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Common sources and composition of phytosterols and their estimated intake by the population in the city of São Paulo, Brazil

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ABSTRACT

Objective: Phytosterols have been used alone, or combined with lipid-altering drugs, to reduce cholesterol levels and the burden of cardiovascular disease. Considerable variation in the composition of phytosterols exists and its consumption, in a regular diet, by the Brazilian population is still unknown. Thus, the aim of the present study was to determine the phytosterols content of the most consumed plant foods and to estimate the phytosterols intake by this population.
Methods: Intake of plant foods of a representative population of the city of São Paulo (n = 1609), randomly selected on the basis of the Brazilian Institute for Geography and Statistics census data (2010), was obtained by a food frequency questionnaire (FFQ). Foods were chosen on the basis of the Consume Expenditure Survey (2002–2003) and from answers to the FFQ. Phytosterols composition of most consumed greens, legumes, cereals, and seeds, fruits, and vegetable oils was determined by gas chromatography (flame ionization detection). Daily phytosterols intake was estimated in terms of mg per 100 g (mg/100 g^{-1}) of edible portion. Underreporters and overreporters were excluded.
Results: Mean (SE) daily phytosterols intake in the diet of the study population was 100.6 (1.2) mg, with β-sitosterol as the largest sterol component (65.4%), followed by campesterol (23.2%), and stigmasterol (10%). No significant changes in daily phytosterols intake were observed after exclusion of underreporters and overreporters. Considerable variation was observed in phytosterols content among the most consumed plant foods.
Conclusions: Analysis of phytosterols composition in most consumed plant foods has shown that phytosterols content varied among food groups. Dietary intake of phytosterols in a large population of the city of São Paulo is in the same range of some countries.

Introduction

Changes in diet are a most effective strategy to reduce the growing incidence of cardiovascular disease. Reduction in the intake of saturated fat and cholesterol combined with increase in fiber consumption are universally recommended and associated with a moderate reduction in the levels of low-density lipoprotein (LDL)-cholesterol. Furthermore, additional reduction in LDL-cholesterol can be achieved when these recommendations are combined with ingestion of adequate amounts of phytosterols, soy protein, and nuts [1].

CMM carried out the clinical and experimental protocol, performed statistical analyses, and drafted the manuscript. FAF conceived of the study, participated in its design and coordination, performed statistical analyses, and drafted the manuscript. CAB helped with data analysis of phytosterol assessment, and also in drafting the manuscript. AMF participated in study design and drafted the manuscript. ADM reviewed the laboratory data and drafted the manuscript. HTG coordinated the standardization and the assessment of plant sterols. MCI conceived of the study, participated in its design and coordination, performed statistical analysis, and drafted the manuscript.

The authors have no competing financial interest to declare.

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Phytosterols are plant sterols with biologic functions similar to those of mammalian cholesterol. However, due to small differences in the chemical structure of phytosterols, they are much less absorbed (2–5%) [2,3] than cholesterol (56%) [4]. Moreover, phytosterols decrease the intestinal absorption of cholesterol and, therefore, they have been proposed as lipid-lowering agents. Recently, a synergistic effect of phytosterols and ezetimibe was demonstrated, providing additional inhibition of cholesterol absorption when compared with each therapy alone [5].

Although a daily consumption of 2 g to 3 g of phytosterol is associated with a 10% to 15% decrease in the levels of LDL-cholesterol [6], such phytosterol intake is not usually observed in a regular diet even among vegetarians.

Legumes, cereals, and seeds, oils, nuts, fruits, and greens are the main sources of phytosterols. Many differences in type and content of plant sterols have been reported for a variety of products in each group of food [7–9].

Whereas few reports were found regarding the mean daily consumption of plant sterols in developed countries [7,9], in developing nations, where food intake habits are markedly different, the available information is still lower.

Considerable variation in the composition of plant food sterols exist and their consumption in a regular diet by the Brazilian population is still unknown. Thus, the aim of the present study was to determine the phytosterol content of the most consumed plant foods and to estimate the phytosterol intake by the population.

Materials and methods

Population study

This cross-sectional study included 1609 individuals of both sexes. Sample-size calculation was based on the Brazilian Institute for Geography and Statistics census data (2010) and included adult individuals living in the city of São Paulo [10]. The study sample was selected using a non-stratified multistage cluster sampling (districts, census tracts, and households). In the first, second, and third stages, 25% of the districts were arbitrarily selected; four census tracts were randomly chosen for each district; and one of two households was selected for interview, respectively. Sixteen interviews per census tracts and 64 per district were performed.

The sample size was based on a population of 7,553,035 adult individuals (population in 2007, SEADE) [11] living in the city of São Paulo. In our calculations, the following equation (OpenEpi 2.0 statistical software) was used: $n = [DEFF^*N^*p(1-p)]/ [(d^2/Z^2_{1-\alpha/2}{}^*(N-1)+p^*(1-p)]$, where N is the population size, p is the expected proportion, $Z^2_{1-\alpha/2}$ is a constant related to the confidence interval (1.96), d^2 is the adopted error (0.05), DEFF is the effect of study design for complex methods, and n is the sample size. The calculated sample size for DEFF = 1 was 384 households; for DEFF = 2, was 768 households. The sample size has been duplicated to increase its representativeness.

The Terraview Social Policies software was used to map the selected households. Maps of the city of São Paulo were built containing information on selected districts with the respective census tracts.

Collection of population data

Interviews were performed by a group of trained dietitians and all procedures were explained to the adult living in the household.

The study protocol was conducted in accordance with the ethical guidelines for human experimentation and was approved by the Ethics Committee of the Federal University of São Paulo (#1328/09). Participants were included after they had signed a written informed consent form.

Self-reported data on socioeconomic characteristics (level of education and incomes), lifestyle (physical activity), body weight and height, and risk factor profile (hypertension, diabetes, hypercholesterolemia, and smoking) were obtained. This procedure was validated for population studies [12–16].

Dietary assessment

A 24-h recall of dietary intake was obtained to estimate the content and type of macronutrients (fat, protein, carbohydrate, cholesterol, fibers) [17]. Although 24-h recalls are easily and rapidly obtained, a single 24-h recall is not a good estimator of the usual diet because the intraindividual diet variability is not taken into account.

To assess the usual consumption of plant-derived foods we used a food frequency questionnaire that has been validated for population studies [18–21]. Common sources of plant sterols were identified and represented as portions for calculation of the daily intake.

To address adequacy of the information obtained in 24-h recalls, the reported energy intake (EI), estimated basal metabolic rate (BMR), and the EI:BMR ratios were obtained. BMR was calculated for gender, weight, height, and age according to the World Health Organization (1995) [22]. The Goldberg cut-off was used to verify underreporters (EI:BMR < 0.76), acceptable reporters (EI:BMR ≥ 0.76 and ≤ 1.24) and overreporters (EI:BMR > 1.24) [23,24]. Additionally, the Black criterion [25] also was used to better detect underreporters (EI:BMR < 0.87) for a single day's record.

The Avanutri software (5.0, for Windows; Rio de Janeiro, RJ, Brazil) was used to calculate the diet composition from 24-h recalls.

Common sources of plant sterols also were chosen on the basis of the Consume Expenditure Survey (CES, 2002–2003) for the city of São Paulo [12], which is a survey of food purchases covering all the economic and geographic sections of the city. For the final estimation of total phytosterol daily intake, we added the phytosterol content of soybean oil (20 mL), the type and amount of vegetable oil most consumed in Brazil according to the CES. The most consumed food plants and oils were then chosen for chemical analyses.

Analyses of plant sterols composition in plant foods

Plant materials

Food samples were obtained from different sources and were submitted to distinct preparations before analysis.

Legumes, cereals, and seeds

Two samples of cereals (brown and polished rice), legumes and seeds (green pea, brown and black bean, chickpea, lentil, soybean, black soybean, linseed) were purchased from a supermarket, and one sample was purchased from the largest warehouse of the city (Company of General Warehouse in São Paulo, CEAGESP, São Paulo, SP, Brazil). Legumes, cereals, and seeds were examined in raw. Food samples (150 g each) were homogenized to form a composed sample, powdered, and packed (100-g bags).

Fruits and vegetables

Fruits (açaí, avocado, pineapple, banana, coconut, guava, orange, apple, papaya, mango, and strawberry) and vegetables (zucchini, eggplant, broccoli, carrot, cauliflower, endive, spinach, white cabbage, tomato, and green bean) were purchased from CEAGESP (Sorocaba, SP, Brazil). Vegetables (800–4000 g) and fruits (650–1400 g) were washed with demineralized water and freeze-dried. Only the edible portion was used after husks and seeds were removed. Samples were crushed and, in some cases (zucchini, eggplant, broccoli, carrot, cauliflower, spinach, white cabbage, and green bean), submitted to a bleaching process, frozen (−30 to −40°C), and freeze-dried in a vacuum camera (759.6 mm Hg) for 20 to 25 h. The samples were then powdered and packed in 100-g bags.

Oils

Canola, coconut, sunflower, corn, soybean, olive, and composed oils were purchased from a supermarket and were examined in *natura*.

Quantification of phytosterols in plant foods

For lipid extraction, dry samples (5 g) or oils (5 mL) were used. Lipids were cold-extracted, as described by Bligh-Dyer [26]. Content analysis of phytosterols was initiated by a saponification step. In a capped test tube, ethanol (95%, 30 mL), KOH (50% in water, 5 mL), and dihydrocholesterol (0.2% in isopropanol, 0.5 mL; as internal standard) were added to the oil extracted from the sample (1.00 ± 0.03 g), and this solution remained in a water bath (100°C, 1 h). After cooling, the tube content was transferred to a separatory funnel, washed with petroleum ether (50 mL), and then shaken vigorously to separate the unsaponified fraction. After separation between the organic and aqueous phases, the petroleum ether fraction was removed and kept in another separatory funnel. This procedure was repeated with petroleum ether (50 mL, three times), by washing and vigorously shaking at each step. The four combined petroleum ether fractions were washed with distilled water until the KOH excess was removed, dried by filtration through anhydrous sodium sulfate and collected into a 250-mL flat-bottom flask. This flask was taken to a rotary evaporator and its content was concentrated to a volume of about 5 mL. This preconcentrated extract was transferred to a previously dried and weighted beaker and was heated in an oven (70°C) for solvent evaporation until dryness.

The unsaponified matter was dissolved in hexane (1 mL) and then applied onto activated (105°C, 30 min) thin-layer chromatography plates (20 x 20 cm, 0.5-mm thickness silica gel as stationary phase; Analtech, Newark, DE, USA)

Table 1
Major characteristics of the study population*, by gender

†	Total N (%)	Women N (%)	Men N (%)	*P*
Age groups (y)				
21–30	232 (14.4)	145 (13.5)	87 (16.5)	0.144
31–40	177 (11.0)	121 (11.2)	56 (10.5)	
41–50	270 (16.8)	185 (17.2)	85 (16.0)	
51–64	462 (28.7)	324 (30.1)	138 (26.0)	
≥65	466 (29.0)	301 (28.0)	165 (31.0)	
Level of education‡				
Illiterate	24 (1.5)	15 (1.4)	9 (1.7)	<0.0001
Elementary	773 (48.1)	547 (50.9)	226 (42.5)	
High school	593 (36.9)	395 (36.7)	198 (37.3)	
University	117 (13.5)	119 (11.1)	98 (18.5)	
Monthly income‡,§ (USD)				
<630	669 (41.6)	485 (45.1)	184 (34.7)	<0.0001
630–1260	621 (38.6)	412 (38.3)	209 (39.4)	
1261–1890	169 (10.5)	96 (8.9)	73 (13.7)	
1891–2520	66 (4.1)	41 (3.8)	25 (4.7)	
>2520	73 (4.5)	35 (3.3)	38 (7.2)	
BMI† (kg/m^2)	26.4 (0.1)	26.5 (0.2)	26.2 (0.2)	0.667

BMI, body mass index; SE, standard error; USD, U.S. dollars
* Self-reported data obtained from interviews.
† Comparisons of categorical data were made by Pearson's χ^2 test; the values for BMI were compared using the Mann–Whitney test.
‡ *P*-values for levels of education and monthly incomes in men (<0.05) were higher than in women.
§ Monthly income was not reported by 0.7% of women and 0.4% of men.

using glass capillaries. A hexane:diethyl ether solution (65:35, v/v) was used as a mobile phase. The separation chamber was previously saturated with the mobile phase (30 min) before the plates were placed in it (40 min). The plates were then dried (room temperature, ca. 10 min), and then 2,7-dichlorofluorescein (0.2% ethanolic solution) was applied to them. Under ultraviolet light, the sterol-containing spots were delimited, using the position of a spot of cholesterol standard that also was applied to the plates as a reference. The sterol spots were removed from the plates and sterol was extracted in chloroform:ethyl eter solution (10:5, v/v). After this procedure, the solvent was evaporated and the residue solubilized with hexane (1 mL). For separation, identification, and quantification of phytosterols in the sterol fraction of food samples, a gas chromatograph (Agilent 6850, Agilent Technologies, Alexandria, VA, USA) with automatic injector and flame-ionization detector (GC-FID) was used. Recording and integration of chromatograms were performed using the Agilent ChemStation Plus software (version A.08). The chromatographic conditions are described in the study of Becker et al [27]. β-sitosterol (60% purity; Sigma, S-5753, USA), campesterol (Sigma, C-5157, USA), stigmasterol (Fluka, 85860, USA), dihydrocholesterol (5α-cholestan-3β-ol; β-cholestanol; 95-97% purity; Sigma, D-6128, USA), cholesterol (5-cholesten-3β-ol; 99% purity; Sigma, C-8,667, USA) were used as chromatographic standards.

Phytosterol amount or concentration (C_P) in the sample was calculated with the following equation, $C_P = [A_P \times C_{IS}]/A_{IS}$, where C_{IS} is the internal standard amount or concentration, A_P is the area of the phytosterol peak, and A_{IS} is the area of the internal standard peak. Results were then extrapolated to the amount of plant food and expressed in terms of mg/100 g^{-1}. All samples were analyzed in duplicates and the variation between analyses did not exceed 5%.

Statistical analyses

For the population study, categorical data are presented as *n* (%). Numerical data are expressed as means (SE) and medians (interquartile range). Categorical variables were compared using the Pearson's χ^2 test; for numerical data, the Mann-Whitney's test was used. Statistical significance was set at a *P*-value <0.05. All analyses were two-tailed, and the SPSS software (17.0, for windows, SPSS Inc, Chicago, IL, USA) was used.

Results

Study population

Major characteristics of the participants are shown in Table 1. We compared demographic characteristics between sexes, and age distribution was found to be similar in the groups; however, there were more men with both higher education levels and higher salary levels than women (Table 1). Body mass index was similar in men and women.

Dietary intake

Levels of daily EI by sex are presented in Table 2. Intake of calories and macronutrients in men was higher than in women ($P < 0.05$), with the exception of carbohydrate ingestion, which was higher in women ($P = 0.01$). Regarding fat intake, we observed an excess of saturated fatty acids and low intake of polyunsaturated fatty acids (PUFA) in both sexes. Ingestion of fibers was below the recommended levels. When data on diet components are distributed over age strata, intake of calories and PUFA in the 21-y to 30-y stratum was higher than in the strata for 51 y to 64 y old and above ($P < 0.002$). Intake of other diet components did not differ among age strata (data not shown).

Mean (SE) ingestion of phytosterol in men and women was 100.8 (1.4) mg/d and 100.4 (1.0) mg/d, respectively, with no difference between sexes. β-sitosterol was the most abundant plant sterol in the diet and accounted for 65.4% of total plant sterols, followed by campesterol (23.2%), stigmasterol (10%), sitostanol (1%), and campestanol (0.4%). No difference was found

Table 2
Daily nutrient intake levels and energy expenditure obtained from the population study, by sex

	Men (n = 531)		Women (n = 1076)		*P*
	Mean (SE)	Median (IQR)	Mean (SE)	Median (IQR)	
EI (kcal/d)	1521 (30)	1406 (1071–1828)	1345 (17)	1287 (993–1599)	<0.0001
Proteins (% EI)	19.9 (0.3)	19.0 (15.4–23.1)	19.0 (0.2)	18.5 (14.3–22.5)	0.015
Carbohydrates (% EI)	44.7 (0.7)	45.8 (34.2–55.7)	47.9 (0.5)	48.3 (36.1–59.9.5)	0.01
Total fat (% EI)	35.5 (0.6)	35.2 (25.3–45.4)	33.1 (0.5)	32.2 (20.3–43.7)	0.002
Cholesterol (mg EI)	224 (8)	178 (109–283)	189 (4)	162 (97–240)	<0.0001
SFA (% EI)	14.6 (0.2)	14.1 (10.1–18.41)	13.9 (0.2)	13.6 (8.7–18.5)	0.052
PUFA (% EI)	8.3 (0.2)	7.5 (4.3–11.0)	7.6 (0.1)	6.2 (3.7–10.6)	0.002
MUFA (% EI)	12.6 (0.3)	11.9 (8.6–16.0)	11.6 (0.2)	10.9 (6.4–15.5)	0.001
Phytosterols (mg)	100.8 (1.4)	95.3 (80.0–113.4)	100.4 (1.0)	93.5 (78.6–112.4)	0.347
Fibers (g)	12.4 (0.3)	10.7 (7.5–16.0)	11.3 (0.2)	9.8 (6.5–14.3)	0.002
BMR (kcal/d)	1680 (10)	1679 (1527–1830)	1370 (5)	1377 (1281–1464)	<0.0001
EI:BMR	0.92 (0.02)	0.85 (0.65–1.12)	1.00 (0.01)	0.93 (0.71–1.20)	<0.0001

BMR, basal metabolic rate; EI, energy intake; IQR, interquartile range; MUFA, monounsaturated fat; PUFA, polyunsaturated fatty acid; SE, standard error; SFA, saturated fatty acid
Data are means (SE) and medians (IQR)
Estimates of macronutrients were based on the 24-h recall; intake of phytosterols was based on food frequency questionnaire
Comparisons were made using the Mann–Whitney test

Table 3
Distribution of UR, AR, and OR, according to Goldberg and Black, by sex

	Men (n = 531)	Women (n = 1076)
Goldberg cut-off [23,24]		
UR (EI:BMR < 0.76)	207 (39)	324 (30)
AR (EI:BMR 0.76–1.24)	227 (43)	523 (48)
OR (EI:BMR > 1.24)	97 (18)	238 (22)
Black cut-off [25]		
UR (EI:BMR < 0.87)	275 (52)	451 (42)
AR (EI:BMR ≥ 0.87)	256 (48)	625 (58)

AR, acceptable reporters; BMR, basal metabolic rate; EI, energy intake; OR, overreporters; UR, underreporters
Data are n (%)
Underreporters, acceptable reporters, and overreporters were obtained according to Goldberg cut-off values for age distribution [23,24] and Black [25]

in the intake of plant sterols in the different age strata or education levels; however, individuals with monthly income of ≥ $1200 USD exhibited higher consumption of plant sterols than those with monthly income < $1200 USD ($P = 0.022$, Mann–Whitney test).

Higher EI and BMR values were observed in men, however, the EI:BMR ratio was higher in women (Table 2).

Applying the Goldberg cut-off, 39% of men and 30% of women were underreporters; acceptable reporters accounted for 43% of men and 48% of women, with an 18% of overreporters in men and 22% in women ($P = 0.002$, Pearson's χ^2 test test) (Table 3). According to Black, 52% of men and 42% of women were underreporters.

After exclusion of underreporters and overreporters (Table 4), the results were not substantially modified, with a non-significant increase in phytosterol intake when the Black cut-off was applied (Table 5).

Composition of sterols in plant foods

Figure 1 shows representative chromatograms obtained for (a) dihydrocholesterol (internal standard), campestanol, campesterol, stigmasterol, β-sitosterol, and sitostanol standards and (b) separation of phytosterols from the sterol phase of lentils.

The content of phytosterols from plant foods is shown in Table 6. Analyses revealed considerable variation in the content (in mg/100 g^{-1}) of plant sterols depending on the type of sample, for example, zucchini (0.64) and chickpeas (56.80); polished (9.23) and brown rice (18.28); and mango (1.34) and avocado (25.75).

The content of plant sterols in plant oils was higher than in other food groups. The highest content (in mg/100 g^{-1}) of plant sterol was found in corn (727), followed by canola (467), sunflower (250), and soybean (231) oils.

Discussion

Our study showed that intake of plant sterols by a large sample population of São Paulo City is approximately 100 mg/d. This amount of phytosterol intake is far from the ingestion recommended to lower LDL-cholesterol and prevent cardiovascular disease in hypercholesterolemic individuals [28,29]. Assessment of consumption is the first step in determining if community interventions in diet composition are necessary. Therefore, our results are both timely and important, and reflect the food intake habits adopted by the population of large cities [30,31].

Rates of underreporting and overreporting in our study did not affect the results of phytosterol intake and other diet components probably because overreporters compensated for underreporters [32,33].

The American Heart Association recommends a daily intake of 2 g of phytosterols [29], and the National Cholesterol Education Program suggests a consumption of 2 g to 3 g of plant sterols [28] to reduce the levels of LDL-cholesterol. These recommendations are based on several studies, including a meta-analysis of 41 clinical trials, showing that ingestion of 2 g of phytosterol can reduce LDL-cholesterol by 10% [34,35]. Mean daily intake of phytosterols varies from 100 mg/d to 400 mg/d, depending on the type of diet and country [7,31,36–42]. In our study, the estimated daily intake of phytosterols was approximately 100 mg, close to the values reported by de Vries [42] for Italy (132 mg) and Japan (137 mg), but lower than for the United States (170 mg), Serbia (181 mg), and Greece (276 mg). A daily intake of phytosterols (close to that for the United States) was reported by Morton et al. [41] for the United Kingdom (163 mg), and average daily intakes were reported by Jiménez-Escrig et al. [7] for Spain (276 mg), the Netherlands (285 mg) [36], Finland (271 mg) [31], China (322 mg) [40], and the highest for Mexico (400 mg) [39].

Table 4
Daily nutrient intake levels and energy expenditure obtained from the population study, by sex with exclusion of underreporters and overreporters, according to Goldberg [23,24]

	Men (n = 227)		Women (n = 523)		*P*
	Mean (SE)	Median (IQR)	Mean (SE)	Median (IQR)	
EI (kcal/d)	1596 (19)	1605 (1354–1797)	1341 (10)	1327 (1190–1478)	<0.0001
Proteins (% EI)	19.5 (0.4)	19.0 (14.9–22.9)	19.5 (0.3)	19.0 (14.7–22.9)	0.943
Carbohydrates (% EI)	40.1 (1.0)	41.8 (29.2–50.0)	45.0 (0.7)	46.1 (34.3–55.9)	<0.0001
Total fat (% EI)	40.4 (0.9)	39.1 (30.1–50.4)	35.4 (0.6)	34.5 (24.8–44.9)	<0.0001
Cholesterol (mg EI)	231 (11)	196 (138–280)	200 (7)	172 (119–242)	0.011
SFA (% EI)	16.8 (0.4)	16.8 (12.5–20.5)	15.1 (0.3)	14.7 (10.7–19.6)	<0.00012
PUFA (% EI)	9.0 (0.4)	8.1 (4.5–11.3)	7.7 (0.2)	6.7 (4.1–10.7)	0.001
MUFA (% EI)	14.6 (0.4)	13.9 (10.0–18.5)	12.7 (0.3)	11.9 (7.5–16.4)	<0.0001
Phytosterols (mg)	102.4 (2.0)	96.6 (80.8–116.6)	99.6 (1.5)	92.0 (78.3–111.5)	0.299
Fibers (g)	13.2 (0.4)	12.3 (8.9–16.5)	11.0 (0.2)	10.4 (6.9–13.7)	<0.0001
BMR (kcal/d)	1658 (15)	1635 (1486–1807)	1373 (7)	1377 (1290–1457)	<0.0001
EI:BMR	0.96 (0.01)	0.95 (0.85–1.07)	0.98 (0.01)	0.98 (0.86–1.09)	0.172

BMR, basal metabolic rate; EI, energy intake; IQR, interquartile range; MUFA, monounsaturated fat; PUFA, polyunsaturated fatty acid; SE, standard error; SFA, saturated fatty acid
Data are means (SE) and medians (IQR)
Estimates of macronutrients were based on the 24-h recall; intake of phytosterols was based on food frequency questionnaire
Comparisons were made using the Mann–Whitney test

Table 5
Daily nutrient intake levels and energy expenditure obtained from the population study, by sex with exclusion of underreporters, according to Black [25]

	Men (n = 256)		Women (n = 625)		*P*
	Mean (SE)	Median (IQR)	Mean (SE)	Median (IQR)	
EI (kcal/d)	2008 (40)	1837 (1632–2257)	1655 (21)	1545 (1342–1807)	<0.0001
Proteins (% EI)	19.1 (0.4)	18.6 (14.6–22.8)	18.6 (0.3)	18.2 (14.2–22.0)	0.271
Carbohydrates (% EI)	38.7 (0.9)	39.8 (29.1–48.2)	41.6 (0.6)	41.0 (31.0–52.0)	<0.027
Total fat (% EI)	42.2 (0.8)	41.7 (35.0–49.7)	39.8 (0.6)	39.4 (29.5–49.7)	<0.024
Cholesterol (mg EI)	287 (12)	252 (158–343)	223 (6)	197 (137–279)	<0.0001
SFA (% EI)	17.4 (0.4)	16.9 (13.2–20.9)	16.5 (0.2)	16.2 (12.1–20.6)	0.092
PUFA (% EI)	10.1 (0.3)	9.7 (5.7–12.9)	9.3 (0.2)	8.5 (5.1–12.3)	0.020
MUFA (% EI)	14.6 (0.4)	13.9 (10.6–18.0)	14.0 (0.3)	13.3 (9.4–18.0)	0.155
Phytosterols (mg)	104.6 (2.1)	97.8 (80.9–110.7)	103.4 (1.4)	94.5 (80.8–115.8)	0.231
Fibers (g)	15.5 (0.5)	13.8 (10.0–19.3)	13.5 (0.3)	12.2 (8.1–17.3)	<0.0001
BMR (kcal/d)	1640 (15)	1635 (1486–1782)	1335 (7)	1342 (1255–1429)	<0.0001
EI:BMR	1.23 (0.02)	1.13 (0.98–1.36)	1.25 (0.02)	1.16 (1.00–1.37)	0.576

BMR, basal metabolic rate; EI, energy intake; IQR, interquartile range; MUFA, monounsaturated fat; PUFA, polyunsaturated fatty acid; SE, standard error; SFA, saturated fatty acid
Data are means (SE) and medians (IQR)
Estimates of macronutrients were based on the 24–h recall; intake of phytosterols was based on food frequency questionnaire
Comparisons were made using the Mann–Whitney test

Important differences in phytosterol content and composition were observed for different food groups. The amount of plant sterols (per 100 g) offered by broccoli (5.6 mg), carrots (16.2 mg), and cabbages (5.2 mg) were higher than that offered by other vegetables. The highest content of phytosterol (per 100 g), was seen in chickpeas (56.8 mg), linseed (44.7 mg), soybean (33.3 mg), and green peas (25.9 mg). Fruits were options with relatively high phytosterol content (per 100 g), namely avocado (25.7 mg), coconut (14.4 mg), and oranges (15.3 mg). Finally, most vegetable oils were identified as important phytosterol sources (Table 4). Differences in composition of plant sterols in vegetables, legumes, cereals, and seeds, fruits, and oils produced in Brazil might be related to climate and soil conditions, not allowing extrapolation of similar data from other countries. Additionally, Brazil is one of the largest producers of merchandises, including a variety of foods and vegetable oils, that are exported to several countries.

Data from the literature reveal a similar composition of plant sterol for carrots [7,9], but lower phytosterol content in cauliflower [7,9]; broccoli shows a huge variation (18–39 mg/100 g^{-1}), depending on the country [7,9]. In our study, chickpeas, soybean, and green beans presented lower values for phytosterols when compared with those of Jiménez-Escrig [7]. Regarding fruits, bananas exhibited phytosterol content similar to that of other study [7]; however, apples and oranges showed higher values. Many types of plant foods that are consumed in other countries are not included in typical Brazilian meals.

The cholesterol-lowering effect of phytosterols is relevant, and even a small reduction in LDL-cholesterol would be beneficial for prevention of cardiovascular disease when applied to the population level [43–46]. A diet rich in phytosterols would be based on legumes, cereals, and seeds, broccoli, carrots, avocado, oranges, and oils, and could be more effective when combined with other lifestyle modifications [47–49].

Phytosterol supplementation to lower LDL-cholesterol levels also can be an interesting alternative when added to statin therapy [50,51]. Meta-analyses of clinical trials, including different dietary and pharmacologic strategies for reduction in LDL-cholesterol, have reported a decrease in cardiovascular events, with no heterogeneity due to therapies [52–54].

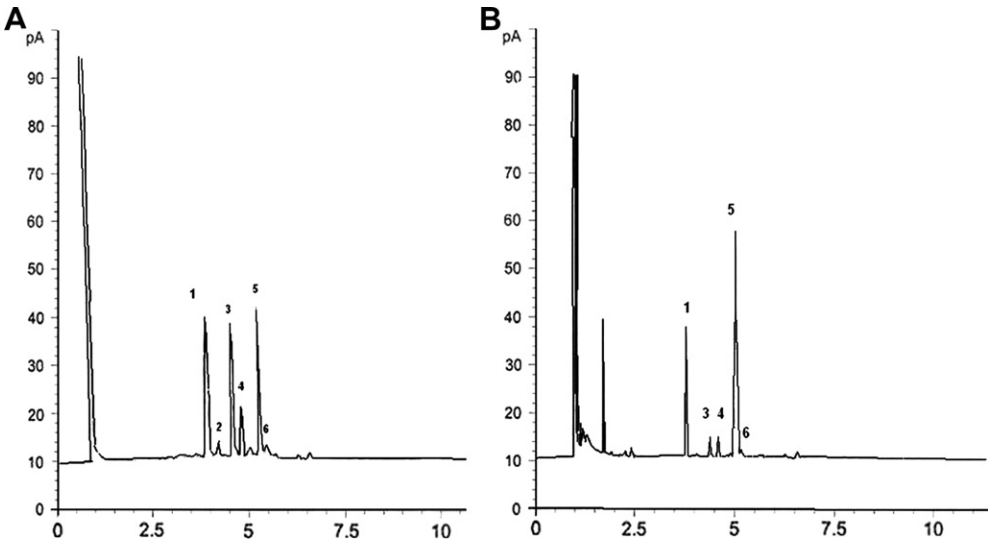

Fig. 1. Representative chromatograms obtained for (A) dihydrocholesterol (internal standard), campestanol, campesterol, stigmasterol, β-sitosterol; and sitostanol standards and (B) separation of phytosterols of the sterol phase of lentils.

Table 6
Plant sterol/stanol composition (mg/100 g−1 of edible portion) in plant-derived foods

Plant-derived foods	Scientific names	Campestanol	Campesterol	Stigmasterol	β-sitosterol	Sitostanol	Total phytosterols	Lipids
Vegetables								
Zucchini	*Cucurbita pepo L.*	0.01–0.01	0.01–0.01	0.01–0.01	0.60–0.60	0.01–0.01	0.63–0.64	0.03–0.04
Eggplant	*Solanum melongena L.*	ND	0.05–0.06	0.18–0.18	0.28–0.28	ND	0.51–0.52	0.03–0.04
Broccoli	*Brassica oleracea var. Italica*	ND	0.64–0.64	2.37–2.41	2.55–2.59	0.03–0.03	5.59–5.67	0.15–0.16
Carrot	*Daucus carota L.*	ND	2.51–2.52	3.52–3.54	10.13–10.17	0.06–0.06	16.22–16.29	0.16–0.18
Cauliflower	*Brassica oleracea var. Botrytis L.*	ND	0.75–0.76	0.25–0.25	2.72–2.73	0.03–0.03	3.75–3.77	0.06–0.06
Endive	*Cichorium endívia L.*	0.01–0.01	0.19–0.19	0.44–0.45	0.58–0.59	0.15–0.15	1.38–1.39	0.05–0.07
Spinach	*Tetragonia expansa*	ND	0.05–0.05	0.08–0.08	0.97–0.98	0.01–0.01	1.11–1.12	0.16–0.17
White cabbage	*Brassica oleracea var. Capitata*	ND	1.42–1.43	0.03–0.03	3.76–3.77	0.01–0.01	5.22–5.24	0.08–0.09
Tomato	*Lycopersicum esculentum Mill.*	0.02–0.02 L.	0.08–0.08	0.15–0.16	1.07–1.08	0.38–0.39	1.70–1.73	0.17–0.18
Green bean	*Phaseolus vulgaris*	0.01–0.01	0.45–0.46	1.76–1.78	2.21–2.24	0.01–0.01	4.46–4.50	0.22–0.23
Legumes, cereals, and seeds								
Brown rice	*Oryza sativa*	0.16–0.16	3.23–3.24	2.85–2.86	11.49–11.52	0.50–0.50	18.24–18.28	1.41–1.53
Polished rice	*Oryza sativa*	0.17–0.20	1.45–1.47	1.12–1.12	6.09–6.10	0.35–0.35	9.21–9.23	0.37–0.38
Green pea	*Pisum sativum*	ND	2.15–2.19	2.08–2.10	20.46–20.66	1.05–1.07	25.80–25.96	1.14–1.37
Brown beans	*Phaseolus vulgaris L.*	ND	1.15–1.18	5.33–5.42	9.07–9.12	1.10–1.13	16.74–16.76	1.17–1.24
Black beans	*Phaseolus vulgaris L.*	ND	1.02–1.07	4.41–4.43	8.79–8.82	1.40–1.43	15.62–15.75	1.30–1.37
Chickpeas	*Cicer arietinum*	7.09–7.11	ND	4.29–4.30	42.30–42.38	2.99–3.04	56.70–56.80	3.52–3.60
Lentil	*Lens esculenta*	ND	2.09–2.11	2.09–2.09	25.06–25.18	1.22–1.24	30.48–30.60	1.02–1.15
Soybean	*Glycine max L. Merr.*	0.18–0.20	6.69–6.71	7.90–7.93	17.64–17.68	0.69–0.69	33.21–33.31	10.64–10.66
Black soybean	*Glycine max L. Merr.*	0.35–0.48	2.93–3.04	3.00–3.14	11.01–11.15	0.39–0.44	17.73–18.20	12.37–12.38
Linseed	*Linum usitatissimum*	1.37–1.38	15.06–15.09	3.35–3.35	21.47–21.49	3.45–3.46	44.72–44.75	22.89–23.00
Fruits								
Acai	*Euterpe oleracea*	ND	0.28–0.29	1.23–1.30	12.93–13.07	ND	14.52–14.58	5.71–5.91
Avocado	*Persea Americana*	ND	2.61–2.65	0.59–0.60	20.76–20.96	1.50–1.54	25.46–25.75	6.07–6.25
Pineapple	*Ananas comosus L. Merril*	ND	0.71–0.72	0.06–0.06	4.12–4.15	0.01–0.01	4.91–4.93	0.03–0.04
Banana	*Musa acuminata x Musa balbisiana*	ND	0.50–0.51	0.41–0.41	2.06–2.08	0.51–0.52	3.48–3.52	0.06–0.07
Coconut	*Cocos nucifera*	0.32–0.32	1.11–1.15	2.35–2.39	5.39–5.45	5.39–5.45	14.24–14.44	23.15–23.25
Guava	*Psidium guajava L.*	0.01–0.01	0.08–0.08	0.01–0.01	3.21–3.23	0.05–0.07	3.36–3.40	0.07–0.08
Orange	*Citrus sinensis*	ND	1.74–1.75	0.86–0.87	12.33–12.40	0.36–0.37	15.29–15.39	0.12–0.14
Apple	*Malus domestica*	ND	0.57–0.58	0.04–0.04	4.59–4.60	0.11–0.11	5.31–5.33	0.11–0.15
Papaya	*Carica papaya*	0.04–0.04	1.92–1.93	0.82–0.83	2.12–2.13	ND	4.91–4.92	0.15–0.18
Mango	*Mangifera indica L.*	0.01–0.01	0.25–0.25	0.10–0.10	0.97–0.97	0.01–0.01	1.34–1.34	0.06–0.06
Strawberry	*Fragaria vesca L.*	ND	0.08–0.08	0.01–0.01	1.35–1.35	0.02–0.02	1.46–1.46	0.08–0.08
Vegetable oils								
Canola (Rapeseed)	*Brassica napus*	ND	156.55–156.77	12.62–12.71	297.41–297.55	ND	466.72–466.89	100
Coconut	*Cocos nucifera*	ND	5.71–5.86	14.34–14.48	52.99–53.07	0.53–0.54	73.57–73.95	100
Sunflower	*Helianthus annuus*	ND	51.22–51.35	26.61–26.74	171.26–171.44	ND	249.09–249.53	100
Corn	*Zea mays*	ND	219.80–220.01	61.28–61.30	445.32–445.56	ND	726.63–726.64	100
Soybean	*Glycine max*	ND	41.85–42.07	48.19–48.30	140.55–140.89	ND	230.59–231.26	100
Olive	*Olea europaea*	ND	28.41–28.42	17.30–17.35	158.05–158.15	ND	203.76–203.92	100
Composed	–	ND	26.10–26.15	14.08–14.11	120.12–120.1	ND	160.36–160.38	100

ND, not detected
All values for plant sterols and stanols are reported as duplicates

Study limitations

As we used a non-stratified multistage cluster sample, our population sample is not equally represented in the age strata. Other bias would have occurred in stratified samples, such as selection of households.

Anthropometry and other clinical variables were self-reported and not measured.

There was a significant rate of misreporters, but the exclusion of these individuals did not change our results.

Conclusions

Analysis of PS composition in most consumed plant foods has showed that PS content varied among food groups. Dietary intake of PS in a large population of the city of São Paulo was in the same range of some countries (~160 mg), and probably has little effect on plasma cholesterol.

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