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# Antioxidant and Hypocholesterolemic Effects of Soy Foods and Cardiovascular Disease

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## 1. Introduction

Cardiovascular disease (CVD) is widespread in the modern world and represents one of the main causes of mortality (Levi et al., 2009). Increased blood cholesterol content (Hozawa, 2011) and oxidative modification of low density lipoproteins (Vogiatzi et al., 2009) represent risk factors for the development of CVD. The right nutrition is considered effective in the prevention and treatment of CVD (Rudkowska & Jones, 2007). The results of epidemiological, clinical and experimental studies show that soy foods decrease blood cholesterol in individuals with hyperlipidemia, as well as mortality rates from CVD both in Asians and Caucasians (Anderson et al., 1995; Borodin et al., 2009; Messina & Messina, 2003). Soy beans contain antioxidants that exert an antioxidant effect when people consume soy foods (Bertipaglia de Santana et al., 2008). This effect may be also beneficial for CVD patients. This review focuses on the discussion of modern data on antioxidant and hypocholesterolemic effects of soy foods, as well as on the potential role of soy foods in the prevention and treatment of CVD in Russia.

## 2. Antioxidant effect of soy foods

The antioxidants of soy beans are represented by isoflavones, tocopherols, ascorbic acid and some other compounds (Barnes, 2010; Borodin et al, 2001). When soy beans are processed into different foods the particular antioxidants content of the produced foods may change depending on the processing procedure (Xu et al., 2010) and storage conditions (Rau De Almeida Callou, 2010) and differ from the initial antioxidant content in the soy beans (Anderson & Wolf, 1995). However, antioxidants of soy beans are also present in soy foods. So, the consumption of soy foods is followed by the antioxidant effect (Bertipaglia de Santana et al., 2008; Borodin et al., 2001). Among the antioxidants of soy beans mainly it is

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isoflavones that had attracted the attention of researches in explaining the health effects of consumption of soy foods (Barnes, 2010; Messina, 2010). Much less attention has been paid to the tocopherols and other soy antioxidants (Borodin et al., 2001; Mikoluc et al., 2009).

## 2.1 Antioxidants of soy beans and some soy foods

Table 1 shows the results of our measurements of some antioxidants content in the species of soy beans, planted in the Far East of Russia, and some locally produced soy foods.

Soy beans and soy foods	Isoflavones*		Tocopherols		β-carotenes μg/g, μg/mL	Ascorbic acid μg/g, μg/mL
	μg/g, μg/mL	mmoles/mole of triglycerides	μg/g, μg/mL	mmoles/mole of triglycerides		
Soy beans	580-3800	10-70	380	3.80	19	70
Full fat soy flour	580-3800	10-70	370	3.37	13	97
Lipoxygenase soy flour	580-3800	10-70	310	2.92	15	101
Semi-fat soy flour	370-2500	10-70	265	3.99	10	110
Soy milk	30-175	9-50	21	3.47	Trace	15
Tofu	-	-	124	4.17	Trace	51.7

Table 1. Isoflavone, tocoferol, β-carotene and ascorbic acid content of soy beans and some soy foods. Tocoferol and β-carotene content was measured in the lipid extracts from soy beans and soy foods by colorimetric methods and ascorbic acid content was measured by titration of acidic extracts with 2,6-dichlorphenolindofenol. \* - data from Anderson R. & Wolf W. (Anderson & Wolf, 1995).

The major antioxidants in soy beans are isoflavones and tocopherols. Total isoflavone content of soy beans (580-3800 μg/g of raw weight or 10-70 mmoles/mole of triglycerides) is 1.5-10 times higher than such of tocopherol content (380 μg/g of raw weight or 3.8 mmoles/mole of triglycerides). In different types of soy flour the content of these antioxidants is nearly the same as in soy beans. Isoflavone and tocopherol content of soy milk and tofu is considerably less if expressed in μg/g or μg/mL of product. However, it is very similar to that of soy beans if expressed in mmoles/mole of triglycerides. Soy bean oil contains 3-4 molecules of tocoferols per 1000 molecules of triglycerides. Despite the fact that isoflavones are polar compounds, which are not soluble in oils, the formal calculation of the total isoflavone content in soy beans and soy foods as mmoles/mole of triglycerides shows that 10-70 molecules of isoflavones correspond to 1000 molecules of triglycerides. β-carotene content of soy beans and soy flour is 20-30 times lower than tocopherol content and 200-300 times lower than isoflavone content. Ascorbic acid content in raw soy beans (70 μg/g) and soy foods (15-110 μg/g) is low and soy foods do not play any substantial role as a source of this vitamin for humans.

Due to the high amounts of antioxidants soy beans and soy foods are resistant to oxidative modification. In the analyzed samples of soy beans and soy foods the primary products of lipid peroxidation, namely, dien conjugates and lipid hydroperoxides have been determined but their content was rather low - from 2 to 7 conjugated double bonds and less than 1 hydroperoxide group per 1000 molecules of triglycerides (table 2).

Soy beans and soy foods	Dien conjugates		Lipid hydroperoxides	
	nmoles/g, nmoles/mL	mmoles/mole of triglycerides	nmoles/g, nmoles/mL	mmoles/mole of triglycerides
Soy beans	1690	7.28	160	0.69
Full fat soy flour	940	3.68	130	0.51
Lipoxygenase soy flour	870	3.53	97	0.39
Partly defatted soy flour	690	4.46	110	0.71
Soy milk	26	1.77	14	0.95
Tofu	122	1.77	34	0.97

Table 2. Dien conjugate and lipid hydroperoxide content of soy beans and some soy foods. Dien conjugate and lipid hydroperoxide content was measured in the lipid extracts from soy beans and soy foods by the absorbance at 233 nm and by the ability to oxidize  $Fe^{2+}$  ions, respectively (Borodin et al, 2001).

## 2.2 Antioxidant effect of soy milk in vitro

It is easy to demonstrate the antioxidant effect of soy milk in vitro by its ability to inhibit the process of lipid peroxidation in a system containing fast oxidizing substrate, for example in liver microsomes (table 3). The rate of lipid peroxidation in liver microsomes consists of  $2.52 \pm 0.31$  nmoles of malonic dialdehyde (MDA)  $min^{-1} mg^{-1}$  of phospholipids in the presence

Type of milk	Ascorbate-dependent lipid peroxidation		NADPH-dependent lipid peroxidation
	Oxidizeability (nmoles of MDA $min^{-1}$ $mL^{-1}$ )	AOA (%)	Oxidizeability (nmoles of MDA $min^{-1}$ $mL^{-1}$ )
Liver microsomes*	$2.52 \pm 0.31$	-	$1.5 \pm 0.18$
Soy milk	$0.48 \pm 0.18$	$57.3 \pm 9.3$	$2.80 \pm 0.09$
Caw milk	$0.28 \pm 0.17$	$10.9 \pm 2.5$ ( $p < 0.05$ )**	$2.63 \pm 0.17$

Table 3. Oxidizeability and antioxidant activity of soy and cow milk at lipid peroxidation in liver microsomes. 1 mL of the incubation mixture contained 0.8 mM ascorbate (or 1mM NADPH); 0.2 mM sodium pyrophosphate; 50 mM tris-HCl buffer, pH 7.4.; 1 mg of microsome protein and 0.1 mL of milk. The time of incubation was 5 min for ascorbate-dependent lipid peroxidation and 20 min for NADPH-dependent lipid peroxidation. Lipid peroxidation was initiated by the addition of  $Fe^{2+}$  ions in final concentration  $2 \mu M$ . The reaction was stopped by the addition of 30% trichloroacetic acid in final concentration 1.5 mM. MDA - malonic dialdehyde. AOA - antioxidant activity. AOA was calculated by the ability of soy and caw milk to inhibit MDA accumulation in the incubation medium at the initiation of lipid peroxidation in liver microsomes (Borodin et al., 2001). MDA content in the incubation medium was determined by the color reaction with thiobarbituric acid (TBA-test) (Borodin et al., 1993). Values are means  $\pm$  SEM. \* - oxidizeability of microsomes was expressed as nmoles of MDA  $min^{-1} mg^{-1}$  of microsome phospholipid; \*\* - p values corresponds to the differences between the AOA of soy and cow milk.

of ascorbic acid and  $\text{Fe}^{2+}$  ions being significantly higher than oxidizability of soy- and cow milk ( $0.48 \pm 0.18$  and  $0.28 \pm 0.17$  nmoles of MDA  $\text{min}^{-1} \text{mL}^{-1}$ , respectively). In contrast to this in the presence of NADPH and  $\text{Fe}^{2+}$  ions soy milk and cow milk oxidize faster ( $2.80 \pm 0.09$  and  $2.63 \pm 0.17$  nmoles of MDA  $\text{min}^{-1} \text{mL}^{-1}$ ) than liver microsomes ( $1.5 \pm 0.18$  nmoles of MDA  $\text{min}^{-1} \text{mg}^{-1}$  of phospholipids). The difference between the oxidizability of soy milk and cow milk in the presence of ascorbic acid and  $\text{Fe}^{2+}$  ions is not statistically significant. In the presence of NADPH and  $\text{Fe}^{2+}$  ions both types of milk oxidize at the same rate. It is interesting to note that in the presence of NADPH soy milk oxidize 5-times faster and cow milk 10-times faster than in the presence of ascorbate. The antioxidant effect of soy milk on ascorbate-dependent lipid peroxidation in liver microsomes is 5-fold higher than the effect of cow milk. To calculate the antioxidant effect of soy and cow milk on NADPH-dependent lipid peroxidation in liver microsomes seems impossible because the rate of the oxidation of microsomes is slower than the rate of oxidation of milk. The results obtained testify to the high antioxidant properties of soy milk, probably due to high isoflavone and tocopherol content. Because of this soy milk oxidizes rather slowly despite the high content of polyunsaturated fatty acids in its lipids (table 4).

### 2.3 Fatty acid composition of soy- and cow-milk

The lipids of cow- and soy milks are characterized by the following marked differences in fatty acid composition, which may be important for their dietary value for CVD patients (table 4). 1) total content of saturated fatty acid in cow milk (63.8%) is 3,7 times higher than in soy milk (17.8%); 2) total monounsaturated fatty acids content in cow milk is also higher and consists of 30,4% versus 21,5% in soy milk; 3) total content of polyunsaturated fatty acids (PUFA) in cow milk (61.2%) is 10,6 times higher than in the cow milk (5,7%); 4) the content of omega-3 PUFA, which is considered favorable for the prophylaxis of CVD (Saravanan et al., 2010), is 4.2 times higher in soy milk than in cow milk (8.53% versus 2.03%, respectively); 5) the fatty acid composition of the lipids in soy milk is less diversified than in cow milk. Short chain fatty acids ( $C < 10$ ) are not represented in the lipids of soy milk and represent minor components of cow milk; 6) the principal fatty acids of soy milk are polyunsaturated linoleic acid (18:2n-6) (52%), monounsaturated oleic acid (18:1n-9) (18%) and saturated palmitic acid (16:0) (12%) while the main fatty acids of cow milk are saturated acids - palmitate (16:0) (25%) and stearate (18:0) (14%) and monounsaturated oleic acid (18:1n-9) (21%); 7) the specific minor fatty acids of soy milk are 18:3n3, 10:1n-9 and 22:0 and cow milk 4:0, 8:0, 10:0, 12:1, 14:1, 15:0-iso, 15:0-antyiso, 16:0-iso, 16:1n-9, 16:2, 17:0-iso, 17:1, 18:1-trans (table 4). High content of PUFA in soy milk and especially of omega-3 family PUFA, represents an important dietary advantage of this milk compared to cow milk for the prevention and treatment of CVD. The striking feature of soy milk in comparison with cow milk is lower triglyceride and higher tocopherol and phospholipid content. As a result, the ratio phospholipids/triglycerides in soy milk is 3 times higher than in cow milk (Borodin et al., 2001), which represents another advantage of soy milk for CVD patients.

### 2.4 Antioxidant effect of consumption of soy milk in vivo

The results presented above show that soy milk contains complex of antioxidants and reveals antioxidant effect both in vitro and in vivo. The consumption of soy milk is followed by an increase in the  $\alpha$ -tocopherol content in the blood (Borodin et al., 2001, 2003). It was therefore of interest to determine to what extent the antioxidant effect of soy milk may be attributed to  $\alpha$ -tocopherol? To answer this question we performed the following study.

Fatty acids	Cow-milk	Fatty acids	Soy-milk
4:0	3.28		
6:0	2.02		
8:0	0.48		
10:0	2.22		
12:0	2.63	12:0	0.11
12:1	0.10	14:0	0.22
14:0-iso	0.19	15:0	0.06
14:0	9.24	16:0	11.80
14:1	0.69	16:1n-7	1.92
15:0-iso	0.38	17:0	0.11
15:0-antiiso	0.68	18:0	4.34
15:0	1.20	18:1n-9	18.05
16:0-iso	0.33	18:1n-7	1.32
16:0	25.37	18:2n-6	51.88
16:1n-9	1.21	18:2n-4	0.82
16:1n-7	0.40	18:3n-6	0.73
17:0-iso	0.56	18:3n-3	7.80
16:2	0.52	20:0	0.32
17:0	0.65	20:1n-9	0.20
17:1	0.28	22:0	0.32
18:0	14.18		
18:1n-9	21.14		
18:1n-7	4.51		
18:1-trans	2.07		
18:2n-6	2.60		
18:2n-4	0.60		
19:0	0.23		
18:3n-3	1.05		
18:4n-3	0.98		
20:0	0.21		

Table 4. Fatty acid composition of lipids of soy- and cow-milk (% from total fatty acids). Fatty acid composition of soy- and cow-milk was measured by the gas-liquid chromatography of methyl esters of fatty acids.

26 healthy young volunteers aged 17-19 years (16 females and 10 males) were recruited on a voluntary basis. The participants were informed in detail about the purpose, advantages and disadvantages of the study, and their rights and duties concerning their lifestyle. Informed consent from all participants was obtained in writing. The protocol was approved by the Amur State Medical Academy Ethics Committee according to the Helsinki Declaration on human studies. The subjects were assigned to two groups with similar age and gender. Participants in the first group received one glass of soy milk daily for 2 weeks. Participants in the control group received 5 mg of tocopherol acetate (pharmaceutical preparation of vitamin E) daily in the form of an oil solution. Exactly that amount of  $\alpha$ -tocopherol is contained in one glass of soy-milk. The subjects were instructed to consume similar amounts of food from day to day and do not consume any vitamins or drugs within the study period. At the beginning and at the end of the study the samples of blood were taken and then the biochemical indices reflecting the state of lipid peroxidation were measured in blood serum. Results are presented in the table 5.

Soy milk was well accepted by all subjects and there were no any complaints. At the beginning there were no significant differences in the values of the biochemical indices between the groups. At the end of the study the increase in the  $\alpha$ -tocopherol content in the control group from  $9.2 \pm 0.71$  to  $10.8 \pm 0.74$  mg/L was not significant ( $p > 0.1$ ). The 30% increase in the  $\alpha$ -tocopherol content in the group receiving soy milk from  $7.8 \pm 0.44$  mg/L at the beginning up to  $10.6 \pm 0.77$  mg/L was statistically significant ( $p < 0.005$ ). The increase in  $\alpha$ -tocopherol content in serum was characteristic for 11 participants of the group receiving soy-milk and only for 7 participants of the group receiving tocopherol acetate. Consumption of soy milk or tocopherol acetate for 2 weeks was followed by a 2-fold or 1,7-fold decrease of serum lipid hydroperoxide content, respectively ( $p < 0.05$ ). However, the decrease of the dien conjugate content from  $31.2 \pm 5.0$   $\mu$ moles/L to  $22.5 \pm 2.1$   $\mu$ moles/L was characteristic only for the experimental group but the changes were statistically insignificant ( $p > 0.1$ ). The consumption of soy milk or tocopherol acetate does not influence the content of TBA-reactive substances and oxidizability of serum. Thus, the consumption of both soy milk and tocopherol acetate for 2 weeks by young healthy people resulted in an antioxidant effect, manifested by a decrease in the lipid hydroperoxide content and an increase in the  $\alpha$ -tocopherol content in blood serum, but the strength of the effect was higher in the group of persons receiving soy milk. The stronger antioxidant effect of soy milk was correlated with higher and statistically significant increase in the  $\alpha$ -tocopherol content in the serum of the participants. The results appear strange because both groups of participants received the same amounts of tocoferol - 5 mg daily. The only difference was the form of tocopherol. In soy beans and soy foods tocopherols are present in a free form, while the pharmaceutical preparation of tocopherol (vitamin E) is tocopherol acetate. In contrast to free tocopherols, tocopherol esters can not express an antioxidant effect because phenol hydroxyl, which is important for this effect, is blocked by fatty acid. This may be the reason for the weaker antioxidant effect of tocopherol acetate. The colorimetric method of determination of tocoferols is based on their ability to reduce  $Fe^{3+}$  ions. Tocopherol acetate is not able to reduce  $Fe^{3+}$  ions and therefore may not be determined by this method. To exert an antioxidant effect in humans and to be determined by the colorimetric method tocopherol acetate would have to be first hydrolyzed by esterase. The other explanation for this finding would be worse intestinal uptake of tocoferol acetate when it is administered in the form of oil solution.

Indices	Groups studied			
	Treated with tocopherol acetate (n=13)		Treated with soy milk (n=13)	
	Before the treatment (1)	After the treatment (2)	Before the treatment (3)	After the treatment (4)
$\alpha$ -Tocopherol (mg/L)	9.2±0.71	10.8±0.74 $p_{1,2}>0.1$	7.8.±0.41 $p_{1-3}>0.1$	10.6±0.77 $p_{3,4}<0.005$ $p_{2-4}>0.1$
Dien conjugates ( $\mu$ moles/L)	23.3±1.42	20.9±2.58 $p_{1,2}>0.1$	31.2±5.0 $p_{1-3}>0.1$	22.5±2.09 $p_{3,4}>0.1$ $p_{2-4}>0.1$
Lipid hydroperoxides ( $\mu$ moles /L)	5.82±1.09	3.10±0.51 $p_{1,2}<0.05$	7.47±1.51 $p_{1-3}>0.1$	3.73±0.80 $p_{3,4}<0.05$ $p_{2-4}>0.1$
TBA-reactive substances ( $\mu$ moles/L)	5.62±0.86	5.96±0.71 $p_{1,2}>0.1$	5.89±0.71 $p_{1-3}>0.1$	6.63±0.74 $p_{3,4}>0.1$ $p_{2-4}>0.1$
Oxidizeability of serum (nmoles of MDA min <sup>-1</sup> mL <sup>-1</sup> )	1.17±0.037	1.22±0.042 $p_{1,2}>0.1$	1.20±0.047 $p_{1-3}>0.1$	1.26±0.056 $p_{3,4}>0.1$ $p_{2-4}>0.1$

Table 5. The influence of soy milk and tocopherol acetate on the content of  $\alpha$ -tocopherol and lipid peroxidation products and oxidizeability of serum of young healthy persons. The methods of the measurements are indicated above in the legend to the tables 1-3. Values are means  $\pm$  SEM. p values correspond to the differences between the values of the index in the indicated groups of persons (t-test for independent samples).

### 3. Hypocholesterolemic effects of soy foods

People, consuming soy foods, have lower blood cholesterol (Anderson et al., 1995; Devell et al., 2006; Zhan & Ho, 2005) and mortality rates from cardiovascular diseases (CVD) (Beaglehole, 1990; Messina & Messina, 2003). It was shown both in Asians (Ho et al. 2000; Nagata et al., 1998) and Westerners (Rosell et al., 2004; Teixeira et al., 2000; Tonstad et al., 2002). Both soy protein and combination of soy protein with isoflavones are effective in reducing blood cholesterol (Dewell et al., 2006). In 1999 the Food and Drug Administration of the USA recommend daily consumption of 25 g of soy protein to control cholesterol content of blood (Food and Drug Administration, 1999). However, consumption of 25 g of soy protein a day may be not enough to low blood cholesterol level and recent meta-analysis showed that favorable results were observed only in the studies with high amounts of soybean protein (up to 60 g a day) (Reynolds et al., 2006) and there were no effects when the participants consumed 25 g of soy protein isolate (SPI) daily for 6 weeks or less (West et al., 2006). These results led some AHA experts to question the effectiveness of soy protein in decreasing blood cholesterol (Sacks et al., 2006). From above it is possible to conclude that that the daily dose of soy protein should be at least 30 g and the duration of study should be more than 6 weeks to achieve favorable changes in lipid concentrations in blood. For longer studies, the problem of acceptability of test foods is very important. If the taste or smell of

foods was not acceptable, participants often failed to follow the study program. It is very common in interventional studies with SPI that a significant proportion of participants do not finish the study. The poor acceptability of SPI to the subjects could have been the reason for the relatively short periods of the previous studies with SPI. The problem of acceptability of the test foods is particularly important for people who are not familiar with soy foods, for example for Russians.

### 3.1 Test foods with soy- and milk protein for interventional studies

Test foods for interventional studies with soy protein should meet both scientific demands and consumer's choice, but it is rather difficult to meet both requirements because they are contradictory. Two types of proteins are usually used in such studies: SPI for the experimental group of participants and milk protein casein for the control group. For scientific reasons, it is better to use pure proteins probably in the form of a drink because other ingredients, such as fats and carbohydrates (especially in high amounts) may substantially increase the energy content of the foods and influence the blood cholesterol level. However, water solubility of SPI and casein is low and drinks with these proteins have a rather bad taste and smell so they may not be consumed by the participants over a long period. Most previous studies did not include a sufficient description of the formulas for the test foods enriched with SPI and casein. The authors simply wrote: "The test proteins were incorporated into a variety of baked products and ready-to-mix beverages" (Teixeira et al., 2000).

We felt it was impossible to prepare drinks with SPI and casein with relatively good taste and smell. To investigate the effect of SPI on blood cholesterol in Russian adults with moderate hyperlipidemia we developed SPI-enriched cookies in cooperation with food specialists (composition per 1 kg of cookies: wheat flour - 333g, SPI - 333g, margarine - 183g, sorbitol - 233g, egg - 33g, salt- 6,7g, baking soda - 3,3g, ammonia carbonate - 1,7g, energy value - 340 kkal in 100g). Thus, the protein content of a cookie was high while the energy content was rather low (340 kcal/100g) due to the use of sorbitol instead of sucrose. The daily total amount of cookies made up 30g in terms of protein content and was divided into 2 or 3 servings throughout the day. SPI (FUJIPRO) was obtained from JILIN FUJI PROTEIN CO., LTD. China. Preparation of cookies with casein was difficult because of its low solubility in water and formation of glue-like structures. So, we used skimmed curd (SMP - skimmed curd milk protein, protein content 18%, fat - 0.6% and carbohydrates - 1.5%) as a source of milk protein for the control group instead of casein powder. The composition of SMP-enriched cookies was identical to the composition of SPI-enriched cookies with the exception of test protein. Skimmed curd was purchased from an authorized local provider. Picture 1 shows images of SPI and SMP-cookies.

### 3.2 The influence of the two-month consumption of 30 g a day of SPI or SMP on blood lipids in Russians with moderate hyperlipidemia\*

\*The results of this study had been published previously in the *J. Nutr. Sci. Vitaminol.* (Borodin et al, 2009).

The aim of this study was to investigate the ability of SPI to decrease blood lipids in Russians, whose dietary habits and life-styles predispose them to low longevity, as a result of a high mortality rate from CVDs (Kharchenko et al., 1997). A cardiologist had elected 14 male and 39 female Russians aged 32-67 years with elevated blood lipids and overweight on a voluntary basis and suggested them to become participants of the study. They were informed in detail about the purpose, the advantages and disadvantages of the study, and their rights and duties concerning their lifestyle. We instructed the subjects to follow their usual life and nutrition

styles, to minimize differences in energy intake from day to day, to maintain their body weight, and to avoid the use of lipid-lowering drugs throughout the study. The physical characteristics, nutrient intake, and physical activities of subjects were documented. The blood samples were taken after a lead-in period of 2-3 weeks. Samples of serum were frozen and analyses of lipids, GPT and GOT were performed. From the total number of subjects thirty (9 males and 21 females) aged 32 - 64 years were selected on the basis of ability to follow the protocol. The inclusion criteria were overweight (BMI 25-34 kg/m<sup>2</sup>), fasting serum total cholesterol 240-330 mg/dL, non-HDL-cholesterol 150-280 mg/dL, HDL-cholesterol 40-70 mg/dL, and triglycerides 100-280 mg/dL. The exclusion criteria were the presence of endocrine, liver, renal and gastrointestinal diseases. The protocol was approved by the Amur State Medical Academy Ethics Committee in accordance with the Helsinki Declaration on human studies. Informed consent from all participants was obtained in writing.



Picture 1. SPI enriched cookies (left) and SMP-enriched cookies (right).

The 30 subjects with hyperlipidemia were randomly assigned to two groups, consisting of 15 persons each. These groups of subjects received either SPI cookies or SMP cookies for 2 months. After a month washout period, the subjects received the opposite test food for another 2 months. So, we used a crossover design in this study, which has apparent advantages for studies with small groups of persons. Twenty-eight participants (19 females and 9 males) could complete the trial. Fasting blood samples were drawn before and after one month and two months of the dietary treatments. Serum samples were frozen and analyzed for lipids, total protein and glucose concentrations, GPT and GOT activities by the conventional laboratory diagnostic methods. A nutrition survey was done every month, including the wash-out period, for 3 non-consecutive days (2 week days and 1 weekend day) by the 24-hour recall method.

The baseline characteristics of the subjects are shown in the table 6.

Subjects were characterized by overweight with average BMI  $29.0 \pm 3.87$ . There were some differences in energy intake and physical activity between male and females participants. The values of serum total cholesterol, non-HDL-cholesterol, HDL-cholesterol, and triglycerides were typical for moderate hyperlipidemia. Both the SPI cookies and SMP cookies were well accepted by the majority of the subjects and twenty-eight subjects successfully completed the trial.

Index	Males	Females
Number of participants	9	21
<b>Physiological indices</b>		
Age (years)	51±2	50±2
Weight (kg)	87.2±3.8	77.6±2.4
Height (cm)	173±2	164±1
BMI	29.6±1.2	28.7±0.9
Energy consumption (kcal per day)	2022±218	1409±93
Nutrients intake (g per day):		
Proteins	107,0±6,7	78,9±5,9
Fats	80,0±7,4	52,5±2,9
Carbohydrates	272,0±20,0	152,7±8,9
Steps per day	9397±1373	8569±642
<b>Biochemical indices</b>		
Total Cholesterol (mg/dL)	301±26	247±7
HDL-Cholesterol (mg/dL)	54±3	63±4
Non HDL-Cholesterol (mg/dL)	247±25	214±9
Triglycerides (mg/dL)	255±33	183±30
Glucose (mg/dL)	80±9	94±8
Total protein (g/L)	78±2	77±1
GOT (U/L)	20±1	20±2
GPT (U/L)	16±2	18±3

Table 6. Characteristics of the participants at baseline (from Borodin et al., 2009). Values are means ± SEM except the number of participants.

The participants consumed similar amounts of food from day to day within the study period. There were no significant differences in BMI, energy, protein, fat and carbohydrate intakes, and physical activity throughout the trial (table 7).

The changes in serum lipid, glucose and total protein concentrations and GPT and GOT activities before, within and after the consumption of the test foods are shown in the table 8. In agreement with the results of the previous studies (Reynolds et al., 2006), in our study one-month SPI consumption was not enough to decrease blood lipids and only triglycerides in blood serum decreased by 10%, while serum cholesterol did not change. In contrast to this, the consumption of SPI for 2 month was followed by the significant changes in serum

lipids, namely, by the reduction of total-cholesterol by 17 mg/dL, of non-HDL-cholesterol by 22 mg/dL, and of triglycerides by 31 mg/dL, and by the increase of HDL-cholesterol by 5,1 mg/dL (table 8). The observed effect of consumption of 30 g a day of SPI should be considered as strong, bearing in mind the results of the recent meta-analysis of the effect of soy protein supplementation on serum lipids, which shows that the overall pooled net effect of soy protein supplementation on serum lipids was -5.26 mg/dL for total-cholesterol, -4.25 mg/dL for LDL cholesterol, 0.77 mg/dL for HDL cholesterol and -6.26 mg/dL for triglycerides (Reynolds et al., 2006). The important results of our study are the clear increase in HDL-cholesterol concentration and clear decrease in non-HDL concentration (table 8). In previous studies, even those with a high SPI administration, only a few studies revealed significant changes in the HDL-cholesterol concentration (Sacks et al., 2006). The established ability of SPI to decrease serum triglycerides and glucose indicates that soy foods may be useful in the prophylaxis and treatment of metabolic syndrome. The consumption of the same amount of SMP did not induce any changes in serum lipids and glucose concentrations.

Indices	Groups	Initial Values	After 1 months	After 2 months
BMI	SPI	28.7±0.7	28.5±0.7	28.8±0.7
	SMP	29.2±0.7	29.0±0.7	28.9±0.7
Energy intake (kcal/day)	SPI	1713±110	1644±115	1670±121
	SMP	1574±92	1612±97	1555±138
Nutrients intake (g/day):				
Protein	SPI	86.9±4,8	89.9±4.8	88.1±4.6
	SMP	82.4±3.0	88.4±3.0	81.7±3.5
Fat	SPI	62.1±3.3	63.1±3.3	67.4±3.8
	SMP	56.6±2.1	58.6±2.1	57.0±3.0
Carbohydrate	SPI	195±7	190±10	182±12
	SMP	185±9	182±9	172±10
Activity* (steps/day)	SPI	8757±955	8629±845	8710±640
	SMP	8910±805	8727±910	9157±702

Table 7. BMI, energy and nutrient intakes and physical activity of the participants at baseline and after 1 and 2 months of the dietary treatments (from Borodin et al., 2009).

\* - Physical activity of the subjects was monitored by the measurement of the number of steps per day with a help of pedometer (Omron HJ-005-E, China). Values are means ± SEM. Total number of participants in each pooled group is 28.

Indices	Groups	Initial Values	After 1 month	After 2 months
Total Cholesterol (mg/dl)	SPI	280±7	281±10	263±9**
	SMP	277±9	282±10	272±9
HDL-Cholesterol (mg/dL)	SPI	57.4±2.5	60.2 ±2.6	62.5±2.9***
	SMP	59.3±3.3	60.1±2.4	56.6±2.6
Non HDL-Cholesterol (mg/dL)	SPI	223±7	221±10	201±8.8***
	SMP	219±10	222±10	215±9
Triglycerides (mg/dL)	SPI	204±23	183.3±2*	173±16*
	SMP	192±17	193±20	201±17
Glucose (mg/dL)	SPI	85.8±4.7	83.4±3.0	79.0±3*
	SMP	87.8±5.5	86.1±4.8	88.6±3.6
Total protein (g/L)	SPI	78.0±1.0	78.3±0.8	78.9±0.8
	SMP	76.2±0.8	78.0±0.7	77.6±1.1
GOT (U/L)	SPI	22.1±2.4	20.8±1.7	22.2±2.1
	SMP	17.2±1.4	20.7±1.4	18.9±1.7
GPT (U/L)	SPI	22.6±3.1	18.3±2.6	21.1±1.9
	SMP	16.5±1.7	16.4±1.7	18.9±1.9

Table 8. Serum lipid and glucose concentrations and GOT and GPT activities at the beginning and after 1 and 2 months of the dietary treatments (from Borodin et al., 2009). Values are means ± SEM. Total number of participants in each pooled group is 28. \* -  $p < 0.05$ ; \*\* -  $p < 0.01$ ; \*\*\* -  $p < 0.005$ . P values correspond to the deference between the initial values of the index and values after 1 or 2 month (t-test for dependent samples)

We see at least three reasons of the observed favorable effects of consumption of SPI in this study. The first reason may be the relatively long administration of the test foods and use of crossover design. In nutrition interventional studies proper care of the subjects is very important and because of this it is not easy to increase the number of participants. Inter-individual variances are usually large and therefore a large number of subjects is required to have significant effects in a parallel-group study, while a relatively small number of participants is sufficient for a study with crossover design because the inter-individual differences are thereby minimized. A review paper by AHA shows that most studies with crossover design were less than 6 weeks (Sacks et al., 2006). In our study, the results at 1 month were not effective but those at 2 months were effective, suggesting that only long studies may be able to decrease blood lipids of hyperlipidemic Russians at 30 g SPI a day. The second reason for the favorable results may be the good acceptability to the subjects of the test diets. Sustainability is always required for these types of studies to show the expected results. The reason for the relatively short periods of the previous studies

mentioned above might have been the poor acceptability and poor sustainability of SPI to the subjects. SPI at 30 g a day may be easy for Asians to accept but not for Westerners and Russians. For this reason, we developed well-accepted test-foods with a high SPI content. The intakes of energy, proteins, fats and carbohydrates were similar at all periods of study: in the initial period, during the first and second months and at the washout period, which may also mean that the cookies were well accepted. The third reason for the favorable results may be the level of hyperlipidemia in our subjects. As reviewed by Anderson et al. (1) the cholesterol-lowering effect of SPI was observed more clearly in subjects with high serum Total- and LDL-cholesterol concentrations than in those with normal and low lipid concentrations. Our inclusion criteria were overweight (BMI 25-34 kg/m<sup>2</sup>), fasting serum total cholesterol 240-330 mg/dL, non-HDL-cholesterol 150-280 mg/dL; HDL-Cholesterol 40-70 mg/dL, triglycerides 100-280 mg/dL.

### **Conclusion**

Consumption of the low-energy SPI-enriched cookies (30 g of SPI a day) for 2 months with a crossover design confirmed the favorable effects of SPI on serum lipids in Russians with moderate hyperlipidemia.

## **4. CVD, soy and soy foods in Russia**

In the 90-th of the last century the situation with the health of Russian people drastically deteriorated and CVD had become the main reason of mortality. Above we discussed the antioxidant and hypocholesterolemic effects of soy foods and our personal results, showing the effectiveness of soy foods in the decreasing of blood cholesterol in Russians with moderate hyperlipidemia. In the last part we will focus attention on the epidemiology of CVD in modern Russia, dietary habits and lifestyle of Russians, which predispose high mortality rate from CVD, and on the possibility of use of soy foods for the prevention and treatment of CVD in Russia.

### **4.1 Epidemiology of CVD in Russia**

An epidemiologic feature of the present Russia is the high mortality rate from CVD. According to the WHO Statistical Information System Mortality Database, the mortality rate per 100,000 from CVD in Russian adults under 65 years increased from 141-161 in 1986-1991 to 233-251 in 2001-2005 (WHO Statistical Information System Mortality Database). The rates were similar to those in the USA (241.8 and 244.4 for males and females, respectively) and England (263 and 235) and higher than those in Japan (136 and 138) and France (172 and 176) (WHO Statistical Information System Mortality Database). The average lifespans of Russians in late 1990s were only 58 years for males and 72 for females, while they were 79 and 86 for Japanese, 75 and 80 for Americans, and 77 and 81 for French individuals (UN Demographic Yearbook 2004). The dietary habits and lifestyle of Russians, namely high consumption of saturated fats (Solodkaia et al., 1998) and abuse of alcohol (Leon et al., 2007), associated with excessive body mass (Konstantinov et al., 2002), predispose individuals to short longevity as a result of a high mortality rate, especially from CVDs (Kharchenko et al., 1997), which is characteristic for the present-day Russians. Therefore, soy foods may be helpful for Russians in the prevention of CVD. Due to climate conditions, Russia is able to produce abundant soybeans but the consumption of soy foods by Russians is very limited

partly because of the low quality of the soy foods produced. Soy foods in Russia are used very infrequently in the treatment and prophylaxis of CVD in contrast to the Asian and European countries and USA.

#### 4.2 Soy planting and soy foods in Russia

Initially, soybean was brought to Russia from northeastern China. Currently, soybean is planted in the Far East of Russia (the Amur region and Prymorie), the Altai region and in the Kuban territory near the Black Sea. (picture 2).



Picture 2. Soy planting in Russia. The regions farming soya are colored in turquoise (blue/green).

Soybean is also planted in some other regions but the crop is small. The annual total crops of soybean in Russia makes up nearly a million metric tons which is very little in comparison with the main producers of soybean in the world. The All Russia scientific research institute of soya is based in the administrative center of Amur region Blagoveshchensk (<http://www.amur.ru/~seray/index.html>). The main task of this institute is the creation of the new species of soya, adapted to the local climatic condition, and the farming techniques of planting soya in these conditions. In Russia, soya traditionally processed into oil, flour and feeds. Soy flour is often associated with meat products – sausage, cutlet etc. One of the main obstacles in the promotion of soy foods in Russia is the image of soy products that, in the opinion of Russians, is not as foods for human but as feed for animals. The other obstacle is association of soya with genetically modified foods. Our 15-year long experience of promotion of soy foods in Russia as healthy foods shows that strong informational support from medical specialists is necessary to create a positive image of these foods in

Russian people. This informational support should be constant and prolonged. Our experience of promotion of soy foods in Russia with a help of specialists from Japan (Fuji Oil co.) shows that when Russian people have a chance to taste modern soy foods produced in Japan, they highly praised these foods and express desire to consume them (picture 3).



Picture 3. Promotion campaign of the Fuji Oil Co (Osaka, Japan) in Russia (2005-2007 years) The visit to the milk plant “Alev” (up left) and the conference with the mass media, medical specialist and cookery experts (up right) in Ulianovsk region; soy field in Amur region (bottom left); meeting with the participants of the study in the biochemistry department of the Amur State Medical Academy –(bottom right).

The campaign of promotion of soy foods had been initiated in Russia in the second part of the 1990s. Many enterprises producing oriental soy foods like soy milk and bean curd– tofu as well as textured soy protein – have flourished. However, the vast majority of these enterprises used just simple equipment and the taste of those products was not good for Russians. As a result, the society lost interest in soy foods. To revive this interest, it is necessary to begin production of good quality soy foods in Russia and request for help from medical specialists in explaining the healthy properties of soy foods to Russian people as was done in European countries and the USA.

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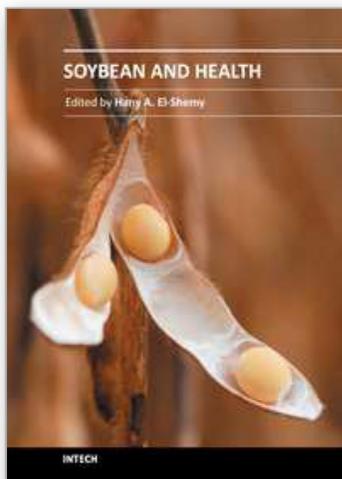
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## **Soybean and Health**

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Worldwide, soybean seed proteins represent a major source of amino acids for human and animal nutrition. Soybean seeds are an important and economical source of protein in the diet of many developed and developing countries. Soy is a complete protein, and soy-foods are rich in vitamins and minerals. Soybean protein provides all the essential amino acids in the amounts needed for human health. Recent research suggests that soy may also lower risk of prostate, colon and breast cancers as well as osteoporosis and other bone health problems, and alleviate hot flashes associated with menopause. This volume is expected to be useful for student, researchers and public who are interested in soybean.

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