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Phytosterol Content and Fatty Acid Pattern of Ten Different Nut Types

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Abstract: Ten different nut kinds (almonds, Brazil nuts, cashews, hazelnuts, macadamias, peanuts, pecans, pine nuts, pistachios, and walnuts) were evaluated for their total oil and phytosterol content as well as their fatty acid composition. The total oil content was the predominant component; mean values oscillated between 45.2 % (cashews) and 74.7 % (macadamias). Mean total phytosterol content ranged from 71.7 mg (Brazil nuts) to 271.9 mg (pistachios) per 100 g oil. ß-sitosterol was the major sterol (mean >71.7 mg/100 g oil) followed by minor contents of campesterol, ergosterol, and stigmasterol. Almonds, cashews, hazelnuts, macadamias, and pistachios were high in monounsaturated fatty acids (MUFA; > 55 %). MUFA- and polyunsaturated fatty acid (PUFA)-rich nuts were peanuts and pecans, whereas Brazil nuts, pine nuts, and walnuts had the highest PUFA content (> 50 %); the high unsaturated/saturated fatty acid ratio ranged from 4.5 to 11.8. However, the fatty acid pattern of every nut is unique.

Key words: phytosterols, fatty acids, almonds, Brazil nuts, cashews, hazelnuts, macadamias, peanuts, pecans, pine nuts, pistachios, walnuts

Introduction

Nuts are basic components of every healthy diet, but particularly of the traditional Mediterranean diet. This diet, which is partly based on a high intake of bioactive compounds, dietary fiber, and unsaturated fatty acids, has been suggested to protect against coronary heart diseases (CHD), insulin resistance, metabolic syndrome, and cancer [1, 2]. Evidence from prospective cohort and controlled intervention studies demonstrate that a regular consumption of nuts is associated with a lower risk of coronary heart disease morbidity and mortality in different populations [3–5]. Epidemiologic studies, like the Adventist Health Study, the Iowa Women's Health Study, the Nurses' Health Study, and the Physicians' Health Study, have consistently shown that frequent consumption of nuts is associated with a reduced coronary heart disease morbidity and mortality in different population groups [6–11]. These results are assumed to be mainly due to the less atherogenic plasma lipid profiles associated with long-term consumption. A pooled analysis of 25 intervention trials underlined this hypothesis, which demonstrated that daily nut consumption, of a quantity ranging from 23 to 132 g, reduced plasma total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), the ratio of LDL-C to HDL-C (high-density lipoprotein cholesterol), and the ratio of TC to HDL-C [12]. Nuts have a favorable fatty acid profile [high in unsaturated fatty acids (UFA) and low in saturated fatty acids (SFA)]. They contain considerable amounts of many beneficial nutrients like plant protein, dietary

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fiber, antioxidant vitamins, minerals, plant sterols, and polyphenols. Kris-Etherton *et al.* reviewed epidemiological as well as clinical studies and found that the cholesterol-lowering diets with nuts exerted about a 25 % greater lipid-lowering effect than the fatty acid profile that would be expected from controlled diets [3, 13]. The seventeen studies under review demonstrated an LDL-reduction of 0.29 mmol/L, with a quite high daily nut dosage expressed in g (ranging from 37 to 100 g nuts/day), or % energy (ranging from 12.5 to 20 % energy) [13].

Different recommendations for daily nut intakes have been made, ranging from 1–2 tablespoons (15–30 g/day) [14] and a "handful" [2], up to 43 g/day for adults [15].

Based on scientific data, the U.S. Dietary Guidelines Advisory Committee issued a statement that unsalted nut consumption (43 g/day) as part of a nutritionally adequate diet has a favorable impact on cardiovascular disease risk factors, when total calorie intake is held constant [15]. However, the evidence for this is modest. One reason may be that 43 g nuts per day contain a very high amount of energy, ranging from 244 kcal (cashews) to 305 kcal (macadamia), and therefore maintaining a reasonable caloric intake when including nuts in the diet requires adequate nutritional information [15]. However, since 2003 the U.S. Food and Drug Administration has originated a health claim for eating nuts, stating that the intake of 43 g/day of especially almonds, hazelnuts, pecans, pistachios, walnuts, and peanuts may lower the risk of CHD [16].

Due to the latter fact, there is a growing interest to determine additional mechanisms for CHD prevention via nut consumption. It is possible that some nuts may have a better effect on human health due to their unique nutritional composition. Therefore, the determination of possible health-supporting components may help to elucidate which nuts are most protective against CHD. There is limited information available in the literature regarding the phytosterol content, fatty acid profile, and fat quality of almonds, Brazil nuts, cashews, hazelnuts, macadamias, peanuts, pecans, pine nuts, pistachios, and walnuts.

The analytical data set presented in this paper was part of a larger determination of the composition of ten different nut types. The first part of the results on tocopherols, carotenoids, unsaponifiable matter, oil content in dry matter, and total phenolics has been already published [17]. The objective of this article is to give a detailed overview of the phytosterol content, fatty acid composition, and important fatty acids of almonds, Brazil nuts, cashews, hazelnuts, macadamias, peanuts, pine nuts, pistachios, and walnuts.

Materials and Methods

Chemicals and reagents

Methanol LiChrosolv, n-hexane p. a, n-hexane HPLC, and sodium sulfate were purchased from Merck (Vienna, Austria). The standards of campesterol, stigmasterol, ergosterol, ß-sitosterol, FAME mixtures, and BF_3 in methanol were purchased from Sigma. All other chemicals were purchased from Riedel de Haën.

Samples and sample preparations

Almonds, Brazil nuts, cashews, hazelnuts, macadamias, peanuts, pecans, pine nuts, pistachios, and walnuts were bought in a local supermarket (Vienna, Austria) and from a market in Ródos (Greece). Additionally, all kinds of nuts were donated from Farmgold, a national company that is one of the largest suppliers of nuts in Austria. Thus, each type of nut was analyzed from three different providers in terms of the phytosterol content. It could not be confirmed that the analyzed nuts originated from the same season. The fatty acid pattern was analyzed only from one provider (Austrian supermarket).

Analytical procedures

Lipid extraction for oil determination was accomplished with a Soxhlet extraction using petroleum ether as solvent. The nuts were chopped in a coffee mill and dried at 103 °C for 1 hour. Nine grams of each sample were placed in an extraction cell and extracted for 6 hours. Thereafter the solvent was evaporated. The remaining oil was dried under a nitrogen stream and placed in an oven at 100 °C for 1 hour. The oil content of the petroleum ether extract was balanced.

Lipid extraction for further chemical analyses was performed according to Kornsteiner *et al.* [17].

Phytosterols were determined by RP-HPLC according to the method of Holen with a slight modification [18]. About 200 mg of oil was placed in a round-bottomed flask. The samples were hydrolyzed with 30 mL 0.8 M KOH in ethanol for about 30 minutes at 80 °C. After saponification, the free phytosterols were extracted twice with 25 mL hexane. The combined extracts were washed with water and dried over Na_2SO_4. The hexane was evaporated to dryness at 40 °C. The unsaponifiable matter was dissolved in methanol and analyzed by RP-HPLC (RP-18, 5 μm) equipped with a Merck Hitachi UV/VIS detector set at 206 nm. The mobile phase was methanol/water (99:1) with a flow rate of 0.65 mL/min.

The fatty acid pattern was determined with GC-FID (Perkin Elmer) according to Kornsteiner *et al.* [19]. About 10 mg of the extracted oil was saponified with 1 mLof 0.5 M methanolic NaOH solution at 100 °C for 5 minutes. BHT was added as an antioxidant (60 mg/L methanolic NaOH solution). The fatty acids were methylated with 1 mLBF_3 at 100 °C for 5 minutes. The fatty acid methyl esters (FAME) were extracted with 2 × 1.5 mLhexane. The hexane phase was abated and evaporated. The remaining samples were dissolved in 2000 μL hexane and 1 μL was injected with a split-ratio 1:50. The FAME were separated by a 30 m × 0.25 mm ID fused silica column (Restek, RTX-2330) and detected with a flame ionization detector (FID). The injector and FID temperatures were set to 250 °C. Initial temperature was 120 °C with an increase to 220 °C at 2 °C/minute. The fatty acids were identified by reference to standard mixtures of known composition run under identical conditions. Quality criterion of the analytical methods was the coefficient of variation (CV): lipid extraction for total oil determination: 1.7; phytosterols: < 5.8; and fatty acid pattern: <3.7.

Results and Discussion

Total oil content

The oil content per edible portion (43 g/day, 1.5 oz/day) and the percentage of oil in nuts are shown in Table I. The mean lipid content in different nut kernels varied from 19.4 g (cashews) to 32.1 g (macadamias) per edible portion. Among all nuts analyzed, walnuts, Brazil nuts, pine nuts, pecans, and macadamias had the highest total oil content. The mean ranged from 61.7 to 74.7 %. For cashews, peanuts, pistachios, almonds, and hazelnuts the oil contents were lower (45.2 – 58.1 %), although oil content and total energy is still very high. The results confirm earlier observations that nuts are an excellent source of energy due to their high oil content [20 – 28]. For this reason nuts have been treated with caution in most of the previous food pyramids [29]. Additionally, the results demonstrate the rather high oil content of an edible portion (43 g/day), which has been claimed to reduce CHD [16]. However, the daily “dosage” of nuts is still subject of controversial discussions [2, 14, 15]. In addition, it is well recognized that excess energy intake and reduced physical activity lead to overweight and/or obesity, which is a risk factor for chronic diseases like type 2 diabetes, dyslipidemia, cardiovascular disease, and stroke [30]. Therefore, in countries with no or little tradition of nut consumption, nutritionists are reserved with regard to the promotion of nut consumption due to their high caloric value and their impact on energy balance and body weight [31]. On the other hand, nuts are not

Table I: Oil content per edible portion (43 g nuts).

nuts	g oil content/43 g edible portion		% oil per nut variety	
	Mean	Range	Mean	Range
Almonds[b]	23.3	21.3 – 24.8	54.3	49.6 – 57.7
Brazil nuts[a]	28.6	27.7 – 29.2	66.4	64.4 – 67.9
Cashews[a]	19.4	18.5 – 20.3	45.2	43.1 – 47.2
Hazelnuts[a]	25.0	23.0 – 28.1	58.1	53.5 – 65.3
Macadamias[a]	32.1	31.1 – 32.9	74.7	72.4 – 76.5
Peanuts[a]	20.8	19.9 – 21.8	48.4	46.3 – 50.6
Pecans[a]	29.8	29.4 – 30.5	69.3	68.3 – 71.0
Pine nuts[a]	28.9	28.4 – 29.5	67.3	66.0 – 68.5
Pistachios[b]	22.1	18.6 – 24.4	51.3	43.3 – 56.7
Walnuts[a]	26.5	26.1 – 26.9	61.7	60.8 – 62.6

[a] Data are expressed as means and range (n = 3). [b] Data are expressed as means and range (n = 6) from nuts with and without skin. The calculation includes the dry matter, which has been analyzed and published by Kornsteiner, Wagner, and Elmadfa (2006)

Table II: Phytosterol content of the investigated nut oils. Data are expressed as mg/100 g nut oil.

	Campesterol		Ergosterol		Stigmasterol		ß-Sitosterol		Total	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Almonds[b]	3.6	2.0–5.8	14.7	10.6–19.3	0.7	nd-2.9	142.8	111.8–168.8	161.8	139.8–182.5
Brazil nuts[a]	nd	nd	nd	nd	nd	nd	71.7	53.2–81.8	71.7	53.2–81.8
Cashews[a]	13.4	12.8–14.1	nd	nd	nd	nd	107.1	104.3–108.6	120.4	117.5–122.4
Hazelnuts[a]	4.7	4.0–5.0	23.5	19.4–26.6	1.0	nd-2.9	86.2	79.5–97.0	115.4	106.3–128.6
Macadamias[a]	5.7	5.0–6.7	nd	nd	nd	nd	113.7	100.3–123.2	119.4	105.6–128.2
Peanuts[a]	16.0	11.5–23.5	nd	nd	9.2	8.0–10.0	100.3	84.5–131.4	125.5	104.4–164.5
Pecans[a]	7.2	6.7–7.5	nd	nd	nd	nd	105.8	97.3–113.5	112.9	104.9–120.2
Pine nuts[a]	11.1	10.6–11.4	nd	nd	nd	nd	108.3	104.5–111.2	119.5	115.0–122.6
Pistachios[b]	16.4	10.5–21.3	nd	nd	0.8	nd-1.7	254.8	202.6–284.7	271.9	214.8–306.0
Walnuts[a]	30.0	29.9–30.2	nd	nd	nd	nd	113.2	96.6–129.8	143.2	126.8–159.7

[a] Data are expressed as means and ranges (n=3). [b] Data are expressed as means and ranges (n=6) from nuts with and without skin. nd, not detectable.

solely characterized by a high energy density; they are also a good source of unsaturated fatty acids, vegetable proteins, fiber, phytosterols, polyphenols, vitamins, and minerals [31]. Therefore, they differ substantially from other high-calorie foods, which can add to chronic overnutrition. Food that is energy-dense and rich in fat, high glycemic/refined carbohydrates, saturated fatty acids, salt and sugar, but low in micronutrients and bioactive compounds, is considered to stimulate intracellular pathways leading to oxidative stress and inflammation, which are key factors in the pathogenesis of non-communicable diseases [30].

A healthy eating pattern is characterized by a limited intake of sodium, saturated fats, added sugars, and refined grains, with a special focus on nutrient-dense foods including vegetables, fruits, whole grains, fat-reduced milk and milk products, seafood, lean meats and poultry, eggs, beans and peas, and nuts and seeds [15].

Phytosterol content

We decided to focus on the most abundant phytosterols, which are ß-sitosterol, campesterol, and stigmasterol [32], as well as ergosterol. The mean total phytosterol content (Table II) in the nuts varied between 71.7 mg (Brazil nuts) and 271.9 mg (pistachios) per 100 g extracted oil. The most predominant phytosterol in all nut types was ß-sitosterol, ranging from about 71.7 mg (Brazil nuts) to 254.8 mg (pistachios) per 100 g extracted oil. Cashews, macadamias, peanuts, pecans, pine nuts, pistachios, and walnuts showed a higher mean concentration of campesterol (5.7 mg-30.0 mg/100 g oil) than ergosterol and stigmasterol. Traces (<6 mg/100 g oil) of campesterol were found in almonds and hazelnuts. Mean ergosterol content was only detected in almonds (14.7 mg/100 g oil) and hazelnuts (23.5 mg/100 g oil). A minor amount of stigmasterol (<11 mg/100 g oil) was present in almonds, hazelnuts, peanuts, and pistachios. The highest mean concentration of stigmasterol was shown in peanuts (9.2 mg/100 g oil). The predominance of ß-sitosterol is in agreement with other publications [20–23, 27, 33–35]. Kaijser *et al.* [23] reported that macadamias had a low content of stigmasterol (<20 μg/g lipids) and 5-avenasterol (<210 μg/g). Further published data [21, 23, 27, 33, 34] have shown that hazelnuts, macadamias, peanuts, and walnuts contain small amounts of 7-avenasterol and 7-stigmasterol, too. Phillips *et al.* described a range of 5-avenasterol amounts in almonds, Brazil nuts, cashews, hazelnuts, macadamias, peanuts, pecans, pine nuts, pistachios, and walnuts, from 2.6 mg/100 g for hazelnuts to 40.3 mg/100 g for pine nuts [35]. Phytosterols are plant compounds structurally related to cholesterol with different side chain configurations, and they cannot be synthesized by humans. The absorption of plant sterols and stanols from the human intestine is low and can vary between 0.04 to 16 % and 0.02 to 0.3 %, respectively, which is highly dissimilar to cholesterol absorption (35 to 70 %) [36, 37]. The detailed cholesterol-lowering mechanism of phytosterols is still not completely understood, but is probably mainly linked to their absorption, which in turn affects the absorption of cholesterol. The cholesterol and phytosterol absorption can only take place when they are incorporated into a mixed micelle, which contains fatty acids, diglycerides, monoglycerides, lysophospholipids, phospholipids, bile salts, free and esterified cholesterol, as well as phytosterols. The high physicochemical affinity of plant sterols for micelles is considered to compete with dietary cholesterol, which results in a reduced absorption in the duodenum and the proximal jejunum. As a consequence, the displaced cholesterol is excreted with the feces [38]. In addition, clinical studies have shown that the intake of 2–3 g/day of plant sterols diminishes cholesterol levels up to 15 % [39]. The minimum effective dose was shown to be 800–1000 mg/day [40]. Due to their cholesterol-lowering properties, phytosterols have been considered as functional foods. At the same time, there is an ongoing debate about the possible long-term adverse effect of unnaturally enriched foods and high dosages of plant sterols, which are able to have pharmacological effects [38, 39]. Nuts are a natural source of plant sterols, which are known to be safe within a wide range of intake. The results showed that nut oils can contribute to a phytosterol intake from 71 mg (Brazil nuts) to 271.9 mg (pistachios) per 100 g extracted oil. This corresponds to 20 mg (Brazil nuts/43 g portion) and to 60 mg (pistachios/43 g portion). The average daily intake of plant sterols is 160–400 mg among different population groups, whereas strict vegetarians can reach up to 700 mg [38]. Regarding the minimum intake of plant sterols for a cholesterol-lowering effect, the analyzed phytosterol content in nuts is well below the dosage in human clinical studies. However, the unique combination of phytosterols with unsaturated fatty acids and other components of the unsaponifiable matter in nuts support the notion that such "low dosages" can have cholesterol-lowering effects. The phytosterol content of the Mediterranean diet has been suggested to play an indirect role in CHD prevention [39].

Fatty acid profile

The fatty acid profile, expressed as a percentage of total fatty acids of extracted oil, and important fatty acid ratios of the nuts are presented in Table III. Palmitic acid was the major saturated fatty acid found, ranging from 5.2 % (hazelnuts) to 16.9 % (Brazil nuts), followed by stearic acid. In the extracted nut samples monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) (Table III) were predominant. A high percentage of oleic acid was detected in hazelnuts (84.0 %), almonds (68.1 %), cashews (62.0 %), macadamias (60.1 %), pistachios (56.7 %), pecans (46.2 %), and peanuts (39.5 %). Macadamias showed a high concentration of palmitoleic acid (19.2 %). The

Table III: Overview of the most important fatty acids and fatty acid ratios.

	Fatty acid content (% of total fatty acids)									
	Almonds[a]	Brazil nuts[b]	Cashews[b]	Hazelnuts[b]	Macadamias[b]	Peanuts[b]	Pecans[b]	Pine nuts[b]	Pistachios[a]	Walnuts
16:0	6.3	16.9	10.1	5.2	9.7	11.0	7.2	6.1	10.0	6.6
18:0	1.5	–	7.0	2.5	3.5	3.7	2.8	3.3	1.1	2.6
16:1	0.5	0.5	0.7	0.2	19.2	0.1	0.1	–	0.5	0.1
18:1n-9	68.1	26.1	62.0	84.0	60.1	39.5	46.2	32.5	56.7	14.3
18:2n-6c	22.9	55.6	18.7	7.5	–	40.5	39.1	53.8	30.3	63.4
18:3n-3	0.1	0.1	0.1	0.1	0.2	0.1	2.3	0.2	0.2	12.5
SFA[c]	8.1	17.6	18.1	7.9	17.8	18.3	11.6	10.8	11.7	9.4
MUFA[c]	68.9	26.7	63.1	84.3	82.0	41.0	46.6	34.6	57.5	14.6
PUFA[c]	23.0	55.7	18.8	7.8	0.2	40.7	41.8	54.6	30.8	76.0
UFA[c]	91.9	82.4	81.9	92.1	82.2	81.7	88.4	89.2	88.3	90.6
PUFA/SFA	2.8	3.2	1.0	1.0	–	2.2	3.6	5.1	2.6	8.1
UFA/SFA	11.4	4.7	4.5	11.8	4.6	4.5	7.6	8.3	7.6	9.7
LA/ALA	229	457	156	68	–	404	17	265	160	5

[a] Data are expressed as means (n = 2) from nuts with and without skin. [b] Data are expressed as single analyses of nuts from the supermarket. [c] The sums and ratios are with traces of fatty acids, which are not included in the table.

main PUFA was linoleic acid (LA), between 7.5 % in hazelnuts and 63.4 % in walnuts, with the exception of macadamias. The greatest level of α-linolenic acid (ALA) was found in walnuts (12.5 %); for all other nuts this value was lower than 2.5 % of total fatty acids (FA) (Table III). Both traces (<2.5 % of total FA) of SFA (myristic, heptadecanoic, arachidic, tricosanoic, lignoceric acid) and unsaturated fatty acids (UFA; heptadecenoic, gadoleic, erucic, nervonic, trans-linoleic, γ-linolenic, eicosadienoic acid) were detected depending on the nut variety (results not shown). Total SFA (Table III) are only a small part of the total fatty acid content of the extracted oil. It ranged between 7.9 % (hazelnuts) and 18.3 % (peanuts). The MUFA content in descending order was hazelnuts > macadamias > almonds > cashews > pistachios > pecans > peanuts > pine nuts > Brazil nuts > walnuts. Walnuts showed the highest PUFA content (76 %), whereas it was negligible (0.2 %) in macadamias. The remaining nuts showed a PUFA amount between 7.8 % (hazelnuts) and 55.7 % (Brazil nuts). The sum of total UFA was very high ranging from 81.7 % (peanuts) to 92.1 % (hazelnuts). The ratio of PUFA/SFA was between 1.0 (cashews, hazelnuts) and 8.1 (walnuts), with the exception of macadamias (no ratio available). Elevated ratios of LA/ALA (>15) showed almonds, Brazil nuts, cashews, hazelnuts, peanuts, pecans, pines and pistachios, whereas the ratio for walnuts was 5. There was no LA/ALA ratio in macadamias, due to the absence of LA. The investigated nuts showed a very heterogeneous fatty acid profile, which probably contributes in a different manner to the cholesterol-lowering effect. For instance, it has been stated by the FAO/WHO Expert Consultation (2008) that "lauric, myristic and palmitic acids raise LDL cholesterol levels compared with stearic acid and there is convincing evidence that substituting SFAs C12:0–C16:0 with PUFA reduces LDL-cholesterol levels and the TC/HDL-cholesterol ratio. This effect can also be observed, but to a lesser extent, by substituting these SFA with MUFA" [41].

Almonds, cashews, hazelnuts, macadamias, and pistachios are rich in MUFA (>55 %). MUFA- and PUFA-rich nuts are peanuts and pecans, whereas Brazil nuts, pines, and walnuts are high in PUFA (>50 %). These results are in agreement with previously published studies [22–24, 27, 28, 33, 42, 43]. For that reason, the cholesterol-lowering properties of nuts are based on the combined effects of fatty acids with phytosterols as a main fraction of the unsaponifiable components of nuts. Nevertheless, it seems that the main observed improvement of the plasma cholesterol content is based on the replacement of SFA in the habitual diet by the increase of UFA, which could be demonstrated in a systematic review of the effects of nuts on blood lipid profiles [4]. In our investigation, walnuts in particular showed a favorable ratio of linoleic to α-linolenic acid (LA/ALA), which is in accordance with published data [22, 33]. The LA/ALA ratio of 5:1 is still recognized as an important parameter, especially in the absence of dietary pre-formed n-3 long-chain polyunsaturated fatty acids (n-3LCPUFA) [44]. This ratio can contribute to an adequate n-3LCPUFA status via the bioconversion of ALA to eicosapentaenoic and docosahexaenoic acid. A recent observational study of Austrian vegetarians and vegans demonstrated that they are able to convert ALA to n-3LCPUFA fatty acids. However, the n-3LCPUFA status of erythrocyte phospholipids is reduced compared with omnivores due to an increased ratio of LA/ALA (10:1) and the negligible intake of pre-formed n-3LCPUFA fatty acids [19]. In this context, it has to be emphasized that the WHO has recently published recommendations for the intake of eicosapentaenoic and docosahexaenoic acid to ensure an adequate n-3LCPUFA status, which is important for physical, mental, and neurological health [41].

Conclusion

This article should provide the readers with a data set of the fatty acid profiles and phytosterol content of popular nuts in addition to the already published literature dealing with other bioactive compounds [17]. Since nuts are a "storehouse" for a large number of phytochemicals, the knowledge of these components is a basic step in the exploration of protective mechanisms, particularly in CHD prevention. However, the energy load of nuts consumed must be taken into consideration in the planning of the daily diet.

Conflict of interest

The authors have declared no conflict of interest.

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