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SHORT COMMUNICATION

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Effects of a new low dose soy protein/β-sitosterol association on plasma lipid levels and oxidation

Summary *Background* High doses of soy protein are able to decrease plasma cholesterolemia significantly, but they unbalance daily protein intake and strongly modify nutritional habits in patients. *Aim of the study* To evaluate the antihypercholesterolemic efficacy of a low dose soy protein product with added β-sitosterol (rapport = 4:1) in 36 moderately hypercholesterolemic subjects. *Methods* The study was divided into 3 separate periods of 40 days each: a stabilization diet period, followed by a treatment period during which all subjects took 10 g of the test product once daily and, finally, a wash out period. The following parameters were monitored: weight, dietary habits, plasma lipid levels, glycemia, uric acid, fibrinogenemia and antibodies against oxidized LDL (ox-LDL Ab). *Results* From the end of the stabilization diet period to the end of the supplementation with the soy protein product with added β-sitosterol we observed a 19.64 ± 20.32 mg/dL, 8.47 ± 54.61 mg/dL, 1.69 ± 10.92 mg/dL, and 7.06 ± 16.66 mg/dL mean ± SD decrease respectively in LDL-C ($p < 0.001$), TG ($p = 0.358$), VLDLs ($p = 0.358$) and apoB ($p = 0.016$) levels, associated with a 1.31 ± 8.08 mg/dL and 1.03 ± 19.09 mg/dL mean increase respectively in HDL-C ($p = 0.251$) and apoAI ($p = 0.749$) plasma concentrations. The dietary supplementation did not influence Lp(a) ($p = 0.984$) and ox-LDL Ab ($p = 0.953$) plasma levels. A statistically significant correlation was observed for LDL-C plasma levels, between the end of the stabilization diet period and the end of the period of supplementation with soy proteins with added β-sitosterols ($p < 0.001$). *Conclusion* Although further long-term clinical studies are necessary before claims can be made regarding the therapeutic effects of the tested formulation, the preliminary findings regarding its efficacy and safety as an antihypercholesterolemic agent are encouraging.

Key words phytosterols – soy bean protein – β-sitosterol – hypercholesterolemia

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Introduction

Among other natural products, soyfoods and phytosterols have received widespread attention for their role in coronary heart disease prevention [1, 2]. Currently, the key issues regarding the use of plant sterols and their derivatives are the effectiveness of different mixtures and the long-term safety, since many studies have well established their benefits and short-term safety [1, 2]. In a meta-analysis carried out by Anderson et al. it was estimated that, after adjustment for initial serum cholesterol concentrations and other variables, the ingestion of 25 g or 50 g of soy protein per day decreases serum cholesterol by 0.23 mmol/dL or 0.45 mmol/dL respectively [3]. Although some studies have demonstrated that phytostanols (saturated plant sterols) are more effective cholesterol lowering agents than phytosterols [4], more recent findings support the conclusions of Anderson and colleagues that all current soy bean derived mixtures are more or less equivalent with regard to their cholesterol lowering properties [5]. The major differ-

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ence remains the quantity to be taken daily in order to achieve similar results, which in various studies ranges from 18 g to 124 g [3].

In a previous report, we tested the synergistic effect of soy proteins and β-sitosterols in reducing plasma LDL-C levels. The tested soy protein/β-sitosterol association was then tested on a small group of moderately hypercholesterolemic patients and it seemed to be able to induce a significant decrease in plasma LDL-C levels at the dose of 10 g/day [6].

The aim of this study was to confirm the previous results on a wider patient sample and on a more complete set of laboratory parameters.

Methods

Thirty-six Caucasian volunteers (M:F = 1:1; mean age = 52±13 years) affected by type IIa hypercholesterolemia according to Fredrickson's classification were recruited in the Atherosclerosis Center of the University of Bologna (Italy). All subjects were submitted to a careful medical examination and laboratory analyses were carried out to confirm the diagnosis of multigenic type IIa hypercholesterolemia. The selection criteria were:

- LDL-C ranging from 130 mg/dL to 190 mg/dL (calculated with Friedewald's formula);
- HDL-C > 45 mg/dL and TG < 200 mg/dL;
- No history of cardiovascular disease (myocardial, angina pectoris, stroke, or revascularization);
- Absence of diagnosed diabetes mellitus, familial combined hyperlipoproteinemia, X-metabolic syndrome or secondary dyslipidemias and any disease or treatment that could affect lipid measurements (including steroids, immuno-suppressors, nicotinic acid > 50 mg per day, lipid lowering drugs) or limit the individual's ability to participate in the study (for example endocrine diseases, acute and chronic infections);
- Absence of laboratory values indicating significant abnormalities defined as: plasma transaminase concentrations (AST, ALT) > 1.5 time of upper limit of normal (ULN); Plasma CPK concentrations > 2 x ULN that could not be explained (for example intense massage or exercise); hyperuricemia (uric acid > 60 mg/L).

For the entire duration of the study, each patient's dietary pattern, through the "Seven-day questionnaire" [7], and weight were monitored by a registered dietician.

The study was divided into three separate periods of forty days each. First, a stabilization diet period, during which all patients received dietary advice (according to Step 1 of the recommendations issued by the American Heart Association) [8] and any nutritional supplement use was eliminated. After this period, all the subjects took a low dose product (10 g once daily) containing isolated soy protein (8 g) and β-sitosterol (2 g) in a ratio of 4:1 (patented and offered by Inpharma SA, Lugano, Switzerland) for a period of forty days. The dose of powder was suggested to be solved in 125 ml of lean yogurt 10 minutes before the main meal of the day. At the end of this forty-day period we collected the empty containers to evaluate patient compliance with treatment, calculated as the number of doses that the patient actually took versus the total amount supplied. The patients then interrupted product use for a wash out period of forty days. At each study stage a complete plasma lipid and lipoprotein assessment was carried out.

The hematochemistry measurements were carried out on venous blood coming from the antecubital arm vein, after 12 hours of fasting. The plasma samples were immediately separated by high-speed centrifugation at 3500 x g for twenty minutes. TC, HDL-C, TG, glucose, and uric acid were determined with standardized enzymatic colorimetric methods [9]. The LDL-C plasma levels were estimated with Friedewald's formula [LDL-C = TC – (TG/5 + HDL-C)], for TG < 4.4 mmol/L. The VLDL plasma levels were then estimated always using Friedewald's formula [VLDL = TC– (LDL-C + HDL-C)]. The prevalence of high small and dense LDLs was estimated using Hattori's formula [10]. For higher TG values, a VLDL and LDL separation by preparative centrifugation and successive measurements of the lipoprotein fraction were carried out. Apolipoprotein AI and B100 and fibrinogen were dosed by immunoturbidimetry as elsewhere described [9]. The Lp(a) concentrations were measured by enzyme-linked immunosorbent assay (ELISA) [11].

The dosage of ox-LDL Ab has been obtained by a solid-phase ELISA method with a commercially available kit (OLAB, BioMedica) [12]. Antibody titer was calculated by construction of a standard curve using the standards included in the kit. All the samples have been doubly analyzed. The intra-assay and inter-assay reproducibilities (coefficients of variation) of the assay were respectively < 5 % and < 10 %.

A normality Shapiro-Wilks test was carried out on all the tested variables, followed, when possible, by the one-way ANOVA, two-tails t-test for paired samples and Tukey's post hoc test. The degree of correlation between baseline plasma lipid levels and the reduction obtained with the treatment were estimated with Pearson's bivariate correlation test followed by linear regression. For all the tests a "p" level below 0.05 was considered as significant.

Results

Weight, body mass index, baseline glucose, uric acid and fibrinogenemia did not significantly change during the various observation periods. No side effects were re-

ported and compliance was complete. The dietary pattern did not significantly change after the stabilization period. A slight, but insignificant decrease in protein intake was observed, without doubt related to the dietician's advice. The main results of the study are reported in Table 1. From the end of the stabilization diet period to the end of the period of supplementation with the soy protein product with added β-sitosterol we observed a 16.03 ± 24.40 mg/dL, 19.64 ± 20.32 mg/dL, 8.47 ± 54.61 mg/dL, 1.69 ± 10.92 mg/dL, and 7.06 ± 16.66 mg/dL mean ± SD decrease respectively in TC (t = 3.94, 35DF, p < 0.001), LDL-C (t = 5.80, 35DF, p < 0.001), TG (t = 0.93, 35DF, p = 0.358), VLDL-C (t = 0.931, 35DF, p = 0.358) and apoB (t = 2.54, 35DF, p = 0.016) levels, associated with a 1.31 ± 8.08 and 1.03 ± 19.09 mg/dL mean increase respectively in HDL-C (t = –1.94, 35DF, p = 0.251) and apoA (t = –0.323, 35DF, p = 0.749) plasma concentrations. The dietary supplementation did not influence Lp(a) (t = –0.115, 35DF, p = 0.984) or ox-LDL Ab (t = –0.196, 35DF, p = 0.953) plasma levels which basically remained unchanged.

A statistically significant correlation was observed for LDL-C plasma levels, between the end of the stabilization diet period and the end of the dietetic supplementation period with the soy protein product with added β-sitosterols (r = –0.602, p < 0.001). A similar result was also obtained for TC (r = –0.531, p = 0.001) and apo B (r = –0.582, p < 0.001) plasma levels. The total and mean (95 %IC) LDL-C – DLDL-C fit line is reported in Fig. 1. No significant difference was found as regards the mean density and volume of LDL particles (t = 1.711, 35DF, p = 0.096).

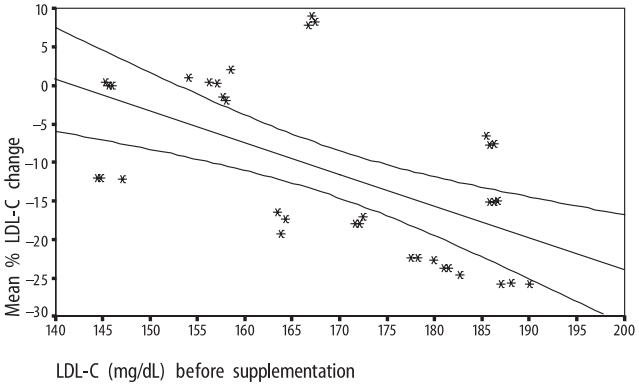

Fig. 1 Linear regression between LDL-C (low density lipoprotein cholesterol) plasma value at the end of the stabilization diet period and the absolute LDL-C variation obtained at the end of the treatment period in relation to the end of the stabilization diet (36 subjects; R Square = 0.299, p < 0.001; fit line and mean regression prediction lines with 95 % confidence intervals)

Discussion

The AHA recommends inclusion of at least four 6.25 g (25 g/day) servings of soy protein in a low fat and cholesterol diet in order to reduce the risk of heart disease [1]. Moreover, it has been estimated (but still not demonstrated) that daily introduction of 2 g of plant sterols could be expected to reduce the risk of coronary heart disease by 25 % [2]. According to such recommendations, low doses of soy protein with added β-sitosterol seem to be a practical and safe alternative for patients seeking modest reductions in LDL-C (< 15 %). Although

Table 1 Mean ± SD weight, daily dietary intake (% on total energy) and plasma lipid parameters (mg/dL) at various study stages. Percent difference from the end of stabilization diet is given as means ± SE (36 subjects; *LDL* low density lipoprotein; *HDL* high density lipoprotein; *VLDLs* very low density lipoproteins)

	Baseline	After 40 days stabilization diet	Soy protein with added β-Sitosterol	Difference from the end of the stabilization diet	After 40 days without soy supplementation
Body Weight (kg)	71.35 ± 9.05	70.12 ± 9.18	69.94 ± 8.54	–0.26 ± 0.13 %	71.91 ± 10.32
Body Mass Index (kg/m^2)	25.32 ± 3.32	25.01 ± 3.37	24.90 ± 3.14	–0.43 ± 0.14 %	24.98 ± 3.79
Daily fat dietary intake	31.18 ± 3.15	29.79 ± 3.02	29.42 ± 2.85	–1.24 ± –0.11 %	29.98 ± 2.04
Daily saturated fat dietary intake	13.13 ± 2.43	11.08 ± 1.11	10.97 ± 0.89	–0.99 ± 0.17 %	10.88 ± 2.36
Daily cholesterol intake (mg)	236.18 ± 15.37	193.74 ± 10.13	193.88 ± 9.51	+0.07 ± 0.14 %	199.54 ± 10.02
Daily protein intake	15.33 ± 1.94	16.54 ± 2.06	17.09 ± 1.85	+3.32 ± 0.55 %	16.72 ± 2.12
Total Cholesterol (mg/dL)	267.53 ± 18.21	257.55 ± 15.44	241.53 ± 20.55	–6.22 ± 3.91 %*	249.25 ± 24.95
LDL-Cholesterol (mg/dL)	165.65 ± 16.53	159.18 ± 15.38	149.54 ± 16.48	–11.61 ± 6.15 %*	154.84 ± 27.54
HDL-Cholesterol (mg/dL)	58.47 ± 16.09	55.86 ± 12.68	57.17 ± 12.53	+2.29 ± 1.11 %	58.03 ± 15.52
Triglycerides (mg/dL)	117.00 ± 48.23	162.56 ± 58.01	154.08 ± 65.22	–5.21 ± 4.97 %	186.89 ± 87.29
VLDLs (mg/dL)	23.40 ± 9.65	32.51 ± 11.60	30.82 ± 13.04	–5.19 ± 4.41 %	33.38 ± 13.46
Apolipoprotein A (mg/dL)	–	117.53 ± 18.52	118.56 ± 19.25	+0.87 ± 1.12 %	121.47 ± 14.87
Apolipoprotein B (mg/dL)	–	118.94 ± 12.11	111.81 ± 12.64	–5.93 ± 0.79 %*	112.87 ± 13.96
Lp(a) (mg/dL)	–	34.13 ± 8.04	34.69 ± 9.01	+0.38 ± 0.29 %	34.08 ± 7.54
Oxidized LDL Ab (mU/dL)	–	344.83 ± 387.09	390.33 ± 378.69	+13.08 ± 2.21 %	381.50 ± 368.27

* Significant difference in relation to stabilization diet (P < 0.05)

the use of soy alone may not allow patients with hyperlipidemia to achieve target lipid parameters, they do not pharmacologically interact with lipid lowering therapy. On the contrary, the combination of plant sterols and a statin seems to improve the cholesterol lowering effect of the drug [13].

Among the advantages of a low-dose soy bean protein formula, two are particularly significant: it does not unbalance daily energy intake in relation to proteins or modify the pattern of dietary habits in any significant manner.

In this study, we tested the antihypercholesterolemic efficacy of a new formula containing soy protein and isolated β-sitosterol in a 4:1 ratio. Good results were obtained with oral administration of only 10 g/day of this formulation: mean 6.22% ($p < 0.001$), 11.61% ($p < 0.001$), and 5.93% ($p < 0.001$) decrease in TC, LDL-C and ApoB plasma levels, respectively. A statistically insignificant, but maybe clinically relevant mean 5.21% and 5.19% in TG and VLDL plasma levels was observed also. This low dose product has the advantage that it does not unbalance daily dietary intake of proteins in relation to other nutrients, while the chosen ratio between mixed soy derived protein and β-sitosterol has the advantage of a higher solubility in both lipids and water and a better palatability [14]. These latter factors are especially important to obtain good patient compliance during long-term treatment, which is essential for the achievement of the desired clinical result. Moreover, contrary to what was observed in a previous clinical trial carried out with high doses of soy proteins [15], the tested formulation did not negatively influence Lp(a) plasma levels.

The tested dietary supplementation had no one significant effect on the ox-LDL Ab plasma level, a marker of oxidative stress. The antihypercholesterolemic effect that is proportional to the baseline cholesterolemia level is important should the tested product be included in foods like yoghurt, beverages, bread or pasta, because it could be taken by all family members without having to worry about excessive cholesterol reduction in healthy subjects. Further long-term clinical trials on a greater number of subjects are required in order to better evaluate the true therapeutic benefits achievable from the use of this formula in relation to a low fat diet and, possibly, in association with a lipid lowering drug.

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