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Calcium Intake in Relation to Diet and Health Indicators in Cretan Primary and High School Pupils, Greece

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Abstract: In recent years, the issue of dietary calcium (Ca) requirements is said to have caused more controversy than requirements for any other nutrient. There is little published data on dietary Ca intake levels in Greek children and relationships with other health indicators. Pupils at twenty primary and ten high schools in Crete, Greece, were examined as part of a wider study of the dietary habits and health status of children and adolescents. A total of 1054 children participated: 583 9- to12-year-olds and 471 14- to 16-year-olds. "High" and "low" Ca intake in each age group was defined using upper and lower Ca intake quartiles for each sex. Multiple regression techniques were used to examine associations after adjustment for energy intake. No statistical association was observed between calcium intake and serum lipoproteins, anthropometric measurements, or physical activity status. Energy-adjusted Ca intakes were positively associated with intakes of protein, saturated fatty acid (SFA), magnesium, phosphorus, vitamin A, and vitamin B_2, whereas higher Ca intakes were associated with lower monounsaturated fatty acid (MUFA) and omega-6 fatty acid intakes. In both age groups, pupils with high Ca intake had higher intakes of the food groups "milk and milk products" and "grains and grain products" than those with low Ca intake, although even "high" Ca intake in older female Cretan pupils (with a 75th percentile cut-off of 999 mg/day) may not be at adequate levels.

Key words: Diet, calcium intake, adolescents, primary school pupils, nutrient

Abbreviation: Ca = calcium, %En = percentage of total energy intake, sd = standard deviation, SE = standard error, MVPA = moderate-to-vigorous physical activity, MUFA = monounsaturated fatty acids, PUFA = polyunsaturated fatty acids, BMD = bone mineral density, BMC = bone mineral content.

Introduction

There has been more recent controversy over calcium (Ca) requirements and allowances than over those of any other nutrient [1]. Calcium is by far the most common mineral in the human body, constituting between 1.5% and 2% of total body weight and is known to be important in building bone mass and strength [2]. Having an optimal amount of bone deposited during adolescence is the most effective strategy for decreasing the risk of osteoporosis later in life because a substantial part of bone accretion occurs between the ages of 11 and 16 years [3, 4]. In addition, a high Ca intake is known to decrease the risk of hypertension, colon cancer, adverse affects of exposure to lead, and also to aid the prevention of kidney disease [5]. Although Ca intake is believed to play a major role in determining bone mass, it is difficult to ascertain the degree of association between adult bone measurements and previous Ca intake, as longitudinal studies have been limited and have produced inconsistent findings [6]. The increase in bone mineral density (BMD) that has been observed in pediatric Ca supplementation trials is thought to be transient, lasting up to six to twelve months, particularly when inorganic Ca is used. On the other hand, a randomized controlled trial in 149 girls aged 6 to 9 years, with a milk-derived Ca supplement of about 850 mg/day, found an increased BMD in the intervention group (as compared to the placebo group) one year after termination of the 48-week supplementation period [7]. A recent literature review by Wosje & Specker [6] found that observational studies assessing relationships between childhood bone measurements and childhood dietary Ca intake have also produced contradictory results. Physical activity has also been shown to contribute to bone development, in addition to the largely unknown hereditary contributions, which are estimated to be in the region of 60% to 80% for bone mass [8].

Much interest has turned of late to the role of dairy products in the human diet, with dietary consumption of four to five servings of milk and milk products per day advised for adolescents in a recent publication of the American Academy of Pediatrics [9] to meet the United States National Institutes of Health Ca recommendations, subsequently endorsed by the American Medical Association [10]. For humans, the primary source of easily absorbed Ca is in the form of dairy foods such as milk, cheese, and yogurt [5]. In addition, foods such as green vegetables, nuts, and cereals can contribute to Ca intake. Calcium needs are also thought to be influenced by genetic and environmental factors such as nutrient-nutrient interactions and physical activity. For example, Ca absorption depends on adequate vitamin D status.

In the United States Department of Agriculture's (USDA) Continuing Survey of Food Intakes by Individuals (CSFII), the percentage of U.S. adolescents not meeting 100% of the 1989 recommended daily allowance (RDA) for Ca was found to be 68% for males and 88% for females ([11] cited in [12]). The U.S. Food and Nutrition Board of the Institute of Medicine (IOM) presented new reference intakes for Ca in 1997 with dietary recommendations (of adequate intake, AI) of 1300 mg/day for 9- to 18-year-olds [13]. These are higher for adolescents than previous recommendations, the 1989 RDAs being 800 mg/day for 7- to 10-year-olds and 1200 mg/day for 11- to 19-year-olds [14] and are higher than the Ca intake of most Americans [15]. The USDA survey and a separate recent study [16] have shown that American children have, in recent years, increased their intake of soft drinks. The surveys showed that adolescents drinking relatively large quantities of soft drinks were much more likely to have a low milk consumption. A study of nutrient intakes of young women aged between 13 and 18 years revealed that women who drank fluid milk had higher levels of essential nutrients than nondrinkers, although the two groups did not differ significantly with respect to their macronutrient intake [17].

Nordic and European recommendations for dietary Ca intake in all age groups published by the European Commission in 1998 are consistently lower than those of the U.S., with 550–700 mg/day recommended for 7- to 10-year-olds and 900–1000 mg/day recommended for 11- to 24-year-olds [18, cited in 19]. It is not certain, however, that U.S. and general European recommendations are appropriate for pupils consuming a Mediterranean diet. There are no reliable national Ca intake recommendations available for Greek children and young adults. There is, in fact, very little published data on estimated dietary Ca intake levels in Greek children and adolescents. No national dietary survey aimed at assessing Ca intake levels in children and adults in Greece has yet been published, although a survey using a stratified sample of 1936 Greek children with a three-day, household-measured dietary record concluded that Greek children have adequate Ca intake [20]. A study in which the seven-day food composite representing the typical diet of a present-day Greek male teenager was chemically analyzed predicts a daily Ca intake of 655 mg [21]. The aim of the present investigation is to assess Ca intake levels in male and female primary school and high school pupils in Crete, Greece, in relation to intakes of other nutrients and food groups. Possible differences in anthropometrics and serum lipid levels between males and females in each age group with low and high Ca intake are also investigated.

Subjects and Methods

Subjects

The data collected for the present analysis were gathered as part of a general cross-sectional study on the dietary habits and general health of children and adolescents in Crete, Greece. The study encompassed three of the four prefectures of Crete, which account for about 75% of its population of approximately half a million. The fieldwork was undertaken in two stages between January and December 1999. Initially a random sample of 10 primary and 10 secondary schools was taken from two Cretan prefectures. Subsequently, a random sample of 10 primary schools was taken in the third prefecture (Agios Nikolaos). As the three prefectures do not display any major differences in primary school children population demographics, records obtained from the younger pupils are considered as one sample. In total, 1824 4th, 5th, and 6th grade (primary school) and 9th or 10th grade (high school) pupils were invited to participate. Of those invited, the 1145 pupils (63%) who returned the signed parental consent form were examined. Of these, 1054 pupils (91%) provided complete dietary, hematological, and anthropometric data. The 583 younger pupils were aged between 9 and 12 years. The 471 ninth and tenth graders were aged between 14 and 16 years. The study protocol was approved by the Medical Ethics Committee of the University of Crete Medical School.

Dietary data and the nutrient database

A single 24-hour dietary recall questionnaire was administered by dieticians to all subjects, with quantities of foods consumed assessed using food-model photographs and household measures. The 24-hour dietary recall method has been previously suggested as a suitable tool for quantifying the dietary intakes of children [22, 23, cited in 24]. Administration of the 24-hour recall questionnaire was followed by a cross-check with regard to what extent the previous day's dietary intake could be regarded as representative of the individual's intake. The cross-check included a direct question on whether the pupil being interviewed considered that their previous day's intake was as usual and if not, why not. The records of those pupils whose intake was judged by the dietician not to be representative of their normal dietary intake were not included in the main analyses.

For validation purposes, three-day weighed food records (completed on two weekdays and one weekend day) were obtained from 157 of the subjects in the younger age group from one prefecture; 275 subjects were examined and their parents were instructed as to how to complete three-day weighed records, if they were willing to do so. The majority of older pupils were not willing to undertake a weighed record, therefore the three-day records were given only to parents of the younger pupils for completion by the parent, with the child's cooperation. Greek primary school hours are such that there is no lunch break (in contrast to Northern European schools), so it may be assumed that the three main daily meals were monitored by the parent or guardian.

The operational food database "Greek Diet" used to obtain dietary nutrient intake estimates is a database containing 490 foods. It was created in 1990 in the Preventive Medicine and Nutrition Clinic of the University of Crete, based on USDA tables and subsequently upgraded using version 11 of the USDA Nutrient Database for Standard Reference issued in 1996 [25]. The chemical composition of twenty Greek foods was determined at Wageningen Agricultural University, The Netherlands, and the detailed fat composition of 105 fat-containing foods, providing 95% of the fats and oils in the usual Greek diet, was determined at TNO Voeding as part of the EU-funded TRANSFAIR project [26, 27]. For the combined foods in the database, recipe calculations were made, with adjustments made for weight changes (e.g. after cooking) where necessary, as described by the Royal Society of Chemistry, U.K. [28]. The "Greek Diet" database has been validated against chemically analyzed food composites [21].

The types of foods consumed were separated into the following thirteen groups, according to the EUROCODE 2 food groupings [29–31]: 1. milk, cheeses, yogurt, and other dairy products; 2. eggs and egg products (including omelettes); 3. meats and meat products (red and white meats); 4. fish and other seafood; 5. fats and oils; 6. grains (including wheat flour, rice, pasta, cheese pies, bread, cakes, rusks, crackers, and cereals); 7. nuts and pulses (including lentils, broad beans, and chick peas); 8. vegetables (including potatoes); 9. fruits; 10. sugars and sweets (including chocolate bars); 11. beverages; 12. soups and sauces and 13. powdered drinks and milks. The food groups were obtained from the 24-hour dietary data and where composite foods were involved, they were split into constituents as far as possible using standard recipes, based on the weight of the portion consumed. In the *Results* section, macronutrient intakes are presented in daily absolute values and also in terms of the percentage of total energy intake per person per day (%En) whilst food group consumption estimates are in grams per day (g/day) per person.

Biochemical and anthropometric measures

Venous blood samples were taken following an overnight fast, using methods previously employed in health inter-

vention studies by Preventive Medicine & Nutrition researchers at the University of Crete (further details can be found in [32]). Briefly, 10 mL of whole blood was obtained from each subject and transferred on ice to the Preventive Medicine and Nutrition Clinic, with temperatures maintained at 3 to 4°C. The blood samples were centrifuged and 1.5 mL aliquots were pipetted into plastic Eppendorf tubes. One aliquot was used for blood analysis on the day of collection whilst the remainder was stored at –80°C to allow for repeat measurements. Triacylglycerol levels were determined using Fossati's method [33] and total cholesterol measurements were obtained using Allain's method [34]. High density lipoprotein (HDL) was measured using the heparin-manganese precipitation method [35] whilst low density lipoprotein (LDL) was calculated using the equation LDL = TC-HDL + TG/5 [36].

Body weight was measured using a digital scale (Seca) accurate to 100 g and standing height was measured to the nearest 5 mm. BMI was calculated as weight (kg) divided by height squared (m^2). Age- and sex-specific International Obesity Task Force (IOTF) cut-offs [37] were used to identify obese pupils.

The extent to which moderate-to-vigorous physical activity (MVPA) was undertaken was estimated for each child using the same standardized activity questionnaire as previously used in a health intervention study performed by our unit (using two consecutive weekdays and one weekend day, as described in [32]). In the present study, completion of the questionnaire was undertaken by pupils in the presence of a research team member.

Statistical analysis

As there were two distinct age groups being considered which were believed a priori to have differing anthropometric measures and energy intake levels, separate nutrient intake analyses were undertaken by age group. The validity of the 24-hour dietary recall was assessed by comparing nutrient intake estimates with those obtained from three-day weighed food records using the Bland & Altman method: differences between the two methods are compared with the mean intake, with the "limits of agreement" between the two methods being defined as the mean difference plus/minus two standard deviations [38]. This method can be used to graphically detect outliers and any trends. Spearman rank correlation coefficients were also calculated as they provide evidence of the degree of relatedness between the two methods (but not whether the two methods actually agree). Finally, Cohen's kappa [39] was used to validate quartile classification. The kappa statistic, which is the proportion of classifications in the same quartile according to either dietary estimation method, corrected for agreement based on chance alone, is often used to compare categories of nutrient intake [40]. The corresponding significance test is based on the null hypothesis that kappa is zero, meaning that there is no additional agreement beyond that which is expected by chance [41].

Male and female subjects in each age group were classed according to quartiles of Ca intake. Initially, the non-parametric Mann-Whitney test was applied to obtain an indication of possible differences in nutrient intake, biochemical measures and the types of foods eaten by subjects with low and high Ca intake by comparing the estimates of those in the upper quartile (Q3) with those in the lower quartile (Q1). Subsequently, a multivariate approach was taken for each age group in order to obtain measures of Ca intake that were independent of the total energy intake (as these two variables were found to be highly correlated: Pearson correlation coefficient 0.54). The first step in this approach was to obtain standardized Ca residuals by regressing Ca intake (response variable) on total energy intake (explanatory variable); this is known as the residual method [42]. The energy-adjusted Ca intake was obtained by adding the predicted Ca intake for the mean energy intake to the standardized residual. The second step was to perform (forced entry) multiple linear regressions of each of the nutrient intakes and biochemical measures against the energy-adjusted Ca intake, controlling for possible age and sex effects. Nutrient intakes were log-transformed where necessary (protein, carbohydrate, polyunsaturated fatty acids, trans fatty acids, cholesterol, fiber, and vitamins A, B_1, B_2, and C) prior to the regression. Model adequacy was checked by plotting model residuals against fitted values. As the MVPA variable had a high proportion of zero values, it was recorded into a binary variable (some MVPA/no MVPA) and logistic regression was applied. The statistical package SPSS 8.0 was used throughout. The Bonferroni correction was applied (to both univariate and multivariate analyses) to adjust for multiple comparisons [43]; the adjustment has been applied to the p-values presented, unless otherwise stated.

Results

Low and high Ca intake in relation to diet and health indicators

A summary of the characteristics and general health indicators of the males and females in each age group are presented in Tables I and II. Eighty seven percent of the younger age group (92% of the females and 81% of the males) and 84% of the older age group (88% of the females as opposed to 80% of the males) were found not to

reach 100% of the Ca daily intake currently recommended in the U.S. (1300 mg), based on the 24-hour dietary recall estimates. The mean Ca intake was estimated to be 930 (sd 432) mg/day for the 9 to 12 year old males and 795 (sd 360) mg/day for the 9 to 12 year old females. For the older age group, the average intakes were similar, being 905 mg/day for males, and 771 (sd 390) mg/day for females. There was no evidence of a difference in average Ca intake between the sexes in either age group, although in both age groups there was strong evidence of a higher

Table I: General health and dietary characteristics of 513 nine- to twelve-year-old pupils in Crete by calcium intake (lower quartile Q1 versus upper quartile Q3) for each sex separately based on a 24-hour dietary recall

	Males (n = 255)						Females (n = 258)					
	Q1: Ca intake < 611 mg/day (n = 64)			Q3: Ca intake > 1201 mg/day (n = 63)			Q1: Ca intake < 558 mg/day (n = 64)			Q3: Ca intake > 977 mg/day (n = 64)		
Measurements	Mean	sd	Median	Mean	sd	Median	Mean	sd	Median	Mean	sd	Median
Age (years)	11.7	7.0	11.0	10.8	0.7	11.0	10.7	0.7	10.8	10.6	0.6	10.5
Weight (kg)	43.4	10.7	41.6	39.3	8.2	38.1	42.9	9.7	42.6	39.6	9.5	37.3
Height (cm)	142	7.1	143	143	8.1	143	145	7.8	145	143	9.3	143
BMI (kg/m^2)	21.2	4.3	20.9	19.2	2.8	18.6	20.3	3.8	19.5	19.1	3.1	18.3
Waist-to-hip-ratio	0.9	0.1	0.9	0.9	0.1	0.9	0.9	0.1	0.9	0.8	0.1	0.8
MVPA (hrs/week)	2.9	3.7	0.5	4.8	4.9	4.0	1.0	1.9	0	0.9	2.6	0
Total serum cholesterol (mg/dL)	169	31.8	169	167	24.8	167	173	31.3	172	170	25.3	168
Triacylglycerol (mg/dL)	62.4	33.9	54.5	61.0	22.9	56.0	75.3	31.2	67.5	70.3	34.6	65.0
High-density lipoprotein HDL (mg/dL)	49.6	11.0	49.0	52.2	13.4	51.0	49.6	11.9	47.0	51.6	13.2	49.0
Low-density lipoprotein LDL (mg/dL)	107	29.8	108	102	22.8	100	110	28.7	105	104	22.5	103
TC:HDL ratio	3.6	1.5	3.4	3.4	0.9	3.2	3.7	1.1	3.4	3.4	0.7	3.4
Energy (kcals)	1427	480	1339	2443	578	2357[a]	1357	392	1347	1997	511	1937[d]
Protein (g/day)	46.7	19.8	41.6	86.9	20.5	83.8[a]	42.5	14.9	42.9	73.7	22.7	71.1[d]
Protein (%En)	13.4	4.7	12.5	14.6	3.2	14.4	12.7	3.7	12.5	15.0	3.8	14.1[e]
Carbohydrate (g/day)	170	63.7	156	291	98.2	280[a]	160	55.5	155	242	72.1	234[d]
Carbohydrate (%En)	48.0	10.9	47.7	47.0	7.5	47.4	47.1	9.3	47.8	48.8	6.8	48.0
Total fat (g/day)	64.2	29.6	55.6	106	28.2	105[a]	62.6	22.8	60.3	84.4	27.3	80.9[f]
Total fat (%En)	39.7	9.6	40.2	39.4	6.4	40.3	41.3	8.3	41.1	37.9	6.5	37.8
Saturated fatty acids, SFA (%En)	12.6	3.8	12.9	14.8	3.5	14.8	13.6	4.2	13.1	14.0	3.1	13.3
Monounsaturated fatty acids (%En)	17.8	6.6	17.5	15.3	4.2	14.7	18.3	5.5	18.1	14.9	4.5	14.3[g]
Polyunsaturated fatty acids (%En)	4.7	1.8	4.6	4.6	1.8	4.3	5.4	3.2	4.5	4.3	1.9	4.0
Trans fatty acids (%En)	0.8	0.7	0.5	0.8	0.4	0.8	0.9	0.8	0.7	0.7	0.5	0.7
Omega-3 fatty acids (%En)	0.3	0.1	0.3	0.3	0.1	0.2	0.3	0.1	0.3	0.3	0.2	0.2
Omega-6 fatty acids (%En)	3.9	1.7	3.9	3.5	1.0	3.4	3.9	1.5	3.6	3.3	1.0	3.3
Dietary cholesterol (g/day)	170	172	120	262	168	222[b]	152	119	113	207	100	184[g]
Fiber (g/day)	12.4	6.2	11.1	20.1	10.5	18.0[a]	11.5	5.7	10.0	16.7	7.5	15.5
Vitamin A (RE*)	351	365	259	921	724	666[a]	367	353	268	1153	1010	649[d]
Vitamin B_1 (mg/day)	2.1	2.6	1.2	2.0	0.8	1.8[b]	2.1	2.6	1.2	2.0	1.2	1.8
Vitamin B_2 (mg/day)	1.1	0.7	1.0	2.4	0.7	2.3[c]	1.0	0.3	1.0	2.1	0.5	2.0[d]
Vitamin B_6 (mg/day)	1.1	0.6	1.1	1.8	0.8	1.6[b]	1.2	0.6	1.1	1.8	0.7	1.7[f]
Vitamin B_{12} (mg/day)	2.0	2.1	1.4	3.6	2.1	3.4[a]	1.5	1.1	1.4	3.6	2.8	2.9[d]
Vitamin C (mg/day)	103	91.3	75.2	171	140	151	108	89.5	85.1	182	150	134
Niacin (mg/day)	12.7	5.8	11.7	17.8	7.2	16.3[c]	11.4	5.1	11.2	16.0	5.8	15.8[g]
Vitamin E (mg/day)	5.2	3.3	5.1	7.9	4.2	7.2[b]	4.9	3.0	4.7	7.0	4.0	5.7
Sodium (mg/day)	1537	724	1543	2814	834	2763[a]	1301	615	1127	2204	705	2113[d]
Calcium (mg/day)	414	136	432	1503	275	1420[a]	389	120	408	1262	310	1188[d]
Magnesium (mg/day)	171	70.8	155	313	84.7	307[a]	159	62.3	147	265	80.2	248[d]
Phosphorus (mg/day)	724	244	681	1613	288	1559[a]	666	195	686	1341	311	1242[d]
Potassium (mg/day)	2056	1135	1919	3058	1214	2873[a]	2043	1096	1886	3012	1211	2764[d]

* RE, retinol equivalents.

All comparisons were made using the non-parametric Mann-Whitney test and all p-values adjusted for multiple comparisons:
[a] Q1 vs Q3 males: $p < 0.0001$; [b] Q1 vs Q3 males: $p < 0.01$; [c] Q1 vs Q3 males: $p < 0.001$; [d] Q1 vs Q3 females: $p < 0.0001$;
[e] Q1 vs Q3 females: $p < 0.05$; [f] Q1 vs Q3 females: $p < 0.001$; [g] Q1 vs Q3 females: $p < 0.01$.

average energy intake in males than females ($p < 0.01$ and $p < 0.0001$ for the younger and older age groups respectively).

The health and dietary indicators are presented by Ca intake quartiles (low, Q1, versus high, Q3) in Tables I and II. For the 9- to 12-year-olds, the low and high Ca intake quartile cut-offs were 611 mg/day and 1201 mg/day for males and 558 mg/day and 978 mg/day for females (Table I). The analogous estimates for the 14- to 16-year-olds are depicted in Table II: the Ca intake quartile cut-offs

Table II: General health and dietary characteristics of 186 fourteen to sixteen year pupils in Crete by calcium intake (lower quartile versus upper quartile) for each sex separately based on a 24 hour dietary recall

	Males (n = 148)						Females (n = 138)					
	Q1: Ca intake < 605 mg/day (n = 37)			Q3: Ca intake > 1253 mg/day (n = 36)			Q1: Ca intake < 472 mg/day (n = 34)			Q3: Ca intake > 999 mg/day (n = 34)		
Measurements	Mean	sd	Median	Mean	sd	Median	Mean	sd	Median	Mean	sd	Median
Age (years	14.6	0.6	14.5	14.6	0.6	14.5	14.5	0.5	14.5	14.4	0.4	14.5
Weight (kg)	65.9	12.6	65.2	62.6	9.4	61.1	57.3	8.8	55.8	51.2	7.5	50.7
Height (cm)	167	7.8	169	167	5.9	169	161	5.9	160	160	6.3	160
BMI (kg/m^2)	23.5	3.8	22.6	22.4	3.2	22.2	22.2	2.8	21.8	20.0	2.5	20.0
Waist-to-hip-ratio	0.9	0.1	0.9	0.8	0.1	0.9	0.8	0.1	0.8	0.8	0.1	0.8
MVPA (hrs/week)	6.0	7.9	0	3.6	5.0	0	0.6	1.7	0	1.0	2.8	0
Total serum cholesterol (mg/dL)	158	34.5	151	162	42.9	154	169	26.4	171	174	27.9	173
Triacylglycerol (mg/dL)	75.3	40.9	67.0	74.4	33.5	68.0	75.7	24.4	75.0	71.7	19.9	66.0
High-density lipoprotein HDL (mg/dL)	41.6	11.3	41.0	44.9	10.7	46.0	47.2	11.2	46.5	54.4	11.0	55.5
Low-density lipoprotein LDL (mg/dL)	1012	28.5	100	103	42.4	94.4	107	22.3	102	106	23.3	107
TC:HDL ratio	4.0	1.2	3.6	3.8	1.6	3.3	3.8	0.9	3.7	3.3	0.6	3.2
Energy (kcals)	1765	684	1644	2489	724	2475[a]	1319	324	1281	2128	618	2058[e]
Protein (g/day)	60.4	19.6	64.1	98.3	30.9	97.6[b]	41.4	14.7	40.0	81.2	21.8	78.4[e]
Protein (%En)	14.3	3.7	13.6	16.1	3.1	16.4	12.9	4.6	12.2	15.7	3.6	15.2[f]
Carbohydrate (g/day)	212	98.2	196	286	107	270[c]	150	47.6	154	239	71.6	240[e]
Carbohydrate (%En)	47.8	7.3	47.5	45.6	8.2	44.9	45.5	10.2	47.2	45.5	8.8	44.7
Total fat (g/day)	76.7	33.5	70.1	107.8	35.7	108.5[a]	63.1	22.1	57.9	95.9	37.9	93.0[g]
Total fat (%En)	38.8	6.8	38.9	39.1	7.1	39.6	42.8	9.3	41.8	39.7	8.8	40.1
Saturated fatty acids, SFA (%En)	11.6	3.1	11.6	15.1	3.4	15.1[a]	13.5	3.9	12.6	14.7	3.3	14.8
Monounsaturated fatty acids (%En)	18.1	4.7	17.3	14.5	4.1	14.8	19.3	6.5	19.1	15.3	5.5	15.4
Polyunsaturated fatty acids (%En)	5.3	3.4	4.3	4.1	1.4	3.7	5.0	2.1	4.7	4.3	1.6	3.9
Trans fatty acids (%En)	0.9	0.8	0.6	1.1	0.6	1.0	1.2	1.1	0.8	0.8	0.4	0.7
Omega-3 fatty acids (%En)	0.3	0.2	0.3	0.3	0.1	0.3	0.4	0.2	0.3	0.3	0.1	0.2
Omega-6 fatty acids (%En)	4.0	1.6	3.5	3.0	1.1	2.9	4.3	2.0	3.8	3.4	1.6	3.2
Dietary cholesterol (g/day)	236	180	178	309	197	240	175	168	125	269	156	224[f]
Fiber (g/day)	13.7	7.4	11.6	19.3	10.2	16.8	11.1	6.4	9.5	15.2	8.8	13.2
Vitamin A (RE*)	344	365	230	1448	1605	771[b]	464	787	263	706	562	540[h]
Vitamin B_1 (mg/day)	1.7	0.8	1.5	3.4	2.9	2.3[c]	2.0	2.9	1.1	2.4	2.1	1.8
Vitamin B_2 (mg/day)	1.6	1.1	1.3	2.7	0.9	2.4[b]	0.9	0.4	0.9	2.3	1.3	2.0[e]
Vitamin B_6 (mg/day)	1.6	0.7	1.7	2.3	1.3	2.1	1.0	0.4	1.0	1.7	0.6	1.5[h]
Vitamin B_{12} (mg/day)	3.0	3.8	1.8	7.4	14.3	3.4	3.2	7.5	1.2	3.6	2.0	3.4[g]
Vitamin C (mg/day)	91.3	87.1	64.7	153.5	130.7	108.8	98.2	91.4	67.2	150.2	131.2	125.6
Niacin (mg/day)	16.7	7.1	18.1	21.6	8.9	21.0	11.4	5.0	10.9	15.0	6.7	14.3
Vitamin E (mg/day)	5.9	3.6	5.1	6.8	4.0	5.9	5.0	3.2	4.4	5.8	3.5	5.4
Sodium (mg/day)	1779	754	1590	3142	1009	3063[b]	1372	787	1091	2322	776	2240[h]
Calcium (mg/day)	420	131	453	1674	608	1537[b]	332	110	371	1313	269	1250[e]
Magnesium (mg/day)	203	91.4	181	311	113	296[d]	153	65.1	145	268	63.7	264[e]
Phosphorus (mg/day)	877	287	856	1760	579	1612[d]	632	171	630	1442	312	1364[e]
Potassium (mg/day)	2548	1361	2331	3052	1599	2421[d]	1847	838	1766	2773	865	2599[e]

* RE, retinol equivalents.

All comparisons were made using the non-parametric Mann-Whitney test and all p-values adjusted for multiple comparisons:
[a] Q1 vs Q3 males: $p < 0.01$; [b] Q1 vs Q3 males: $p < 0.001$; [c] Q1 vs Q3 males: $p < 0.05$; [d] Q1 vs Q3 males: $p < 0.0001$;
[e] Q1 vs Q3 females: $p < 0.0001$; [f] Q1 vs Q3 females: $p < 0.05$; [g] Q1 vs Q3 females: $p < 0.01$; [h] Q1 vs Q3 females: $p < 0.001$.

used were 605 mg/day and 1253 mg/day for males, and 472 mg/day and 999 mg/day for females. From both tables it can be seen that the high Ca consumers (both male and female) tend to be those pupils with higher energy intakes, and high intakes of all macronutrients (absolute values). In terms of macronutrient intake as a proportion of energy intake, some evidence was found of a difference between average protein intake of those in Q1 vs Q3 for both the younger females (Q1 median 12.5%En, Q3 median 14.1%En, $p < 0.05$) and older females (Q1 median 12.2%En, Q3 median 15.2%En, $p < 0.05$). In the 9- to 12-year-old females there was also evidence of a difference in the average monounsaturated fatty acid (MUFA) intake (Q1 median 18.1%En, Q3 median 14.3%En, $p < 0.01$). The only micronutrients for which there do not appear to be significant intake differences in either young or old male and female pupils in Q1 versus Q3 are vitamin C and vitamin E (apart from vitamin E intake in the young males, for which there is some evidence of a difference between Q1 and Q3, with medians 5.1 mg/day and 7.2 mg/day respectively, $p < 0.01$). In general, the older age group displays more differences in terms of individual nutrient intakes between the lower and higher Ca consumers (both for males and females) than the younger age group, as can be seen from Tables I and II. No evidence was found in either age group of a difference in either growth or biochemical data between those with low and high Ca intake. Using the IOTF obesity cut-offs, however, of the 100 pupils (9.5%) classified as being obese, 30% were low Ca consumers and only 9% were high Ca consumers. The evidence of an association between low and high Ca intake and obesity status was highest in the young male group (13 obese pupils observed in Q1, 7 expected using a chi-square test, $p = 0.003$). No evidence was found in either age group or sex of a difference in MVPA levels between those with low and high Ca intake.

Using multiple regression procedures, no evidence of a significant Ca intake effect was obtained with regard to the anthropometric data, serum lipids, or physical activity (MVPA) measurements (i.e. adjusted p-values > 0.05 for both age groups). For the younger age group, there was evidence that Ca intake increases as protein intake (both g/day on log scale and %En), saturated fatty acid (SFA) intake (%En), magnesium (mg/day), phosphorus (mg/day), vitamin A (RE on log scale), and vitamin B_2 intakes (mg/day, on log scale) increase, with an (unadjusted) relative increase in energy-adjusted Ca intake per unit increase in nutrient intake (95% CI) of 0.6 (0.4, 0.8), $p < 0.0001$, 0.09 (0.07,0.11), $p < 0.0001$, 0.06 (0.04,0.08), $p < 0.0001$, 0.001 (0.001,0.002), $p = 0.002$, 0.001 (0.001, 0.002), $p < 0.0001$, 0.4 (0.3, 0.5), $p < 0.0001$ and 0.2 (0.1, 0.3), $p < 0.0001$ respectively, controlling for age and sex. Similarly, there was evidence that Ca intake decreases as MUFA intake (%En), and omega-6 fatty acid intake (%En) increase with estimated regression coefficients (95% CI) of –0.05 (–0.06, –0.03), $p < 0.0001$ and –0.16 (–0.22, –0.10), $p < 0.01$, respectively, controlling for age and sex. For the older age group, there was evidence that Ca intake increases as protein intake (both g/day on log scale and %En), SFA intake (%En), magnesium (mg/day), phosphorus (mg/day), vitamin A (RE on log scale), vitamin B_2 intakes (mg/day on log scale), and vitamin B_{12} intakes (mg/day on log scale) increase, with relative increase in energy-adjusted Ca intake per unit increase in nutrient intake (95% CI) of 0.6 (0.4, 0.8), $p < 0.0001$, 0.09 (0.07, 0.11), $p < 0.0001$, 0.08 (0.06, 0.10), $p < 0.0001$, 0.002 (0.001, 0.003) $p = 0.002$, 0.001 (0.001, 0.001) $p < 0.0001$, 0.32 (0.24, 0.40), $p < 0.0001$, 0.73 (0.55, 0.92), $p < 0.0001$, and 0.19 (0.09, 0.28), $p < 0.01$ respectively, controlling for age and sex. Similarly, there was evidence that Ca intake decreases as MUFA intake (%En), PUFA intake (%En on log scale), and omega-6 fatty acid intake (%En) increase with estimated regression coefficients (95% CI) of –0.04 (–0.06, –0.03), $p < 0.0001$, –0.66 (–0.88, –0.43), $p < 0.0001$ and –0.19 (–0.25, –0.14) $p < 0.0001$ respectively, controlling for age and sex.

Foods eaten by low and high calcium consumers

For the younger age group, details of the types of foods eaten by each sex and within each sex by those with low and high Ca intake are presented in Table III and similarly for the older age group in Table IV. For both males and females, there was strong evidence of a difference in the quantity of milk and milk products consumed by those in the low as opposed to the high Ca intake quartile; 9- to 12-year-old males in Q1 had a median intake of 43 g/day whilst females in Q1 had a median intake of 60 g/day, with corresponding Q3 values being 360 g/day and 318 g/day respectively ($p < 0.0001$ for both sexes). Similar results are seen in the older pupils (Table IV). The only other food group in which there was found to be a difference in average intake between low and high Ca consumers was the grains group; young Q1 males had a median intake of 168 g/day versus the Q3 median of 410 g/day ($p < 0.0001$), and young Q1 females had a median intake of 188 g/day versus the Q3 median of 266 g/day ($p < 0.0001$), with similar results for the older age group (with $p < 0.05$, see Table IV). It is interesting that for both sexes in the younger age group, there is a slightly higher median consumption of vegetables and vegetable products by those in Q1 as compared to Q3 (although the difference is not statistically significant). No evidence was found of a difference in the types of foods eaten by 9- to 12-year-old males as compared to females.

Table III: Daily consumption of foods (EUROCODE 2 food groups) by 513 nine- to twelve-year-old pupils in Crete, presented for each sex separately and by calcium intake (lower quartile versus upper quartile) based on a 24-hour dietary recall

	Males								Females							
	All (n = 255)				Q1: Ca < 611 mg/day (n = 64)		Q3: Ca > 1201 mg/day (n = 63)		All (n = 258)				Q1: Ca < 558 mg/day (n = 64)		Q3: Ca > 977 mg/day (n = 64)	
Food groups (g/day)+	Mean	sd	Median	% cons*	Median	% cons*	Median	% cons*	Mean	sd	Median	% cons*	Median	% cons*	Median	% cons*
Milk	254	191	250	90	43	69	360[a]	99	216	169	168	89	60	58	318[b]	100
Eggs	15	47	0	13	0	19	0	8	12	41	0	14	0	14	0	14
Meat	69	89	40	60	55	69	30	52	62	87	30	55	36	58	30	55
Fish, mollusks, reptiles & crustaceans	13	46	0	10	0	8	0	13	9	32	0	10	0	8	0	16
Oils & fats	6	11	0	39	0	36	0	43	6	10	0	41	0	34	0	45
Grains	298	194	260	97	168	72	410[a]	100	253	170	203	98	188	94	266[b]	98
Pulses, seeds, kernels & nuts	51	129	0	20	0	19	0	22	34	94	0	15	0	11	0	14
Vegetables	161	172	120	74	113	72	105	76	159	150	138	77	148	73	133	78
Fruits	127	226	0	42	0	36	65	54	142	183	65	54	0	37	140	66
Sugar, chocolate & confectionery	14	33	0	44	0	36	5	51	10	27	0	40	0	41	2	50
Beverages (non-milk)	223	277	200	57	0	48	250	63	181	214	150	57	110	53	240	66
Miscellaneous, soups, sauces, snacks	31	85	0	25	0	34	0	21	38	108	0	31	0	34	0	27
Foods for special nutritional use	6	40	0	32	0	20	0	30	3	15	0	24	0	22	0	33

+ Each food group consists of the named foods and their products; * % consumers; [a] Q1 vs Q3 males: Mann-Whitney test, adjusted $p < 0.0001$; [b] Q1 vs Q3 females: Mann-Whitney test, adjusted $p < 0.0001$.

Table IV: Daily consumption of foods (EUROCODE 2 food groups) by 286 fourteen to sixteen year pupils in Crete presented by sex and calcium intake (lower quartile Q1 versus upper quartile Q3) based on a 24 hour dietary recall

	Males								Females							
	All (n = 148)				Q1: Ca < 605 mg/day (n = 37)		Q3: Ca > 1253 mg/day (n = 36)		All (n = 138)				Q1: Ca < 472 mg/day (n = 34)		Q3: Ca > 999 mg/day (n = 34)	
Food groups (g/day)+	Mean	sd	Median	% cons*	Median	% cons*	Median	% cons*	Mean	sd	Median	% cons*	Median	% cons*	Median	% cons*
Milk	199	200	163	93	30	70	235[b]	70	171	152	130	85	0	44	275[d]	44
Eggs	21	61	0	17	0	19	0	19	27	67	0	20	0	15	0	15
Meat	129[a]	131	92	79	120	86	82	86	87	104	50	32	48	62	45	62
Fish, mollusks, reptiles & crustaceans	26	76	0	16	0	19	0	19	13	40	0	14	0	15	0	15
Oils & fats	8	13	0	49	3	59	0	59	6	11	0	43	0	35	0	35
Grains	316[a]	209	273	97	200	95	345[c]	95	243	198	201	96	155	88	285[e]	88
Pulses, seeds, kernels & nuts	55	143	0	15	0	11	0	11	45	104	0	20	0	18	0	18
Vegetables	197[a]	181	168	80	140	76	170	76	136	145	100	69	110	71	130	71
Fruits	89	165	0	37	0	30	0	30	91	197	0	38	0	35	0	35
Sugar, chocolate & confectionery	14	35	0	45	0	32	5	32	16	34	0	46	5	53	13	53
Beverages (non-milk)	263	297	222	68	220	65	290	65	184	237	136	62	86	65	211	65
Miscellaneous, soups, sauces, snacks	21	67	0	32	0	30	0	30	29	82	0	34	0	29	0	29
Foods for special nutritional use	1	2	0	15	0	3	0	3	8	43	0	22	0	18	0	18

+ Each food group consists of the named foods and their products; * % consumers; All comparisons were made using the non-parametric Mann-Whitney test and all p-values adjusted for multiple comparisons: [a] Males vs females: $p < 0.05$;
[b] Q1 vs Q3 males: $p < 0.0001$, [c] Q1 vs Q3 males: $p < 0.05$, [d] Q1 vs Q3 females: $p < 0.0001$; [e] Q1 vs Q3 females: $p < 0.05$.

Validation of 24-hour recall against three-day weighed food record

In a comparison of the energy intakes estimated by the dietary recall to those estimated using the weighed three-day records, non-significant correlation coefficients were obtained for both males and females (0.11 for males, n = 79, p > 0.05 and 0.15 for females, n = 76, p > 0.05). The correlation coefficients for the Ca intake estimates were higher: a correlation coefficient of 0.34 was obtained for males (p = 0.002, n = 79) and for females 0.24 (p = 0.033, n = 76). The Bland and Altman plots (Fig. 1 and 2) did not indicate any obvious relationship between the difference and the mean. There were no major 24-hour recall biases evident in either plot, although the limits of agreement were wide. The mean differences (estimated bias) between the 24-hour recall and the three-day weighed record were –159 (sd 753) kcal, and 53 (sd 473) mg Ca for males, and –301 (sd 625) kcal, and –70 (sd 382) mg Ca for females. The 95% CI for the estimated bias in Ca intake was –55 mg to 161 mg in males and –157.6 to 17.8 mg in females. Cohen's kappa was found to be 0.42 (estimated se 0.13, p = 0.004) for the energy intake quartile classification comparison and 0.52 (estimated se 0.13, p < 0.0005) for the Ca intake quartile classification comparison.

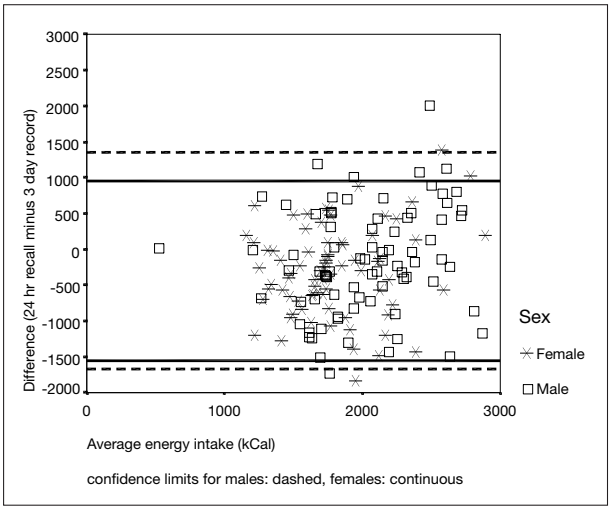

Figure 1: Individual differences in energy intake between 24 hour recall and 3 day weighed records plotted against their mean (n = 155) by sex.

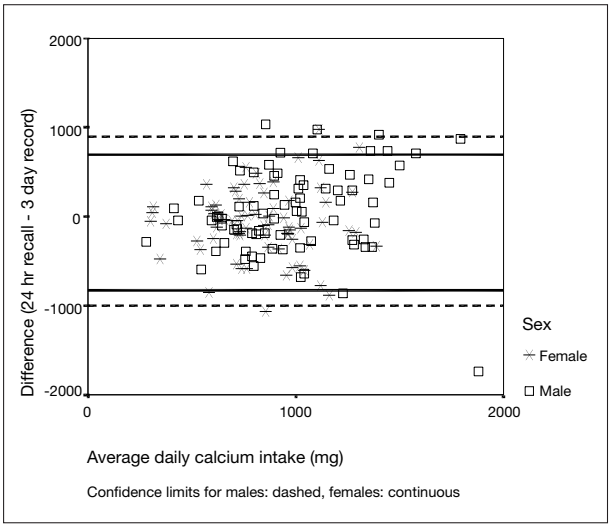

Figure 2: Individual differences in calcium intake between 24-hour recall and three-day weighed records plotted against their mean (n = 155) by sex.

Excluded dietary records

Dietary records were obtained from 583 primary school children and 471 high school children. Of these, 70 pupils (12%) from the younger age group and 185 (39%) from the older age group gave reasons why the 24-hour recall could not be taken as being representative of their usual diet. The four most common reasons given for this were that a) the diet was different because the subject knew they had a medical examination the following day (31% of the 255 pupils); b) one main meal (lunch or dinner) was skipped (25%); c) the subject was dieting (13%) and d) the subject was ill (11%). In the younger age group no pupils stated "dieting" as a reason (the most common reason being the examination the following day), but in the older age group 23% of the females and 6% of the males state dieting as a reason. The anthropometric measurements of those pupils whose 24-hour recalls were not included, however, did not differ significantly from their "usual intake" counterparts, when comparing each sex within each age group. The BMI values, for example, were for those pupils whose dietary records were not included in the analyses 19.5 (sd 3.1) kg/m^2, 19.2 (sd 3.1) kg/m^2, 22.6 (sd 4.1) kg/m^2, 22.3 (sd 3.5) kg/m^2 for the 9- to 12-year-old males, 9- to 12-year-old females, 14- to 16-year-old males and 14- to 16-year-old females respectively.

Discussion

The findings of the present study indicate that the average intake of Ca in Cretan adolescents may be well below current U.S. and European recommendations, particularly for older female adolescents. These results are in line with a recent review that indicates a risk of deficiency of Ca in Southern European adolescents [44]. Appropriate Ca in-

take recommendations for Greece, and Crete in particular, may well be different from those of central Europe and the U.S. The traditional Cretan diet has been found by chemical analysis to have, for example, low average animal protein intake [21]. Certain published studies have shown that it would be difficult to obtain sufficient Ca intake with an exclusively plant-based diet. One reason for this is that the bioavailability from plant foods can be affected by their contents of oxalate and phytate, which are inhibitors of Ca absorption [45]. On the other hand, a high vegetable intake provides plant storage carbohydrates such as oligofructose and inulin which are present in a number of plants and vegetables, such as wheat, onions, bananas, garlic, and chicory [46]; Europeans eating a Western diet have been postulated to have an inulin and oligofructose intake of between one and 10 g/day [47]. Ingestion of inulin in a clinical study involving nine healthy young men was shown to significantly increase the apparent absorption and balance of Ca [48] and a second human study involving twelve healthy adolescent males found an 11% increase in Ca absorption with ingestion of 15 g per day of oligofructose [49]. It should be noted, however, that there are as far as we know no published studies showing increased BMD with increased inulin consumption. Dietary vitamin D intakes were not assessed but it is expected that as vitamin D is largely derived from sunlight, which is abundant in Southern Europe and in Crete in particular, the availability of vitamin D will to a great extent reflect the exposure to sunlight.

It has been shown that high intakes of protein and sodium result in an increase in urinary Ca and thus increase the need for Ca: an increase of one gram in animal protein consumption increases urinary Ca by one milligram and each ingested gram of sodium increases the urinary Ca by about 15 mg [1]. The kinetics of Ca absorption imply that the obligatory loss through skin, kidneys, and bowel, which is thought to be over 40 mg/day, needs to be replenished by a dietary intake of about 200 mg [1]. Data have been published showing that the association of high fracture rates with high Ca intakes may be indicative of high animal protein intakes with subsequent increase in the excretion of urinary Ca [1]. However, an increased urinary Ca loss with increased protein intake does not imply that bone density will be affected. It is believed that the absorption efficiency of Ca accounts for more of the variability in calcium balance than the actual intake of Ca [50]. *Heaney* (2000) in an observational study on 191 nuns using the double-tracer method to estimate Ca absorption, found "no hint of a relation between calcium absorption efficiency and either protein or phosphorus intake". In fact, it is believed that increased protein intake may enhance bone health. A cross-sectional study on 215 young women found both calcium and protein intakes to be positively correlated with radius and spine BMD. The authors further state that increasing protein intake had a positive effect on total body BMD and bone mineral content (BMC) at a low Ca intake (600 mg/day), but when Ca intakes increased (up to 1400 mg/day), a high protein intake tended to have a negative effect on BMD and BMC [51].

It was found that Cretan children with lower Ca intakes were likely to have lower intakes of protein, SFA, magnesium, phosphorus, vitamin A, and vitamin B_2, even after adjusting for possible differences in energy intake, whereas there appeared to be negative relationships between Ca intake and both MUFA and omega-6 fatty acid intakes (in both age groups). A MEDLINE search did not produce any articles reporting either associations or the lack of them between spontaneous Ca intake and dietary fat or serum lipid measurements in children or adolescents. In an 18-month randomized, controlled milk-supplementation study in 12-year-old girls in the U.K., however, no significant differences were found in energy, fat, or carbohydrate intakes or body composition variables between the intervention group (who consumed a pint of milk a day resulting in a 52% increase in Ca intake above baseline Ca intake) and the control group (who were asked to continue their habitual diet). A significantly greater bone mineral acquisition throughout the 18 months was found in the group given the milk supplement than in the control group [52]. The milk-supplemented group had significantly higher intakes of phosphorus and magnesium than the control group, a finding similar to ours (high Ca consumers have higher phosphorus and magnesium intakes than low Ca consumers in both age groups). In a study of the effects of Ca supplementation with dairy products to 1200 mg Ca/day on the bone and body composition of 48 girls aged 9 to 13 years, it was found that after 12 months the dairy group had increased intakes of phosphorus, vitamin D, and protein [53]. The Ca supplementation did not result in increased total fat intake, saturated fat intake, or body fat. Also, there was no evidence of a difference in weight gain between the intervention and control groups.

The lack of a difference in anthropometric and serum lipid measurements between those with relatively high and low Ca intakes may be attributed to the properties of Ca, which has an extremely slow turnover in the body and displays very gradual effects of deprivation and replenishment [1]. Similar findings to ours have been reported in other studies. For example, in a study of BMD in 200 adolescent girls (aged 11–15 years) in Southern Italy (100 girls with low and 100 with high Ca intake chosen from the lower and upper ends of the Ca intake distribution for 722 girls), weight measurements and calculated BMI was seen to be somewhat higher in the low Ca intake group.

Girls with high Ca intake had a mean reported weight of 53.3 kg (se 1.4) and BMI of 21.1 kg/m^2 (se 0.3) as compared to 55.0 kg (se 1.2) and 22.5 kg/m^2 (se 0.4) for those with low Ca intake [54], although these differences perhaps do not reach statistical significance. The longitudinal assessment of children's food consumption in relation to body composition was the aim of a recent study on 53 white children in the U.S [55]. The findings of this study, similar to the results of studies using animal models, were that higher longitudinal intakes of calcium and dairy products were associated with lower body fat. When we used obesity cut-offs to compare obesity status by Ca intake quartile, we also found a higher overall proportion of obese subjects in the low Ca intake quartile than in the high quartile, this difference being statistically significant in the case of young males. Not all findings are similar to ours, however. Within a randomized, controlled Ca-supplementation trial, when comparisons were made between high and low spontaneous Ca consumers in both intervention and control groups there was, however, evidence of a greater height and body weight of subjects classed as high Ca consumers as compared to the low Ca consumers [7]. Attention has also turned recently to the beneficial role of physical activity in the first two decades of life in attaining eventual optimal bone mass and skeletal size [8]. In our study, however, there was no evidence of a difference in the levels of physical activity between those with low and high calcium intakes.

There are several reasons why the present study might be considered as providing a qualitative rather than quantitative summary of Ca intake levels in Cretan schoolchildren. Firstly, it may be argued that the results obtained from the dietary data would have been more representative of the populations studied had no exclusions been made after completion of the interviews. The aforementioned omitted 24-hour recalls were, however, independently checked by a dietician and were indeed not found to represent a life-sustaining diet (all being cases of major "under-reporting"). We find it disturbing that almost 50% of the 14- to 16-year-old girls stated reasons why the previous day's intake was not representative of their usual intake, with the highest proportion of these adolescent girls stating that "currently on a diet" is the reason why the intake is not their usual one. It is known that in the U.S. eating disorders are common in adolescent females; results from a recent national survey on 5th to 12th grade pupils indicated that 45% of females have at some point been on a diet (and 13% reported disordered eating), as compared with 20% of the male pupils, whilst about 24% of the population is overweight [56]. It is postulated that the prevalence of eating disorders is increasing in Greece. In the present study, weights and BMI, however, did not differ significantly between the females included and those excluded. We believe that this issue is in itself worthy of further study.

Secondly, the fact that it was only possible to compare 24-hour recalls with three-day records for the younger age group must also be seen as a limitation, as indeed should the wide limits of agreement in the Bland & Altman validation process. The limitations of the 24-hour dietary recall are well known but it remains a quick and economical application that is considered important in describing average intakes in large groups [57]. The single 24-hour recall may produce large errors in the estimation of micronutrients. As estimates of micronutrients such as Ca and vitamin A are likely to be of several orders of magnitude greater than other micronutrients (which can be seen in Tables I and II), Ca- and vitamin-A associated errors may not be as large as those of micronutrients such as iron or zinc. A recent comparison of the 24-hour recall versus a food frequency questionnaire found that the 24-hour recall gave estimates of vitamin A intake closely associated with vitamin A status even though micronutrients and vitamins in particular are known to have high within-person variability [58]. In the present study, the differences between the 24-hour recall and the three-day weighed record for the nutrient of major interest (i.e. Ca intake) were smaller than those for energy intake. Also, the kappa statistics obtained indicated a fair/good agreement between the two dietary estimation methods.

Thirdly, the present study formed part of a larger investigation into the health and dietary habits of Cretan adolescents and as such it may be viewed as being subject to certain limitations with regard to the issue of Ca intake. Ideally, Ca intake could be related to bone mineral measurements using dual energy x-ray absorptiometry. Another limitation is that no information was obtained on the prevalence of lactose intolerance in the sample. Perceived lactose intolerance causes sufferers to reduce or eliminate milk and other dairy products from the diet. A previous clinical study on lactose intolerance in healthy Greek school children, indicated that whilst many children reported milk-related lactose maldigestion symptoms, true malabsorption and intolerance of lactose was rare for children between 5 and 12 years of age; the prevalence of lactose intolerance after ingestion of milk was 8.6% in 12-year-olds [59]. Indeed, scientific evidence suggests that, in general, the prevalence of lactose intolerance might be highly overestimated [60]. Recent recommendations by the U.S. IOM [13] suggest that methods to increase Ca intake should include fortified foods and dietary supplements. Ca supplement intake information was obtained: none of the participants reported taking any dietary supplements. The proportion of Cretan prepubescent and adolescent populations taking Ca tablets is believed to be negligible.

Finally, in the present study it was not possible to ob-

tain estimates of Ca balance and Ca absorption efficiency. It is known that Ca absorption and retention are never complete, with one study of 9 to 14 year old children citing the percentage of dietary Ca absorbed as 25% in females and 27% in males [61]. Our "Greek Diet" database does not provide information regarding bioavailability or the absorption rate of Ca from various foods. For example, the entry for spinach in our "Greek Diet" dietary database gives 136 mg of Ca per 100 g but spinach also contains oxalate, which is known to inhibit the absorption of Ca, resulting in about 5% of the Ca in spinach being absorbed, as compared to about 30% of the 119 mg Ca in pasteurized full-fat fresh milk [62]. Certain studies have shown a genetic link between Ca absorption and vitamin D receptor polymorphisms [63, cited in 64]. These issues could be considered further through more specific studies in this area.

Despite the qualitative nature of the present observational study, due in the main to the limitations of the available dietary intake estimation method, we believe that the dietary intakes presented reflect patterns between Ca and other nutrient intakes and health indicator factors in male and female pre-adolescent and adolescent pupils in Crete. As such, the study is of wide interest: it offers the first insight into Ca intake associations with dietary and health indicators in schoolchildren living in this typical Mediterranean region.

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