Bakery Products Enriched with Phytosterol Esters, α-Tocopherol and β-Carotene Decrease Plasma LDL-Cholesterol and Maintain Plasma β-Carotene Concentrations in Normocholesterolemic Men and Women[1]

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ABSTRACT The hypocholesterolemic effects of phytosterols have not been evaluated in bakery products, and the addition of liposoluble antioxidants to the carrier has never been tested. We investigated the effects of consuming croissants and *magdalenas* (Spanish muffins) enriched with sterol esters, α-tocopherol and β-carotene on plasma lipid and fat-soluble antioxidant concentrations in normocholesterolemic, habitual consumers of bakery products following their usual diet and lifestyle. Using a randomized, double-blind, placebo-controlled design, the control (C) group ($n = 29$) received two pieces daily (standard croissant and muffin) and the sterol ester (SE) group ($n = 28$), the same products with sterol esters added (3.2 g/d) for 8 wk. Total and LDL cholesterol (LDL-C) decreased in the SE group by 0.24 mmol/L ($P < 0.01$) and 0.26 mmol/L ($P < 0.005$), respectively, whereas these variables did not change in the control group. The total difference in total and LDL-C changes between groups was 0.38 mmol/L (8.9%) and 0.36 mmol/L (14.7%), respectively ($P < 0.001$). Within-group changes in HDL cholesterol, triacylglycerol or lipoprotein(a) concentrations did not differ. Similarly, within-group changes over time in plasma tocopherol and carotenoid concentrations did not differ between groups. Our findings suggest that bakery products are excellent carriers for phytosterols, and their consumption is associated with a decrease in total and LDL-C concentrations, with no changes in α-tocopherol and β-carotene. The ability of bakery products to include sufficient quantities of β-carotene to compensate for a potential deficiency, and the fact that their efficacy was not associated with the time of day at which they were consumed, are interesting findings. J. Nutr. 133: 3103–3109, 2003.

KEY WORDS: • *phytosterols* • *cholesterol* • *LDL cholesterol* • *bakery products* • *β-carotene*

Hypercholesterolemia is an important risk factor for cardiovascular diseases, which are the main causes of mortality in developed countries. The high intakes of saturated fatty acids and cholesterol in these countries are associated with an increase in plasma LDL cholesterol (LDL-C),[3] which can promote atherogenesis and atherosclerosis (1). Dietary changes are the preferred way of improving plasma lipid variables in primary prevention. Nevertheless, these dietary changes may be insufficient to meet the LDL-C target in people with moderate hypercholesterolemia and those who adhere poorly to dietary recommendations.

Certain diet components such as phytosterols can help to improve the blood lipid status because they interfere with both dietary and biliary cholesterol absorption (2). These compounds are related and structurally similar to cholesterol and include both plant sterols and stanols, their saturated form. They occur naturally in vegetable foods and oils; a Western diet provides between 170 and 360 mg/d, depending on dietary habits and geographical location (3). In recent years, great interest has been shown in their cholesterol-lowering properties, because phytosterol consumption > 1.5 g/d reduces plasma LDL-C by 8–15%. As a result, cardiovascular risk is also reduced (4). It has been shown that this effect occurs in both normocholesterolemic and hypercholesterolemic subjects (5) and can be caused by sterols as well as stanols (6,7). Apart from a few cases of subjects suffering from sitosterolemia, a rare inborn error of metabolism, no side effects have been found in people consuming sterols or stanols other than a decreased concentration of some fat-soluble antioxidants, such as carotenoids. Some studies have observed no significant differences in carotenoid concentrations after adjusting for plasma cholesterol concentrations (8,9). Other studies, however, have found that standardized β-carotene decreases (10,11), a diet rich in carotenoids has been recommended when phytosterols are consumed (12). Fat spreads are the main food products used for this purpose, but yogurt (13,14), salad dressings (11), chocolate (15) and ground beef (16) have also been used as carriers. Sterols in their esterified form are widely used because

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[3] ABC, ATP-binding cassette; apo, apolipoprotein; C, control; HDL-C, HDL cholesterol; LDL-C, LDL cholesterol; SE, sterol ester.

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TABLE 1

Baseline characteristics of the subjects[1]

	Control group	Sterol ester group
Men, *n*	12	13
Women, *n*	17	15
Age, *y*	30.9 ± 7.2	31.0 ± 6.7
Weight, *kg*	62.9 ± 11.0	67.4 ± 13.0
BMI, *kg/m²*	22.9 ± 3.8	23.8 ± 3.8
Smokers, *n*	14	14
Blood pressure, *mmHg*		
Systolic	120.9 ± 9.3	120.9 ± 8.8
Diastolic	80.3 ± 6.2	80.1 ± 8.1

1 Values are means ± SD.

they present an increased lipid solubility that facilitates their incorporation into the fat phase of foods.

In countries with reduced consumption of margarine spread, such as Spain, this product is not the best carrier for phytosterols. Bakery products are important food components at breakfast and for mid-morning and afternoon snacks. Similarly, the complex flour-water-fat of which they are composed is suitable for incorporating phytosterols, fat-soluble vitamins and also carotenes, because the yellow-orange color of these products is well accepted. The aim of the present study was to examine the effect of croissants and muffins enriched with sterol esters equivalent to 3.2 g/d of free sterols, 5.3 mg/d α-tocopherol and 0.9 mg/d β-carotene, on plasma lipid, fat-soluble antioxidant (tocopherols and carotenoids), phytosterol and cholesterol precursor (lathosterol and desmosterol) concentrations in normocholesterolemic men and women with no changes in diet and lifestyle. We also investigated these effects when bakery products were ingested as a part of a meal or between meals.

MATERIALS AND METHODS

Subjects. Normocholesterolemic individuals ($n = 70$) were recruited for the study; 3 wk before the start, they were invited for a screening visit. The inclusion criteria were as follows: global cardiovascular risk according to the European Society for Atherosclerosis < 20% (17), fasting plasma total cholesterol concentration ≤ 6.28 mmol/L (240 mg/dL), fasting plasma triglyceride concentration ≤ 2.26 mmol/L (200 mg/dL), BMI (weight/height^2) < 40 kg/m^2, no alcohol abuse (<60 g ethanol/d), no medication or diet known to affect plasma lipids, no psychiatric disorders or alterations that make follow-up difficult, no metabolic, infectious, inflammatory or tumoral diseases and no antecedents of sitosterolemia. Neither pregnant nor breast-feeding women were accepted; finally, subjects were included only if they were habitual consumers of bakery products.

Of the 70 recruits, 61 met the criteria and were included in the study. Four subjects dropped out during the study, three in the first period for personal reasons and one at the end for poor compliance with the general protocol. Altogether, 57 subjects completed the assay, 29 in the control (C) group and 28 in the sterol ester (SE) group. Baseline population characteristics are presented in **Table 1**. The aim of the study was explained to all the subjects and they gave their informed consent before the start. The protocol was approved by the Ethics Committee of the Hospital Universitari de Sant Joan de Reus.

Experimental design and products. The study used a randomized, double-blind, placebo-controlled, repeated-measures design. Each person was assigned to the C or SE group to balance the groups in gender and smoker distribution. The study was conducted for 8 consecutive weeks.

During the intervention period, each subject received one croissant and one muffin every day. Frozen products were defrosted and cooked daily from Monday to Friday, placed in numbered plastic bags and delivered to each person. The products to be consumed on the weekend were cooked and delivered on Friday. The C group was given standard croissants and muffins, and the SE group was given the same products with sterol esters added. Subjects were allowed to eat the two pieces separately at any time of the day in place of their usual consumption of bakery products. They were also encouraged to maintain the same lifestyle and dietary habits.

Dietary intake was assessed at the beginning (wk –1), at the half-way stage (wk 4) and at the end of the study (wk 8) by means of a 7-d food record and by measuring body weight. Subjects were asked to record detailed descriptions of all food and beverages consumed (ingredients, methods of preparation, cooking) and to give quantities using weights or household measurements from a standardized list. A dietician checked the food and beverage records in each phase in the presence of the subjects. Intake was evaluated by means of food photographs (18) and the composition of diet and energy by French (19) and, in some cases, Spanish food composition tables (20). Dieticians also checked compliance by interviewing the participants and counting the number of muffin packs returned. A return < 70% was considered to be poor and participants were excluded from the study. The plasma concentration of phytosterols was also used to evaluate the compliance of the SE group subjects.

The four different products, croissants or muffins in C or SE recipes, were all produced in a single batch, immediately deep-frozen and stored at –18°C (Europastry S.A., Barcelona, Spain) until they were defrosted and cooked as described above. The final weight was 68 g for croissants and 54 g for muffins. The compositions of these products are shown in **Table 2**; the main difference between C and SE products was the partial substitution of fat by sterol esters from soy sterols esterified with canola oil fatty acids (Raisio Group, Raisio, Finland). Furthermore, experimental croissants and muffins were enriched with α-tocopherol and β-carotene (Roche, Basel, Switzerland). The yellow-orange color of SE bakery products was mimicked in C products by the addition of tartrazine and ponceau-4R colorants

TABLE 2

Composition of the products[1]

	Group[2]	Croissant	Muffin
		unit/serving	
Weight, *g*		68	54
Energy, *kJ*	C	1251	954
	SE	1188	962
Carbohydrates, *g*	C	28.0	26.2
	SE	28.0	26.2
Protein, *g*	C	5.1	3.0
	SE	5.1	3.1
Fat, *g*	C	18.5	12.3
	SE	16.8	12.5
Total phytosterols, *mg*	C	49	52
	SE	1579	1734
β-Sitosterol, *mg*	C	24	26
	SE	714	817
Campesterol, *mg*	C	12	5
	SE	418	472
Stigmasterol, *mg*	C	2	6
	SE	213	234
Other phytosterols, *mg*	C	11	15
	SE	234	211
α-Tocopherol, *mg*	C	2.8	3.8
	SE	5.6	6.3
γ-Tocopherol, *mg*	C	0.5	0.4
	SE	0.5	0.4
β-Carotene, *mg*	C	ND[3]	ND
	SE	0.33	0.59

1 α-Carotene and lycopene were not detected in any products.
2 C, control group; SE, sterol ester group.
3 ND, not detected.

(Iproa, Barcelona, Spain). In addition to color, SE and C products were similar in appearance and taste to preserve the double-blind design.

Analyses

Blood samples. Blood samples were taken twice at the beginning of the experimental period (d −2 and 0) and twice at the end (d +54 and +56) after a 12-h fast to decrease the intraindividual variability. Plasma was obtained by centrifugation at 2000 × g for 10 min at 4°C directly after sampling, and snap-frozen and stored in small portions at −80°C. All analyses were carried out at the same time after the plasma samples had been defrosted.

Lipids, lipoproteins and apolipoproteins. Total cholesterol was analyzed by the CHOD/PAP method, as was HDL cholesterol (HDL-C) after apolipoprotein (apo) B-containing lipoproteins had been precipitated by adding phosphotungstic acid and magnesium ions. Triacylglycerols were determined by the GPO-Trinder method. All kits were supplied by Roche Diagnostics Systems. The intra-assay CV were <1.6% for total cholesterol, <5.0% for HDL-C and <3.2% for triacylglycerols. LDL-C was calculated with the Friedewald equation (21). The analyses were performed in a Cobas Mira autoanalyzer (Basel, Switzerland) and the cholesterol results validated with a standard certified by the Centers for Disease Control (Atlanta, GA) and the National Cholesterol Education Program (Bethesda, MD).

Apo A-1 and apo B were measured using an immunoturbidimetric reaction (UNI-KIT Apo A-1 and UNI-KIT Apo B; Roche Diagnostics Systems). The intra-assay CV were <2.7% for apo A-1 and <4.0% for apo B. Plasma lipoprotein(a) concentrations were analyzed by ELISA, using a commercial kit [Lp(a) SPQ II System, DiaSorin, Stillwater, MN]. The intra-assay CV was <5.4%. All samples from one subject were analyzed in one assay at the end of the study. Plasma lipid, lipoprotein and apolipoprotein results from d –2 and 0 and from d +54 and +56 were averaged for data analysis.

Plasma sterols. Plasma sterols were measured as described by Philips et al. (22). Briefly, 0.7 mL of plasma was added to a centrifuge tube, and spiked with 1 mL of 5α-cholestane internal standard solution (10 μg/L). Total lipid was extracted using chloroform/methanol 2:1 followed by a 9 g/L sodium chloride solution. The chloroform phase was filtered and evaporated completely. Then, 8 mL of ethanol with 30 g/L pyrogallol and 1 mL of saturated potassium hydroxide solution were added to the extract. Samples were saponified at 87°C for 30 min, and the nonsaponifiable fraction was extracted twice with cyclohexane. After solid-phase extraction to achieve a cleaner extract, samples were derivatized with 50 μL bis-(trimethyl-silyl) trifluoroacetamide containing 10 g/L trimethyl-chlorosilane and 50 μL pyridine.

Samples were injected into a Shimadzu GC-17A GLC coupled to a QP-5050A MS detector, with a 30-m capillary column SAC-5 (Supelco, Bellefonte, PA). Sterols were identified by comparison with standard compounds supplied by Sigma Chemical (St. Louis, MO) and HPLC grade reagents by Panreac (Barcelona, Spain). The intra-assay CV were <8.0% for desmosterol, <7.0% for lathosterol, <6.6% for campesterol and <7.8% for β-sitosterol. For purposes of standardization, all of the results were adjusted for plasma total cholesterol concentrations (μmol/mmol total cholesterol).

Tocopherols and carotenoids. Tocopherols (α-tocopherol and γ-tocopherol) and carotenoids (α-carotene, β-carotene and lycopene) were determined as described (23). Briefly, 500 μL of ethanol containing an internal standard (25 mg/L of α-tocopheryl acetate) was added to 500 μL of plasma in a 10-mL centrifuge tube and mixed for 30 s. Then, 2 mL of n-hexane was added and the tube was shaken again for 1 min. After centrifugation at 2000 × g for 10 min, the mixture was extracted twice with 2 mL of n-hexane. The solvent in the upper phase was removed under nitrogen steam and the dry residue was immediately dissolved again in 500 μL of n-hexane.

Samples were injected into a Shimadzu HPLC system with a variable wavelength UV-visible SPD-10A detector and a Tracer Extrasil ODS-2 column (250 × 4.0 m i.d., 5-μm particle size) protected by an ODS guard cartridge system (Teknokroma, Barcelona, Spain). Samples were isocratically eluted with a methanol/n-hexane mixture (85:15, v/v), with the detector at 292 nm for detecting tocopherols and at 450 nm for carotenes. The intra-assay CV were <4.5% for tocopherols, <3.0% for β-carotene, <7.0% for α-carotene and <8.2% for lycopene. Standards were supplied by Sigma Chemical and HPLC grade reagents by SDS (Peypin, France) and Scharlau Chemie SA (Barcelona, Spain). Results are presented as absolute values (μmol/L) or standardized by adjusting for plasma total cholesterol concentrations (μmol/mmol total cholesterol).

Statistical analyses. Results are presented as means ± SD. Statistical analyses were performed with the statistical software SYSTAT 10 for Windows (SPSS, Chicago, IL). Normal distribution of variables was verified before further statistical analyses with the Kolmogorov-Smirnov one-sample test. Many variables did not follow normal distribution criteria, and nonparametric tests were used for comparison. The paired mean changes within each group (final vs. baseline) were analyzed with the Wilcoxon signed-rank test. Differences in changes over time between groups were analyzed with the nonparametric Mann-Whitney U-test. The relationships between variables were tested using Spearman's correlation. Differences were considered significant when $P \leq 0.05$.

Sample size was calculated assuming an 8% change in LDL-C concentrations, with a power of 90% and an α risk of 5%. The calculation also assumed a potential drop-out rate of 15%.

RESULTS

The groups did not differ in gender, age, weight, BMI, blood pressure, smoking habits or plasma lipid concentrations at the beginning of the study (data not shown).

Dietary intake and side effects. The changes in the percentage of energy derived from carbohydrate, protein, fat and saturated fat during the study were similar in the two groups. The two groups differed in their changes in total energy and cholesterol intakes (**Table 3**). No side effects were mentioned by the subjects or observed in medical checks, except for one case of diarrhea in a subject from the C group, which was not considered attributable to the intervention. Body weight and blood pressure did not change within either group during the study. The bakery products were very well accepted in all cases and after the study, when asked about what kind of product they had consumed during study, most subjects were not able to identify their group. Therefore, the double-blind design was preserved.

Plasma lipids, lipoproteins and apolipoproteins. During the 8-wk trial, plasma total cholesterol did not change in the C group, whereas it decreased in the SE group (−0.24 mmol/L; 95% CI: −0.06, −0.42; $P = 0.001$) (**Table 4**). The changes in total cholesterol induced by the two interventions differed by 8.9% ($P < 0.001$). A similar trend was observed for the LDL-C, i.e., no change in the C group and a decrease in the SE group (−0.26 mmol/L; 95% CI: −0.09, −0.43; $P = 0.002$). The changes in LDL-C differed by 14.7% between interventions ($P = 0.001$).

Changes in the total cholesterol/HDL-C ratio and the apo B concentration also differed between the groups ($P = 0.002$ and $P = 0.005$, respectively), whereas changes in triacylglycerols, HDL-C, apo A and lipoprotein(a) (data not shown) did not.

The degree of reduction in LDL-C was positively related to the baseline concentration in the SE group ($r = 0.42$, $P < 0.05$), but did not correlate with BMI or the fat or cholesterol consumption.

When individuals who ate bakery-modified products as part of a meal ($n = 14$) were compared with those who ate them separately from meals ($n = 14$), the decrease in LDL-C did not differ between the two types of consumption (−0.26 ± 0.53 vs. −0.26 ± 0.34 mmol/L, respectively).

Plasma sterols and cholesterol precursors. The consumption of bakery products enriched in plant sterols increased

TABLE 3

Estimated daily intakes at baseline and during the study intervention in normocholesterolemic subjects[1]

	Energy	Carbohydrates	Protein	Fat	SFA[2]	Cholesterol
	kJ/d	% energy				*mg/d*
C group (n = 29)						
Baseline	9498 ± 2686	43.1 ± 5.0	15.6 ± 2.2	40.0 ± 4.5	13.7 ± 2.1	402 ± 155
During study	9937 ± 2741	41.5 ± 3.9	14.4 ± 1.9	43.0 ± 3.7	16.7 ± 1.7	497 ± 125
Change	439 ± 1602	−1.6 ± 4.6	−1.3 ± 1.6*	3.0 ± 4.8*	3.1 ± 1.7*	95 ± 89*
SE group (n = 28)						
Baseline	9820 ± 2372	41.9 ± 5.4	16.8 ± 1.7	39.6 ± 5.0	13.3 ± 2.3	464 ± 127
During study	9489 ± 2105	41.6 ± 4.3	14.6 ± 1.4	42.4 ± 4.4	16.3 ± 1.9	497 ± 104
Change	−331 ± 1280	−0.2 ± 4.3	−2.2 ± 2.1*	2.8 ± 3.9*	2.9 ± 2.1*	33 ± 98*
Differences between groups in changes (SE − C)						
Difference,[3] (%)	−770[a] (−7.9)	1.4 (3.2)	−0.9 (−4.9)	−0.2 (−0.5)	−0.2 (−0.4)	−62[b] (−16.6)

[1] Values are means ± SD. The values "During study" represent the mean of the half-way stage (wk 4) values and the end (wk 8) values. * Change within group (final vs. baseline), $P < 0.01$ (Wilcoxon Test). [a,b] Difference between groups in changes, [a] $P < 0.05$, [b] $P < 0.01$ (Mann-Whitney Test).
[2] SFA, saturated fatty acids; C control; SE, sterol ester.
[3] Represents the addition of the % change from baseline in each group.

plasma sterol concentrations (**Table 5**). Standardized campesterol and sitosterol concentrations increased considerably in the SE group ($P < 0.001$) and the changes in campesterol and sitosterol concentrations during the study differed significantly between groups ($P < 0.001$). Plasma concentrations of cholesterol precursors, desmosterol and lathosterol, increased in the SE group ($P < 0.005$), but did not change in the C group. The changes in desmosterol and lathosterol concentrations induced by the two interventions differed significantly ($P < 0.001$ and $P = 0.003$, respectively).

Fat soluble antioxidants. Both the absolute and standardized plasma α-tocopherol concentrations increased in the C group (0.98 μmol/mmol cholesterol; 95% CI: 0.62, 1.35; $P < 0.001$), and SE group (0.57 μmol/mmol cholesterol; 95% CI: 0.24, 0.90, $P = 0.005$) (**Table 6**), whereas γ-tocopherol did not change in either group.

Absolute and cholesterol-standardized carotenoid concentrations did not change in group C. In the SE group, the carotenoids not added to the bakery products, α-carotene and lycopene, tended to decrease ($P = 0.10$ and $P = 0.07$, respectively). In contrast, the absolute and corrected values (0.013 μmol/mmol cholesterol; 95% CI: −0.005, 0.031) of the plasma concentrations of β-carotene, which was added to the bakery products, did not fall and even tended to increase ($P = 0.22$ and $P = 0.19$, respectively) in the SE group. Nevertheless, there were no significant differences in the changes observed in tocopherols or carotenoids between groups.

DISCUSSION

The effect of administering both the free and esterified forms of phytosterols was evaluated previously with different carriers, mainly fat spreads. This is the first study to analyze how bakery products enriched with phytosterols can affect the plasma lipid profile.

Our results show that enriching bakery products with 3.2 g/d of sterols (1.55 g/croissant and 1.68 g/muffin) reduces total cholesterol by 5.5% and LDL-C by 10.4%, and does not

TABLE 4

Effects of 8-wk sterol ester supplementation in bakery products on plasma lipids, lipoproteins and apolipoproteins in normocholesterolemic subjects[1,2]

	TAG	TC	HDL-C	LDL-C	TC/HDL-C	Apo A-I	Apo B
	mmol/L					*g/L*	
C group (n = 29)							
Baseline	1.11 ± 0.25	4.18 ± 0.59	1.28 ± 0.30	2.40 ± 0.54	3.40 ± 0.77	1.35 ± 0.19	0.61 ± 0.12
Final	1.16 ± 0.35	4.32 ± 0.72	1.29 ± 0.32	2.50 ± 0.62	3.48 ± 0.83	1.38 ± 0.21	0.62 ± 0.12
Change	0.05 ± 0.30	0.14 ± 0.40	0.02 ± 0.13	0.10 ± 0.35	0.08 ± 0.34	0.03 ± 0.09	0.01 ± 0.06
SE group (n = 28)							
Baseline	1.16 ± 0.30	4.33 ± 0.66	1.30 ± 0.33	2.50 ± 0.70	3.55 ± 1.12	1.39 ± 0.21	0.63 ± 0.14
Final	1.13 ± 0.36	4.09 ± 0.62	1.33 ± 0.28	2.24 ± 0.66	3.25 ± 1.04	1.40 ± 0.19	0.59 ± 0.13
Change	−0.03 ± 0.27	−0.24 ± 0.47*	0.03 ± 0.15	−0.26 ± 0.43*	−0.30 ± 0.59*	0.02 ± 0.12	−0.03 ± 0.07*
Differences between groups in changes (SE − C)							
Difference,[3] (%)	−0.08 (−7.1)	−0.38[b] (−8.9)	0.01 (0.9)	−0.36[a] (−14.7)	−0.38[a] (−10.7)	−0.01 (−0.8)	−0.04[a] (−7.1)

[1] Values are means ± SD. * Change within group (final vs. baseline), $P < 0.01$ (Wilcoxon Test). [a,b] Difference between groups in changes, [a] $P < 0.01$, [b] $P < 0.001$ (Mann-Whitney Test).
[2] TAG, triacylglycerol; TC, total cholesterol; HDL-C, HDL cholesterol; LDL-C, LDL cholesterol; apo, apolipoprotein; C, control; SE, sterol ester.
[3] Represents the addition of the % change from baseline in each group.

TABLE 5

Effects of 8-wk sterol ester supplementation in bakery products on plasma concentrations of cholesterol precursors and plant sterols in normocholesterolemic subjects[1,2]

	Desmosterol	Lathosterol	Campesterol	β-Sitosterol
	µmol/mmol cholesterol			
C group (n = 29)				
Baseline	0.96 ± 0.35	1.95 ± 0.65	1.82 ± 1.07	1.55 ± 0.76
Final	0.97 ± 0.33	1.86 ± 0.69	1.91 ± 0.93	1.64 ± 0.68
Change	0.00 ± 0.23	−0.08 ± 0.55	0.09 ± 0.47	0.09 ± 0.31
SE group (n = 28)				
Baseline	0.94 ± 0.28	2.32 ± 1.56	1.62 ± 0.71	1.44 ± 0.53
Final	1.16 ± 0.42	2.76 ± 1.57	4.92 ± 1.97	2.40 ± 1.49
Change	0.22 ± 0.25*	0.44 ± 0.99*	3.29 ± 1.49*	0.97 ± 1.24*
Differences between groups in changes (SE − C)				
Difference,[3] (%)	0.22[b] (23)	0.52[a] (15)	3.20[b] (199)	0.89[b] (61)

[1] Values (standardized by total cholesterol) are means ± SD. * Change within group (final vs. baseline), $P < 0.01$ (Wilcoxon Test). [a,b] Difference between groups in changes, [a] $P < 0.01$, [b] $P < 0.001$ (Mann-Whitney Test).
[2] C, control; SE, sterol ester.
[3] Represents the addition of the % change from baseline in each group.

significantly modify triacylglycerol and HDL-C concentrations in normocholesterolemic subjects. This effect is consistent with previously published studies that showed a reduction between 8 and 15% in LDL-C when between 1.5 and 3 g/d of sterol or stanol esters was administered (5,7,13,14,24,25).

It could be argued that bakery products are not the best carrier for phytosterols because of their fat composition, but the same could be said for margarines, the carrier approved by the Scientific Committee on Food in Europe (26). As mentioned, we chose croissants and muffins as carriers because they are commonly consumed in Mediterranean countries that are not habitual consumers of fat spreads.

In our study, we tried to diminish the intraindividual variability in plasma lipid concentrations by analyzing two plasma samples collected within 48 h. In spite of this, the interindividual variability of the response was considerable, as described by others (13). Genetic factors may be one explanation for this variability. To date, only differences in apo E geno-

TABLE 6

Effects of 8-wk sterol ester supplementation in bakery products on plasma tocopherols and carotenoids in absolute values and after total cholesterol standardization[1,2]

	α-Tocopherol	γ-Tocopherol	α-Carotene	β-Carotene	Lycopene
	µmol/L				
C group (n = 29)					
Baseline	26.6 ± 4.5	2.96 ± 0.99	0.130 ± 0.114	0.65 ± 0.43	0.34 ± 0.14
Final	30.6 ± 5.5	2.88 ± 0.89	0.122 ± 0.092	0.62 ± 0.40	0.33 ± 0.17
Change	4.0 ± 3.8*	−0.08 ± 0.86	−0.008 ± 0.073	−0.03 ± 0.20	−0.01 ± 0.09
SE group (n = 28)					
Baseline	27.7 ± 4.0	2.88 ± 0.67	0.127 ± 0.067	0.69 ± 0.44	0.42 ± 0.37
Final	30.0 ± 4.0	2.89 ± 0.74	0.114 ± 0.047	0.73 ± 0.40	0.37 ± 0.24
Change	2.1 ± 3.4*	0.02 ± 0.48	−0.013 ± 0.041	0.05 ± 0.19	−0.05 ± 0.15
Differences between groups in changes (SE − C)					
Difference[3] (%)	−1.9 (−7.4)	0.10 (3.3)	−0.005 (−4.2)	0.08 (12.0)	−0.04 (−10.6)
	µmol/mmol Cholesterol				
C group (n = 29)					
Baseline	6.42 ± 0.85	0.716 ± 0.246	0.031 ± 0.027	0.155 ± 0.096	0.082 ± 0.034
Final	7.40 ± 1.25	0.702 ± 0.260	0.029 ± 0.021	0.149 ± 0.094	0.080 ± 0.041
Change	0.99 ± 0.96*	−0.014 ± 0.199	−0.002 ± 0.018	−0.006 ± 0.050	−0.001 ± 0.023
SE group (n = 28)					
Baseline	6.40 ± 0.92	0.669 ± 0.178	0.029 ± 0.014	0.157 ± 0.095	0.097 ± 0.081
Final	6.96 ± 1.09	0.675 ± 0.196	0.026 ± 0.010	0.171 ± 0.099	0.085 ± 0.054
Change	0.57 ± 0.85*	0.005 ± 0.106	−0.003 ± 0.09	0.013 ± 0.047	−0.011 ± 0.034
Differences between groups in changes (SE − C)					
Difference (%)[2]	−0.42 (−6.5)	0.019 (2.8)	−0.001 (−3.9)	0.019 (12.4)	−0.010 (−10.1)

[1] Values are means ± SD. * Change within group (final vs. baseline), $P < 0.01$ (Wilcoxon Test).
[2] C, control; SE, sterol ester.
[3] Represents the addition of the % change from baseline in each group.

types have been associated with the effects of sterol consumption (7). However, to establish the genetic causes of this variability, future research must study other genotypes involved in lipid absorption and metabolism such as ATP-binding cassette (ABC) transporters (27).

The main undesirable effect of administering phytosterols is that they can interfere with the absorption of carotenoids. Although some studies with phytosterols have shown no differences in plasma carotenoid concentrations (8,9,25), others have demonstrated that the reduction in the intestinal absorption of cholesterol also affects the carotenoids and significantly reduces the post-treatment β-carotene/cholesterol ratio (5,10,14,24,28). Controlled diets (29) or diets providing high quantities of carotenoids (30) could compensate for this side effect. Therefore, the FDA suggests that the regular consumption of fruits and vegetables should be encouraged when phytosterols are consumed (31). However, it seems reasonable not to subject the safety of phytosterols to external dietary factors. For these reasons we investigated the enrichment of bakery products with β-carotene (0.9 mg/d), taking into account that its bioavailability when added as a pure product to fat-rich foods is one order of magnitude higher than when it is consumed as a natural product, such as in whole fruits or vegetables (32). This moderate amount of β-carotene is widely used as a natural colorant by the food industry.

In our study, the consumption of bakery-enriched products is accompanied by an 8.5% increase in β-carotene plasma concentration. The final difference in β-carotene changes between the groups was 12% ($P = 0.11$). The decrease in plasma concentrations of those carotenoids that were not added to the bakery products (i.e., α-carotene and lycopene) suggests that adding β-carotene has a positive effect on maintaining plasma concentrations. Similarly, it is to be expected that adding other carotenoids such as natural extracts of carotenoids, with α- and β-carotene or lycopene, will give good results; however, further research is required to confirm this.

A practical aspect of the response to treatment with phytosterols is the frequency of consumption within the day. Because their primary mechanism of action is that they reduce the micellar solubility of cholesterol, it was assumed that to be effective, phytosterols should be ingested at the same time as meals (33). However, a study of consumption frequency (9) revealed that a single daily dose is sufficient to achieve the desired effect, which is not significantly different from the effect obtained when the same quantity is distributed into three daily doses. Other work with ground beef as the carrier, consumed in a single daily dose, corroborates this fact (16). Our study also clarifies the role of consumption time. In our trial, although the participants were instructed to eat the two daily pieces separately, the results show that there were no differences in the reduction of LDL-C between those who usually consumed them at the same time as meals and those who ate them between meals. This also confirms that the effect of phytosterols is not linked only to their simultaneous ingestion with cholesterol; the fat content of bakery products consumed between meals may promote sufficient biliary secretion to inhibit biliary cholesterol absorption. This may also be explained by the long-term effect of phytosterols hypothesized by Plat and Mensink (34), who proposed a new mechanism based on increased ABCA1 expression and decreased cholesterol absorption in enterocytes after plant sterol intake.

As expected, consumption of phytosterol enriched-bakery products increases plasma concentrations of campesterol and sitosterol. Compared with previous studies (7), we found relatively high concentrations of β-sitosterol (campesterol/sitosterol ratio $\sim$1), but this is consistent with the consumption of olive oil in the Spanish diet, in which β-sitosterol represents $\geq$85% of the sterol fraction. Under these conditions, although the increase in both sterols was significant at the end of the study in the SE group, campesterol tripled its baseline value (campesterol/sitosterol ratio $\sim$2). Apart from these considerations, these data indicate that the compliance of the voluntary participants was very good. In addition, we observed a significant increase in the plasma concentrations of cholesterol precursors, desmosterol and lathosterol, associated with phytosterol consumption. This increase can be explained by the increase in endogenous cholesterol synthesis because intestinal absorption was inhibited by phytosterol intake.

In conclusion, the results of the present study show that the consumption of croissants and muffins enriched with sterol esters, α-tocopherol and β-carotene significantly decreases the plasma levels of total and LDL-C and does not affect the α-tocopherol and β-carotene concentrations in normocholesterolemic subjects under normal lifestyle conditions. Moreover, our study showed no differences in LDL-C changes induced by phytosterol-enriched bakery between different daily patterns of consumption. Our findings suggest that bakery products are excellent carriers for phytosterols and are a practical option in helping to control cholesterol in a healthy population normally consuming these products.

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