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ORIGINAL CONTRIBUTION

Effects of Mediterranean diets with low and high proportions of phytate-rich foods on the urinary phytate excretion

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Abstract
Background Important health benefits have been reported recently to phytate intake. This includes the prevention of pathological calcifications such as renal calculi, dental calculi and cardiovascular calcification, due its action as crystallization inhibitor of calcium salts, and as preventive of cancer.
Aim of study The aim of this study was to establish a relation between the intake of phytate, through consumption of typical components of the Mediterranean diet (including nuts), and its excretion in urine.
Methods This study recruited participants from subjects included in a larger trial (PREDIMED) of food habits, that were assigned to one of two diet groups: (1) the Mediterranean diet with low proportion of phytate-rich food group, where participants were asked to maintain their usual diet; and (2) the Mediterranean diet with high proportion of phytate-rich food group, where participants were asked to increase phytate-rich foods in their diet. Phytate intake was assessed on the basis of a food frequency questionnaire. Urinary phytate excretion was determined in 2-h urine samples.
Results The overall phytate consumption of the Mediterranean diet with high proportion of phytate-rich food group (672 ± 50 mg) was significantly higher than the Mediterranean diet with low proportion of phytate-rich food group (422 ± 34 mg), representing a 59% difference. Urinary phytate excretion was also significantly higher (54%) in the Mediterranean diet with high proportion of phytate-rich food group (1,016 ± 70 μg/L) than the Mediterranean diet with low proportion of phytate-rich food group (659 ± 45 μg/L).
Conclusions Mediterranean diets high in whole cereals, legumes and nuts compared to Mediterranean diets low in these phytate-rich foods increase the urinary phytate excretion in humans.

Keywords Phytate · Food consumption · Urinary excretion

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Introduction

Compared to north European and American diets, the traditional Mediterranean diet includes a larger amount of plant food. Large European cohort studies [9, 33, 34, 46] suggest that a high degree of adherence to the Mediterranean diet is associated with reduced mortality and other health benefits. Common components of this diet include monounsaturated and polyunsaturated fatty acids, α-tocopherol, phenolic compounds, phytoesterols, folic acid and other antioxidants [1, 30, 35, 47], and whole grain cereals (unrefined), legumes and nuts are typical food ingredients. Phytate (myo-inositol hexaphosphate) is a naturally occurring compound ingested in significant

quantities by humans that consume diets rich in whole grains, legumes and nuts. Phytate is present in plant seeds, where it occurs as the calcium–magnesium salt phytin and acts as a phosphate store, but it is not homogenously distributed in the seed. Phytate is concentrated in the germ and the aleuronic layers of the kernel, and in the bran or combined aleurone layer and pericarp [41, 42]. While large amounts of phytate are found in whole cereals and in other edible vegetable seeds such as legumes and nuts, food refinement processes (such as the removal of seed coats) and reduced fiber consumption have resulted in human diets gradually becoming poorer in phytate. Mellamby [39] demonstrated that the addition of high levels of phytate (as the sodium salt) to dog diets resulted in reduced calcium absorption and induced rickets. A number of studies have subsequently attributed "anti-nutrient" properties to consumption of large amounts of phytate as the sodium salt [10, 11]. However, recent studies have indicated that dietary phytate (mainly as phytin) comprising approximately 0.1% of a balanced diet has no adverse effects on mineral bioavailability [16, 20, 36]. Human trials have indicated that a phytin intake of 2 g/day does not affect mineral balances when mineral intake levels are adequate [5, 48]. Important health benefits recently reported to be associated with the intake of phytate include the prevention of pathological calcifications such as renal calculi [13, 14, 22], dental calculi [25] and cardiovascular calcification [21, 23, 24], and as an antioxidant [12, 41] and a preventive of colo-rectal cancer [44].

A direct relationship has been found between urinary excretion of phytate and its presence in biological fluids (such as blood) and tissues [16, 17]. Hence, the aim of this study was to relate the Mediterranean diet intake with low and high proportions of phytate-rich foods on phytate excretion in urine.

Materials and methods

Study design

The study was a cross-sectional analysis performed on a subset of 81 participants who were recruited into the PREDIMED trial. The details of this trial have been reported elsewhere [9]. The Institutional Review Board of the Hospital Clinic at Barcelona, Spain approved the study protocol. From October 2006 to March 2007 potential participants were selected by physicians in primary care centers. Eligible subjects were community members (age 55–80 years for men, and 60–80 years for women). Exclusion criteria were a previous history of allergy or intolerance to nuts.

Participants and recruitment

The primary care physicians based participant selection on a review of the patient's clinical record and a screening visit. A list of candidates was obtained from computer-based records of patients regularly attending each of the participating centers. Potentially eligible candidates were contacted by telephone and invited to attend a screening visit. Ninety-five percent of eligible candidates who met entry requirements consented to participate in the study. The recruited participants were assigned to one of two diet groups: (1) the Mediterranean diet with low proportion of phytate-rich food (MD-LP) group ($n = 40$), which comprised participants who were asked to maintain their usual diet; and (2) the Mediterranean diet with high proportion of phytate-rich foods (MD-HP) group ($n = 41$), which comprised participants who were asked to increase specific foods in their diet during the study period (1 year), including more vegetables, olive oil, wine and nuts, the latter provided to the participants at a rate of 0.5 kg walnuts/month. The MD-HP group also received informative talks about typical Mediterranean foods, meal plans and recipes.

Measurements and food consumption

The baseline participant examination included personal data. Height and weight were measured with participants wearing light clothing and no shoes. The body mass index (BMI) was calculated as the weight (kg) divided by square of the height (m^2). Food consumption was determined by a previously validated semi-quantitative 137-item food frequency questionnaire (FFQ) [38].

Estimation of phytate consumption

Phytate consumption was estimated from consumption of ten FFQ items that included the most important sources of phytate (whole cereals, legumes and nuts; Table 1), the serving size for each item [26], and the phytate concentration estimated for each food, the latter based on a variety of published sources [4, 26, 28, 31, 37, 40–42]. The phytate concentration in food is highly variable and influenced by environmental factors such as growing location, irrigation conditions, type of soil, fertilizer applications, plant variety, and harvest and food processing methods [42]. In addition, measured food phytate values are influenced by the method used for phytate determination [40]. The estimation of food phytate contents is of fundamental significance for nutrition research and recently this problem was comprehensively discussed [43]. Estimates of the phytate content of the ten selected

Table 1 Estimated phytate intake for standard serving sizes, based on the reported phytate content for selected items in the food frequency questionnaire (FFQ)

Food	Estimate mg phytate/100 g edible	Serving size (g)	Phytate per serving (mg)
Green beans	180	200	360
Almonds, peanuts, hazelnuts, pistachio or pine seed	1,000	30	300
Walnuts	1,600	30	480
Lentils	400	150	600
Beans (pinto, kidney or lima)	700	150	1,050
Chickpeas	400	150	600
Beans and broad beans	600	150	900
Whole bread	350	75	263
Whole cereals: muesli, oatmeal, all-bran	300	30	90
Wholemeal cookies	300	50	150

FFQ items are shown in Table 1, along with estimates of the phytate value, the serving size of each food, and the estimated phytate content per serving.

Determination of phytate excretion

Urine samples for phytate analysis were obtained from fasting study participants approximately 2 h after they had voided (about 07:00 h) the urine accumulated overnight. The urine sample was acidified with HCl (1:1, v:v) to pH 3–4, and an aliquot (5.0 ml) was quantitatively transferred to a column (inside diameter 4 mm) containing 0.2 g of anion exchange resin (AG 1-X8, 200–400 mesh; BioRad, Hercules, CA). The column was washed with 50 ml of HCl (50 mM) and all the eluted material was discarded. The column was then washed with 3 ml of HNO_3 (2 M) to elute the phytate, the concentration of which in the eluate was determined by direct phosphorus analysis using an inductively coupled plasma atomic emission spectrometer (ICP-AES; Perkin Elmer Model 2000) and the appropriate calibration curve, the co-efficient of variation intra-assay was 2.4% [19] and inter-assay 5.8% (unpublished data), the recovery of phytate during this procedure was 97–105%. All chemicals used were analytical grade reagents.

Statistical analysis

The mean and standard error (SE) were calculated for each of the selected food items. Student's t tests were used to assess differences between means. Conventional Windows software was used for statistical computations. A p value < 0.05 was considered significant.

Results

The age (years), height (m), weight (kg), body mass index (kg/m^2) and waist (m) data for the study participants are shown in Table 2. The waist value for the MD-HP group was slightly greater than for the MD-LP group.

Table 2 Personal data for the Mediterranean diet with low proportion of phytate-rich food (MD-LP) and Mediterranean diet with high proportion of phytate-rich foods (MD-HP) groups

	(MD-LP)	(MD-HP)
Age (years)	65 (1.0)	67.0 (1.2)
Height (m)	1.6 (0.01)	1.6 (0.02)
Weight (kg)	77.2 (1.7)	78.3 (2.6)
Body mass index (kg/m^2)	29.8 (0.5)	29.0 (0.5)
Waist (m)	99.7 (0.3)	101.8 (1.7)*
n	40	41

Data are expressed as mean (SE)

* $p < 0.05$ by Student's t test

Consumption of the ten phytate-rich food items (grams consumed per day) are shown in Table 3. In the MD-HP group there was a significant increase (161%) in the consumption of almonds, peanuts, hazelnuts, pistachio or pine seed, and also an increase in consumption of walnuts (151%) and chickpeas (41%). This group also had a non-significant increase in consumption of beans, whole bread and whole cereals, and a decrease in consumption of wholemeal cookies. Table 4 shows the estimated daily consumption of phytate (mg/day) by the MD-LP and MD-HP groups, and the percentage increase in phytate consumption for each food group. Changes in phytate consumption followed the same pattern as that observed (Table 3) for consumption of food rich in phytate.

Figure 1 shows the estimated overall phytate consumption (mg phytate/day) from the ten phytate-rich FFQ food items and the urinary phytate excretion (μg phytate/L) for the MD-LP and MD-HP groups. The overall phytate consumption of the MD-HP group (672 ± 50 mg) was significantly higher than the MD-LP group (422 ± 34 mg), representing a 59% difference. Urinary phytate excretion was also significantly higher (54%) in the MD-HP group

Table 3 Dietary intake by the Mediterranean diet with low proportion of phytate-rich food (MD-LP) and Mediterranean diet with high proportion of phytate-rich foods (MD-HP) groups for ten selected phytate-rich items in the food frequency questionnaire (FFQ)

Food	Food consumed (g food/day)		
	(MD-LP)	(MD-HP)	Increase (%)
Green beans	31.0 (2.4)	36.6 (4.4)	18
Almonds, peanuts, hazelnuts, pistachio or pine seed	3.3 (0.6)	8.6 (1.0)*	161*
Walnuts	5.5 (1.0)	13.8 (1.6)*	151*
Lentils	5.9 (0.5)	6.2 (0.5)	5
Beans (pinto, kidney or lima)	3.0 (0.39	4.2 (0.5)	40
Chickpeas	4.4 (0.4)	6.2 (0.5)*	41*
Beans and broad beans	2.5 (0.3)	2.5 (0.7)	0
Whole bread	41.6 (6.4)	54.4 (11.2)	31
Whole cereals: muesli, oatmeal, all-bran	0.4 (0.4)	0.7 (0.5)	75
Wholemeal cookies	6.4 (1.6)	4.3 (2.2)	−33

Data are expressed as mean (SE)

* $p < 0.05$ by Student's t test

Table 4 Estimated daily phytate consumption by participants in the Mediterranean diet with low proportion of phytate-rich food (MD-LP) and Mediterranean diet with high proportion of phytate-rich foods (MD-HP) groups

Food	(MD-LP) (mg phytate/day)	(MD-HP) (mg phytate/day)	Increase (%)
Green beans	55.9 (4.3)	65.8 (7.8)	17.8
Almonds, peanuts, hazelnuts, pistachio or pine seed	33.3 (6.6)	85.5 (9.9)*	157.1*
Walnuts	88.4 (15.7)	221.5 (25.3)*	150.7*
Lentils	23.9 (2.1)	24.7 (2.2)	3.2
Beans (pinto, kidney or lima)	21.3 (2.8)	29.2 (3.3)	37.0
Chickpeas	17.8 (1.4)	24.7 (2.0)*	38.5*
Beans and broad beans	15.2 (1.9)	15.2 (4.0)	0.0
Whole bread	145.7 (22.7)	190.4 (36.7)	30.7
Whole cereals: muesli, oatmeal, all-bran	1.1 (1.1)	2.1 (1.4)*	87.7
Wholemeal cookies	19.2 (4.9)	12.9 (6.4)	−32.6

Data are expressed as mean (SE)

* $p < 0.05$ by Student's t test

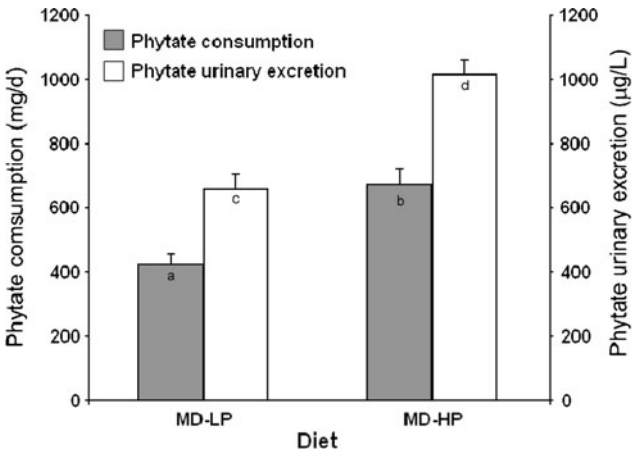

Fig. 1 Estimated phytate consumption (mg/day) and phytate urinary excretion (µg/L) for the Mediterranean diet with low proportion of phytate-rich food (MD-LP) and Mediterranean diet with high proportion of phytate-rich foods (MD-HP) groups. *a*, *b* and *c*, *d* are significantly different $p < 0.05$ by Student's t test

(1,016 ± 70 µg/L) than the MD-LP group (659 ± 45 µg/L) (Fig. 1), indicating a clear relationship between phytate consumption and excretion.

Discussion

Intake of phytate is difficult to estimate because the phytate content of food varies as a function of seed treatment (e.g., dehulling or germ separation) and storage, the part of the seed used, and the processing of food containing seeds [43]. In this study, estimates of phytate intake were calculated from reported values of phytate content in food [4, 26, 28, 31, 37, 40–42]. These calculations did not consider the effect of food processing. Participants in the MD-LP group ingested 422 ± 34 mg phytate/day, which is a low/medium value (Table 5) relative to values reported for other countries (range approximately 200–1,500 mg/day). With respect to phytate-rich foods, there was significantly higher consumption of nuts and chickpeas by the MD-HP group than the MD-LP group, and the former also showed a non-significant increase in consumption of beans and whole bread (Table 3). Thus, consumption of walnuts, other kinds of nuts and chickpeas by the MD-HP group represented an increase of 151, 161 and 41%, respectively, relative to the MD-LP group. It has been shown that phytate in blood, urine and biological fluids of mammals depends on the dietary

Table 5 Estimated phytate intake in selected countries

Country	Phytate (mg/day)		Condition	Reference
	Mean	Range		
UK	1,436	681–2,191	Male (>40 years)	[29]
Sweden	1,146	500–2,927	Male–female (35–76 years) vegetarian diets	[2]
South Korea	839	439–1,239	Male (21–70 years)	[31]
South Korea	752	345–1,159	Female (21–70 years)	[31]
UK	750	600–800	Male–female	[6]
USA	750		Average American (bw, 75 kg)	[7]
USA	746	714–762	Male omnivorous (self-selected)	[27]
UK	676	504–848	Male–female	[49]
USA	631	590–734	Female omnivorous (self-selected)	[7]
USA	538	253–1,352	African-American males–females	[8]
South Korea	496	244–748	Female (64–75 years)	[32]
Sweden	369	230–532	Male–female western-type diets	[2]
Italy	219	112–1,367	Male–female	[3]
Sweden	180		Male–female	[45]

intake of phytate [15, 18], and that consumption by humans and rats of a phytate-Mediterranean diet with low proportion of phytate-rich food (MD-LP) decreases the urinary excretion of phytate by about 50% after 36 h [15, 18]. The results of the present study are consistent with previous studies, as the data indicated an average increase of 250 mg of dietary phytate/day and an increase of 0.3 mg/L in urinary phytate. Based on published data for animals [15], it appears that there is a maximum amount of phytate that can be absorbed (20.9 mg/kg per day), beyond which no further absorption occurs. Extrapolation of these data to a 70-kg human suggests that the minimum intake of dietary phytate necessary to achieve maximum absorption is 1,463 mg phytate/day. This intake would require a Mediterranean diet richer in whole cereals, legumes, and nuts than was consumed by the participants in the present study. However, as noted in the introductory comments, this level of intake would not affect mineral bioavailability but would have important health benefits including the prevention of pathological calcifications [13, 14, 21–25] and colo-rectal cancer [44], and enhance antioxidant action [12, 41].

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