Effects of Two-Month Consumption of 30 g a Day of Soy Protein Isolate or Skimmed Curd Protein on Blood Lipid Concentration in Russian Adults with Hyperlipidemia

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Summary
Recently the American Heart Association has reported that favorable effects of soy protein on blood lipids were characteristic only for high amounts of soy protein and not observed for an intake less than 30 g/d. However, the period of the studies with the smaller amount was 4–6 wk and we thought a longer study was necessary for the conclusion. The death rate by heart disease is very high in Russia; therefore, we have done this study in Russian subjects with hyperlipidemia. Prior to the study we tried to find a favorable method for subjects to take 30 g protein a day from soybean protein isolate (SPI) or skimmed curd protein (SMP) and decided to use Russian style cookies. Thirty subjects with hyperlipidemia were recruited; however, due to the 5-mo long study 28 of them (19 females and 9 males aged 50±2 y) could complete the trial. They were randomly assigned to two groups and were given either cookie for 2 mo separated by a month-long washout interval in a crossover design. Fasting blood samples were drawn before and after the dietary treatments. Fasting blood samples at 1 mo were also measured as a health check and to observe the trends of the blood parameters in the middle of the study period. Serum samples were used for the lipid and other biochemical measurements. Every month for 3 non-consecutive days, energy and nutrient intakes were assessed and physical activity was estimated by pedometer. With the consumption of SPI for 2 mo, concentrations of total-cholesterol changed from 280±7 to 263±8 mg/dL (−6.5%, p=0.0099), HDL-cholesterol from 57.4±2.5 to 62.6±2.9 mg/dL (9%, p=0.0047), non-HDL-cholesterol (total-cholesterol−HDL-cholesterol) from 223±7 to 201±8 mg/dL (−11%, p=0.0023) and triglycerides from 204±23 to 173±19 mg/dL (−18%, p=0.022). There were no significant changes with SMP (p>0.05). Thus, administration of 30 g SPI a day for 2 mo confirmed its favorable effects on serum lipids in Russians with hyperlipidemia.

Key Words soy protein isolate, skimmed milk curd, hyperlipidemia, Russian

It is well established that soy protein foods can decrease blood cholesterol (1–3) and mortality rates from cardiovascular diseases (CVD) (4, 5) both in Asians (6, 7) and Westerners (8–10). 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 (11, 12). However, this conclusion is based mainly on studies with whole soy foods, which contains several components of soy beans that influence blood lipids (13). 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 (13) and that there were no effects when the participants consumed about 25 g of SPI daily for 6 wk (14, 15). These findings suggest that at least 30 g of soy protein and more than 6 wk 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 international journals there are no publications on the effect of soy protein foods on blood lipids in Russians, whose dietary habits and life-style, i.e. high consumption of saturated fats (16) and abuse of alcohol (17–19), associated with excessive body mass (20), predispose to short longevity, as a result of a high mortality rate especially from CVDs (21), which is characteristic of present-day Russians. According to the WHO Statistical
Information System Mortality Database, the mortality rate per 100,000 from CVDs in Russian adults under 65 in 1986–1991 was 146–161 and subsequently gradually increased, reaching 232–251 in 2001–2005 (22). The mortality rates from CVDs for males and females in other countries were 136.3 and 138.0 respectively in Japan, 241.8 and 244.4 in the USA, 262.7 and 235.2 in England and 172.4 and 176.0 in France (22). In the late 1990s the average life-spans of Russians were only 58.4 for males and 72.8 for females (21), while they were 79.0 and 85.8 for Japanese, 75.2 and 80.4 for Americans, and 76.6 and 81.0 for Frenchmen (23). This study was aimed at investigating whether SPI (30 g/d) is effective in controlling serum lipids and preventing heart disease in Russians. 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. To promote the use of soybean and soy products, production of good quality products is necessary as well as scientific evidence that SPI is good for the health of Russians.

MATERIALS AND METHODS

**Subjects.** Fifty-three male and female subjects aged 32–67 were recruited by a cardiologist on a voluntary basis from people visiting a private diagnostic center. These subjects were instructed 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. Their physical characteristics, nutrient intake, and physical activities were documented. After a lead-in period of 2–3 wk blood samples were taken. Serum samples were frozen until analyses of lipids, total protein and glucose concentrations, and GPT and GOT activities were made. Of these 53 subjects, 30 subjects (9 males and 21 females) aged between 32 and 64 were selected on the basis of their ability to follow the protocol. The inclusion criteria were overweight (BMI 25–34 kg/m²), 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.

**Ethics.** In accordance with the Helsinki Declaration on human studies, the protocol was approved by the Amur State Medical Academy Ethics Committee. The participants were informed in detail about the purpose, the advantages and disadvantages of the study, and their rights and duties concerning their lifestyle. Informed consent from all participants was obtained in writing.

**Study design.** This study was done by a crossover design. Subjects were given either SPI- or skimmed curd protein (SMP)-enriched cookies (protein content 30%) for the first 2 mo. After a month-long washout period, the subjects received the opposite test food for another 2 mo.

**Intervention.** We tried to make various kinds of foods with 30% SPI or SMP in cooperation with local food specialists and assessed their acceptability with our associates. We finally decided on cookies with a low energy content (340 kcal/100 g) due to the use of sorbitol instead of sucrose. Special SPI-enriched cookies (protein content 30%) were supplied by the local authorized confectioner and used as the test food for the experimental group (composition per 1 kg of cookies: wheat flour 333 g, SPI 333 g, margarine 183 g, sorbitol 233 g, egg 33 g, salt 6.7 g, baking soda 3.3 g, ammonium carbonate 1.7 g, energy value 340 kcal in 100 g). The daily total amount of cookies made up 30 g 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 and its tendency to form glue-like structures. Therefore we used SMP (protein 18%, fat 0.6% and carbohydrate 1.5%). Originally the producer suggested the following formula for skimmed curd-enriched cookies: skimmed curd 500 g, wheat flour 150 g, sorbitol 100 g, egg 33 g, salt 3 g, baking soda 2 g, ammonium carbonate 1 g. However, the composition of these cookies (ratio protein : fat : carbohydrates) was not identical to the composition of SPI-enriched cookies. SMP content in these cookies was insufficient, and storage time was limited. For this reason, we allowed the subjects to prepare SMP cookies by themselves by mixing the daily amount of skimmed curd (170 g) with a 33 g of wheat flour, 1 g of egg solids, 23 g of sorbitol, salt 0.7 g, baking soda 0.3 g, ammonium carbonate 0.2 g, and frying the obtained dough. The composition of these SMP-enriched cookies was identical to the composition of SPI-enriched cookie with the exception of test protein. For the convenience of participants the mixtures of minor ingredients for daily portions of skimmed curd cookies were prepared by the confectionery producer, packed in small packets and delivered to the participants. Skimmed curd was purchased from an authorized local provider.

**Nutrition survey.** A nutrition survey was done every month, including the wash-out period, for 3 non-consecutive days (two weekdays and one weekend day) by the 24-h recall method. A specialist instructed the subjects on how to record their food on the day prior to the interview.

**Physical activity monitoring.** To monitor physical activity of the subjects, we measured the number of steps per day using a pedometer (Omron HJ-005-E, China).

**Chemical analysis.** Blood samples were drawn in the morning after overnight fasting (10 h) before the beginning of the intervention, as well as after the first and second month. Serum samples were frozen until analyses. TC was measured by the cholesterol-oxidase method, HDL-C by the same method after precipitating low density and very low density lipoproteins, TG by the lipoprotein lipase and glycerophosphate-oxidase method, glucose by the glucose-oxidase method, TP by the biuret colorimetric method, GOT and GPT by the enzymatic kinetic methods.
Among the 21 women, 5 were pre-menopausal. They had regular menstrual cycles, and blood was withdrawn monthly on the same day after menses.

**Statistical analysis.** The statistical analysis was performed by the StatSoft, Inc. (2001) STATISTICA (data analysis software system), version 6. To evaluate the statistical significance of the observed changes in the studied indices in the same group of participants between the initial and final values (after 2 mo of the dietary intervention) and between the SPI and SMP groups of participants at the end of the trial, we used parametric criteria: paired \( t \)-test for dependent samples and unpaired \( t \)-test for independent samples, respectively. The statistically significant level was set at \( p \) less than 0.05.

Table 1 shows the baseline characteristics of the subjects. All subjects were overweight (average BMI 29.0±3.87). The average values of serum total cholesterol, non-HDL-cholesterol, HDL-cholesterol, and triglycerides were typical for moderate hyperlipidemia. Six subjects had serum cholesterol higher than 300 mg/dL. Glucose and total protein contents, GPT and GOT activities were normal.

The 30 subjects were assigned to two groups, consisting of 15 persons each. These groups received SPI cookies and skimmed curd cookies for 2 mo. After a month’s washout period, the subjects received the opposite test food for another 2 mo.

### RESULTS

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The 30 subjects were assigned to two groups, consisting of 15 persons each. These groups received SPI cookies and skimmed curd cookies for 2 mo. After a month’s washout period, the subjects received the opposite test food for another 2 mo.
Both the SPI and SMP cookies were well accepted by the majority of the subjects. Among the 30 subjects enrolled in the study, only 2 did not finish. One woman failed to consume the SPI enriched cookies because she developed an intestinal upset, and the other finished only the first half of the trial and was lost to follow-up for personal reasons. Twenty-eight subjects successfully completed the trials. Energy and nutrient intakes, physical activity, and physical characteristics are shown in Table 2. 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, or physical activity throughout the trial.

Table 3 shows the changes in serum lipid, glucose and total protein concentrations and GPT and GOT activities at the beginning and after 1 and 2 mo of the dietary treatments.

Table 3. Serum lipid and glucose concentrations and GOT and GPT activities at the beginning and after 1 and 2 mo of the dietary treatments.

| Indices                        | Groups | Initial values | After 1 mo  | After 2 mo  |
|-------------------------------|--------|----------------|-------------|-------------|
| 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 ± 2.8***|
|                               | SMP    | 59.3 ± 3.3     | 60.1 ± 2.4  | 56.6 ± 2.6  |
| Non-HDL-cholesterol (mg/dL)   | SPI    | 22.7 ± 7       | 22.1 ± 10   | 201 ± 8.8***|
|                               | SMP    | 219 ± 10       | 222 ± 10    | 215 ± 9     |
| Triglycerides (mg/dL)         | SPI    | 204 ± 23       | 183.3 ± 20* | 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  |

1 Values are means ± SE. Total number of participants in each pooled group was 28.

*p < 0.05, **p < 0.01, ***p < 0.005. p values correspond to the difference between the initial values of the index and values after 1 or 2 mo (t-test for dependent samples).

DISCUSSION

This study was aimed at investigating 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, which is characteristic for today’s Russia. Although older studies, in which subjects were given high doses of SPI, showed favorable results and Anderson et al. summarized from their meta-analysis that 31–47 g SPI can significantly decrease serum total- and LDL-cholesterol concentrations (1), recent studies with 25 g of the protein for 6 wk did not show such effects (14, 15). This was the reason for the American Heart Association experts to question the effectiveness of SPI in decreasing blood cholesterol (24). Thus, the daily amount of SPI and the duration of the study seem to be very important for the effect of SPI on blood lipids. In this study with a crossover design we have shown that by consuming 30 g of SPI daily for 2 mo serum concentrations of total-cholesterol, non-HDL-cholesterol and triglycerides decreased, and HDL-cholesterol concentration increased in Russians with moderate hyperlipidemia (Table 3).

In agreement with the results of the majority of studies (13), in our study a 1-mo 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, 2-mo SPI consumption was followed by statistically significant changes in serum total protein content or in GPT and GOT activities, thereby indicating that consumption of the test foods with a daily SPI amount of 30 g is safe for the liver. The serum glucose contents in the experimental group decreased slightly by 6.8 mg/dL (−8.6%, p = 0.048), significantly lower (−9.6 mg/dL, −10.8%, p = 0.046) compared with the milk curd group. We also measured the various blood parameters after 1 mo on dietary intervention as part of a health check and to observe the trends of the blood parameters at mid study period.
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 mg/dL (Table 3). The observed effect 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 (13). 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) (Table 3). Among previous reports, even those with a high SPI administration, only a few studies observed significant changes in the HDL-cholesterol concentration (24). Incidentally, we discovered that consumption of SPI was followed by a decrease in serum glucose level by 6.8 mg/dL (Table 3). 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 or glucose concentrations.

The first reason why we could observe favorable effects in this study with 50 g SPI may be the relatively long administration of the experimental diets with a cross-over design. In this type of study, proper care of the subjects is very important. 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 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 the American Heart Association shows that most cross-sectional studies lasted less than 6 wk (24). In our study, the results at 2 mo were effective, suggesting that a study longer than 2 mo may be able to decrease blood lipids of hyperlipidemic Russians at 30 g SPI/d. The second reason for the favorable results of SPI may be the good acceptability of the test diets by the subjects. 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 by the subjects. SPI at 30 g a day may be easy for Asians to accept but not for Westerners (12). In most of the previous studies, there was not 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” (8). Drinks with SPI and casein have rather a bad taste and smell and may not be consumed by the participants over a long period. For this reason, we tried to develop some well-accepted foods with a high SPI content (30%). Both SPI- and SMP-enriched cookies were well accepted by the majority of the subjects. Of the 30 subjects enrolled in the study, only 2 did not finish. One woman failed to consume SPI cookies because of intestinal upset, and the other could finish only the first half of the trial because of personal reasons. 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 wash-out 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/m²), 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.

In conclusion, the present study showed that the administration of 30 g SPI for 2 mo has favorable effects on serum lipid concentrations in Russians with hyperlipidemia.

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HM initiated this study; SY and KT designed the study; EAB, MAS, HM, TY, SY and KT invented the formula for preparation of SPI and skimmed curd-enriched cookies; IGM and VAD coordinated the subject recruitment and data collection; NAF performed the nutritional survey and coordinated measurement of the physical activity; EAB and MAS supervised the laboratory analyses; EAB, VAD and SY performed the statistical analyses; EAB, TY, SY, KT and HM interpreted the results of the study; EAB wrote the manuscript; TY, SY, KT and HM assisted in the manuscript preparation. None of the authors had any personal or financial conflict of interest.

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