Effects of Soy Protein Isolate (SPI) and Casein on the Postprandial Lipemia in Normolipidemic Men

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Summary To elucidate the effects of soybean protein and casein on postprandial lipemia, oral fat load tests were performed before and 3 weeks after the administration of soy protein isolate (SPI) and casein supplement to normolipidemic men. Eleven normolipidemic male subjects on otherwise identical controlled diets were assigned to either a 20 g/d soy protein isolate (SPI) dietary supplement or a casein dietary supplement for three weeks in a crossover design. Fat load tests with 40 g/m² of bovine milk fat were carried out before and after 3 weeks on the experimental dietary supplements. Fasting plasma concentrations of lipids and apolipoproteins were not significantly different from baseline levels before or after the administration of SPI or casein supplemented diets. Neither SPI nor casein supplement affected the fasting plasma concentrations of lipids and apolipoproteins. The areas under the incremental curve (AUIC) of triglyceride (TG) and remnant-like particles triglyceride (RLP-TG) after both experimental diets were not significantly different from those before the experimental diets. However, the AUIC of remnant-like particles cholesterol (RLP-C) showed a tendency (p = 0.07) to decrease after administration of the diet supplemented with SPI than before the diet. The AUIC of RLP-C was significantly (p < 0.05) lower after the diet supplemented with SPI than after administration of the diet supplemented with casein. These results suggest that 3 weeks of 20 g/d SPI dietary supplement favorably affects the postprandial remnant lipoprotein response as compared to the casein dietary supplement.

Key Words soybean protein, casein, postprandial lipemia, remnant lipoprotein, normolipidemic male subjects

Western diets, rich in animal protein, total fat, saturated fatty acid and cholesterol, are associated with an increase in mortality from cardiovascular and
coronary artery disease (CAD). The elevation of low-density lipoprotein cholesterol (LDL-C) and reduction of high-density lipoprotein cholesterol (HDL-C) are great risk factors for CAD (1). However, many individuals with normal fasting plasma lipid levels have CAD. Plasma lipids levels in individuals who eat 3 meals a day are altered throughout almost two-thirds of the day by the fat contained in the meals. Many previous studies indicate that the magnitudes of postprandial plasma triglyceride (TG), TG-rich lipoproteins (TRL) and apoB-48 responses after a fat-rich meal are greater in patients with CAD than in those without CAD (2–9). Thus, postprandial lipemia is considered to be associated with the development of CAD.

The substitution of vegetable proteins, mainly soybean protein, for animal protein reportedly decreases the fasting plasma levels of total cholesterol (TC), TG and LDL-C in humans (10–13). Decreased levels of TC, TG and LDL-C are believed to be associated with a decreased risk of CAD. Several mechanisms have been proposed whereby soybean protein reduces plasma TC: (1) it raises LDL receptor activity (14–17); (2) it reduces intestinal bile acid and cholesterol absorption (18–20); (3) it raises lipoprotein lipase (LPL) activity (21); and (4) it alters insulin levels (22). In the postprandial state, intestine-derived chylomicrons are hydrolyzed by LPL and converted to cholesterol-rich chylomicron remnants. Thereafter, chylomicron remnants are removed by the liver through the LDL receptor pathway and so-called remnant receptor pathway (23). Additionally, LPL is an insulin-dependent enzyme (24). Therefore, the factors mentioned above are all known to affect the metabolism of TG-rich lipoproteins (TRL). Although the relationship between dietary protein and postprandial lipemia has not been examined yet, it seems reasonable to assume that soybean protein could have some effect. Thus, the purpose of this study is to elucidate the effects of soybean protein on postprandial lipemia, especially remnant lipoprotein.

Recently, a monoclonal antibody to apoB-100 (JI-H) has been used to isolate and determine chylomicron-derived lipoprotein (25, 26). Since this monoclonal antibody recognizes an epitope just distal to the C-terminus of apoB-48, it fails to recognize apoB-48-containing lipoprotein (27). Remnant-like particles (RLP) designate the lipoprotein unbound to the immunoaffinity chromatography that consists of monoclonal antibody to apoB-100 (JI-H), and monoclonal antibody to apoA-1. Lipoproteins not bound to this immunoaffinity mixed gel are mainly remnants of chylomicron and a minor portion of very-low-density lipoprotein (VLDL) (27–29). Utilization of this immunoaffinity mixed gel enables us to isolate RLP from plasma in a single step (9, 28–31). In this study, RLP-lipids were measured as markers of remnant lipoprotein.

MATERIALS AND METHODS

Subjects. Eleven normolipidemic male volunteers, mean age 32 y, were selected for this study. None showed any chronic ailments such as hepatic, renal, thyroid
or cardiac dysfunction, lactose intolerance or malabsorption, and none were taking any drugs known to affect plasma lipid levels. Characteristics of the subjects are shown in Table 1.

**Dietary supplements.** Nakamura et al previously reported that 20 g/d of soybean protein substitution for two weeks significantly decreased the levels of plasma and LDL cholesterol in Japanese subjects (32). In this study, therefore, we chose to give 20 g/d of soy protein isolate (SPI) for 3 weeks. Soybean protein-enriched powder and casein-enriched powder were obtained from Fuji Oil Co. (Osaka, Japan). Soybean protein-enriched powder (100 g/d) containing 80 g/d of powdered corn soup and 20 g/d SPI, and providing 438 kcal/d (53.5% as carbohydrate, 17.5% as fat and 29% as protein), was dissolved in 450 mL of hot water to prepare the soup for a day; the P/S ratio of this powder was 1.12. An alternative soup enriched with casein (20 g/d) instead of SPI, and other contents being exactly the same, was also prepared by Fuji Oil Co. During the first one-week baseline period, subjects consumed 150 mL of casein-enriched soup 3 times with daily meals. During the following 3-week experimental period, subjects consumed 150 mL of either the SPI or casein soups 3 times with daily meals. In addition to the SPI or casein soups, subjects were provided with 7 types of pre-cooked package foods (Nichirei Food Co., Tokyo, Japan) to serve as major dishes. Subjects were required to consume the pre-cooked meals in a one-week cycle menu. The pre-cooked packaged foods contained standard Japanese dishes without soybean products (e.g., without tofu, miso or natto). During the four-week experimental periods, all subjects were required to eat 2 of their 3 daily meals (breakfast and lunch) at the National Defense Medical College Hospital; the third meal, the evening dinner, could be eaten at home but had to be soup plus one of the pre-cooked meals.

The energy requirements of each subject were estimated during an initial interview. Body weights were monitored daily and all subjects were asked to maintain a steady body weight (i.e., refrain from unusual energy splurges). Food intake was checked by diet questionnaires throughout the experimental period, and alcohol intake was prohibited during the entire study period. Mean energy intake was 1,980 kcal/d (55% as carbohydrate, 26% as fat and 19% as protein, P/S ratio 1.36)

| Table 1. Subject characteristics. |
|-----------------------------------|
| Age (y)                           | 32.6 ± 6.4 |
| BMI (kg/m²)                       | 24.6 ± 2.8 |
| Total cholesterol (mmol/L)        | 5.19 ± 0.57 |
| TG (mmol/L)                       | 1.41 ± 0.59 |
| LDL-cholesterol (mmol/L)          | 3.38 ± 0.51 |
| HDL-cholesterol (mmol/L)          | 1.17 ± 0.35 |

Mean ± SD (n = 11).

BMI, body mass index; TG, triglyceride; LDL, low-density lipoprotein; HDL, high-density lipoprotein.
and mean cholesterol intake was 210 mg/d, including the experimental soup.

**Study design.** The design of the study, which covered 10 weeks, is shown in Fig. 1. The subjects were randomly assigned to one of two groups. During the first week (week 0), members of both groups consumed the casein-based soup, had breakfasts and lunches at the National Defense Medical College Hospital and evening meals at their own home, and ate pre-cooked food. Over the next 3 weeks (weeks 1–3), the diets of one group were supplemented with the SPI-based soup while the diets of the other group were supplemented by the casein-based soup; otherwise, their diets were exactly the same. This was followed with a 2-week ‘rest’ period during which subjects ate ad lib. The following week was a repeat of week 0, wherein all subjects followed the same routine (breakfasts and lunches at the hospital and evening meals at their home eating pre-cooked foods supplemented with casein-based soup). Then, in the final 3 weeks, both groups followed the same routines as in weeks 1–3, except that the dietary supplements were switched so that the first group received the casein-enriched soup while the second group received SPI-enriched soup. In the evening of the day preceding blood collections, subjects were required to have identical meals at 08:00 PM at the National Defense Medical College Hospital. Fasting blood was collected in vacutainer tubes containing 1 mg/mL of EDTA once a week at 08:00 AM after a 12 h fast.

Oral fat load tests were performed before (at the end of week 0) and after (at the end of week 3) administration of the 3-week experimental diets. In the test,

![Fig. 1. Study design. Subjects were randomly assigned to one of two groups, and members of both groups had identical meals supplemented with casein-enriched soup during week 0 as baseline period. From weeks 1–3, the meals of one group were supplemented with soy protein isolate (SPI)-enriched soup while the meals of the other group were supplemented with casein-enriched soup; otherwise, diets were exactly the same. The week following a 2-week ad lib. diet interval was a repeat of week 0, wherein all subjects had identical meals supplemented with casein-enriched soup. Then, during the final 3 weeks, both groups followed the same routines as in weeks 1–3 except that the dietary supplements were switched so that the first group received the casein-enriched soup while the second group received SPI-enriched soup. Oral fat load tests were performed before (at the end of week 0) and after (at the end of week 3) the 3-week experimental diets. CA, casein-enriched soup supplement diet (20 g/d); SPI, SPI-enriched soup supplement diet (20 g/d); INT, 2-week interval; F, oral fat load test.](image-url)
40 g/m\(^2\) body surface area (BSA) of bovine milk fat emulsion (Fresh Cream, Meiji Milk Co., Tokyo, Japan) containing 110 mg/m\(^2\) BSA of cholesterol was ingested after a 12-h fast. Blood samples were collected before and 2, 4 and 6 h after the oral fat load intake.

The experimental protocol was approved by the Ethical Committee of the National Defense Medical College. All subjects gave written informed consent for participation in this study.

**Assay procedure.** Fasting and post fat-load plasma lipids, HDL cholesterol and apolipoproteins were determined in the National Defense Medical College Hospital Central Laboratory. This laboratory has documented traceability to the Cholesterol Reference Method Laboratory Network of the Center for Disease Control and Prevention in Atlanta, Georgia, USA. Fasting and postprandial plasma lipids were determined enzymatically (Determiner L, Kyowa, Tokyo, Japan). HDL-C was determined after precipitation of apoB-containing lipoproteins with dextran sulfate and magnesium chloride. Apolipoproteins were determined by immunoturbidimetric assay (Apo Auto, Daiichi Chemical, Tokyo, Japan). Lipoproteins(a) (Lp(a)) was measured by enzyme immunoassay (TintElize Lp(a), Biopool, Sweden).

**Measurement of remnant-like particles (RLP) lipids.** Five microliters of plasma was added to the mixture of 50 µL of CNBr Sepharose 4B which contained 125 µg of anti-apoA-1 Mab, 125 µg anti-apoB-100 Mab (JI-H) and 300 µL of reaction suspension. The mixture was shaken gently for 60 min to mix and let sit for 10 min. Thirty microliters of clear supernatant was taken, and cholesterol and TG levels of RLP were determined enzymatically.

**Phenotyping of apoE.** ApoE phenotypes were determined by isoelectric focusing on 5% polyacrylamide gels containing 3 M urea at pH 4.5–8 (Joko, Tokyo, Japan).

**Statistical analysis.** Concentrations of fasting plasma lipids and apolipoproteins before both experimental diets were compared and analyzed by paired t-test. Concentrations of fasting plasma lipids and apolipoproteins during both experimental diet phases were compared by repeated measures ANOVA. Postprandial increments of plasma lipids and apolipoproteins before both experimental diets were compared by two-way repeated measures ANOVA. Area under the incremental curve (AUIC) of the lipids and apolipoprotein values above baseline plotted against times were calculated by the trapezoidal method, and expressed as mmol-h/L. In this calculation, plasma concentrations that fell under baseline levels were calculated as zero. The AUIC values before both diets were compared by paired t-test, and the AUIC values before and after the experimental diets were compared by paired t-tests. Statistical analyses were carried out using StatView 4.5 software.

**RESULTS**

All subjects (six men in group A and five men in group B) completed the entire
Table 2. Fasting plasma lipid, apolipoprotein and Lp(a) concentrations.

|                      | SPI supplement |
|----------------------|----------------|
|                      | -1 W           | 0 W          | 1 W         | 2 W          | 3 W          |
| TC (mmol/L)          | 5.22 ± 0.57    | 5.02 ± 0.57  | 4.96 ± 0.68 | 5.00 ± 0.68  | 4.99 ± 0.59  |
| TG (mmol/L)          | 1.38 ± 0.59    | 1.39 ± 0.61  | 1.40 ± 0.54 | 1.45 ± 0.55  | 1.32 ± 0.51  |
| HDL-C (mmol/L)       | 1.17 ± 0.35    | 1.12 ± 0.32  | 1.11 ± 0.22 | 1.10 ± 0.22  | 1.17 ± 0.26  |
| LDL-C (mmol/L)       | 3.38 ± 0.51    | 3.29 ± 0.52  | 3.23 ± 0.61 | 3.20 ± 0.61  | 3.21 ± 0.61  |
| apoA-I (g/L)         | 1.38 ± 0.19    | 1.32 ± 0.17  | 1.29 ± 0.14 | 1.31 ± 0.17  | 1.34 ± 0.16  |
| apoA-II (g/L)        | 0.36 ± 0.04    | 0.35 ± 0.03  | 0.35 ± 0.03 | 0.35 ± 0.04  | 0.36 ± 0.03  |
| apoB (g/L)           | 0.98 ± 0.15    | 0.97 ± 0.14  | 0.97 ± 0.18 | 0.97 ± 0.16  | 0.95 ± 0.12  |
| apoC-II (g/L)        | 0.038 ± 0.015  | 0.038 ± 0.014| 0.041 ± 0.018| 0.037 ± 0.015| 0.038 ± 0.011|
| apoC-III (g/L)       | 0.098 ± 0.034  | 0.098 ± 0.038| 0.100 ± 0.033| 0.098 ± 0.020| 0.105 ± 0.036|
| apoE (g/L)           | 0.050 ± 0.011  | 0.047 ± 0.010| 0.047 ± 0.008| 0.049 ± 0.006| 0.047 ± 0.009|
| Lp(a) (g/L)          | 0.043 ± 0.030  | 0.046 ± 0.022| 0.050 ± 0.030| 0.045 ± 0.024| 0.039 ± 0.020|

10-week study and none complained of diarrhea or other symptoms of malabsorption. The apoE phenotype of 9 subjects was E3/E3, 1 subject E3/E4 and 1 subject E4/E4. The postprandial lipid response of 2 subjects with apoE4 phenotype did not differ from that of the subjects with apoE3/3 phenotype. Body weights of the subjects changed less than 1 kg each during the study period. Fasting plasma lipids, apolipoproteins and Lp(a) during this study are shown in Table 2. Plasma concentrations of lipids, apolipoproteins and Lp(a) prior to administration of the soybean protein supplement did not differ significantly from those prior to administration of the casein supplement. Neither SPI nor casein supplements significantly affected the concentrations of fasting plasma lipids, apolipoproteins or Lp(a).

Before the SPI or casein dietary supplements were implemented (measures obtained at the end of week 0), postprandial increments of serum lipids or apolipoproteins after oral fat load did not show significant differences. At the end of week 0, postprandial increments of TG (ΔTG), RLP-TG (ΔRLP-TG) and RLP-C (ΔRLP-TC) from fasting concentrations were highest 4 h after oral fat loading, as
Table 2. (continued)

| Casein supplement | -1W | 0W | 1W | 2W | 3W |
|-------------------|-----|----|----|----|----|
| TC (mmol/L)       | 5.07 ± 0.81 | 5.19 ± 0.56 | 4.90 ± 0.52 | 5.11 ± 0.8 | 5.17 ± 0.85 |
| TG (mmol/L)       | 1.48 ± 0.55 | 1.49 ± 0.73 | 1.52 ± 0.56 | 1.49 ± 0.73 | 1.42 ± 0.56 |
| HDL-C (mmol/L)    | 1.12 ± 0.3 | 1.18 ± 0.34 | 1.13 ± 0.34 | 1.09 ± 0.32 | 1.15 ± 0.35 |
| LDL-C (mmol/L)    | 3.36 ± 0.73 | 3.32 ± 0.57 | 3.04 ± 0.52 | 3.28 ± 0.75 | 3.23 ± 0.82 |
| apoA-I (g/L)      | 1.42 ± 0.24 | 1.36 ± 0.26 | 1.31 ± 0.18 | 1.29 ± 0.18 | 1.31 ± 0.21 |
| apoA-II (g/L)     | 0.36 ± 0.04 | 0.36 ± 0.04 | 0.34 ± 0.03 | 0.35 ± 0.04 | 0.35 ± 0.03 |
| apoB (g/L)        | 1.01 ± 0.2 | 0.99 ± 0.16 | 0.96 ± 0.18 | 1.00 ± 0.23 | 0.97 ± 0.18 |
| apoC-II (g/L)     | 0.037 ± 0.010 | 0.040 ± 0.018 | 0.037 ± 0.016 | 0.041 ± 0.020 | 0.039 ± 0.013 |
| apoC-III (g/L)    | 0.105 ± 0.038 | 0.109 ± 0.036 | 0.114 ± 0.040 | 0.106 ± 0.041 | 0.105 ± 0.040 |
| apoE (g/L)        | 0.050 ± 0.007 | 0.050 ± 0.009 | 0.048 ± 0.009 | 0.050 ± 0.008 | 0.048 ± 0.009 |
| Lp(a) (g/L)       | 0.031 ± 0.019 | 0.036 ± 0.019 | 0.035 ± 0.019 | 0.039 ± 0.015 | 0.041 ± 0.024 |

Mean ± SD (n=11).
TC, total cholesterol; TG, triglyceride; HDL, high-density lipoprotein; LDL, low-density lipoprotein; apo, apolipoprotein; Lp(a), lipoprotein(a).

expected (Fig. 2). ΔRLP-C at 2 and 4 h after oral fat loading were significantly (p < 0.05) lower when diets were supplemented with SPI than those of each hour after oral fat loading when diets were supplemented with casein (Fig. 2, lower).

The AUIC of postprandial response before and after both experimental diets are shown in Table 3. The AUIC of RLP-C before both experimental diets did not differ significantly. The AUIC of RLP-C after 3 weeks of SPI supplement showed a tendency to decrease (p = 0.07) as compared to that before the SPI diet, but did not reach significance. On the contrary, the AUIC of RLP-C after 3 weeks of casein supplement was significantly (p = 0.03) larger than before administration of the casein supplement. Further, the AUIC of RLP-C after 3 weeks of administering the SPI diet supplement was significantly (p = 0.02) smaller than after 3 weeks of administering the casein diet supplement.

The slopes of postprandial response before and after both experimental diets were also calculated (Table 4). The 0–2, 2–4 and 4–6 h slopes for TG, RLP-TG...
Fig. 2. Effects of SPI and casein supplements on postprandial increments of plasma TG, RLP-TG and RLP-C from baseline after oral fat load tests. Lines show mean ±SD (mmol/L) ΔTG, ΔRLP-TG and ΔRLP-C. Left panels, subjects had been on diets supplemented with SPI; right panels, subjects had been on diets supplemented with casein. *p<0.05, after administration of SPI supplement as compared to that at each hour of after administration of casein supplement.

and RLP-C did not differ significantly between week 0 and week 3 of the SPI supplement diet. These slopes also did not differ significantly between week 0 and week 3 of the casein supplement diet. The 0–2h slope for RLP-C after 3 weeks of administering SPI supplement was significantly (p = 0.04) smaller than that after 3 weeks of administering casein supplement.
Table 3. Area under the incremental curve of postprandial lipids and apolipoproteins before and after the experimental diets.

|                | SPI supplement              | Casein supplement            |
|----------------|----------------------------|------------------------------|
|                | Before | After | p value | Before | After | p value |
| TG (mmol-h/L)  | 5.18±3.79 | 5.12±3.37 | 0.534 | 4.88±2.57 | 5.12±2.60 | 0.55 |
| RLP-TG (mmol-h/L) | 4.74±3.78 | 4.10±4.23 | 0.51 | 4.61±3.98 | 4.71±2.74 | 0.86 |
| RLP-C (mmol-h/L) | 0.54±0.16 | 0.34±0.34* | 0.07 | 0.48±0.40 | 0.62±0.38 | 0.03 |
| apoA-I (g-h/L)  | 0.58±0.36 | 0.30±0.36 | 0.083 | 0.43±0.28 | 0.27±0.22 | 0.13 |
| apoB (g-h/L)    | 0.59±0.36 | 0.42±0.33 | 0.09 | 0.48±0.22 | 0.49±0.16 | 0.84 |
| apoE (g-h/L)    | 0.02±0.02 | 0.01±0.01 | 0.11 | 0.01±0.01 | 0.02±0.02 | 0.06 |

Mean ± SD (n=11).

*p = 0.02 after administration of SPI supplement as compared to after administration of casein supplement.

TG, triglyceride; RLP, remnant-like particles.

DISCUSSION

The design of this study was cross-over, and the clearance of lipoprotein remnant particles from the circulation after fat load testing was studied after two baseline periods of one week, during which casein was added to the diet, and after supplementation with SPI and casein for three weeks. The AUIC of the postprandial RLP-C response tended to decrease after 3 weeks of administering the SPI supplement as compared to that of before giving the SPI supplement, but did not reach significance. Furthermore, the AUIC of the postprandial RLP-C response after administering casein supplement was significantly larger than that before giving the casein supplement. The AUIC of postprandial RLP-C response after administering the SPI supplement was significantly smaller than that after administering the casein supplement. These data suggest that 3 weeks of administering 20 g/d of SPI supplement could favorably affect the postprandial remnant lipidoprotein response as compared to casein supplement, while fasting plasma lipid and apolipoprotein concentrations were not affected.

Many previous studies indicate that the magnitudes of postprandial plasma triglyceride (TG), TG-rich lipoproteins (TRL) and apoB-48 after a fat-rich meal are greater in patients with CAD than in those without CAD (2–9). Therefore, postprandial lipemia is believed to be associated with the development of CAD. Recently, immunoaffinity chromatography using Mab against apoB 100 (JI-H) has been developed to isolate apoB-48 containing lipoprotein. RLP, not bound to the immunoaffinity chromatography that consists of JI-H and monoclonal antibody to apoA-1, contains chylomicron and a minor portion of VLDL. Utilization of this immunoaffinity mixed gel enables us to isolate RLP from plasma in a single process (9, 28–31). We previously indicated that the magnitude of RLP-C and TG increments
Table 4. Slope of postprandial plasma TG, RLP-TG and RLP-C levels after oral fat loading.

|                  | SPI supplement | Casein supplement |
|------------------|----------------|-------------------|
|                  | 0 W            | 3 W              | 0 W            | 3 W    | p value | 0 W            | 3 W              | p value |
| TG (mmol/L/h)    |                |                  |                |        |         |                |                  |         |
| 0–2 h            | 0.47 ± 0.33    | 0.43 ± 0.29      | 0.37 ± 0.22    | 0.45 ± 0.21 | 0.25    |
| 2–4 h            | 0.33 ± 0.30    | 0.26 ± 0.44      | 0.30 ± 0.25    | 0.30 ± 0.28 | 0.97    |
| 4–6 h            | −0.33 ± 0.35   | −0.34 ± 0.34     | −0.14 ± 0.26   | −0.27 ± 0.45| 0.38    |
| RLP-TG (mmol/L/h)|                |                  |                |        |         |                |                  |         |
| 0–2 h            | 0.35 ± 0.28    | 0.33 ± 0.30      | 0.32 ± 0.24    | 0.38 ± 0.22 | 0.31    |
| 2–4 h            | 0.32 ± 0.33    | 0.22 ± 0.49      | 0.27 ± 0.32    | 0.26 ± 0.27 | 0.84    |
| 4–6 h            | −0.32 ± 0.36   | −0.28 ± 0.47     | −0.10 ± 0.22   | −0.31 ± 0.39 | 0.76    |
| RLP-C (mmol/L/h) |                |                  |                |        |         |                |                  |         |
| 0–2 h            | 0.038 ± 0.025  | 0.022 ± 0.024*   | 0.031 ± 0.024  | 0.053 ± 0.032 | 0.06    |
| 2–4 h            | 0.031 ± 0.036  | 0.021 ± 0.029    | 0.028 ± 0.030  | 0.016 ± 0.034 | 0.24    |
| 4–6 h            | −0.014 ± 0.02  | −0.001 ± 0.023   | 0.003 ± 0.034  | −0.007 ± 0.035 | 0.53    |

Mean ± SD (n = 11).
* \( p = 0.04 \) after administration of SPI supplement as compared to after administration of casein supplement.

TG, triglyceride; RLP, remnant-like particles.
after oral fat load are greater in normolipidemic patients with angiologically defined CAD than in those without CAD (9). Furthermore, RLP were taken up by macrophages, which resulted in cholesterol accumulation (9). Thus, postprandial RLP-C increment might be one of the risk factors for CAD.

In this study, we have clearly indicated that the AUIC of RLP-C was significantly smaller after the 3-week administration of SPI supplement as compared to that after the 3-week administration of casein supplement. This data suggests that SPI might reduce the risk of CAD as compared to casein.

The mechanisms whereby SPI decreases the AUIC of the postprandial lipemia were not investigated in this study. The SPI dietary supplement decreased the concentrations of the plasma RLP-C at 2 and 4h after fat load as compared to those after casein dietary supplementation (Fig. 2). Furthermore, the 0–2 slope for RLP-C after administering the SPI supplement was significantly smaller than that after administering the casein supplement (Table 4). RLP-C expresses the cholesterol levels of chylomicron-derived lipoprotein. Therefore, the AUIC of RLP-C after fat load is believed to reflect the increment of plasma cholesterol levels of chylomicron-derived lipoprotein. Furthermore, the 0–4 h slopes could reflect the rate of cholesterol absorption. These data suggest that 3 weeks of SPI supplement might decrease the rate of cholesterol absorption as compared to casein supplement. Soybean protein is reported to selectively inhibit the absorption of cholesterol in experimental animals, while it does not affect the absorption of TG (19). In this study, the AUIC of the postprandial RLP-TG, which reflects the increment of plasma TG concentration of the chylomicron-derived lipoprotein, was not affected by either SPI or casein supplements. The 0–4 h slopes in RLP-TG, which could reflect the rate of TG absorption, were also not affected by either SPI or casein supplements. These data indicate that the SPI supplement could not affect the intestinal absorption of TG and suggest the possibility that soybean protein could selectively inhibit the intestinal absorption of cholesterol in humans. Furthermore, this selective inhibition of intestinal cholesterol absorption could not affect the fasting plasma cholesterol concentration because the mean intake of daily cholesterol was only 210 mg, a very small amount.

Many previous studies reported the mechanism by which soybean protein reduces fasting plasma lipids. Soybean protein, compared to casein, raises hepatic LDL receptor activity (14–17), alters insulin level (22) and raises LPL activity (21). These factors are all known to affect the metabolism of TG-rich lipoproteins. Previous studies also reported that soybean protein decreased the fasting plasma lipid concentration when lipids levels of subjects were elevated by dietary or genetic factors, or by chronic diseases (high fat intake, familial hypercholesterolemia (17, 33), renal disease (34), nephrotic syndrome (34, 35)). In this study, however, all the subjects were normolipidemic, and the fasting levels of plasma lipids and apolipoproteins were not affected by SPI or casein intake. Therefore, these factors might not be responsible for the difference in the magnitude of postprandial lipemia.

Fasting plasma TG and HDL-C concentrations themselves are also known to
be important determinants of the magnitude of postprandial lipemia (36). In this study, however, fasting plasma lipid and apolipoprotein concentrations did not change after the ingestion of diets supplemented with either SPI or casein. This study was conducted on normolipidemic subjects, and neither the administration of SPI nor casein supplement affected fasting plasma lipid concentrations. We can neglect the influences of the difference in fasting plasma TG and HDL-C concentrations to the magnitude of postprandial lipemia. Therefore, the decrement of RLP-C response after fat loading was not due to the changes in fasting plasma TG or HDL-C concentrations.

The mechanism by which SPI exerts lipid-lowering effects is unknown. In this study, we did not investigate the mechanism affecting the postprandial response of RLP-C. Many previous reports showed the components of soybean protein which would affect plasma lipid levels. The lower lysine/arginine ratio of soybean protein (37–39), and soybean globulins (40) are considered to be responsible for the reduction of serum lipid levels. Recently, isoflavonic phytoestrogen in soybean protein was reported to favorably affect serum lipids and lipoprotein in cynomolgus monkeys (41). These components might also affect the postprandial RLP-C response.

In this study, we found that the administration of 20 g/d of SPI supplement decreased the magnitude of postprandial lipemia, especially remnant lipoprotein, in normolipidemic males as compared to that after the administration of casein supplement. Neither casein nor SPI affected fasting plasma lipid or plasma apolipoprotein concentrations. This study suggests that 20 g/d of SPI, which does not affect fasting plasma lipid levels, favorably affects postprandial lipemia and thereby decreases the risk of CAD.

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