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Ultra heat treatment destroys cholesterol-lowering effect of soy protein

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Abstract
A randomized, placebo-controlled, double-blind clinical study was performed to investigate the dose-dependent response of serum cholesterol after consuming an ultra-heat-treated milk containing a soy protein preparation. Eighty hypercholesterolemic subjects were assigned to one of four study groups receiving 12.5 or 25 g soy protein (active treatment) or casein (placebo) daily over a period of 4 weeks. The trial substances were provided as ready-made, ultra-heated milk preparations. Before and after the treatment, serum concentrations of total, low-density lipoprotein, and high-density lipoprotein cholesterol were determined. Unexpectedly, at the end of the study, low-density lipoprotein cholesterol concentrations were significantly increased compared with baseline in all study groups. The magnitude of this increase (17–19%) was similar in all active and placebo study groups. Soy protein supplements previously shown to be effective in reducing serum cholesterol had in this study no such lipid-lowering effect after ultra heat treatment.

Keywords: *Soy, protein, cholesterol, ultra heat treatment*

Introduction

Cardiovascular diseases cause approximately 40% of all deaths in industrialized countries. Control of this major public health problem includes early recognition of the related risk factors. It is widely accepted that dyslipidemia, in particular hypercholesterolemia, is one of the major risk factors for atherosclerosis and coronary heart disease (Riesen 1998). A 10% decrease of cholesterol in 35-year-old to 44-year-old men has been estimated to reduce the heart attack risk by half (Law et al. 1994). This is a figure of high clinical relevance for all affluent societies. In Germany, for instance, 60–70% of the adult population exceeds the currently recommended blood cholesterol concentrations (Forschungsverband DHP 1998; Wood et al. 1998).

Established lipid-lowering drugs, although effective, may be accompanied by serious adverse reactions (Shepherd et al. 1996). As an alternative, early dietary interventions are recommended, such as the restriction of saturated fatty acids

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(Müller-Bohn 1996), increased intake of dietary fibers (Glore et al. 1994) and of protein-rich legumes, preferably soy (Messena and Erdman 1995).

The mechanism by which soy products exert a lipid-lowering effect is as yet unknown. The possibilities under discussion include the activation of the low-density lipoprotein (LDL) receptor by bioactive small peptides or essential amino acids from soy protein (Sirtori et al. 1998; Anderson 2003; Gianazza et al. 2003)—namely the α′ subunit of β-conglycinin, an oligomeric 7S globulin, the stimulation of fecal bile acid excretion by soy fiber (Tsai et al. 1983), and the inhibition of endogenous cholesterol synthesis (Tham et al. 1998). Numerous clinical studies have demonstrated that soy protein can cause significant reductions in serum concentrations of total and LDL cholesterol and triglycerides (Anderson et al. 1995, Baum et al. 1998, Wong et al. 1998, Crouse et al. 1999, Washburn et al. 1999, Merz-Demlow et al. 2000, Teixeira et al. 2000, Hermansen et al. 2001, Puska et al. 2002, Tonstad et al. 2002). Published results, however, remain controversial, which may in part be attributed to different study designs with treatment durations ranging from 5 to 24 weeks and daily soy protein intake from 17 to 124 g (Anderson et al. 1995). Furthermore, soy products differ considerably in their composition, containing protein of varying structure and various quantities of other soy ingredients, for which clinical effects on serum lipid concentrations have been shown, such as isoflavones (Crouse et al. 1999, Washburn et al. 1999, Merz-Demlow et al. 2000, Wangen et al. 2000), cotyledon fibers (Lo et al. 1986, Brown et al. 1999) or phospholipids (Dewailley et al. 1985, Ovesen et al. 1985, Sirtori et al. 1985, Kirsten et al. 1994). A preparation combining isolated soy protein of standardized isoflavone content with soy-derived phospholipids and fiber has been proven to have superior cholesterol-lowering efficacy compared with isolated soy protein alone (Hoie et al. 2005).

A randomized, double blind, placebo-controlled study was performed among hypercholesterolemic subjects to investigate the cholesterol-lowering effect of different dosages of a soy preparation in a chocolate flavored milk beverage that was ultra heat treated.

Subjects and methods

The study enrolled 80 adults (51 women and 29 men), who met the following inclusion criteria: total serum cholesterol concentration, 200–300 mg/dl (5.2–7.8 mmol/l); age, 20–70; and written informed consent. The exclusion criteria were severe cardiovascular, gastrointestinal, hepatic, renal or endocrine diseases; hypertension ($>$160/100 mmHg); myocardial infarction or apoplexy; familiar hypercholesterolemia; diabetes mellitus type 1 and type 2 under medical treatment; obesity (body mass index $\geq$35 kg/m^2); use of lipid-lowering drugs 4 weeks prior to the study; clinically significant lactose intolerance; drug or alcohol abuse; HIV infection and clinically manifest AIDS; plans to reduce weight during the study period; pregnancy and breast-feeding; participation in a clinical trial 4 weeks prior to the study; or potential compliance problems due to insufficient knowledge of the German language. Concomitant medications, which could not be expected to notably influence study results, were permitted.

The study was performed as a four-armed, randomized, placebo-controlled, double-blind design. For an individual period of 4 weeks, participants were assigned to one of the following four groups: Active treatment 1 (AT1; receiving 1 l

chocolate-flavored milk containing 24.4 g soy protein and 30.4 g milk protein daily) or placebo 1 (P1; receiving 1 l chocolate-flavored milk containing 43.3 g milk protein daily), and active treatment 2 (AT2; receiving 0.5 l chocolate-flavored milk containing 12.2 g soy protein and 15.2 g milk protein daily) or placebo 2 (P2; receiving 0.5 l chocolate-flavored milk containing 21.7 g milk protein daily). The soy protein product (containing SuproSoy® isolated soy protein and Fibrim® soy fiber from Solae, St Louis, MN, USA, and soy phospholipids from Degussa, Germany) and placebo preparation, both produced by Contract Foods (Birmingham, UK) (for a list of ingredients see Table I), were premixed with low-fat (0.2%) chocolate-flavored milk and ultra heat treated (142°C for 4–6 sec), and were distributed at the regular examinations.

Assessments took place at baseline and after 2 and 4 weeks, and involved clinical evaluation and blood sampling to determine serum concentrations of total, LDL and high-density lipoprotein (HDL) cholesterol. To evaluate tolerance, the clinical data and safety parameters were recorded at the initial and final evaluations and the physical condition and side-effects were recorded during each assessment.

The placebo-controlled study was conducted by Phytopharm Research (Berlin, Germany). It complied with Good Clinical Practice guidelines of the European Union and was approved by the Ethics Committee of the Humboldt University Medical School (Charité), Berlin. Written informed consent was obtained from all participants prior to the study. Block randomization was carried out separately for the two dosage levels of the active and placebo preparations. Data were recorded on dBase IV® (Borland, Scottsvalley, CA, USA) by double data entry and were evaluated with SPSS® for Windows™ (SPSS Inc., Chicago, IL, USA) according to the full-analysis procedure. In addition to variance analysis and the F-test, the t-test for coupled observations was applied.

Table I. Composition of study preparations (contents per 100 g).

	Active	Placebo
Energy (kcal)	61.7	62.6
Energy (kJ)	257.9	261.7
Total carbohydrate (g)	9.15	11.18
Lactose (g)	4.12	6.21
Sucrose (g)	4.55	4.55
Fat (g)	0.48	0.49
Total protein (g)	5.47	4.33
Protein (g)		
Soy	2.44	–
Skim milk	2.83	2.83
Dried skim milk	–	1.29
Cocoa powder	0.21	0.21
Total fiber (g)	0.76	0.29
Soy fiber (g)	0.47	–
Isoflavonoids (mg)	8.28	–
Total phospholipids (mg)	102	–
Phosphatidylcholine (mg)	42	–
Sodium (g)	0.09	0.06

Results

All of the 80 subjects enrolled in the placebo-controlled study completed treatment according to the study plan. Table II summarizes basic characteristics, which along with further clinical data and lifestyle assessments appear to provide sufficiently homogeneous baseline values for all four study groups.

Serum concentrations of total, LDL and HDL cholesterol are summarized in Table III. In all groups, LDL cholesterol increased significantly during the course of the study independently of dosage or composition of the study preparations (see Figure 1 and Table III). In comparison with baseline values, LDL cholesterol increased in the AT1 group by 24.9 mg/dl (18.6%) and in the P1 group by 22.7 mg/dl (16.5%). In the AT2 group, there was an increase of 26.4 mg/dl (17.4%) and in the P2 group an increase of 31.0 mg/dl (20.7%). The last value was strongly influenced by one participant, who had an increase of 81 mg/dl. Without this patient, the difference between the initial and final examination was 28.3 mg/dl (18.7%). HDL cholesterol values did not change significantly during the study (Table III). At the final assessment, total serum cholesterol concentration, as compared with baseline, decreased by 1.0 mg/dl (0.4%) in the AT1 group and by 4.0 mg/dl (1.6%) in the P1 group; in the AT2 group, values were decreased by 7.9 mg/dl (3.0%), while a slight increase by 1.6 mg/dl (0.6%) was observed in the P2 group. However, this last value was strongly influenced by one participant who experienced an increase of 180 mg/dl; without this subject, the difference between initial and final examination in the P2 group was a decline of 7.8 mg/dl (3.0%). These results were accordingly revealing no difference between active treatment and placebo groups.

Clinical data such as body weight, heart rate, blood pressure, body temperature or hematological profiles did not change during the study, nor did these data significantly differ between study groups. All study preparations showed good tolerability. The only unwanted event experienced during the study was experienced by one subject in the placebo group who had an increase of γ-glutamyl transferase (GGT) at the final examination, which was unlikely to be related to the intake of the study preparation.

Table II. Baseline characteristics of the analyzed study population.

	Ultra-heat-treated milk (1.0 l/day with ISP)			Ultra-heat-treated milk (0.5 l/day with ISP)		
Characteristic	Active (AT1)	Placebo (P1)	*P*	Active (AT2)	Placebo (P2)	*P*
Sex (*n*)						
Male	5	8		9	7	
Female	15	12		11	13	
Age (years)			0.96			0.39
Mean±SD	51.4±12.7	51.6±9.4		54.2±8.5	56.8±10.7	
Range	30–69	31–66		37–65	36–70	
Height (cm)			0.06			0.14
Mean±SD	166.2±10.2	171.7±7.5		171.4±8.0	167.2±9.5	
Range	152–191	158–185		155–183	150–185	
Weight (kg)			0.20			0.96
Mean±SD	72.0±13.9	77.3±11.8		73.9±13.6	74.1±14.0	
Range	52–102	59–96		53–102	50–100	

ISP, isolated soy protein; SD, standard deviation.

Table III. Serum cholesterol concentrations.

	Ultra-heat-treated milk (1.0 l/day with ISP)			Ultra-heat-treated milk (0.5 l/day with ISP)		
	Active (AT1)	Placebo (P1)	*P*	Active (AT2)	Placebo (P2)	*P*
Total cholesterol (mg/dl)						
Baseline	240.5±24.5	246.5±28.7	0.48	260.4±25.3	261.3±26.3	0.91
2 weeks	235.8±24.3	245.0±38.2	0.37	251.7±32.0	254.2±31.9	0.81
4 weeks	239.5±26.8	242.5±28.2	0.73	252.5±29.8	262.9±49.8	0.43
Δ Baseline – second week	4.7±21.5	1.6±22.9		8.8±27.3	7.2±22.3	
P value	0.34	0.77		0.17	0.17	
Δ Baseline – fourth week	1.0±16.1	4.0±18.7		8.0±24.5	−1.6±46.0	
P value	0.78	0.35		0.16	0.88	
LDL cholesterol (mg/dl)						
Baseline	134.2±21.2	137.3±24.4	0.68	151.5±20.1	150.0±19.9	0.81
2 weeks	153.9±24.9	157.9±31.7	0.66	171.3±25.4	168.6±27.2	0.75
4 weeks	159.1±30.8	160.0±28.8	0.92	177.9±22.8	181.0±23.0	0.67
Δ Baseline – second week	−19.7±13.9	−20.6±15.9		−19.8±17.8	−18.6±15.5	
P value	<0.001	<0.001		<0.001	<0.001	
Δ Baseline – fourth week	−24.9±13.3	−22.8±13.4		−26.4±16.0	−31.0±15.8	
P value	<0.001	<0.001		<0.001	<0.001	
HDL cholesterol (mg/dl)						
Baseline	60.7±15.6	65.8±20.2	0.38	58.6±15.8	54.1±9.4	0.28
2 weeks	60.5±16.5	64.9±21.5	0.47	60.1±16.7	53.9±9.5	0.16
4 weeks	60.7±17.3	63.7±18.5	0.59	59.6±16.1	52.9±9.2	0.12
Δ Baseline – second week	0.2±8.0	0.9±6.6		−1.6±5.6	0.2±5.5	
P value	0.91	0.54		0.23	0.90	
Δ Baseline – fourth week	0.5±5.4	2.1±4.2		−1.0±4.4	1.2±7.6	
P value	0.97	0.04		0.32	0.49	

ISP, isolated soy protein.

Discussion

A meta-analysis of 38 studies on the effect of soy protein on serum lipids (Anderson et al. 1995) gave an average reduction of total and LDL cholesterol concentrations of approximately 9.3% and 12.9%, respectively, without detailed consideration for the amount of soy protein (mean 47 g/day) and study duration. However, this review also showed that a reduction of LDL cholesterol could not be achieved in every study and furthermore that a clear dosage–effect ratio cannot be unequivocally established. Neither could later trials (Teixeira et al. 2000, Tonstad et al. 2002) clearly ascertain such a relationship, which may depend on the study design and on the preparation; for example, the amount and quality of soy protein or isoflavones (Crouse et al. 1999). For example, in one study of a 3-week duration, a significant cholesterol reduction was only observed with daily dosages of 40 g soy protein or more (Teixeira et al. 2000).

The changes of cholesterol parameters found in this study do not appear to be related to any effects of soy protein since they do not differ significantly between the respective study groups. Unexpectedly, LDL cholesterol concentrations increased significantly compared with baseline both in the active and placebo groups. Similar findings have been observed with regards to trans fatty acids, which significantly

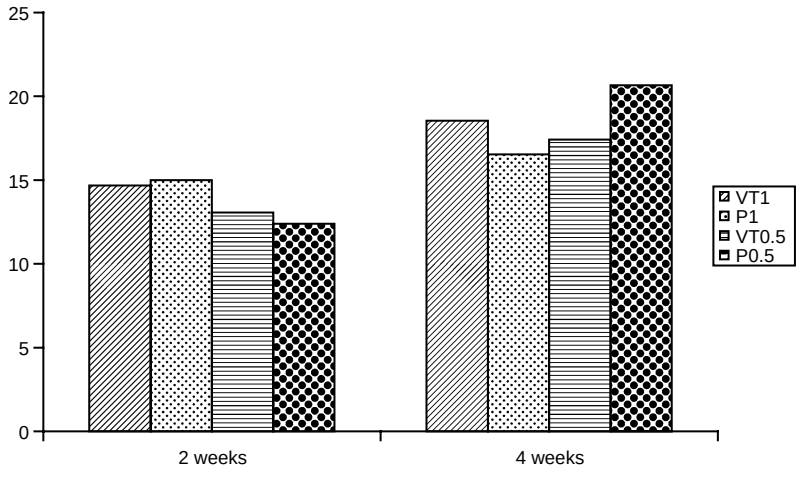

Figure 1. Relative changes in LDL cholesterol concentrations (% of baseline).

increase LDL cholesterol, and thereby increase the risk for heart disease (Mauger et al. 2003).

As we could not detect any reasons for this outcome in our study design or population, we assume that the technological process might be responsible for the lack of efficacy observed. The active treatment preparation significantly reduced serum cholesterol concentrations in previous clinical trials. In this study, however, it had for the first time been applied in pre-mixed chocolate-flavored milk drinks manufactured using ultra heat treatment processing. The main components in the active treatment preparation are soy protein and isoflavones. Soy protein usually contains isoflavones in the form of their β-glucosides, which may be malonylated, acetylated or unesterified in the 6″-OH position. It has been shown that treatment of soy flour or protein concentrate with high temperatures of up to 190°C does not alter the total isoflavone content (Coward et al. 1998, Mahungu et al. 1999). It does, however, decrease the portion of the 6″-*O*-malonyl derivates due to decarboxylation, which may have an effect on the bioavailability. On the other hand, it is known that heating denatures soy proteins: the globular structure opens up, and the resulting long-chain proteins will form insoluble aggregates (Arrese et al. 1991; Petrucelli and Anon 1995). A high degree of aggregation consequently reduces the amount of potentially bioactive peptides that can be absorbed through the intestinal wall. A plausible explanation for the loss of lipid-lowering efficacy might thus be heat-induced damage that changes those constituents of soy protein that are responsible for serum cholesterol reduction (i.e. soy peptides from the 7S globulins). Based on the findings of this study, we have developed a new non-denatured isolated soy protein that has proven twice as effective to lower LDL and total cholesterol compared with the best hitherto commercially available isolated soy protein.

The results of this placebo-controlled study indicate that ultra heat treatment of soy protein in milk may destroy its cholesterol-lowering activity. Further studies are needed to show whether health claims should be recommended for beverages containing ultra-heat-treated soy protein.

Epilogue

Without ultra heat treatment of the soy protein preparation, a significant lowering of LDL and total cholesterol was observed

The unexpected result of the placebo-controlled study gave rise to a non-controlled follow-up study using the same preparation without preceding heat treatment. Over a period of 4 weeks, 20 hypercholesterolemic subjects (seven women and 13 men), who met the above listed inclusion and exclusion criteria, received 25.2 g soy protein daily from a nutritional supplement preparation, which they stirred into 1 l cold low-fat milk (0.3%), that was consumed in two portions with morning and evening meals. One subject was excluded due to non-compliance, and three subjects terminated the study by their own choice after the second examination (after 2 weeks of treatment). In the course of the 4-week treatment period, total cholesterol decreased by 4.3% (10.8 ± 18.3 mg/dl) and LDL cholesterol by 9.9% (16.3 ± 16.9 mg/dl). Both reductions were significant compared with baseline ($P = 0.020$ and $P = 0.001$). Especially during the first half of the study, reductions were observed (4.6% for total and 17.9% for LDL cholesterol). HDL cholesterol was also decreased (by 3.3%) but this change was not statistically significant ($P = 0.13$). It was thereby ascertained that the soy protein preparation does possess cholesterol-lowering properties if it is not subjected to ultra heat treatment processing.

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