Effects of a phytosterol-enriched dairy product on lipids, sterols and 8-isoprostane in hypercholesterolemic patients: A multicenter Italian study

E. Mannarino a,*, M. Pirro a, C. Cortese b, G. Lupattelli a, D. Siepi a, A. Mezzetti c, S. Bertolini d, M. Parillo e, R. Fellin f, A. Pujia g, M. Averna h, C. Nicolle i, A. Notarbartolo h

a Medicina Interna, Angiologia e Malattie da Arteriosclerosi, Università di Perugia, Perugia, Italy
b Medicina Interna, Università di Roma Tor Vergata, Roma, Italy
c Medicina e Scienze dell’Invecchiamento, Università di Chieti, Chieti, Italy
d Medicina Interna, Ospedale San Martino, Genova, Italy
e Azienda Ospedaliera S. Sebastiano di Caserta, Caserta, Italy
f Medicina Clinica e Sperimentale, Università di Ferrara, Ferrara, Italy
g Medicina Interna, Università di Catanzaro, Catanzaro, Italy
h Medicina Clinica e Patologie Emergenti, Università di Palermo, Palermo, Italy
i Danone Research, Palaiseau, Cedex, France

Received 1 January 2008; received in revised form 21 March 2008; accepted 31 March 2008

KEYWORDS
Hypercholesterolemia; Phytosterols; Isoprostanes

Abstract  Background and aims: Plant sterols, added to several food sources, lower serum cholesterol concentrations. Plant sterol-induced cholesterol lowering is paralleled by a mild decrease in plasma levels of the antioxidant β-carotene, the amount of this decrease being considered clinically non-significant. Whether the effect on lipid profile of daily consumption of plant sterol-enriched low-fat fermented milk (FM) is paralleled by a concomitant variation in a reliable marker of the oxidative burden like plasma isoprostane levels is unresolved.  Methods and results: The effect of plant sterol consumption on plasma lipid and isoprostane levels of hypercholesterolemic patients was evaluated in a multicenter, randomized double blind study. Hypercholesterolemic patients consumed a FM daily for 6 weeks. Subjects were randomized to receive either 1.6 g of plant sterol-enriched FM (n = 60) or control FM product (n = 56). After 6 weeks of plant sterol-enriched FM consumption, LDL cholesterol was reduced from 166.2 ± 2.0 to 147.4 ± 2.8 mg/dL (p = 0.01). A significant reduction was observed for...
total cholesterol (from 263.5 ± 2.6 to 231.0 ± 3.2 mg/dL, p = 0.01). There was greater LDL cholesterol lowering among hypercholesterolemic patients with higher LDL cholesterol at baseline. We found a reduction of plasma 8-isoprostane in patients taking plant sterol-enriched FM (from 43.07 ± 1.78 to 38.04 ± 1.14 pg/ml, p = 0.018) but not in patients taking the control product (from 42.56 ± 2.12 to 43.19 ± 2.0 pg/ml, p = NS). Campesterol and β-sitosterol levels were not influenced by phytosterol consumption.

Conclusions: Daily consumption of low-fat plant sterol dairy product favourably changes lipid profile by reducing LDL-cholesterol, and may also have an anti-oxidative effect through a reduction of plasma isoprostanes.

© 2008 Elsevier B.V. All rights reserved.

Introduction

The benefits of cholesterol-lowering treatment on the risk of coronary heart disease and mortality have been clearly established in large trials involving the use of statins [1-5]. Statins greatly reduce LDL cholesterol levels and are currently the first choice of a cholesterol-lowering drug treatment. Phytosterols (PS) have become products of increasing importance since it was reported that the addition of sterols to a cholesterol-enriched diet prevented increases in plasma cholesterol level and significantly reduced the incidence of atherosclerotic plaque in the chick aorta [6]. More recently, the Adult Treatment Panel III of the National Cholesterol Education Program (NCEP ATP-III) [7] underlined the importance of lifestyle modifications as the initial step for cholesterol levels reduction and recommended the use of phytosterols to lower plasma LDL cholesterol levels by ~10% [7]. It has been shown that phytosterols enhance the cholesterol lowering effect of a low saturated fat diet [8]. Thus, the use of phytosterols in addition to low fat diet may be useful in those forms of diet-responsive hypercholesterolemia. Moreover, since a statin mono-therapy may be insufficient for reducing blood cholesterol levels to target levels in clinical practice, especially in hypercholesterolemic patients with increased intestinal cholesterol absorption [9], a combination of dietary phytosterols to statin treatment may represent an additional tool to lower plasma cholesterol levels [10]. Accordingly, combined treatment with a statin and dietary phytosterols has led to significantly greater reductions in LDL cholesterol compared to statin mono-therapy [11-13].

Although a number of theories have been advanced concerning the mechanism by which phytosterols lower serum cholesterol, it is generally accepted that phytosterols mainly act by inhibition of intestinal cholesterol absorption [14]. Particularly, phytosterols reduce absorption of cholesterol in the digestive tract by interfering with the solubilisation of the cholesterol in the intestinal micelles. Moreover, phytosterols are believed to be internalized by the Niemann-Pick C1-Like 1 (NPC1L1) into the enterocytes, and to enhance a process in which cholesterol is pumped back out of enterocytes into the lumen of small intestine by ATP-Binding Cassette (ABC) transporter [14]. Phytosterols can also interfere with the absorption of carotenoids; in fact, long-term use of phytosterol-ester enriched spreads results in a reduction in the serum levels of the most lipophilic carotenoids, but at current levels of intake this is unlikely to result in reductions in carotenoids that are of biological significance [15].

The isoprostanes are a unique series of prostaglandin-like compounds formed in vivo via a non-enzymatic mechanism involving the free radical-initiated peroxidation of arachidonic acid [16]. Several studies carried out over the past decade have shown that these compounds are extremely accurate measures of oxidant injury in vivo. Experimental and clinical data suggest a role for isoprostanes in atherogenesis; these compounds, once released from cell membranes by phospholipases, may induce vasoconstriction, platelet aggregation and cell proliferation [17]. Although phytosterols may contribute to the reduction in the serum levels of lipophilic carotenoids, thus possibly suggesting a hypothetical pro-oxidative effect, the potential effects of daily phytosterol consumption on a reliable marker of in vivo oxidation like plasma isoprostanes is still unknown.

The aims of the present study were to investigate the effects of daily consumption of plant sterols-enriched low fat fermented milk on plasma lipid profile of hypercholesterolemic patients; in addition, we examined the possible effects of phytosterols intake on plasma isoprostanes levels, as a measure of global in vivo oxidative burden.

Methods

Study design and subjects

This is a multicentric (7 centers), randomised stratified by centre and by statin treatment, parallel, double-blind, test product vs control product study. Four weeks before the study treatment allocation (Visit 1), uniformisation of subjects’ diet was obtained by the use of the NCEP-ATP III dietary recommendations [7]. During 14 consecutive days (run-in period), all subjects were to take one low fat dairy product within the main meals (lunch or dinner). During the 6 consecutive weeks (treatment period), the subjects were asked to take either:

- the test product (PS), 1 bottle of 100 ml of a fresh dairy product, low fat fermented milk enriched with natural plant sterol esters (DANACOLa);
- the control product (FM), 1 bottle of 100 ml of a fresh dairy product, low fat fermented milk, without plant sterol esters.
The compositions of the plant sterol ester-enriched and control products are presented in Table 1. Plant sterols were extracted from tall oil and were esterified with rapseed oil. The plant sterol ester-enriched fermented milk contained mainly β-sitosterol (75%) and campesterol (8.4%).

Laboratory measurements were conducted at the screening visit of the study (Visit 0) to ensure normal health status. Fasting blood samples were taken: (1) at Visit 1 (4 weeks before treatment randomization); (2) at the beginning of the experimental treatment period (day 0, Visit 2); and (3) after 3 (Visit 3) and 6 weeks (Visit 4) of product consumption. Potential side effects were recorded at each visit.

Subjects were eligible for the study if male or female, aged 20–75 years, with body mass index between 19 and 30 kg/m² and with stabilized hypercholesterolemia (LDL cholesterol between 130 and 190 mg/dL since more than 3 months), willing to follow dietary recommendations for hypercholesteremic patients and used to consume dairy products. Subjects on statin therapy could participate in the study only if on stable statin treatment for at least 3 months before the study beginning. Subjects were not included in case of: plasma triglycerides levels >350 mg/dL, previous cardiovascular event within the last 6 months, use of any drug affecting lipid metabolism other than statin monotherapy, diabetes, allergy or hypersensitivity to milk proteins or soy, history of metabolic or gastrointestinal disease, current diarrhoea or constipation, irritable bowel syndrome, renal failure, use of laxatives during previous weeks, refusal to stop consumption of plant sterol-enriched products other than study product, systemic or topical treatment likely to interfere with the study parameters evaluation. Subjects were excluded if they smoked >20 cigarettes/day.

Evaluation of the subject’s compliance to dietary recommendations and to the consumption of active and control products during the study was evaluated with the help of a subject’s diary observation booklet and accounting for unused products returned to the clinic. The compliance was to be over 80%.

On the basis of these criteria, 116 subjects were included and randomised: 60 to the "active" dairy product containing phytosterols and 56 to the group assuming low fat fermented dairy product without phytosterols. Compliance to protocol was very good; all subjects were over 80% compliant to dietary recommendations and to the study product. Six subjects (5.2%) presented major protocol deviations (three subjects in each study group). All participants gave their written informed consent and the study was approved by the Umbria ethics committee (Perugia, Italy).

Blood sampling

The biological samples collected from the subjects on each of the seven sites, were kept refrigerated or frozen before they were sent to the central laboratory in charge of the analyses (University of Tor Vergata, Rome, Italy). Venous blood samples were obtained after the subjects had fasted overnight. Samples were drawn from the forearm vein into EDTA-treated and plain tubes. Plasma samples were analyzed enzymatically for total cholesterol and HDL cholesterol after precipitation of apolipoprotein B-containing lipoproteins. LDL cholesterol was calculated with the use of the Friedewald equation. Plasma concentrations of β-carotene were measured by reversed-phase HPLC. Plasma β-sitosterol and campesterol concentrations were measured by APPI-LC-MS/MS. The standard curves were linear throughout the calibration range for campesterol and β-sitosterol with an r > 0.99. Mean accuracy throughout the calibration range was between 95 and 118% for campesterol and between 81 and 119% for β-sitosterol. Plasma lathosterol was measured by Gas Chromatography coupled to Mass Spectrometry (GC-MS) with multiple selected ion monitoring (SIM) according to Ahmida et al. [18]. Direct measurement of free plasma 8-isoprostane was performed in duplicate on 50 μl of serum (without hydrolysis) with an EIA commercial kit (8-isoprostane EIA Kit, Cayman, USA), according to the manufacturer’s instruction.

Table 1 Composition of plant sterol-enriched (test) and control fermented milk

<table>
<thead>
<tr>
<th>Product (100 g)</th>
<th>Lipids (g/100 g)</th>
<th>Carbohydrates (g/100 g)</th>
<th>Proteins (g/100 g)</th>
<th>Energy (kcal/100 g)</th>
<th>Active substance (g/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test</td>
<td>1.2 ± 0.1</td>
<td>10.5 ± 0.5</td>
<td>3.2 ± 0.1</td>
<td>66</td>
<td>1.6 equivalent as free sterol</td>
</tr>
<tr>
<td>Control</td>
<td>1.2 ± 0.1</td>
<td>10.5 ± 0.5</td>
<td>3.2 ± 0.1</td>
<td>66</td>
<td>–</td>
</tr>
</tbody>
</table>

Statistical analysis

The sample size was calculated at 56 subjects per group to provide 80% statistical power to detect a difference between the values of the primary efficacy criterion (LDL cholesterol variation) equal or greater than 14 mg/dL between both groups, assuming a common standard deviation of 21 mg/dL and a drop-out rate of 10%. Finally 116 subjects were randomised. Descriptive statistics are presented as mean ± standard error of the mean (SEM) or median/quantiles with 95% confidence intervals (CI) for continuous data or as percentage for qualitative variables. All analyses were performed according to the intention-to-treat and per-protocol principle. To test the differences between the two groups, statistical tests using significance level of 5% (two-tailed) with appropriate methods according the distribution (parametric and/or no parametric) were performed. The comparison between 2 product groups of continuous data was analysed with mixed ANCOVA, including the baseline values, the study product consumption and stratification factors (center, statin therapy level). For those variables not following parametric description, statistical analyses were performed on transformed data (logarithmic transformation) or on rank data. The comparison between 2 product groups of qualitative data was analysed using a Chi-square/Fisher Exact test or logistic regression analysis with a binary response or Cochran–Mantel–Haenszel test. Data analyses were performed using SAS version 9.1 (SAS Institute, Cary, NC, USA).
Results

The baseline clinical characteristics of 116 patients randomized to either PS-enriched or control fermented milk are reported in Table 2. Treatment randomization allowed a balanced distribution of baseline characteristics, the two groups of hypercholesterolemic patients being homogeneous for age, gender, BMI, lipid values. Also statin therapy was uniformly distributed in the two arms of treatment.

Six patients (3 in each group) were excluded from the analysis due to major protocol deviations for the following reasons: one subject withdrew for personal reasons, two subjects reported mild adverse effects, and three subjects did not attend the scheduled visit. Of the remaining 110 participants, 57 were randomized in the PS-enriched product arm and 53 in the control product arm. The results obtained by per-protocol and intention to treat analyses were comparable.

Plasma lipid levels after consumption of PS-enriched or control FM for 3 and 6 weeks are shown in Table 3. After the first 3 weeks of active treatment, LDL cholesterol reduction was reached in the PS-enriched group, but not in the FM control group, the difference between groups being significant ($p < 0.001$). After 6 weeks of treatment with the PS-enriched product, plasma LDL cholesterol was still reduced, but not in the FM control group, the difference between groups being still significant ($p < 0.05$) (Table 3).

At the end of the study treatment, 91% subjects who consumed the PS-enriched product experienced a LDL cholesterol reduction: 76% patients above 5%, 52% above 10%, and 29% above 15%.

Subgroup analysis in those patients who were on stable statin therapy before randomization to PS-enriched or FM control product, showed a similar beneficial effect of the PS-enriched product on LDL cholesterol as that observed in those patients not taking a statin before randomization ($-10.9 \pm 2.0\%$ vs $-11.5 \pm 1.6\%$).

LDL cholesterol absolute changes were then calculated in function of tertiles of baseline LDL cholesterol levels. After 6 weeks of the PS-enriched product consumption, those hypercholesterolemic patients who were in the 1st tertile of baseline LDL cholesterol experienced a $12.4 \pm 3.1 \text{mg/dL} (8.4 \pm 2.1\%)$ reduction of plasma LDL cholesterol; the reduction of plasma LDL cholesterol was even higher in those patients in the 2nd tertile of baseline LDL cholesterol ($21.2 \pm 4.3 \text{mg/dL} ; 12.6 \pm 2.5\%)$ and in those patients in the 3rd tertile of baseline LDL cholesterol ($23.4 \pm 4.8 \text{mg/dL} ; 12.8 \pm 2.6\%)$. The control dairy product consumption was not associated with LDL cholesterol reduction in the 3 tertiles of baseline LDL cholesterol ($0.4 \pm 3.9 \text{mg/dL} ; 9.5 \pm 3.8 \text{mg/dL} ; 0.9 \pm 4.6 \text{mg/dL}$, respectively). Also total cholesterol levels showed a significant decrease in the PS-enriched group, but not in the FM control arm, the difference between the two groups being significant ($p < 0.05$). HDL cholesterol and triglycerides did not change significantly during the consumption of either PS-enriched or FM control product (Table 3). The PS-enriched product led to a significant reduction in the LDL cholesterol/HDL cholesterol ratio compared to the FM control product ($-12.7 \pm 1.9\%$ vs $-5.1 \pm 1.4\%$, $p < 0.001$).

Table 4 shows plasma non -cholesterol sterol levels at baseline and after 6 weeks of either PS-enriched or FM control product consumption. After 6 weeks of treatment no significant variations were observed for both plasma lathosterol, campesterol and $\beta$-sitosterol concentrations.

![Table 2](image.png)

<table>
<thead>
<tr>
<th></th>
<th>PS-enriched fermented milk N = 60</th>
<th>Control fermented milk N = 56</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>50.3 (1.3)</td>
<td>49.9 (1.5)</td>
</tr>
<tr>
<td>Male/female, (%)</td>
<td>45/55</td>
<td>43/57</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>24.9 (0.3)</td>
<td>25.1 (0.3)</td>
</tr>
<tr>
<td>Smokers, n.</td>
<td>8</td>
<td>12</td>
</tr>
<tr>
<td>Cigarettes, n.</td>
<td>8.3 (2.0)</td>
<td>8.6 (1.4)</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>263.5 (2.6)</td>
<td>260.0 (3.2)</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>126.8 (6.8)</td>
<td>125.4 (7.1)</td>
</tr>
<tr>
<td>LDL cholesterol, mg/dL</td>
<td>166.2 (2.0)</td>
<td>163.7 (2.1)</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dL</td>
<td>51.6 (1.9)</td>
<td>50.7 (1.9)</td>
</tr>
<tr>
<td>Glucose, mg/dL</td>
<td>85.3 (1.4)</td>
<td>86.6 (1.3)</td>
</tr>
<tr>
<td>Insulin, μU/mL</td>
<td>7.4 (1.1)</td>
<td>8.3 (0.8)</td>
</tr>
<tr>
<td>Statin therapy n. (%)</td>
<td>8 (13)</td>
<td>7 (13)</td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>128.6 (1.9)</td>
<td>127.5 (1.3)</td>
</tr>
<tr>
<td>DBP, mmHg</td>
<td>77.3 (1.0)</td>
<td>77.8 (1.1)</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM and as percentage. PS, phytosterol; SBP, systolic blood pressure; DBP, diastolic blood pressure. To convert mg/dL of total cholesterol, LDL cholesterol and HDL cholesterol to mmol/L multiply by 0.0258. To convert mg/dL of triglycerides to mmol/L multiply by 0.011. To convert mg/dL of glucose to mmol/L, multiply by 0.055.

![Table 3](image.png)

<table>
<thead>
<tr>
<th></th>
<th>PS-enriched fermented milk N = 60</th>
<th>Control fermented milk N = 56</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol mg/dL</td>
<td>Baseline 263.5 (2.6)</td>
<td>260.0 (3.2)</td>
</tr>
<tr>
<td>3 weeks</td>
<td>226.9 (3.3)*</td>
<td>242.5 (3.5)</td>
</tr>
<tr>
<td>6 weeks</td>
<td>231.0 (3.2)*</td>
<td>243.1 (4.2)</td>
</tr>
<tr>
<td>Triglycerides mg/dL</td>
<td>Baseline 126.8 (6.8)</td>
<td>125.4 (7.1)</td>
</tr>
<tr>
<td>3 weeks</td>
<td>117.0 (5.5)</td>
<td>125.6 (7.0)</td>
</tr>
<tr>
<td>6 weeks</td>
<td>131.6 (9.1)</td>
<td>128.5 (7.6)</td>
</tr>
<tr>
<td>LDL cholesterol mg/dL</td>
<td>Baseline 166.2 (2.0)</td>
<td>163.7 (2.1)</td>
</tr>
<tr>
<td>3 weeks</td>
<td>148.7 (3.1)*</td>
<td>160.1 (2.8)</td>
</tr>
<tr>
<td>6 weeks</td>
<td>147.4 (2.8)*</td>
<td>160.5 (3.1)</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dL</td>
<td>Baseline 51.6 (1.9)</td>
<td>50.7 (1.9)</td>
</tr>
<tr>
<td>3 weeks</td>
<td>51.9 (1.9)</td>
<td>52.7 (2.1)</td>
</tr>
<tr>
<td>6 weeks</td>
<td>53.4 (2.2)</td>
<td>52.7 (2.1)</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SE. *p ≤ 0.001 for between groups comparisons. To convert mg/dL of total cholesterol, HDL cholesterol and LDL cholesterol to mmol/L multiply by 0.0258. To convert mg/dL of triglycerides to mmol/L multiply by 0.011.

Please cite this article in press as: Mannarino E et al., Effects of a phytosterol-enriched dairy product on lipids, sterols and 8-isoprostane in hypercholesterolemic patients: A multicenter Italian study, Nutr Metab Cardiovasc Dis (2008), doi:10.1016/j.numecd.2008.03.012
Phytosterols, lipid profile and oxidative burden

Table 4  Plasma sterols at baseline and after 6 weeks of product consumption

<table>
<thead>
<tr>
<th></th>
<th>PS-enriched fermented milk N = 60</th>
<th>Control fermented milk N = 56</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lathosterol, mg/L</strong></td>
<td>Baseline  2.50 ± 0.19</td>
<td>2.30 ± 0.14</td>
</tr>
<tr>
<td></td>
<td>6 weeks   2.45 ± 0.19</td>
<td>2.27 ± 0.13</td>
</tr>
<tr>
<td><strong>Campesterol, mg/L</strong></td>
<td>Baseline  5.68 ± 0.27</td>
<td>5.67 ± 0.25</td>
</tr>
<tr>
<td></td>
<td>6 weeks   6.11 ± 0.26</td>
<td>6.30 ± 0.43</td>
</tr>
<tr>
<td><strong>Sitosterol mg/L</strong></td>
<td>Baseline  4.92 ± 0.24</td>
<td>4.63 ± 0.26</td>
</tr>
<tr>
<td></td>
<td>6 weeks   5.94 ± 0.31</td>
<td>5.41 ± 0.32</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM.

At the end of the study treatment, plasma β-carotene levels were reduced by 12.1 ± 3.6% in the PS-enriched group and by 0.8 ± 5.5% in the control group, with a statistically significant difference between the 2 groups of 72 ng/mL (p = 0.022). After plasma β-carotene levels were normalized to LDL cholesterol concentration (β-carotene/LDL cholesterol ratio), the absolute change in the β-carotene/ LDL cholesterol ratio was comparable in the two arms of treatment (−0.13 ± 0.15 × 10⁻⁴ vs −0.03 ± 0.14 × 10⁻⁴).

Finally, plasma 8-isoprostane levels showed a significantly greater decrease in the PS-enriched group than in the FM control group (−5.03 ± 1.64 pg/ml vs 0.63 ± 1.71 pg/ml, p = 0.002); in particular, plant sterol-enriched FM consumption was associated with a significant plasma 8-isoprostane lowering (from 43.07 ± 1.78 to 38.04 ± 1.14 pg/ml, p = 0.018) but not the control FM product consumption (from 42.56 ± 2.12 to 43.19 ± 2.0 pg/ml, p = NS).

Discussion

The results of the present study showed a positive effect of phytosterol daily intake on LDL cholesterol levels in subjects with moderate hypercholesterolemia, the magnitude of this effect being already evident since the 3rd week of PS consumption and still present after 6 weeks of active PS intake. Moreover, daily consumption of PS-enriched low-fat fermented milk was paralleled by a concomitant reduction of plasma 8-isoprostane levels, which have been referred as a reliable marker of global in vivo oxidative burden.

The hypocholesterolemic effects of phytosterols have been documented in animal models since 1951 [19]; thereafter, due to their poor water solubility and bioavailability, the use of these substances was partially abandoned. Their consume as hypocholesterolemic agents in humans restated later on, when the esterification of plant stanols with fatty acids increased lipid solubility allowing their incorporation in various foods, such as margarine, cream cheese, salad dressing and yoghurt [20]. Phytosterols were also emulsified with lecithin and delivered in non-fat food (bread, cereals, orange juice) or low fat food (milk and yoghurt), their efficacy being higher when added to low fat milk than to bread and cereals [21-23]. A meta-analysis [23] of small scale trials with phytosterols added to high fat foods (i.e. margarines, butter, mayonnaise, olive oil) showed that the reduction of LDL cholesterol obtained with a phytosterol intake above 2 g/day ranged from 13 to 20 mg/dl [23]. Other studies with low fat dairy products as vectors for phytosterols had a variable influence on plasma LDL cholesterol [21,24-27]. In general, a phytosterol daily consumption of less than 1.2 g was not effective in reducing LDL cholesterol levels, whereas a significant hypocholesterolemic effect was achieved with 1.6–3.2 g/day [22,28]. Accordingly, the NCEP ATP-III recommended a phytosterol daily consumption of 2 g [7].

A recent multicenter, randomized controlled trial with a low fat dairy product added with 1.6 g/day phytosterol subdivided in two servings [29], showed a significant absolute 8.5% reduction of plasma LDL cholesterol after 3 weeks of plant sterol enriched yoghurt consumption, this reduction being comparable (8.6%) after 6 weeks. Interestingly, in this study the authors used the same low-fat dairy product and the same daily amount of phytosterols used in our study. However, the two studies partially differed in terms of LDL cholesterol reduction, the absolute LDL cholesterol decrease after 3 and 6 weeks of active PS consumption being slightly greater in the present study (10.5% at the 3rd week and ~12% at the 6 week of PS-enriched yoghurt consumption). Baseline cholesterol levels were also higher in the present study compared to those in the study by Hansel et al. [29]. Thus, the different baseline cholesterol levels between the two studies may account for the different degree of LDL cholesterol reduction observed after 1.6 g/day phytosterol consumption. This assumption may be supported by previous observation that plant stanol esters in low-fat milk are more effective in reducing cholesterol in patients with higher baseline cholesterol levels [30]. Also in the present study we found that the beneficial effects of phytosterol consumption on plasma LDL cholesterol levels was greater in those patients with higher baseline cholesterol concentrations (2nd and 3rd tertiles of baseline LDL cholesterol), which might suggest a more important reduction of cholesterol absorption in patients with higher cholesterol levels. Although most individuals absorb roughly 50% of dietary cholesterol, there is a large interindividual difference in cholesterol absorption, which ranges from 20 to 80% [31]. Actually, patients most likely to respond better to phytosterols should be those with higher levels of serum cholestanol to cholesterol, an index of cholesterol absorption [13]. The latter assumptions are motivated by the mechanism by which phytosterol reduce plasma cholesterol levels. Plant sterols are solubilized and incorporated in intestinal micelle, thus competitively displacing cholesterol from micelles and limiting the amount of cholesterol available for absorption [14]. Plant sterols once entered in the enterocyte are very efficiently transported back into intestinal lumen by sterolin 1 and 2, these proteins being expressed not only in the mucosal cells but also in the canalicular system, thus resecreting phytosterols also from the liver to bile [14,32,33]. Moreover, in cultured cells sitostanols increased the expression of ABCA1 messenger RNA, suggesting a further contribute to the decrease of cholesterol absorption [34]. Another finding is the similar beneficial effect of the PS-enriched product on LDL cholesterol in those patients taking or not taking a statin before randomization. This result is however
preliminary because the number of subjects under statin treatment in the present study was low to make definitive conclusions on this issue.

The present study, in agreement with the most trials with phytosterols, did not demonstrate any effect on triglycerides and HDL cholesterol; anyway, as a consequence of the reduction of LDL cholesterol, also LDL cholesterol/HDL cholesterol ratio, which is a good predictor of cardiovascular risk in hypercholesterolemic patients [35], showed a significant reduction in the PS-enriched group (−12.7 ± 1.9%), compared to FM control group (−5.1 ± 1.4%, p < 0.001).

Several studies had pointed out the importance of a possible decrease of liposoluble vitamins during phytosterol therapy [36]. Actually, in our study absolute plasma β-carotene levels showed a decrease after 6 weeks of phytosterol consumption; however, after normalization for LDL cholesterol reduction, the decrease of plasma β-carotene levels was no longer significant. Since the decrease of vitamins and antioxidants during phytosterol consumption could represent a possible determinant of an increased oxidative burden, we measured plasma 8-isoprostane variation during the PS-enriched treatment. Isoprostanes are prostaglandin-like compounds formed in vivo via a non-enzymatic mechanism involving the free radical-initiated peroxidation of arachidonic acid [16]; thus, these compounds are extremely accurate measures of oxidant injury in vivo. In the present study, we found that 6-week phytosterol consumption was associated with a significant plasma 8-isoprostane reduction, thus suggesting possible anti-oxidative instead of pro-oxidative properties of phytosterols. Since isoprostane levels are increased in smokers and are influenced by abstinence from smoking [37], the presence of smoking subjects in the present study might have had an influence on plasma 8-isoprostane; however, no change in the consumption of cigarettes occurred after baseline, thus lessening the possible confounding effect of smoking status on 8-isoprostane variations.

Importantly, the levels of β-sitosterols and campesterol have been considered potentially atherogenic even in non-sitosterolemic patients [38]; actually in sitosterolemia their levels are 10–20 folds higher than in healthy or hypercholesterolemic subjects. In our study no statistically significant increase in plasma phytosterols was recorded during PS-enriched product consumption; moreover, plasma phytosterol absolute levels were very low and far from levels observed in patients with sitosterolemia. Also plasma lathosterol levels were not affected by the 6-week phytosterol consumption, thus suggesting that the reduced cholesterol absorption was not paralleled by a concomitant increase in cholesterol synthesis among hypercholesterolemic patients consuming the phytosterol-enriched fermented milk.

In conclusions, 6-week phytosterols consumption with low-fat fermented milk accounts for a significant 12% reduction of plasma LDL cholesterol levels, an effect that may be reached after just only 3 weeks of active consumption without adverse effects. The use of phytosterols may thus represent a simple and safe tool to reduce plasma cholesterol in patients with mild cholesterol elevations, thus enhancing the attainment of LDL cholesterol goal in hypercholesterolemic patients [39]. Their use in cholesterol “hyperabsorbers” might probably further amplify their therapeutic effect. However, large long-term studies are needed to confirm results obtained in small scale studies.

Acknowledgements

This study was supported by a grant from Danone Research, France. We would like to kindly thank A. Simonnet, P. Rondeau, S. Doat and B. Rumo for their contribution to this study. We also thank all the volunteers who participated.

References

Phytosterols, lipid profile and oxidative burden


Please cite this article in press as: Mannarino E et al., Effects of a phytosterol-enriched dairy product on lipids, sterols and 8-isoprostane in hypercholesterolemic patients: A multicenter Italian study, Nutr Metab Cardiovasc Dis (2008), doi:10.1016/j.numecd.2008.03.012