

## VEGETABLE PROTEIN DECREASES BLOOD CHOLESTEROL

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A role of vegetable proteins in reducing blood cholesterol and coronary artery disease risk was postulated as long ago as 1909 in Russia by Ignatowski (1). The earliest study demonstrating significant cholesterol-lowering effect of soy-protein-based diet was carried out in 1967 by Hodges et al. (2), who demonstrated serum cholesterol decrease from 7.7 mmol/L to about 5.0 mmol/L in six subjects with hypercholesterolemia after 4 wk of treatment. Several investigators have studied the potential of soybean proteins to help lower plasma cholesterol in normocholesterolemic and hypercholesterolemic individuals. Most of the experiments have involved a partial or total replacement of animal proteins in the diet with soy protein preparations that vary in the degree of purity. Diets have been fed generally for 3-6 wk in a crossover design, in which the patients serve as their own control subjects (1). The meta-analysis of 38 controlled clinical trials revealed that ingestion of soy protein was associated with the following net changes in serum lipid concentrations: total cholesterol, a decrease of 23.2 mg per deciliter or 9.3 percent; low-density lipoprotein (LDL) cholesterol, a decrease of 21.7 mg per deciliter or 12.9 percent; and triglycerides, a decrease of 13.3 mg per deciliter or 10.5 percent. The changes in serum cholesterol and LDL cholesterol concentrations were directly related to the initial serum cholesterol concentration, it was clearly established that the reduction in plasma cholesterol was inversely related to the baseline degree of hypercholesterolemia (3). The Food and Drug Administration of the USA and the American Heart Association recommend daily consumption of 25 g of soy protein to control blood cholesterol (4). However, this conclusion is based mainly on studies with whole soy foods, which contains several components of soy beans that influence blood lipids (5). The results of studies with soy protein isolate (SPI) are less consistent and recent meta-analysis shows that favorable results were observed only in studies with quite high amounts of soybean protein (5) and that there were no effects when the participants consumed 25g of SPI daily for 6 weeks (6,7). These findings suggest that at least 30g of soy protein and more than 6 weeks administration are necessary to achieve favorable changes in blood lipid concentrations. From the practical point of view the amount of soy protein that should be consumed daily is very important. If the amount is too high, few people can follow a program in daily life and the results will remain only as research findings without practical substance. This is especially important for people who are not familiar with soy foods and in particular for Russians.

In a crossover design study with a group of 30 subjects (9 males and 21 females) aged between 32 and 64 with mild hyperlipidemia (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.) we have shown that by the consumption of 30 g of SPI for 2 months, concentrations of total-cholesterol changed from  $280 \pm 7$  to  $263 \pm 8$  mg/dl (-6.5%), HDL-cholesterol from  $57.4 \pm 2.5$  to  $62.6 \pm 2.9$  mg/dl (+9%), non-HDL-cholesterol from  $223 \pm 7$  to  $201 \pm 8$  mg/dl (-11%) and triglycerides from  $204 \pm 23$  to  $173 \pm 19$  mg/dl (-18%) (8).

In agreement with the results of the majority of studies (5), 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, two-month SPI consumption was followed by statistically 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. The important results in this study were the clear increase in HDL-cholesterol concentration ( $P < 0.001$ ) and clear decrease in non-HDL concentration ( $P < 0.001$ ). In previous reports, even those with a high SPI administration, only a few studies observed significant changes in the HDL-cholesterol concentration (9). The reason why we could observe favorable effects in this study with 30 g SPI may be the relatively long administration of the experimental diets with a cross-over design. A large number of subjects is required to have significant effects in a parallel study, while a relatively small number of participants is sufficient for a cross-sectional study because the inter-individual differences are thereby minimized. A review paper by American Heart Association shows that most of cross-sectional studies were less than 6 weeks (9). In our study, the results at 1 month were not effective but those at 2 months were effective, suggesting that a study longer than 2 months may be able to decrease blood lipids of hyperlipidemic Russians at 30 g SPI/day. The other reasons for the favorable results of SPI may be the good acceptability of the test diets by the subjects and the level of hyperlipidemia in our subjects.

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## **THE INFLUENCE OF THE TREATMENT OF RATS WITHIN A LIFE WITH ENRICHED SOYA DIET ON LIPIDS OF BLOOD, LIVER AND BRAIN**

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Nervous tissue is extremely rich in lipids, [18] which changes, and especially their oxidative modification [3,4], play an important role in the functioning of the brain during various stages of ontogenesis and in particular during the aging period. [2] Vegetable oils are the main source of unsaturated lipids and fat-soluble antioxidants for the organism of animals and humans [11]. The most common vegetable oil is soy, it accounts for half of all vegetable oils produced in the world [19]. The literature discusses the ability of soy products to reduce cholesterol in the blood [20] and prevent a decline in cognitive function in postmenopausal women [13,15]. In experiments on laboratory animals [1] and clinical studies [7], we demonstrated antioxidant [1,8] and hypocholesterolemic effects [7] from the intake of soy products. We present the results of the study of the effect of long-term soy intake on the content of some lipids and products of their oxidative modification in the blood, liver and brain of rats, and attempts are made to use them to explain the ability of soya that we have established to prevent a decrease in the cognitive abilities of rats in adulthood growth [6].

The study was performed on white laboratory rats (18 males and 24 females). The animals of the control group received a standard diet, and the experimental three days a week the standard diet, and four days boiled soya. A detailed description of the conditions of feeding of rats was described earlier [6]. The animals were removed from the experiment at the age of 15 month. Blood plasma, erythrocyte mass, liver and brain tissue were analyzed. Biochemical parameters of blood plasma were determined with the help of standard reagent sets on the biochemical semi-automatic analyzer Stat Fax 1904+. Lipids were extracted from the erythrocyte mass, liver and brain samples of the rats using Folch method [12]. In the lipid extracts, the content of total lipids was determined gravimetrically, cholesterol, phospholipids, diene conjugates and hydroperoxides with color reactions [1]. UV absorption spectra of lipid extracts were recorded on a UNICO 2804 spectrophotometer [9]. The fatty acid composition of brain lipids in the combined extracts of the control and experimental groups of rats was studied by gas-liquid chromatography of methyl esters of fatty acids on the gas-liquid chromatogram Crystal 2000M, column DB-23, carrier gas-purity 0.9999. Chromatogram analysis was performed using the Chromatec Analytic 2.5 program [5].

Feeding rats with large quantities of soy for a lifetime did not affect the rates depending on the nature of the diet, and the animals receiving the enriched diet did not differ in the content of total protein, glucose, cholesterol, triglycerides, and activity of alanine aminotransferase (ALAT) in the blood plasma with *vivo*-control group. The sex of the animals did not matter.

There were no differences in the content of total lipids, cholesterol, phospholipids and vitamin E in erythrocytes, depending on the sex of the animals. The only changes in the lipid composition and oxidation state of the erythrocyte lipids in the experimental and control group included a moderate increase in cholesterol by 28% and a decrease in the content of diene conjugates also by 28% in the erythrocytes of males that received soy.

In the group of animals treated with soya a marked increase in the content of vitamin E in the liver, in both females and males, was found 1.3-1.5 times, respectively, which is understandable taking into account the high content of this antioxidant in the soybean used consisting of 380 µg/g wet weight [8]. Other changes in the lipid composition and the degree of oxidation of liver lipids in animals on soybean diet depended on sex. In particular, a statistically significant increase in the phospholipid content by 18% and an antioxidant effect, manifested in a decrease in the content of diene conjugates (by 25% based on phospholipids), conjugated trienes and ketodienes (A278nm) (by 37%) in animals, treated with soya, were characteristic only for females. The content of lipid hydroperoxides in the control and experimental groups of rats was  $2.81 \pm 0.4$  and  $1.88 \pm 0.21$  nmol/mg phospholipid, respectively, but due to the large variation in the values in the control groups, the differences did not reach statistical significance. The reason for the dependence of the antioxidant effect of soya on the gender of animals may be associated with greater oxidation of liver lipids in the control group of rats in adulthood compared to males, as indicated by a higher content of conjugated trienes and ketodienes (by 40%) and lipid hydroperoxides (1,5 times in terms of the wet weight of the liver and 2 times in terms of phospholipids) in the liver of females in relation to males.