Effects of Soy Protein on Levels of Remnant-like Particles Cholesterol and Vitamin E in Healthy Men

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Summary We determined the effects of soy protein isolate (SPI) intake on remnant-like particles (RLP), lipolytic enzymes, lipid transfer protein, transaminases, sex hormones, iron, calcium, and vitamin E in healthy men. In the first randomized, crossover experiment, 14 men were given either 20 g per day of SPI or nothing (control) for each 4-week segment. After 3 weeks of SPI intake, TG and RLP cholesterol levels were significantly lower than the baseline by 13.4% (p<0.05) and 9.8% (p<0.05), respectively. However, no significant change was found in total and low-density lipoprotein (LDL) cholesterol levels or the activities of lipoprotein lipase, hepatic lipase, cholesteryl ester transfer protein, and lecithin cholesterol acyltransferase. Although the levels of transaminases, testosterone, iron, and calcium did not change, the vitamin E level was reduced from the baseline by 9.7%, a significant decrease (p<0.01). In the second study, we attempted to determine the effect of vitamin E supplement taken with SPI. For each 3-week segment, 12 men were given 20 g per day of SPI, either with or without 200 mg per day of vitamin E, in a randomized crossover design. The vitamin E level was reduced by 9.2%, a significant decrease (p<0.05), after SPI intake for 3 weeks, and vitamin E supplement increased vitamin E level significantly (p<0.05). These results demonstrate that SPI intake reduces remnant lipoproteins, TG, and the plasma level of vitamin E, although vitamin E supplementation compensates for the reduction of vitamin E. Therefore the supplementation of vitamin E may be required in subjects with long-term and abundant intake of soy protein.

Key Words soy protein isolate, lipid, remnant lipoproteins, sex hormones, vitamin E

It is well-known that lipids and atherosclerotic disease have a close relationship. Therefore a diet aimed at improving the lipids profile is believed to be important. Many studies have shown that soy protein intake reduces the levels of total cholesterol, low-density lipoprotein (LDL) cholesterol, and triglyceride (TG) (1–3). It has also been reported that soy protein isolate (SPI) might reduce total cholesterol without affecting TG (4, 5). Recently, remnant-like particles (RLP), which is the lipoprotein unbound to the immunoaffinity chromatography that consists of monoclonal antibodies to apoB-100 and to apoA-1, has been used as a marker of remnant lipoproteins (6). We have previously reported that 20 g per day of SPI intake for 3 weeks improved the metabolism of postprandial remnant lipoproteins after an oral fat load compared with casein intake and slightly reduced the fasting of RLP cholesterol, which was not significant (7). Therefore the effect of soy protein on TG or remnant lipoproteins is unclear. Moreover, its effect on enzymes associated with lipoprotein metabolism, such as lipoprotein lipase (LPL), hepatic lipase (HL), cholesteryl ester transfer protein (CETP), and lecithin cholesterol acyltransferase (LCAT), is also unknown.

The hypocholesterolemic action of soy protein is thought to enhance bile acid excretion and activation of the LDL receptor (8). Sugano et al. reported that undigested soybean protein inhibits reabsorption of bile acid in intestine (9). We speculate that the intestinal absorption of vitamin E, calcium, and iron may be affected by this action. Moreover, the isoflavones in soy protein are phytoestrogens, which are structurally similar to estrogen and can bind to estrogen receptors (10). On the other hand, sex hormones are derived from cholesterol, so reduced blood cholesterol level may affect the blood levels of sex hormones. It is important, therefore, to determine the effects of long-term and abundant intake of soy protein on sex hormones in young adult men.

In the present study, we determined the effects of SPI intake on remnant lipoproteins and certain enzymes associated with lipid metabolism and its adverse effects in healthy men. We also determined whether oral supplementation with vitamin E compensates for the reduction in vitamin E caused by SPI intake.

METHODS

Subjects. Fourteen healthy male volunteers parti-
pated in the first experiment. Their ages and body mass indexes (mean±SD) were 31±4 y and 24.8±2.9 kg/m², respectively. For the 12 healthy male volunteers who participated in the second experiment, the ages and body mass indexes (mean±SD) were 30±2 y and 22.4±2.4 kg/m², respectively. None of the volunteers had chronic hepatic, renal, or thyroid disease or malabsorption, and they were taking no drugs.

Protocol. The present study consisted of two separate clinical experiments. In the first randomized, crossover experiment, 14 healthy men were given 20 g of SPI per day (SPI period) or no SPI (control period) for 4 weeks each (experiment 1). A one- to two-month washout period was used between the two test periods. The subjects drank 20 g of SPI (Fuji Oil Co., Osaka, Japan) mixed with milk or yogurt in the control period and only milk or yogurt in the control period. More than 12 h of fasting blood samples were collected before the experiment and at 2, 3, and 4 weeks during it. Postheparin plasma was also collected 10 min after an intravenous injection of heparin (30 U/kg) to measure the activity of LPL and HL before the experiment, and at 4 weeks during the experiment.

In the second experiment, a randomized crossover design. 12 men were given 20 g of SPI per day, either with or without 200 mg of vitamin E (tocopherol acetate, Juvela, Eizai Co., Tokyo, Japan) for each 3-week segment, at a one- to two-month washout period (experiment 2). Fasting blood samples were collected at the baseline 10 days and 3 weeks during the experiment. All participants were requested to maintain their lifestyles and body weights for the duration of the experiment. All subjects gave written informed consent for participation in each experiment. The experimental protocol was approved by the Ethical Committee of the National Defense Medical College of Japan.

Biochemical Analysis. Plasma TG, total cholesterol, LDL cholesterol, high-density lipoprotein (HDL) cholesterol, and glucose were measured enzymatically (Determiner L, Kyowa Medics, Tokyo, Japan). ApoA-I and apoB were measured by immunoturbidimetry (Daichi Pure Chemicals Co., Tokyo, Japan). The RLP cholesterol was isolated from the other lipoproteins as an unbound fraction in immunoaffinity mixed gels containing monoclonal apoA-I and apoB-100 antibodies (6, 7). In brief, 5 µL of plasma was added to the mixture of 50 µL of CNBr Sepharose 4B, which contained 125 µg of anti-apoA-I monoclonal antibody, 125 µg anti-apoB-100 monoclonal antibody, and 300 µL of reaction suspension. The mixture was shaken gently for 60 min to mix it and was then left alone for 10 min. Thirty microliters of clear supernatant was taken, and the cholesterol level of RLP was determined enzymatically. LCAT activity was determined by the method of Nagasaki and Akanuma (11). The CETP mass was determined by a sandwich-enzyme immunoassay (12). The activity of LPL and HL in postheparin plasma was measured by the method of Nozaki et al. (13). The level of vitamin E in plasma was determined by high-performance liquid chromatography (HPLC). In brief, vitamin E was extracted with ethanol and hexane; the hexane phase was evaporated under N₂ gas, and the residue was dissolved in ethanol. Vitamin E was separated by reversed-phase HPLC on C18 columns (TSK gel ODS-80Ts, Tohso, Tokyo, Japan), and was eluted with ethanol/distilled water (92:8, vol/vol) at 1.0 mL/min as the mobile phase and monitored at 295 nm in a UV detector (UV-8000, Tohso, Tokyo, Japan). Transaminases were measured enzymatically (SRL, Inc., Japan). Testosterone and estradiol were determined by radioimmunoassay (SRL, Inc.). The calcium level was determined by using O-cresolphthalein complexone (SRL, Inc.). Plasma iron was measured by using 2-Nitroso-5-[N-n-propyl-N-(3-sulfopropyl)amino]phenol (SRL, Inc.).

Statistics. Values are presented as mean±SD. Various parameters of fasting in both experimental groups were compared by paired t tests. Parameters from both groups were compared by repeated measures of analysis of variance. The Pearson correlation was calculated between vitamin E levels and lipids or lipoprotein levels. P<0.05 was considered to be statistically significant.

RESULT

Experiment 1

The changes in serum lipids, lipoproteins, andapolipoproteins are shown in Table 1. These changes were most apparent after 3 weeks of SPI intake, but they returned to the baseline after 4 weeks. After 3 weeks of SPI intake, total cholesterol, LDL cholesterol, TG, and RLP cholesterol levels decreased from baseline values by 4.3%, 4.5%, 13.4%, and 9.8%, respectively. The changes in TG and RLP cholesterol were statistically significant (p<0.05, and p<0.05). The CETP mass and the activities of LCAT, LPL, and HL did not change in either group (Table 2). The levels of testosterone, transaminases, calcium, and iron did not significantly change during the experiment, though compared with the baseline, vitamin E concentrations decreased significantly after 2, 3, and 4 weeks of an SPI intake by 6.4% (p<0.05), 9.7% (p<0.01), and 6.0% (p<0.05), respectively (Table 3). The correlations of vitamin E with lipids and lipoproteins in the value before the experiment and in the change after 3 weeks of SPI intake are shown in Table 4. The vitamin E level was significantly correlated with total cholesterol, TG, LDL cholesterol, and RLP cholesterol, but not with HDL cholesterol before this experiment. After 3 weeks of SPI intake, the change of vitamin E was correlated with TG and RLP cholesterol, but not with TC or LDL cholesterol.

Experiment 2

Serum lipids, lipoproteins, testosterone, estrogen, and vitamin E concentrations during the experiment were shown in Table 5. No difference was noted in the lipid profile of subjects who took SPI plus vitamin E compared with those who took only SPI. Neither testosterone nor estrogen levels changed in either group. Vitamin E levels after 3 weeks of SPI intake decreased significantly by 9.2% (p<0.05), which was similar to the result of the first experiment. Vitamin E levels after
### Table 1. Change of blood lipid levels during the first experiment.

|                  | Control   |            |            |            | SPI        |            |            |            |
|------------------|-----------|------------|------------|------------|-----------|------------|------------|------------|
|                  | Before    | 2 wk       | 3 wk       | 4 wk       | Before    | 2 wk       | 3 wk       | 4 wk       |
| Total cholesterol| 193±29    | 191±23     | 188±28     | 198±24     | 199±27    | 195±23     | 189±22     | 198±26     |
| Triglyceride     | 115±78    | 109±53     | 120±50     | 107±48     | 145±82    | 123±68     | 119±60*    | 122±61     |
| HDL cholesterol  | 53±15     | 54±17      | 55±18      | 58±17      | 52±14     | 53±15      | 54±18      | 54±18      |
| LDL cholesterol  | 124±27    | 124±25     | 118±24     | 128±22     | 127±28    | 128±27     | 121±24     | 129±28     |
| RLP cholesterol  | 4.5±3.4   | 3.8±1.8    | 4.9±2.2    | 4.5±1.7    | 5.8±4.1   | 4.6±2.1    | 4.4±2.0*   | 5.5±2.8    |
| ApoAI            | 131±23    | 133±26     | 135±28     | 142±26     | 131±21    | 131±24     | 133±24     | 133±27     |
| ApoB             | 93±23     | 94±23      | 89±21      | 94±18      | 97±26     | 97±24      | 93±22      | 96±23      |

Values are mean±SD (mg/dL). SPI indicates soy protein isolate; HDL, high-density lipoprotein; LDL, low-density lipoprotein; RLP, remnant-like particles; Apo, apolipoprotein.

* Significantly different from the value before p<0.05.

### Table 2. Change of levels of enzymes during the first experiment.

|                  | Control   |            |            |            | SPI        |            |            |            |
|------------------|-----------|------------|------------|------------|-----------|------------|------------|------------|
|                  | Before    | 4 wk       |            |            | Before    | 4 wk       |            |            |
| LCAT (nmol/mL/h) | 103±20    | 103±21     |            |            | 107±23    | 103±14     |            |            |
| LPL (µmol·FFA/mL/min) | 0.48±0.05 | 0.48±0.06  |            |            | 0.48±0.06 | 0.47±0.07  |            |            |
| HL (µmol·FFA/mL/min) | 0.3±0.06  | 0.32±0.07  |            |            | 0.33±0.07 | 0.33±0.08  |            |            |
| CETP (µg/mL)     | 2.7±0.4   | 3.4±0.3    |            |            | 2.9±0.7   | 3.1±0.6    |            |            |

Values are mean±SD. LCAT indicates lecithin cholesterol acyltransferase; LPL, lipoprotein lipase; HL, hepatic lipase; CETP, cholesteryl ester transfer protein; FFA, free fatty acid. No significant difference is found between groups or from the previous value.

### Table 3. Change of testosterone, transaminases, vitamin E, iron, and calcium levels during the first experiment.

|                  | Control   |            |            |            | SPI        |            |            |            |
|------------------|-----------|------------|------------|------------|-----------|------------|------------|------------|
|                  | Before    | 2 wk       | 3 wk       | 4 wk       | Before    | 2 wk       | 3 wk       | 4 wk       |
| Testosterone (ng/dL) | 541±172  | 492±151    | 577±207    | 505±128    | 578±221  | 527±145    | 551±162    | 523±179    |
| AST (unit/L)     | 23±6      | 23±5       | 23±7       | 28±14      | 26±10     | 26±12      | 25±5       | 24±7       |
| ALT (unit/L)     | 26±12     | 24±10      | 25±15      | 36±32      | 30±15     | 29±16      | 28±15      | 25±10      |
| Vitamin E (mg/dL) | 1.43±0.41 | 1.36±0.31  | 1.38±0.38  | 1.41±0.24  | 1.46±0.28 | 1.35±0.22* | 1.30±0.18** | 1.36±0.22* |
| Iron (mg/dL)     | 111±48    | 107±36     | 128±48     | 89±31      | 117±31    | 109±45     | 114±34     | 111±42     |
| Calcium (mg/dL)  | 9.6±0.1   | 9.6±0.1    | 9.3±0.3    | 9.5±0.3    | 9.7±0.3   | 9.6±0.4    | 9.4±0.3    | 9.4±0.3    |

SPI indicates soy protein isolate; AST, aspartate aminotransferase; ALT, alanine aminotransferase. Values are mean±SD.

* Significantly different from the values before p<0.05 and p<0.01, respectively.

### Table 4. Correlations of vitamin E with lipids and lipoproteins in value before the experiment and in change after 3 weeks of SPI intake.

|                  | Values at before experiment (n=28) | Change after 3 wk of SPI (n=14) |
|------------------|-----------------------------------|----------------------------------|
|                  | r       | p       | r       | p       |
| Total cholesterol| 0.771   | 0.0007  | 0.231   | 0.4357  |
| Triglyceride     | 0.691   | 0.0048  | 0.619   | 0.0163  |
| HDL cholesterol  | 0.541   | 0.0448  | 0.004   | 0.9896  |
| LDL cholesterol  | -0.241  | 0.4141  | -0.117  | 0.6961  |
| RLP cholesterol  | 0.729   | 0.0021  | 0.731   | 0.0020  |

SPI indicates soy protein isolate; HDL, high-density lipoprotein; LDL, low-density lipoprotein; RLP, remnant-like particle.
Table 5. Change of lipids, sex hormones, and vitamin E levels during the second experiment.

|                     | SPI Before | SPI 10 d | SPI 3 wk | SPI + vitamin E Before | SPI + vitamin E 10 d | SPI + vitamin E 3 wk |
|---------------------|------------|---------|----------|------------------------|--------------------|---------------------|
| Total cholesterol (mg/dL) | 202±30     | 189±30  | 192±10   | 198±25                 | 194±26             | 196±33              |
| Triglyceride (mg/dL)  | 102±57     | 77±34   | 79±36    | 87±34                  | 81±34              | 80±28               |
| HDL cholesterol (mg/dL)| 60±13      | 58±14   | 61±13    | 62±14                  | 62±12              | 64±10               |
| LDL cholesterol (mg/dL)| 120±28     | 117±30  | 115±26   | 118±26                 | 119±28             | 114±32              |
| Testosterone (ng/ml)   | 545±122    | 550±100 | 562±118  | 503±99                 | 562±112            | 506±110             |
| Estrogen (ng/ml)       | 35.2±6.0   | 35.0±12.1 | 36.9±11.5 | 38.5±13.6             | 32.6±13.6          | 35.6±15.1           |
| Vitamin E (mg/dl)     | 1.30±0.19   | 1.26±0.22 | 1.18±0.23* | 1.31±0.22             | 1.65±0.37**        | 1.56±0.38**         |

SPI indicates soy protein isolate; HDL, high-density lipoprotein; LDL, low-density lipoprotein. Values are mean±SD.
* Significantly different from the values before p<0.05 and p<0.01, respectively.
** Significantly different from the values before p<0.001 and p<0.01, respectively.

DISCUSSION

In the present study, SPI intake significantly reduced RLP cholesterol and TG with no change in the levels of enzymes, such as LPL, HL, CETP, and LCAT. SPI intake did not affect sex hormones, transaminases, iron, or calcium levels, though it significantly reduced the vitamin E level by about 10%. Moreover, a vitamin E supplement taken with SPI could compensate for the decrease in the vitamin E level.

In a meta-analysis of previous studies that used soy protein, Anderson et al. showed that an average intake of 47 g of soy protein decreased total cholesterol, LDL cholesterol, and TG levels by 9.3%, 12.9%, and 10.5%, respectively (3). A recent study showed that at least 20 g per day of soy protein for 6 weeks could reduce total cholesterol and non-HDL cholesterol by 1.8% and 2.6%, respectively, without affecting the TG level (5), though the initial TG level was higher than in our study. The total cholesterol, LDL cholesterol, and TG levels in our study after 20 g SPI for 3 weeks decreased by 4.3%, 4.5%, and 13.4%, respectively, in the first experiment, and 4.4%, 1.7%, and 18.5%, respectively, in the second. These changes might be comparable to the previous studies, but lipid profiles after 4 weeks of SPI intake returned to levels that were similar to baseline in our first experiment, and the cholesterol levels were apparently unchanged in our second experiment. Thus there might be limitation in the efficiency of soy protein intake on lipid profiles.

RLP particles consist mainly of remnants of chylomicron and apoE-rich VLDL (6). Recently, RLP cholesterol has been shown to be an independent marker of the development of atherosclerosis (14–17). In vitro, it has also been reported that RLP are easily uptaken by macrophages and fibroblasts without modifications (18, 19). Moreover, it has been demonstrated that RLP inhibit the production of nitric oxide in endothelial cells, enhance the platelet aggregation, and induce the expression of intercellular adhesion molecule-1, vascular cell adhesion molecule-1, and tissue factor in endothelial cells (20–22). We therefore believe that the reduction of RLP is necessary, if elevated. In our first experiment, RLP cholesterol level was reduced by SPI intake for 3 weeks. The metabolism of TG-rich lipoproteins is affected by enzymes such as LCAT, CETP, LPL, and HL. In the first experiment, these enzymes did not significantly change after 4 weeks of SPI intake, though we had better measure after 3 weeks of SPI intake, when RLP cholesterol level decreased maximally. So SPI intake might inhibit the synthesis and secretion of TG-rich lipoproteins from liver and intestine, or it might activate the receptor of these lipoproteins. Potter suggested that the main hypocholesterolemic mechanisms of soy protein are the enhancement of bile acid excretion and the activation of LDL receptor (8). Some TG-rich lipoproteins can also be uptaken by LDL receptors with apoE as a ligand. Thus part of the decrease in RLP that enrich apoE might be caused by an activation of the LDL receptor by SPI intake. Shige et al. reported that in comparison with casein, SPI intake improved RLP clearance after an oral fat load, and RLP cholesterol showed a tendency to decrease after SPI intake for 3 weeks (7). A decrease in RLP cholesterol by SPI intake might therefore also be due to an improvement of postprandial lipemia.

Soy isoflavones are phytoestrogens, and they are thought to be effective in the treatment of various diseases, such as atherosclerosis, cancer, osteoporosis, and menopausal symptoms (10, 23). Using food questionnaires, Nagata et al. found an inverse correlation between soy protein consumption and testosterone level in men (24). We also thought that soy protein might affect sex hormones, especially in men, but 20 g per day of SPI for 3 weeks did not affect testosterone and estrogen levels in our study. It has been reported that the consumption of soy protein for one month affects transaminases in rats (25). In the present study, however, there was no clear effect of SPI intake on transaminases. It is thus possible that 20 g per day of SPI may not affect sex hormones and transaminases in healthy men.

In this study, SPI intake did not affect iron and calcium levels, but it did decrease the vitamin E level significantly by about 10%. It might be speculated that a de-
crease in the VLDL or LDL particles that contain most of the vitamin E causes a decrease in plasma vitamin E. In fact, the vitamin E level was significantly correlated with TG and RLP cholesterol, but not with LDL cholesterol. Moreover, the percentage change of vitamin E significantly correlated with TG and RLP cholesterol but not LDL cholesterol in only the SPI intake group. Thus the decrease in TG-rich lipoprotein particles containing RLP might be one cause of the decrease in the vitamin E level. After 4 weeks of SPI intake, however, vitamin E was significantly lower from the baseline, but at the same time lipids were not different from the baseline. We speculate that SPI might change the content of vitamin E in lipoproteins, besides decreasing the TG-rich lipoproteins. So we consider it important to determine whether an oral supplement of vitamin E can compensate for the reduction in vitamin E caused by SPI intake.

In our second study, vitamin E supplementation was effective at increasing or maintaining the vitamin E level during SPI intake. This causes us to believe that a recommendation for soy protein intake should be accompanied by encouraging a vitamin E supplement.

In conclusion, the present study shows that (1) 20 g per day of SPI intake decreases not only TG, but also RLP cholesterol; (2) SPI intake lowers the vitamin E level by about 10%; (3) a vitamin E supplement compensates for the decrease in vitamin E during SPI intake. For patients to enjoy the beneficial and protective effects of soy protein intake, the addition of a vitamin E supplement may be required.

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