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ORIGINAL CONTRIBUTION

Comparable reduction in cholesterol absorption after two different ways of phytosterol administration in humans

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Abstract
Purpose Consumption of phytosterols is a nutritional strategy to reduce cholesterol absorption, but the efficacy of various phytosterol intake modalities remains uncertain. The main objective was to investigate the effects of phytosterol esters (PE) provided either as a spread (dispersed in fat) during a mixed meal or as a minidrink (micro-dispersed in liquid form) after a meal.
Methods In a randomized, single-blinded crossover design, 12 healthy intubated volunteers tested three different liquid meal sequences with and without PE. The liquid meal (500 mL, Fortisip) contained an oral dose (80 mg) of deuterium-enriched cholesterol (D7C). The intubation was stopped at 240 min, and the fate of sterols was determined in the different phases of duodenal content samples as function of time. A second solid fat-containing meal without sterols was consumed at 270 min. D7C was quantified in chylomicrons and plasma for 8 h. The conditions tested were as follows: (1) no PE added (control), (2) PE in a spread added into a liquid meal (PE-spread meal) and (3) PE given 30 min after a liquid meal as 100-g yoghurt drink (PE-minidrink meal).
Results Addition of PE decreased the incorporation of cholesterol into the duodenum aqueous phase including micelles. PE added as a spread or as a minidrink significantly and comparably lowered meal cholesterol occurrence in chylomicrons (−40 % for PE-spread and −54 % for PE-minidrink, $p < 0.0001$) compared with the control meal.
Conclusions PE either dispersed in fat during a meal or micro-dispersed in a liquid form after a meal resulted in a markedly reduced occurrence of meal-derived cholesterol in the circulation at a comparable extent.

Keywords Phytosterols · Cholesterol absorption · Clinical trial · Duodenum · Micelle · Stable isotope

Abbreviations

PE	Phytosterol esters
CE	Cholesterol esters
P	Phytosterol
C	Cholesterol
D7C	Deuterium-enriched cholesterol

Introduction

Phytosterols and phytostanols [both further referred to as phytosterols (P)] are structurally similar to cholesterol and

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exist as such or as their conjugated forms [esters (PE) and glycosides]. Because of their structural homology with cholesterol, and thus their competition with dietary/biliary cholesterol for intestinal absorption, phytosterols display hypocholesterolemic effects in humans [1–3]. Racette et al. [4] recently reported that phytosterols taken at a moderate feasible dose (459 mg/day) through a healthy diet without supplementation reduced intestinal cholesterol absorption. However, the consumption of P-enriched foods became common, resulting from the demonstration of their hypocholesterolemic effects in different populations and related approved health claims [5]. We recently showed in healthy subjects that adding PE into a meal does not alter fat hydrolysis in the duodenum nor chylomicron triglyceride release, but specifically reduces free cholesterol availability for intestinal absorption and subsequent cholesterol resecretion into chylomicrons [6]. Besides solid spreads enriched with phytosterol/stanol esters [7, 8], new dietary sources are now available and marketed through a variety of enriched low-fat food products such as yoghurt, milk preparation and beverages [3, 9–11]. However, the efficacy of various ways of PE intake remains questionable [12]. The present study in humans thus aimed to determine the capacity of two different ways of PE administration to reduce intestinal cholesterol absorption. These two options have been selected to mimic daily life and were likely combinations of both available PE-enriched foods (solid spread or liquid yoghurt) and intake opportunities (during a meal or separately after a meal). Thus, we compared the cholesterol processing in the duodenum content and its resulting absorption after a control sequence (meal and yoghurt without PE) and two PE ways of administration, that is, as a PE-spread within a meal or a PE-micro-dispersed liquid drink after a meal. The intestinal absorption of meal cholesterol as a function of time was quantified by mass spectrometry using hepta-deuterated cholesterol (D7C) through its occurrence in postprandial intestinally derived chylomicrons.

Subjects and methods

Study subjects and design

Study subjects, recruitment and selection

Twelve healthy male subjects were included in the study. Inclusion criteria were the following: age 18–45 year, body mass index 18–25 kg/m^2, good health with no medical conditions affecting the gastrointestinal tract or liver and no use of medication known to affect cholesterol or bile acid metabolism. Fasting plasma total cholesterol, LDL cholesterol, triglycerides and glucose concentrations were <5.0, 3.1, 1.5 and 6.3 mmol/L, respectively. Subjects with high alcohol intake (>140 g/w), non-conventional diets or high physical activity (>4.5 h/w) were excluded.

The study protocol has been reviewed and approved by the local medical ethics committee (CCPPRB Marseille 2; ref N°206,030; July 17, 2006), and each subject approved and signed an informed consent. There was no clinical trial registration given the recruitment has been done in 2006.

Study design External calibration has been done with the following standards: cholesterol, cholesteryl palmitate, cholesteryl oleate, beta sitosterol, campesterol and stigmasterol.

In a randomized, single-blinded crossover design, 12 healthy volunteers tested three different meal sequences at the Clinical Investigation Center, as shown in Fig. 1: (1) a control sequence with no PE added into meal and yoghurt ingested 30 min later, (2) a PE-enriched spread added into the meal (PE-spread meal) and followed by a plain yoghurt and (3) a meal without PE and a yoghurt drink enriched with PE (PE-minidrink meal). A washout period of 1 month occurred between meal sequences. The procedure started around 7.00 a.m. by intubation of the volunteers after an overnight fast. A single lumen radiopaque nasoduodenal tube with 1 sampling point (OD 4.7 mm, 108 cm, ref 8888-264846, Kendall Argyle, Sherwood Medical, Tullamore, Ireland) was located in the small intestine at the ligament of Treitz (30 cm from the pylorus) under X-ray control as previously described [13, 14]. After the tube was fitted, the subjects were sat until the end of the protocol to limit variations in gastric emptying rates. An antecubital vein was then catheterized with an intravenous cannula (5066 20G, Optiva Medex, UK). Volunteers drunk within 15 min a 500-mL liquid meal warmed at 37 °C added with the control spread or PE-enriched spread. It contained a dose (80 mg) of deuterium-enriched cholesterol D7C ([$^2H_7$]-cholesterol) previously dispersed in the spread as before [13] to trace meal cholesterol. After

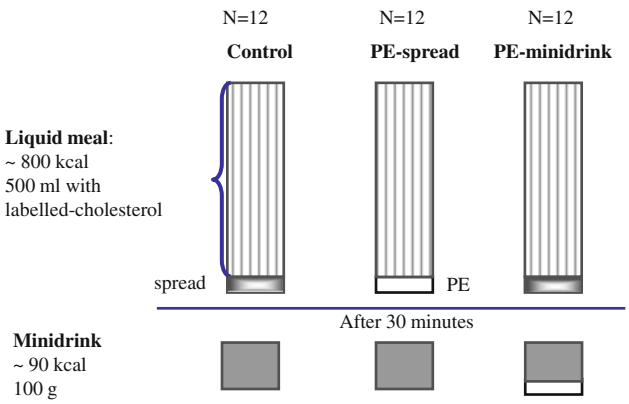

Fig. 1 Scheme of the experimental design

30 min, 100-g yoghurt minidrink with or without PE was consumed. After 240 min, the nasoduodenal tube was removed. To allow for monitoring D7C absorption and occurrence into the plasma compartment, a second meal was consumed at 270 min [13]. This solid meal was made of commercial foods (lasagna, wheat bread, egg, yoghurt, sugar and water, providing 1,820 kJ and 22 g fat) and contained 17.2 mg total phytosterols. The protocol ended after 480 min.

Meal preparation and compositions

All meals were prepared in the kitchen of the Clinical Investigation Centre. The three sequences tested were as follows:

- *Control meal sequence* 500-mL liquid meal (3,595 kJ, 41 g fat, 31 g protein, 92 g carbohydrate) made of Fortisip (Nutricia, UK) + 26.7 g Becel regular spread (no PE, 9.3 g fat, Unilever), and after 30 min, 100-g minidrink (342 kJ, 1.5 g fat) (Actimel, Danone, France) without PE.
- *PE-spread meal sequence* 500-mL liquid meal made of Fortisip (Nutricia, UK) + 26.7 g Becel pro.activ with PE (containing 3.2 g PE, equivalent to 2.0 g P, and 9.3 g fat, Unilever, the Netherlands), and after 30 min, 100-g minidrink (Actimel, Danone, France) without PE.
- *PE-minidrink meal sequence* 500-mL liquid meal made of Fortisip (Nutricia, UK) + 26.7 g Becel regular spread (9.3 g of fat), and after 30 min, 100-g Becel pro.activ minidrink (360 kJ, 2.9 g fat) (Unilever) with PE (containing 3.2 g PE, equivalent to 2.0 g phytosterols). The study products were manufactured under factory conditions. Mixed soya beans and tall oil sterol esters (tall oil sterols esterified with fatty acids from sunflower oil) were used. The PS composition of the products was as follows (wt% of total sterol content, in descending order): sitosterol (78.7 %), sitostanol (8.9 %), campesterol (7.7 %), stigmasterol (1.1 %), campestanol (1.0 %), D5-avenasterol (0.6 %), brassicasterol (0.1 %) and other sterols (1.4 %). The lipid droplet size in the minidrink was in the range 6–7 μm, and the fatty acid composition was as follows: 1.0 g PUFA, 0.8 g MUFA and 1.1 g SFA.

Duodenum content, blood sampling and chylomicron isolation

The duodenum content samples (mostly 5–10 mL) were taken by aspiration using a syringe at 0, 20, 40, 60, 90, 120, 150, 180 and 240 min after the three meal sequences. This duration corresponded to the liquid meal digestion process [14–16]. Immediately after collection, a protease, lipase and microbial inhibitor cocktail was added as previously described [15], and samples were put in the refrigerator.

Blood samples were collected in tubes containing EDTA at 0, 60, 120, 180, 240, 390 and 480 min. The first three time points allowed for the determination of plasma excursion of triglycerides after digestion of the meal sequences as previously done after a test meal in healthy subjects [15, 17]. The two subsequent samplings were performed to allow for the accurate follow-up of plasma or chylomicron D7C as previously determined [13]. Blood samples were centrifuged within 1 h after collection at 1,700×*g* for 10 min, and collected plasma was stored at −80 °C until use.

Chylomicrons were isolated from fresh plasma (5 mL) by ultracentrifugation as previously described [17]. The floating creamy layer was carefully collected and stored at −80 °C until analysis.

Measurements

Duodenum analysis

Duodenal phase separation A careful separation process has been used based on previous studies with intubated volunteers [14–16, 18, 19]. To 4-mL aliquots of duodenum content, 7 mL ultrapure water was added and centrifuged for 50 min at 50,500×*g* and 4 °C using a Sorvall WX100 Ultracentrifuge (Thermo Scientific, France) and a SW Ti 40 rotor (Beckmann, France), in similar conditions as previously published [18, 19], to avoid a full breakage of emulsion droplets [15, 16]. The floating creamy layer (subsequently called as usual oil phase) was first collected and then the clear infranatant (aqueous phase), and finally the precipitated material at the bottom of the tube was re-dispersed in 1 mL 0.9 % NaCl (pellet). All fractions were stored at −80 °C until analysis.

Extraction, separation and quantification of sterols The concentrations of sterols in the three collected duodenal fractions have been determined. Free cholesterol (C), free phytosterols (P), cholesterol esters (CE) and phytosterol esters (PE) have been separated and quantified by gas–liquid chromatography during a 60-min run. Briefly, 0.7 mL of aqueous phase or 0.5 mL pellet phase or 0.3 mL of oil phase was extracted two times with hexane (1:1; v:v) and once with diethyl ether (1:1; v:v). The extracts were evaporated and silylated with BSTFA (Macherey–Nagel, Hoerdt, France) using pyridine as solvent (Sigma, France) before analysis by gas–liquid chromatography. An Autosystem XL Perkin Elmer apparatus was used and equipped with a Chrompack, CP-Sil5 10-m column (CP7730, Varian, Les Ulis, France). A programed temperature on column (POC) injector was used. Temperature gradient was from 80 to 360 °C at 10°/min, and detector temperature was 370 °C. External

calibration has been done with the following standards: cholesterol, cholesteryl palmitate, cholesterol oleate, β-sitosterol, campesterol and β-sitostanol (Sigma, France). CE, PE, C and P peaks were integrated, and their concentrations in each phase were expressed as mmol/L duodenum content. PE were the sum of the main phytosterols and stanols (i.e., β-sitosterol, campesterol and β-sitostanol).

Determination of D7C D7C concentrations in the three duodenal phases have been determined by gas chromatography–mass spectrometry using a HP 6890 series II gas chromatograph fitted with a HP 7673 automatic sampler and interfaced to a HP 5973 A mass spectrometer, as previously reported [13, 20]. Single ion monitoring was performed on the following fragments: D7C, ion $m/z = 336$; epicoprostanol, ion $m/z = 370$; endogenous free cholesterol, ion $m/z = 329$. D7C was expressed as µmol/L duodenum content.

Chylomicrons and plasma analysis

Determination of D7C in chylomicrons and plasma D7C concentration was determined from 0.2 mL chylomicron fraction. Total lipids (mostly triglycerides) were extracted by the Bligh and Dyer method [15] from 0.6 mL chylomicron fraction and measured gravimetrically after solvent evaporation.

Calculations and statistical analysis

The data are presented as mean ± SEM of 12 subjects. The area under the curve (AUC) for sterol species (mmol/L) versus time (AUC60, AUC240 or AUC480 for 60, 240 or 480 min, respectively) was calculated using the trapezoidal method. For each dependent variable, the normality of distribution was validated using the Kolmogorov–Smirnov test. The dependent variables at each time point and AUCs were statistically compared between meal groups using a univariate general linear model. Because subjects were their own control, each model was adjusted for the subject effect. The longitudinal meal group effect on dependent variables (P, PE, C, CE and D7C) over time was analyzed using a mixed linear model with a one-order autoregressive covariance structure. Bonferroni's adjustments were applied when multiple two-by-two comparisons were made. All statistical tests were performed using the SAS software package. All results with a p value <0.05 were considered statistically significant.

Results

Distribution of sterols in the duodenum phases

The distribution of sterols in the three phases (aqueous phase, oil phase and pellet) of the duodenum contents has been determined for the overall process studied using the calculated AUC_{240} (Table 1) after the three meal sequences. Whatever the meal sequence, C was found in the three phases at higher level in the aqueous phase than in oil phase and in pellet. For meal sequences with phytosterols (PE), AUC_{240} for C in the aqueous phase tended to be lower than control,

Table 1 AUC 240 min (mean ± SEM, expressed in mmol min/L) of sterol species (C, P, CE and PE) in the three duodenal phases (aqueous and oil phases, pellet) after three meals [containing PE (as spread or yogourt minidrink) or not (control)]

Sequence	Control meal	PE-spread meal	PE-minidrink meal	PE-spread versus control	PE-minidrink versus control	PE-minidrink versus PE-spread
Aqueous phase						
C	32.1 ± 6.6	23.3 ± 4.2	22.9 ± 4.2	p 0.074	p 0.066	ns
P	–	11.9 ± 1.8	14.7 ± 1.7			ns
CE	<lq	<lq	<lq			
PE	–	<lq	<lq			
Oil phase						
C	18.3 ± 2.3	15.5 ± 2.1	19.8 ± 2.2	ns	ns	ns
P	–	11.6 ± 1.5	18.1 ± 3.3			p 0.059
CE	16.7 ± 3.7	16.5 ± 1.6	24.5 ± 4.6	ns	ns	ns
PE	–	152.4 ± 17.7	198.9 ± 30.8			ns
Pellet						
C	15.6 ± 2.2	15.8 ± 2.8	17.8 ± 4.6	ns	ns	ns
P	–	7.7 ± 1.6	10.9 ± 2.1			ns
CE	4.2 ± 1.1	5.5 ± 1.1	5.9 ± 1.9	ns	ns	ns
PE	–	14.9 ± 1.1	18.5 ± 6.6			ns
$C_{aqueous/pellet}$	2.06	1.47	1.29	ns	ns	ns

<lq limit of quantification

with a comparable extent for the two PE food matrices tested (−27.4 % for PE-spread meal and −28.7 % for PE-minidrink meal). No difference in AUC_{240} in the pellet and oil phases was found for C AUC_{240} between the three meals. As free cholesterol, free phytosterols occurred in the three phases. AUC_{240} for P in the oil phase tended to be higher after the PE-minidrink than PE-spread. In contrast, PE were concentrated in the oil phase and at comparable levels after the two meal sequences containing PE, with minor amounts found in the pellet and traces in the aqueous phase. CE displayed a comparable distribution pattern as for PE and the CE level in the oil phase found after the control meal was not significantly different after the PE-spread meal but was noticeably higher (+47 %) after the PE-minidrink meal. The $C_{aqueous/pellet}$ AUC_{240} ratio tended to be lower after the PE-spread (−36 %) and the PE-minidrink (−28 %) meals than after the control meal (Table 1).

Duodenum, chylomicron and plasma D7C

In the duodenum content, the calculated $D7C_{aqueous\ phase/pellet}$ ratio AUC_{240} tended to be higher (+26 %, but not significantly) with the meals providing PE than control (data not shown). In chylomicrons, the occurrence of D7C added as a single dose into the three meals (time 0) has been determined until 480 min, with an intermediate identical meal without D7C and without PE at 270 min to get optimal chylomicron response as before [21, 22]. The data are presented in Fig. 2. As expected [13], after the control meal, D7C slightly increased in chylomicrons after 180 min and decreased after 240 min, and further after the second meal, markedly increased after 360 min, then coming down at 480 min. After the two PE meals, comparably lower chylomicron D7C increases were observed after 180 min, and more markedly, after 360 and 480 min. As shown in Fig. 2 insert, the different kinetics resulted in significantly different chylomicron D7C AUCs over 480 min, with comparably lower figures ($p < 0.001$) after the PE-minidrink (−59 %) and the PE-spread (−43 %) sequence compared with the control sequence without PE. The occurrence of D7C in the plasma was measured at 480 min. The tracer/tracee (isotopic enrichment) ratio of plasma cholesterol (Fig. 3) showed a significant reduction in plasma cholesterol enrichment after both the PE-minidrink (−51.6 %, $p < 0.0001$) or the PE-spread (−58.3 %, $p < 0.0001$) compared with the control sequence (Fig. 3 inset). The individual values showed differences between volunteers: the subject numbered 12 behaves as a high responder compared to the low responder numbered 8. Except the volunteer numbered 8, whose the both PE treatments gave a different response, all volunteers got a reduction in plasma cholesterol enrichment after both PE-minidrink and PE-spread administration. Notably, the volunteers (3, 7, 8, 10 and 12) with the lowest tracer/tracee values with the control meal were the lowest responders

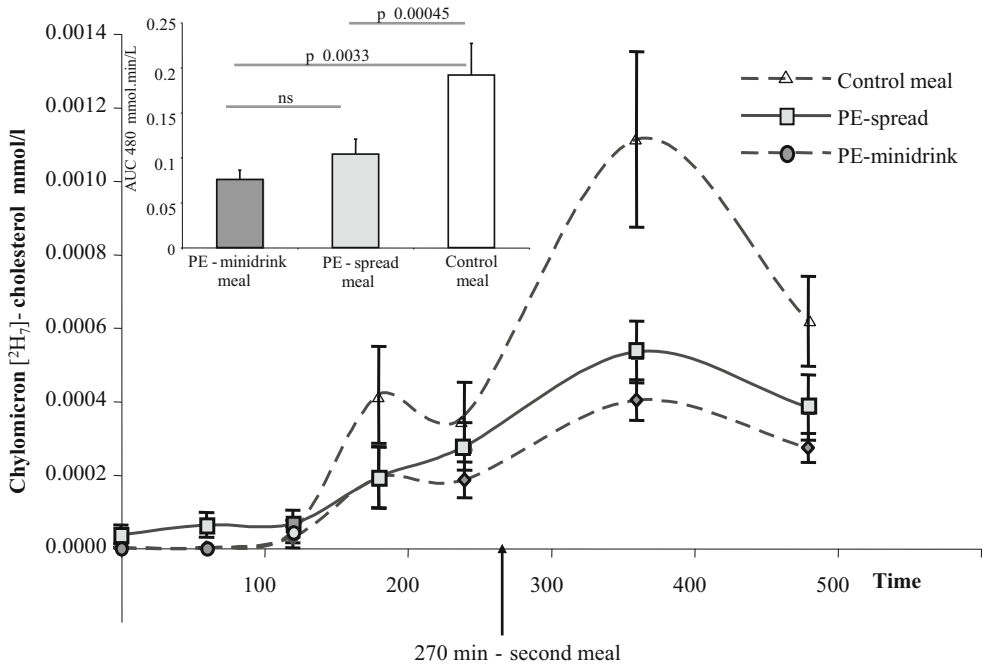

Fig. 2 Chylomicron D7C absorbed cholesterol kinetics and D7C AUCs over 480 min (insert) after intake of two PE-enriched food matrices within meals. PE-minidrink (*dark gray*), PE-spread (*light gray*), control meal without PE (*white*). A solid meal given at 270 min; *ns* non-significant; PE meals significantly different from control meal $p < 0.0001$

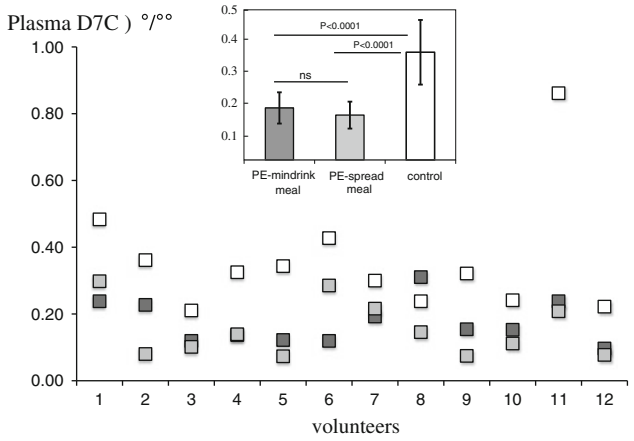

Fig. 3 Individual and integrated isotopic (*inset*) enrichment, ‰ (tracer D7C/tracee ratios) of plasma cholesterol after intake of two PE-enriched food matrices within meals. PE-minidrink (*dark gray*), PE-spread (*light gray*), control meal without PE (*white*). PE meals significantly different from control meal $p < 0.0001$

compared to those (1, 2, 4, 5, 6, 9 and 11) with the highest values.

Discussion

The present study shows the overall comparable effects of PE addition under two realistic ways of administration (as a PE-enriched spread within a meal or a PE-enriched drink 30 min after a meal) on the handling and distribution of plant sterol and cholesterol in the intestinal lumen. Furthermore, our data link these effects to a comparable and significantly reduced cholesterol absorption and resulting appearance into the chylomicron/plasma compartment.

Our data show that PE provided as the two PE-containing sequences mainly accumulated into the oil phase given their hydrophobicity, which is in agreement with previous data obtained with phytostanols [23] or during our recent study on phytosterols [6]. This distribution pattern seems to be independent of the PE-containing matrix although the ingested matrices, that is, fat spread or minidrink, were physicochemically very different. The first one is a non-emulsified solid fat, and the second one is a microdispersed homogenized liquid formula. This suggests marked molecular redistributions within the stomach and duodenum content. PE that are poorly soluble in aqueous phase cannot directly interfere with free cholesterol [22]. To be efficient, PE are hydrolyzed into free phytosterols (P) in the duodenum content. In the present study, this apparent hydrolysis rate was about 10 % during digestion in the duodenum (using a single sampling area). Higher figures (40–88 %) have been reported by others using a design based on constant lipid infusion and collection of jejunal samples [23, 24] or ileal output in ileostomized subjects [25]. In contrast to their esters (PE), amphipathic free phytosterols (P) reached comparable concentrations in the oil and aqueous phases as well as in pellet after the two PE-enriched meals. Cholesterol (C) in its free form behaves similarly as P, because they have very close structural and physicochemical properties [26, 27]. Indeed, C also reached overall comparable levels in oil and aqueous phases and in pellet after the two PE-enriched meals. Nevertheless, it is noteworthy that the C concentration tended to be lower in the aqueous phase after the two PE-containing sequences (−28.3 and −27.1 % for PE-minidrink and PE-spread, respectively) than after the control one. These figures are even lower using the aqueous/pellet C AUC ratios, especially at 240 min with values reduced by 36 % lower after the PE-minidrink and 28 % lower after the PE-spread compared with the control sequence. A lowering of about 34 % of free cholesterol level in the jejunum aqueous phase has already been reported by others when phytostanols were added [23]. Our in vivo observations confirmed those made in vitro where free sitostanol or sitosterol not only were interchangeable with free cholesterol during mixed micelles formation but effectively compete with cholesterol already solubilized in mixed micelles [26, 27]. Indeed, the free phytosterols (P) generated upon PE hydrolysis reached concentration levels in the same range as free cholesterol (C) in the aqueous phase and to lesser degree in the pellet. It has already been shown that cholesterol uptake by enterocytes is lower from small vesicles than mixed micelles [28]. While in the present study the true micellar cholesterol availability cannot be exactly determined (the aqueous phase is a mix of vesicles and micelles), the present data clearly show that C availability for intestinal mucosal uptake from the aqueous phase is markedly reduced when PE are added into the two meal sequences studied, whatever the initial food matrix and presence of a meal. We observed, in agreement with data from others [23, 24], that about 1/4–1/3 of the cholesterol present is in the form of CE, whereas the major source of C would be free C from bile. As no CE is present in the meal product, the CE is likely produced in the gut or secreted directly from the gut wall.

Because chylomicron cholesterol output reflects cholesterol absorption rate from the small intestine, the likely result of the observations made in the duodenum content phases would be a reduction in the overall extent of meal cholesterol absorption after the two PE meal sequences. This was studied using a specific approach by assaying meal-derived D7C in plasma as previously done [6, 13, 29]. This was done during 8 h after the tested meal intakes, a duration allowing most dietary cholesterol to occur into the plasma and close to the steady-state equilibrium reached after about 24 h [6, 13]. Using this methodology, the data

of the present study clearly show that addition of PE into a separate minidrink or a spread/meal significantly and comparably lowered the extent of meal cholesterol occurrence into the chylomicrons (−59 and −43 %, respectively) and the plasma (−40 and −54 %, respectively) compared with the control sequence. These figures are very close to the ones previously obtained by others for lowering of cholesterol absorption in ileostomized patients (−32 % with plant stanol or sterol esters [30] or −40 % with plant stanol esters [31], −34 to 37 % with sitostanols and lecithins given to healthy subjects [29]).

In conclusion, the present study showed in healthy subjects that addition of PE under two different forms (as part of a fat spread added to a meal or added in a microdispersed form 30 min after fat meal intake) increased P levels in the duodenum phases and comparably reduced C availability in the aqueous phase. Thus, the reduction in cholesterol availability in the duodenum after PE intake, whatever the condition of intake, resulted in a comparable markedly reduced occurrence of cholesterol in the circulation, that is, chylomicrons and plasma.

The present data support the idea that PE-enriched foods taken under two different formats (spread or yoghurt), during or after a meal, can elicit overall comparable reduction in intestinal cholesterol absorption and resulting plasma cholesterol-lowering effect.

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Conflict of interest None of the authors had a personal or financial conflict of interest.

References

1. Ostlund RE Jr (2002) Phytosterols in human nutrition. Annu Rev Nutr 22:533–549
2. Tapiero H, Townsend DM, Tew KD (2003) Phytosterols in the prevention of human pathologies. Biomed Pharmacother 57:321–325
3. Jones PJ, AbuMweis SS (2009) Phytosterols as functional food ingredients: linkages to cardiovascular disease and cancer. Curr Opin Clin Nutr Metab Care 12:147–151
4. Racette SB, Lin X, Lefevre M, Spearie CA, Most MM, Ma L, Ostlund RE Jr (2010) Dose effects of dietary phytosterols on cholesterol metabolism: a controlled feeding study. Am J Clin Nutr 91:32–38
5. EFSA So (2009) Plant stanols and plant sterols and blood LDL-cholesterol. Scientific opinion of the panel on dietetic products nutrition and allergies on a request from the European Commission and a similar request from France in relation to the authorization procedure for health claims on plant stanols and plant sterols and lowering/reducing blood LDL-cholesterol pursuant to Article 14 of Regulation (EC) No 1924/20061. EFSA J 1175:1–9
6. Amiot MJ, Knol D, Cardinault N, Nowicki M, Bott R, Antona C, Borel P, Bernard JP, Duchateau G, Lairon D (2011) Phytosterol ester processing in the small intestine: impact on cholesterol availability for absorption and chylomicron cholesterol incorporation in healthy humans. J Lipid Res 52:1256–1264
7. Law M (2000) Plant sterol and stanol margarines and health. BMJ 320:861–864
8. Lee YM, Haastert B, Scherbaum W, Hauner H (2003) A phytosterol-enriched spread improves the lipid profile of subjects with type 2 diabetes mellitus—a randomized controlled trial under free-living conditions. Eur J Nutr 42:111–117
9. Volpe R, Niittynen L, Korpela R, Sirtori C, Bucci A, Fraone N, Pazzucconi F (2001) Effects of yoghurt enriched with plant sterols on serum lipids in patients with moderate hypercholesterolaemia. Br J Nutr 86:233–239
10. Mensink RP, Ebbing S, Lindhout M, Plat J, van Heugten MM (2002) Effects of plant stanol esters supplied in low-fat yoghurt on serum lipids and lipoproteins, non-cholesterol sterols and fat soluble antioxidant concentrations. Atherosclerosis 160:205–213
11. Spilburg CA, Goldberg AC, McGill JB, Stenson WF, Racette SB, Bateman J, McPherson TB, Ostlund RE Jr (2003) Fat-free foods supplemented with soy stanol–lecithin powder reduce cholesterol absorption and LDL cholesterol. J Am Diet Assoc 103:577–581
12. Demonty I, Ras RT, van der Knaap HC, Duchateau GS, Meijer L, Zock PL, Geleijnse JM, Trautwein EA (2009) Continuous dose-response relationship of the LDL-cholesterol-lowering effect of phytosterol intake. J Nutr 139:271–284
13. Beaumier-Gallon G, Dubois C, Senft M, Vergnes MF, Pauli AM, Portugal H, Lairon D (2001) Dietary cholesterol is secreted in intestinally derived chylomicrons during several subsequent postprandial phases in healthy humans. Am J Clin Nutr 73:870–877
14. Armand M, Borel P, Dubois C, Senft M, Peyrot J, Salducci J, Lafont H, Lairon D (1994) Characterization of emulsions and lipolysis of dietary lipids in the human stomach. Am J Physiol 266:G372–G381
15. Armand M, Borel P, Pasquier B, Dubois C, Senft M, Andre M, Peyrot J, Salducci J, Lairon D (1996) Physicochemical characteristics of emulsions during fat digestion in human stomach and duodenum. Am J Physiol 271:G172–G183
16. Armand M, Pasquier B, Andre M, Borel P, Senft M, Peyrot J, Salducci J, Portugal H, Jaussan V, Lairon D (1999) Digestion and absorption of 2 fat emulsions with different droplet sizes in the human digestive tract. Am J Clin Nutr 70:1096–1106
17. Dubois C, Beaumier G, Juhel C, Armand M, Portugal H, Pauli AM, Borel P, Latge C, Lairon D (1998) Effects of graded amounts (0–50 g) of dietary fat on postprandial lipemia and lipoproteins in normolipidemic adults. Am J Clin Nutr 67:31–38
18. Hofmann AF, Borgstroem B (1964) The intraluminal phase of fat digestion in man: the lipid content of the micellar and oil phases of intestinal content obtained during fat digestion and absorption. J Clin Invest 43:247–257
19. Hernell O, Staggers JE, Carey MC (1990) Physical-chemical behavior of dietary and biliary lipids during intestinal digestion and absorption. 2. Phase analysis and aggregation states of luminal lipids during duodenal fat digestion in healthy adult human beings. Biochemistry 29:2041–2056
20. Beaumier-Gallon G, Dubois C, Portugal H, Lairon D (1998) Postprandial studies on dietary cholesterol in human subjects using stable isotopes and gas chromatography–mass spectrometry analysis. Atherosclerosis 141(Suppl 1):S81–S85
21. Beaumier-Gallon G, Lanfranchi J, Vergnes MF, Lairon D, Pastor J, Pauli AM, Portugal H (1998) Method for simultaneous measurements of traces of hepta deuterated cholesterol and cholesterol by gas chromatography-mass spectrometry: application in humans. J Chromatogr B Biomed Sci Appl 718:23–32

22. Brown AW, Hang J, Dussault PH, Carr TP (2010) Phytosterol ester constituents affect micellar cholesterol solubility in model bile. Lipids 45:855–862
23. Nissinen M, Gylling H, Vuoristo M, Miettinen TA (2002) Micellar distribution of cholesterol and phytosterols after duodenal plant stanol ester infusion. Am J Physiol Gastrointest Liver Physiol 282:G1009–G1015
24. Nissinen MJ, Vuoristo M, Gylling H, Miettinen TA (2007) Respective hydrolysis and esterification of esterified and free plant stanols occur rapidly in human intestine after their duodenal infusion in triacyl- or diacylglycerol. Lipids 42:603–612
25. Normen L, Ellegard L, Janssen HG, Steenbergen H, Trautwein E, Andersson H (2006) Phytosterol and phytostanol esters are effectively hydrolysed in the gut and do not affect fat digestion in ileostomy subjects. Eur J Nutr 45:165–170
26. Ikeda I, Tanabe Y, Sugano M (1989) Effects of sitosterol and sitostanol on micellar solubility of cholesterol. J Nutr Sci Vitaminol (Tokyo) 35:361–369
27. Mel'nikov SM, Seijen ten Hoorn JW, Eijkelenboom AP (2004) Effect of phytosterols and phytostanols on the solubilization of cholesterol by dietary mixed micelles: an in vitro study. Chem Phys Lipids 127:121–141
28. Haikal Z, Play B, Landrier JF, Giraud A, Ghiringhelli O, Lairon D, Jourdheuil-Rahmani D (2008) NPC1L1 and SR-BI are involved in intestinal cholesterol absorption from small-size lipid donors. Lipids 43:401–408
29. Ostlund RE Jr, Spilburg CA, Stenson WF (1999) Sitostanol administered in lecithin micelles potently reduces cholesterol absorption in humans. Am J Clin Nutr 70:826–831
30. Normen L, Dutta P, Lia A, Andersson H (2000) Soy sterol esters and beta-sitostanol ester as inhibitors of cholesterol absorption in human small bowel. Am J Clin Nutr 71:908–913
31. Miettinen TA, Vuoristo M, Nissinen M, Jarvinen HJ, Gylling H (2000) Serum, biliary, and fecal cholesterol and plant sterols in colectomized patients before and during consumption of stanol ester margarine. Am J Clin Nutr 71:1095–1102