Milk enriched with phytosterols reduces plasma cholesterol levels in healthy and hypercholesterolemic subjects

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Abstract

The consumption of plant sterols has been shown to decrease plasma concentrations of cholesterol without adverse effects in human subjects. To evaluate if milk would be a good vehicle for phytosterols to lower plasma levels of cholesterol, we performed a randomized blind study with healthy subjects (n = 22) and hypercholesterolemic patients (n = 19), both groups treated with phytosterol-enriched milk (2 g/d). Another hypercholesterolemic group (n = 15) was used as a control group. Lipid profile and biochemical, hematologic, and hemorheological parameters were determined at the beginning and after 15 and 30 days of milk beverage intake. After 15 days of treatment, healthy individuals showed lowered total cholesterol and LDL-C levels, by 8.31% (P < .05) and 11% (P < .05), respectively. After 30 days of the trial, these values did not change significantly. Hypercholesterolemic patients treated with phytosterol-enriched milk demonstrated significantly diminished levels of total cholesterol and LDL-C concentrations, by 9.62% (P < .05) and 12.20% (P < .05), respectively. After 30 days, an increase in the total cholesterol and LDL-C levels was observed for hypercholesterolemic subjects, 6.69% (P < .05) and 8.68% (P < .05), respectively. In the hypercholesterolemic control subjects, no difference was found between plasma levels of total cholesterol, high-density lipoprotein cholesterol, and LDL-C. Only healthy subjects showed significant changes during the intake of phytosterol-enriched milk. The results obtained indicate that phytosterol-enriched milk is a good vehicle for reducing plasma cholesterol in hypercholesterolemic subjects.

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1. Introduction

Atherosclerosis is a complex process involving several risk factors, including a high level of low-density lipoprotein cholesterol (LDL-C). Deposition of cholesterol, cholesteryl esters, and others lipids in the artery wall leads to narrowing and hardening of the artery, which, in turn, may lead to atherosclerosis and increased risk of forming blood clots (thrombosis) [1]. In an atherogenic milieu, oxidized LDL infiltrates the intima, where it stimulates inflammation, endothelial dysfunction, and eventually, atherosclerosis. Although it is true that very high LDL-C levels (>200 mg/dL) are strongly associated with coronary heart disease (CHD) risk, atherosclerosis in not uncommon even in those with relatively normal levels (90-130 mg/dL) [2,3]. It is known that atherosclerosis progression varies directly with increases in LDL-C. A recent study demonstrated that
atherosclerosis does not progress when LDL-C levels are 67 mg/dL or below [2], and people with LDL-C levels between 70 and 120 mg/dL, which is considered normal, can potentially suffer a CHD event.

Drug therapy to inhibit cholesterol biosynthesis is effective but is not without some potential negative consequences. Blood LDL-C levels may be reduced by incorporating into the diet sterol esters, which reduce the absorption of cholesterol [2]. The trials using drug therapy have demonstrated remarkable reductions in CHD events, especially in patients with hypercholesterolemia [4,5]. This reduction in LDL-C levels represents only a partial treatment, and the effect appears after 6 to 8 weeks of treatment. Recent studies show that more robust reduction occurs when the therapy is accomplished with an increased consumption of phytosterols in the daily diet [1,6-8]. Most strategies for lowering serum cholesterol require unpopular dietary restrictions or the use of drugs. The prospect of lowering cholesterol levels by consuming foods fortified with natural phytonutrients is much more attractive. Phytosterols (including plant sterols and stanols) are natural components of edible vegetable oils [9]. The structures of these phytosterols are closely related and resemble that of cholesterol [10]. The mechanism of action is not clear, but it is accepted that phytosterols are thought to displace cholesterol from bile acid micelles and/or coprecipitate cholesterol in the intestinal lumen, thereby limiting its uptake [1,10].

The plasma cholesterol–lowering properties of plant sterols have been known since the 1950s. Early studies examined the cholesterol-reducing potential of plant sterols using up to 25 g/d consumed in solid crystalline form. Over the years, however, it became clear that sterols dissolved in edible fat products are more effective at reducing blood cholesterol levels than sterols in crystalline form [1]. Functional foods appear to help in the treatment of some diseases in concert with medical therapy. The enrichment of foods such as margarines with plant sterols and stanols is one of the most recent developments in functional foods for enhancing the cholesterol-lowering ability of traditional food products [9]. Investigations on mechanisms, uses, and properties of plant phytosterols have been tested in animal models that respond to phytosterols, as well as other known cholesterol-lowering agents [10-14].

Previous work in humans indicate that a combined therapy of cholesterol-lowering drugs and cholesterol-lowering bread spreads provides an extra 10% to 12% reduction in LDL-C levels [8]. These findings may lead to new therapeutic options for treating hypercholesterolemia. Hypercholesterolemia is an independent risk factor for CHD, and the use of drugs to control disease is important [4].

Shin et al [15] recently investigated the effect of a beverage containing plant sterols on serum concentrations of triacylglycerol and cholesterol in hypercholesterolemic subjects on a low-cholesterol diet. After 8 weeks of trial, the results obtained showed a significant decrease in serum concentrations of total cholesterol and LDL-C by 4.38% ($P < .05$) and 8.28% ($P < .05$), respectively [15]. The search for a new alternative for hypercholesterolemia treatment and the efficacy of phytosterol-containing food have been a common interest in recent years [13,15-17]. Plant sterols have been a useful additive therapy in the treatment of patients with hypercholesterolemia with no apparent adverse effects [13,15,16,18-20]. The effect of plant sterols has not been evaluated in a Portuguese population, which generally has a medium-high dietary cholesterol intake. The aim of the current study is to investigate the effect of phytosterols added into milk on plasma concentrations of total cholesterol, high-density lipoprotein cholesterol (HDL-C), and LDL-C, and on biochemical, hematologic, and hemorheological profiles in healthy and hypercholesterolemic subjects in Portugal.

2. Methods and materials

2.1. Materials

All reagents were of analytic grade and were purchased from Sigma Aldrich, Co (St. Louis, MO).

2.2. Subjects

Eligible subjects, men and women between 25 and 75 years of age, were evaluated in this study. Exclusion criteria included familiar hypercholesterolemia, CHD risk factors, diabetes, cancer, and renal and hepatic disease. The inclusion criterion for the hypercholesterolemic group was baseline plasma concentration of LDL-C of more than 130 mg/dL and less than 125 mg/dL for healthy subjects (HSs).

2.3. Study design

The study was a randomized trial evaluating the effects of phytosterol-enriched milk on biochemical, hematologic, and hemorheological parameters over a treatment period of 30 days. The subjects were assigned to 2 principal groups: one group of 22 healthy subjects (10 women, 12 men, aged 27-50 years) and a second group of 34 hypercholesterolemic subjects (aged 40-72 years), with 15 patients given non-enriched milk as a control (12 women, 3 men) and 19 patients (11 women, 8 men) given phytosterol-enriched milk. The hypercholesterolemic subjects were randomly assigned to 1 of 2 types of milk: phytosterol-enriched milk or non-enriched milk. Hypercholesterolemic patients received medication (reductase inhibitors) and continued follow-up examination by their physician. The subjects gave their informed consent for the study. The ethics committee of the Faculty of Medicine, University of Lisbon, Lisbon, Portugal, approved the study protocol for human use.

The healthy subjects (HSs) and the hypercholesterolemic test group (HTG) were fed sterol-containing milk (2 g/d) for 30 days. Phytosterol concentration was the same during the study for all subjects. The hypercholesterolemic control group (HCG) was given the same milk but without phytosterols in a blinded manner. The subjects were
informed to maintain the same dietary intake during the study. The hypercholesterolemic patients were advised to continue their prescribed medications. The procedures followed were in accordance with good clinical practice.

2.4. Blood collection and analyses

All subjects were subjected to an overnight fast (12 hours) before blood collection on days 0, 15, and 30 of the trial. Plasma concentrations of lipids (total cholesterol, HDL-C, LDL-C), biochemical parameters (creatinine, urea, aspartate aminotransferase, alanine aminotransferase), and hematologic (hemoglobin, hematocrit, carboxyhemoglobin, metahemoglobin, red blood cells) and hemorheological (plasma viscosity and erythrocyte aggregation) parameters were determined.

The β-carotene determinations were achieved by a spectrophotometric method described by Bradley and Hornbeck [21] but modified to a microprocedure. The quantification was done by using a Genesys 10UV Thermo-spectronic Spectrophotometer (Rochester, NY), and the optical density was read at $\lambda = 450$ nm. Serum samples of 500 μL were mixed with an equivalent quantity of absolute ethanol and extracted with 750 μL of n-hexane; the contents were vortex for 2 minutes and centrifuged for 10 minutes at 3000 rpm and 10°C. The absorbance of the upper organic phase containing β-carotene was measured at 450 nm.

2.4.1. Plasma viscosity

Blood samples, treated with the anticoagulant K3EDTA, were centrifuged at 1200 rpm for 1 minute. The resulting plasma was collected for the determination of plasma viscosity by the Harkness method [22].

2.4.2. Erythrocyte aggregation

Erythrocyte aggregation was determined using the MA1 aggregometer from Myrenne (Roetgen, Germany). The MA1 aggregometer consists of a roto-cone plate aggregometer, which disperses the sample by high shear stress (600/s), and a photometer that determines the extent of aggregation. Light intensity exerted by a light-emitting diode was measured after transmission through the blood sample using a photodiode. The aggregation was determined after dispersion of the blood sample [23].

2.5. Statistical methods

Fig. 1. Mean ± SD of lipid levels of plasma total cholesterol, HDL-C, and LDL-C after 15 days of the phytosterol-enriched milk intake for HFSs. Open bars represent mean values at 0 day of phytosterol-enriched milk intake (base value), and shaded bars represent the mean value after 15 days. ns indicates not significant.

Fig. 2. Mean ± SD of plasma total cholesterol (A), HDL-C (B), and LDL-C (C) after 15 and 30 days of treatment with nonphytosterol and phytosterol-enriched milk intake for hypercholesterolemic subjects. Open bars represents mean values at 0 day, and shaded bars represent the mean value at 15 days and 30 days of dietary treatment. ns indicates not significant.
3. Results and discussion

Fig. 1 presents the change in cholesterol levels in HSs (mean ± SD) after 15 days of phytosterol-enriched milk. Total cholesterol levels decreased from 198 to 182 mg/dL \((P = .05)\). The same effect was obtained for LDL-C levels (decreased from 120 to 107 mg/dL, \(P < .05\)), but the HDL-C levels did not change from the values at 0 day. After 30 days of the study, there were no differences in these measurements in the subjects (data not shown).

The hypercholesterolemic patients included 2 groups: the HCG that received no phytosterols and the HTG that received the phytosterol-enriched milk. The HTG demonstrated significant decreases in total cholesterol and LDL-C but no change in HDL-C level in blood (Fig. 2). In the HCG, all blood cholesterol values remained the same after the 30-day study. In the HTG subjects, total cholesterol levels decreased from 248 to 224 mg/dL after 15 days and from 245 to 229 mg/dL after 30 days of phytosterol-enriched milk intake.

This study was accompanied by an evaluation of the plasmatic biochemical, hematologic, and hemorheological parameters in all groups (Table 1). The results obtained indicate significant changes in the HS group. During the trial, the HCG and HTG did not show significant changes in their biochemical, hematologic, and hemorheological parameters. After 30 days of the trial, the HS group had increased hemoglobin values, from 13 to 13.5 g/dL \((P < .05)\) (Table 1). The observed values for all parameters were maintained between the reference values, and few differences were statistically significant.

Phytosterols such as \(\beta\)-sitosterol, campesterol, and stigmasterol are constituents of many edible plants and may account for 20% to 25% of total dietary sterol. Although phytosterols are absorbed less efficiently than cholesterol, the mechanism of absorption is similar; however, phytosterols inhibit cholesterol absorption by competition with cholesterol for micellar solubilization. In addition, because of their hydrophobicity and poor absorbability, the phytosterols remain in intestinal micelles and continuously interfere with the micellar solubility of cholesterol [18].

In the present study, the effectiveness of milk as a dietary vehicle for phytosterols was examined on cholesterol lowering and changes in the hematologic and hemorheological parameters in healthy and hypercholesterolemic subjects. Other studies have evaluated the use of spreads and beverages as dietary vehicles for phytosterols. For example, Jong et al [8] reported that a combined treatment including a spread containing phytosterols and drugs was effective therapy in the treatment of healthy subjects. In our study with HS subjects, we observed a decrease of 8.3% in total cholesterol and 11% in LDL-C levels after 15 days of consumption of phytosterol-enriched milk but no difference at 30 days. In the HCG, no changes in blood cholesterol measurements were found. In contrast, the HTG had a decrease in blood cholesterol levels; the total cholesterol concentration for the HTG was 9.6% after 15 days and 6.7% after 30 days of treatment compared with the baseline values. Moreover, the LDL-C concentration was reduced by 12.2% after 15 days and 8.7% after 30 days of treatment.

The effect of phytosterols on plasma lipid levels has been tested in animals [10-12] and humans [13,15-20]. The phytosterols exhibit relatively little toxic effects because they are poorly absorbed. These compounds inhibit cholesterol absorption by competing with cholesterol for micellar solubilization, and, due to their hydrophobicity and poor absorption, they remain in intestinal micelles and continuously interfere with the micellar solubility of cholesterol [18].

Abnormalities in blood rheology, in particular, elevated plasma fibrinogen level and blood viscosity, are regarded as a risk factors for the progression of atherosclerosis, the

<table>
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<th>Group</th>
<th>Parameters</th>
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<tbody>
<tr>
<td></td>
<td>Hb (g/dL)</td>
</tr>
<tr>
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<td>13.5 ± 1.0</td>
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<tr>
<td></td>
<td>13.5 ± 1.3</td>
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<tr>
<td></td>
<td>30 d 13.5 ± 1.1*</td>
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<tr>
<td>HCG</td>
<td>14 ± 1</td>
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<td>13.7 ± 1.2</td>
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<td>30 d 13.9 ± 1.3</td>
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<tr>
<td>HTG</td>
<td>14.5 ± 1.3</td>
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<td></td>
<td>14.3 ± 1.5</td>
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<td></td>
<td>30 d 14.4 ± 1.1</td>
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Hb indicates hemoglobin; Ht, hematocrit; CarbHb, carboxyhemoglobin; MetHb, metahemoglobin; RBC, red blood cell; PV, plasmatic viscosity; EAI, erythrocyte aggregation; AST, aspartate aminotransferase; ALT, alanine aminotransferase.

* Significant difference at \(P < .05\).
occurrence of cerebrovascular disorders, and ischemic heart disease [24]. It has been reported that the values of hemorheological parameters increase in patients with hyperlipoproteinemia. Tsuda et al [24] studied the changes in plasma fibrinogen level and blood rheology in patients with type II hyperlipoproteinemia before and after administration of 2 reductase inhibitors, and the results were compared with those obtained for healthy subjects. The changes encountered depend on drug therapy, and the beneficial and the negative effects depend on the hydrophilic or lipophilic and binding capacity with the plasma protein of the drug [24]. The results of the effects on hemorheology are rather controversial at present. That is, the hemorheological effects induced by the different cholesterol-lowering therapies may vary depending on the drug [24-26]. In the present study, biochemical, hematologic, and hemorheological parameters were evaluated to identify any adverse effects resulting from the use of phytosterol-enriched milk for the treatment of hypercholesterolemia.

The hemorheological disturbance in patients with cardiovascular diseases is due to changes in the protein, lipid, and electrolyte composition of the blood plasma. Deterioration of the rheological properties of blood significantly influences the degree of microcirculatory disturbances and the development of complications of ischemic heart disease [27]. These disturbances were not observed in the present study. Only the hemoglobin levels in the HS group after 30 days of phytosterol intake were modified.

In recent years, the research on phytosterols has focused on their cholesterol-lowering properties. The only side effect observed in humans was a decrease in the levels of some fat-soluble antioxidants, such as carotenoids. Because cholesterol reduction is mainly due to an inhibition of absorption, phytosterols may interfere with the absorption of fat-soluble vitamins. The effect of phytosterols on fat-soluble vitamin absorption is controversial [28,29]. Antioxidants, such as β-carotene, are assumed to reside in the hydrophobic part of LDL particles, protecting the polyunsaturated fatty acids from oxidation [24]. This interaction between β-carotene and the LDL particle has been used to monitor the interactions of LDL directly in plasma [29]. Raeni-Sarjaz et al [30] did not observe changes in serum fat-soluble vitamins or carotenoid concentrations with the consumption of plant sterol/stanol esters in a controlled diet. Others studies conclude that sitostanol esters reduce serum β-carotene levels [31]. In the present study, β-carotene levels were lower in the HCG and HTG compared with those in the HS group; however, within these 2 hypercholesterolemic groups, the levels before and after the trial were not different.

Many studies have shown that phytosterols lower blood cholesterol levels. The main result of the current study is that a phytosterol-enriched milk reduced plasma levels of total cholesterol and LDL-C in healthy and hypercholesterolemic subjects. Although with a small sample size, our study did not reveal any side effects, and the results confirm previous findings. The evaluation of the different biochemical, hematologic, and hemorheological parameters indicate that the combination of a drug therapy and phytosterol-enriched milk was safe in hypercholesterolemic subjects.

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