8	54·7	12·8	55·1	11·3	0·812	55·2	11·8	54·6	12·5	55·6	11·5	0·746	56·4	11·6	53·5	12·1	55·5	12·0	0·046
Height (cm)	162·2	7·6	161·9	8·6	163·0	7·9	0·385	162·5	7·9	161·8	8·0	162·8	8·3	0·452	163·2	7·7	161·0	8·3	163·0	7·9	0·010
Parents' years of schooling (*n*, %)																					
≤6 years	53	24·2	40	21·5	17	9·1		56	23·5	34	17·7	20	12.4		50	25·5	41	20·6	19	9·7	
7–9 years	53	24·2	42	22·6	22	11·8		61	25·6	37	19·3	19	11·8		50	25·5	39	19·6	28	14·3	
10–12 years	64	29·2	52	28·0	58	31·2		65	27·3	57	29·7	52	32·3		59	30·1	56	28·1	59	30·1	
>12 years	49	22·4	52	28·0	89	47·8	<0·001	56	23·5	64	33·3	70	43·5	<0·001	37	18·9	63	31·7	90	45·9	<0·001
Physical activity (*n*, %)																					
Never	22	10·0	19	10·2	16	8·6		27	11·3	18	9·4	12	7·4		18	9·2	25	12·6	14	7·1	
≤1 time/week	42	19·2	26	14·0	28	15·0		42	17·6	32	16·7	22	13·7		32	16·3	32	16·1	32	16·3	
2–3 times/week	60	27·4	66	35·5	65	35·0		66	27·7	68	35·4	57	35·4		55	28·1	64	32·2	72	36·7	
>3 times/week	84	38·4	73	39·2	69	37·1		94	39·5	72	37·5	60	37·3		83	42·4	77	38·7	66	33·7	
Missing information	11	5·0	2	1·1	8	4·3	0·280	9	3·8	2	1·0	10	6·2	0·156	8	4·1	1	0·5	12	6·1	0·038
Total energy intake (kJ/d)	9761	3330	10 452	264	11 757	2615	<0·001	10 594	3167	10 862	3163	10 326	2653	0·259	10 778	3222	10 698	3059	10 343	2807	0·314
Total energy intake (kcal/d)	2333	796	2498	644	2810	625	<0·001	2532	757	2596	756	2468	634	0·259	2576	770	2557	731	2472	671	0·314
Ca intake (mg/d)	993	405	1119	360	1364	407	<0·001	1009	382	1201	422	1295	414	<0·001	1077	419	1152	413	1219	420	0·004
Ca intake (mg/4184 kJ (1000 kcal))	435	139	458	126	488	112	<0·001	404	114	468	119	528	123	<0·001	425	133	455	117	497	125	<0·001

P_{25}, 25th percentile; P_{75}, 75th percentile.

Table 2 Bone mineral density (BMD) at 13 years old (baseline evaluation, 2003/2004) and at 17 years old (first follow-up evaluation, 2007/2008) according to tertile of adherence to the Mediterranean diet (MD) pattern, to the Dietary Approaches to Stop Hypertension (DASH) diet and to the Oslo Health Study (OHS) dietary index in Portuguese girls and boys, EPITeen cohort

	Girls (13 years old)																		
	MD pattern							DASH diet							OHS dietary index					
	1st tertile (*n* 247)		2nd tertile (*n* 232)		3rd tertile (*n* 194)			1st tertile (*n* 272)		2nd tertile (*n* 204)		3rd tertile (*n* 197)			3rd tertile (*n* 226)		2nd tertile (*n* 224)		1st tertile (*n* 223)		
	Mean	SD	Mean	SD	Mean	SD	*P*	Mean	SD	Mean	SD	Mean	SD	*P*	Mean	SD	Mean	SD	Mean	SD	*P*
Baseline BMD (g/cm^2)	0·360	0·059	0·361	0·055	0·361	0·060	0·996	0·359	0·059	0·361	0·057	0·362	0·056	0·811	0·355	0·054	0·365	0·059	0·361	0·059	0·179
	Girls (17 years old)																				
	MD pattern							DASH diet							OHS dietary index						
	1st tertile (*n* 192)		2nd tertile (*n* 189)		3rd tertile (*n* 168)			1st tertile (*n* 215)		2nd tertile (*n* 166)		3rd tertile (*n* 168)			3rd tertile (*n* 170)		2nd tertile (*n* 185)		1st tertile (*n* 194)		
	Mean	SD	Mean	SD	Mean	SD	*P*	Mean	SD	Mean	SD	Mean	SD	*P*	Mean	SD	Mean	SD	Mean	SD	*P*
Follow-up BMD (g/cm^2)	0·431	0·052	0·437	0·051	0·437	0·053	0·405	0·434	0·056	0·438	0·050	0·434	0·050	0·720	0·429	0·052	0·442	0·053	0·434	0·051	0·064
	Boys (13 years old)																				
	MD pattern							DASH diet							OHS dietary index						
	1st tertile (*n* 219)		2nd tertile (*n* 186)		3rd tertile (*n* 186)			1st tertile (*n* 238)		2nd tertile (*n* 192)		3rd tertile (*n* 161)			3rd tertile (*n* 196)		2nd tertile (*n* 199)		1st tertile (*n* 196)		
	Mean	SD	Mean	SD	Mean	SD	*P*	Mean	SD	Mean	SD	Mean	SD	*P*	Mean	SD	Mean	SD	Mean	SD	*P*
Baseline BMD (g/cm^2)	0·338	0·047	0·347	0·052	0·347	0·053	0·132	0·342	0·051	0·344	0·052	0·346	0·048	0·720	0·342	0·051	0·344	0·050	0·344	0·050	0·860
	Boys (17 years old)																				
	MD pattern							DASH diet							OHS dietary index						
	1st tertile (*n* 173)		2nd tertile (*n* 147)		3rd tertile (*n* 154)			1st tertile (*n* 187)		2nd tertile (*n* 154)		3rd tertile (*n* 133)			3rd tertile (*n* 151)		2nd tertile (*n* 168)		1st tertile (*n* 155)		
	Mean	SD	Mean	SD	Mean	SD	*P*	Mean	SD	Mean	SD	Mean	SD	*P*	Mean	SD	Mean	SD	Mean	SD	*P*
Follow-up BMD (g/cm^2)	0·450	0·074	0·448	0·075	0·458	0·076	0·510	0·455	0·074	0·452	0·079	0·448	0·072	0·679	0·456	0·076	0·451	0·076	0·450	0·074	0·748

Table 3 Linear regression coefficients (95 % confidence intervals) for the cross-sectional and prospective associations between adherence to the Mediterranean diet (MD) pattern, to the Dietary Approaches to Stop Hypertension (DASH) diet and to the Oslo Health Study (OHS) dietary index and bone mineral density (BMD) in mg/cm^2 among Portuguese girls and boys, EPITeen cohort

	Outcome: BMD_{13} (mg/cm^2)											
	MD pattern				DASH diet				OHS dietary index			
	Crude coefficient	95 % CI	Adjusted coefficient*	95 % CI	Crude coefficient	95 % CI	Adjusted coefficient*	95 % CI	Crude coefficient	95 % CI	Adjusted coefficient†	95 % CI
Girls	−0·010	−0·215, 0·194	−0·019	−0·191, 0·154	0·042	−0·055, 0·140	−0·005	−0·082, 0·072	−0·598	−1·406, 0·210	−0·293	−0·932, 0·347
Boys	0·192	−0·008, 0·392	0·248	0·052, 0·444	0·070	−0·020, 0·161	0·082	−0·002, 0·166	−0·266	−0·963, 0·431	−0·597	−1·278, 0·084
	Outcome: BMD_{17} (mg/cm^2)											
	MD pattern				DASH diet				OHS dietary index			
	Crude coefficient	95 % CI	Adjusted coefficient†	95 % CI	Crude coefficient	95 % CI	Adjusted coefficient†	95 % CI	Crude coefficient	95 % CI	Adjusted coefficient†	95 % CI
Girls	0·142	−0·062, 0·346	0·046	−0·158, 0·250	0·022	−0·077, 0·121	−0·004	−0·096, 0·089	−0·782	−1·604, 0·040	−0·538	−1·311, 0·235
Boys	0·061	−0·265, 0·388	0·158	−0·185, 0·501	−0·019	−0·170, 0·131	−0·001	−0·147, 0·146	0·821	−0·380, 2·022	0·405	−0·806, 1·617
	Outcome: annual BMD variation (mg/cm^2 per year)											
	MD pattern				DASH diet				OHS dietary index			
	Crude coefficient	95 % CI	Adjusted coefficient†	95 % CI	Crude coefficient	95 % CI	Adjusted coefficient†	95 % CI	Crude coefficient	95 % CI	Adjusted coefficient†	95 % CI
Girls	0·028	−0·025, 0·081	0·028	−0·025, 0·081	0·002	−0·023, 0·027	−0·002	−0·026, 0·022	−0·049	−0·256, 0·158	−0·039	−0·240, 0·162
Boys	−0·019	−0·091, 0·053	−0·012	−0·092, 0·068	−0·026	−0·059, 0·007	−0·026	−0·060, 0·008	0·254	−0·010, 0·518	0·204	−0·078, 0·487

BMD_{13}, bone mineral density (mg/cm^2) at 13 years old; BMD_{17}, bone mineral density (mg/cm^2) at 17 years old.
Further adjustment of the linear regression coefficients for regular practice of physical activity and mean Ca intake did not change the results appreciably (data not shown).
*Adjusted for height and weight at 13 years of age, total energy intake, parental educational level and, in girls, age at menarche.
†Adjusted for height and weight at 17 years of age, total energy intake, parental educational level and, in girls, age at menarche.

regression coefficients and 95 % confidence intervals. Both crude and adjusted linear regressions coefficients confirm the lack of clear associations between the *a priori* dietary patterns studied and BMD at 13 years and longitudinal changes in BMD at 17 years in this sample of adolescents. Among males, higher adherence to the MD pattern was significantly associated with higher BMD at 13 years ($P = 0{\cdot}013$) but not to its annual variation. Further adjustment of the linear regression coefficients for adolescents' regular practice of physical activity and mean Ca intake did not change the results appreciably (data not shown).

Discussion

In the present study we observed no significant difference in annual variation of BMD, between 13 and 17 years of age, by adherence to the different *a priori* dietary patterns. However, among boys, a significant linear trend towards increased BMD at 13 years with increasing adherence to the MD pattern was observed.

Little research has been done examining the relationship between *a priori*-defined dietary patterns and bone quality[20,31–34]. To our knowledge, the present study is the first one to explore both cross-sectional and prospective associations between *a priori*-defined dietary patterns and BMD during the adolescent growth spurt.

We identified two studies examining the association between adherence to the MD pattern and BMD[31,33]. Adherence to the MD was not significantly related to total body bone mineral content or to BMD at the lumbar spine in adult women[31]. Also no evidence of an association between BMD or bone mineral content and adherence to this pattern was found in a sample of young adults[33]. The association between adherence to the DASH diet and bone metabolism was studied as a 3-month DASH intervention study in adults and this pattern significantly reduced bone turnover[18].

The MD and DASH diets are characterized by high intakes of fruit and vegetables, wholegrain bread and cereals, pulses, nuts, dairy products and fish[29]. Although these patterns value the consumption of alkali-forming food groups that have been associated with higher BMD (i.e. fruit and vegetables)[35–37], they also include increased quantities of acid-forming foods (i.e. cereals, pulses, nuts and dairy products). The latter can change the acid–base balance and may preclude expression of the beneficial effects of fruit and vegetables[38,39].

One of the main limitations of the *a priori* dietary patterns approach is related to the use of dietary guidelines that generally are not disease specific and the adherence to them may reduce the risk of some diseases but not others[40]. Therefore, we additionally studied the association between BMD and the OHS dietary index. Although this pattern was associated with BMD in a sample of adults[20], our study found no indication of such an association among adolescents.

In our study, the ascertainment of usual diet was done using an FFQ and, as with any method to assess dietary intake, under-reporting of usual intake is possible because of social desirability bias[41,42], particularly in overweight and obese individuals[43,44]. Differential under-reporting of diet components is a relevant limitation to pattern analysis[41]. For example, diet under-reporting of foods high in fat or sugar, such as fast foods, pastries or sweetened beverages, will be reflected in higher scores in the MD and DASH indices and in lower scores in the OHS dietary index. Additionally, there could be inherent imprecision associated with the option of not quantifying food portion sizes. However, research has shown that the majority of variation in food intakes is captured by frequency of consumption[45]. The lack of validation of the FFQ in this adolescent population may also be a limitation. However, the FFQ had been previously validated in the adult population of the same city[46].

Moreover, in our study dietary intake was evaluated at baseline and we do not know whether adherence to dietary patterns changed throughout the follow-up. Although some studies have been providing evidence that variations of dietary patterns occur during adolescence[47,48], it is also likely that dietary patterns remain relatively constant over time[49–52].

Taking into account the weak and few significant associations found in the present study, we hypothesize that dietary patterns may not be the primary factor in determining BMD in this age group. It is possible that dietary patterns may not have the impact on BMD in adolescents as they do in adult populations. One possible explanation may be the fact that, contrary to what happens in heterogeneous samples of adults, our sample of adolescents is very homogeneous, since they were born in the same year and share the urban environment of Porto city, making it much more difficult to find consistent associations between diet and BMD.

In our study we observed gender differences in the association between adherence to the MD pattern and BMD, possibly attributable to sex-specific difference in BMD response to biological and environmental determinants at this age[53]. Indeed, it is possible that the environmental determinants of bone development during adolescence differ according to sex as was previously described for the relationship between physical activity and BMD[54].

In order to optimize feasibility and to minimize radiation exposure we used forearm BMD measured by DXA. The DXA-derived BMD is based on the two-dimensional projected area of a three-dimensional structure which does not capture true volumetric density or bone geometry[55]. However, BMD remains a valid index of bone quality and DXA-derived measures have been shown to predict fracture risk accurately, which is ultimately the goal of bone quality assessment[56]. Since we studied bone quality only by examining forearm BMD measured with DXA, we cannot rule out the possibility that other measures of bone quality are associated with the dietary patterns considered.

One possible confounder of the association between dietary patterns and BMD is body size. Although we did not find substantial variation in mean BMI, weight and height between different classes of adherence to the dietary patterns studied, which may be related to the aforementioned limitations of the dietary assessment method, there is a well-documented important weight-dependent positive association between adiposity and bone strength[57] that has been found in a previous work evaluating the girls of this cohort[58]. For this reason we found it essential to account for confounding by body size in the present analysis.

Owing to the difficulty in ensuring privacy at school, adolescents' pubertal development by the Tanner criteria[28] was not assessed. This may be a limitation since it is possible that the higher height observed in boys of the third tertile of the MD pattern may reflect greater maturity. However, for a sub-sample of 121 boys, we identified no significant difference in mean level of serum collagen type 1 cross-linked C-telopeptide, a marker of bone resorption that has been associated with skeletal maturation[59], according to tertile of MD pattern adherence. In girls, age at menarche was recorded as an indicator of pubertal development in girls.

Physical activity is also a possible confounder of the association studied, but in the present study it was not consistently associated with either dietary patterns or BMD (data not shown). One possible explanation is the low validity of self-reported information to assess physical activity. However, the most probable reason is the previously described high levels of sedentary behaviour in this population[22], which probably situated the vast majority of the sample below the threshold level for exercise-induced bone formation. In fact, there is evidence that light or moderate physical activity is not associated with bone properties[60]. Although we cannot exclude that physical activity might modify the effect between diet and BMD, the further adjustment to this confounder did not change the results.

As with any observational analysis, it is possible that other confounders that were not measured in the study or error in the measurement of the existing confounders could lead to residual confounding that could not be accounted for. However, we controlled for other potential confounders, which did not substantially affect our estimates.

Another limitation is the large number of comparisons made in the present study, which makes it difficult to rule out the role of chance in any one result. However, adjustment for multiple comparisons by the Bonferroni method did not substantially change the results[61].

Although the dietary patterns approach has certain advantages over traditional methods of examining the relationship between diet and health outcomes, results can be more challenging to interpret. In fact, more than the food combinations consumed, dietary patterns probably reflect individual food preferences modulated by a mix of genetic, cultural, social, health, environmental, lifestyle and economic determinants[12]. As a result of this complexity, mechanisms to explain the observed associations are not clarified by the present results. In fact, more healthy dietary patterns are often reported with a constellation of other desirable health behaviours, thus confounding the pattern and health association[12].

Conclusions

We did not find consistent or strong associations between dietary patterns defined *a priori* and forearm BMD in early or late adolescence. The selected dietary patterns may not capture the elements of diet that are truly important in determining adolescent bone quality or, given the overall adequacy of nutrient intake in high-income populations, dietary patterns may not add substantially to other determinants of BMD at this age.

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