Variable lipemic response to dietary soy protein in healthy, normolipemic men1–3

Karin Nilausen and Hans Meinertz

ABSTRACT We found previously that dietary soy protein, compared with casein, reduced plasma LDL cholesterol and increased HDL cholesterol concentrations in healthy women and men. However, there was considerable variation among individuals. The aim of this study was to characterize the lipoprotein responsiveness of individuals to examine whether different response patterns could be identified. Nine normolipemic men consumed 2 liquid-formula diets of identical composition except that the protein component was either soy protein or casein. After 1 mo of consuming each diet, the subjects’ plasma HDL cholesterol (P < 0.01) and apolipoprotein (apo) A-I (P < 0.05) concentrations were increased by the soy-protein diet whereas the ratio of LDL cholesterol to HDL cholesterol was decreased (P < 0.01); total cholesterol, triacylglycerol, LDL cholesterol, apo B and apo A-II were insignificantly affected. In 5 individuals, however, soy protein reduced mean LDL cholesterol, LDL2 cholesterol, and LDL2 apo B concentrations by 26% and plasma apo B by 16%, whereas HDL cholesterol increased by 11%. In 3 other individuals, soy protein increased mean HDL cholesterol by 17% and plasma apo A-I by 12%, but did not lower LDL. In 1 subject, soy protein decreased LDL2 cholesterol by 11% and increased plasma triacylglycerol by 40%, but neither HDL cholesterol nor apo A-I increased. We identified 3 types of lipemic responses to dietary soy protein involving a reduction in atherogenic LDL and increase in antiatherogenic HDL. In most subjects, the effects on both LDL and HDL were favorable, although fewer experienced either an increase in HDL or a decrease in LDL.

INTRODUCTION

The low-fat diets recommended by the National Cholesterol Education Program (NCEP) (1) are designed to lower LDL cholesterol (LDL-C) concentrations and thus reduce the risk of new and recurrent cardiovascular disease. The preventive effects of such diets may, however, be less than expected. Apart from inadequate compliance, lack of a sufficient effect can in some individuals be due to unresponsiveness to the dietary changes (2–4). A further problem may be that the cholesterol-lowering diets tend to reduce HDL cholesterol (HDL-C), increase triacylglycerol concentrations (3, 5), and shift the LDL particle size distribution from larger and buoyant to smaller and dense (5), all of which may diminish the beneficial effect of the LDL reduction. A solution to some of those problems might be the incorporation of dietary components with beneficial lipid effects of their own into low-fat diets; such a combination of several dietary modifications may achieve a more comprehensive reduction in the lipid risk factors. Because soy protein not only lowers LDL-C, but, in addition, decreases triacylglycerol (6, 7) and increases HDL-C concentrations (8), it might play a useful role in such a combination of dietary changes.

The incorporation of soy protein into cholesterol-lowering diets may thus have the potential of further reducing cardiovascular risk. Before the use of soy protein in such diets can be generally recommended, more studies are required to resolve the following problems:

1) Because the cholesterol-lowering effect of soy protein increases with the amount consumed (6), high intakes will be more effective than low intakes, which raises the question of the long-term safety of high soy-protein intakes (9).

2) A high intake of soy-protein preparations results in a high intake of their nonprotein constituents, such as isoflavones and other compounds, which may have beneficial effects, as suggested by the favorable changes in lipids observed in rhesus monkeys (10), but which also might have adverse effects.

3) In human studies of dietary soy protein there has been a marked variability in the lipemic response, which has not been adequately explained (11).

4) In a previous study (8), LDL-C concentrations did not decrease and HDL-C did not increase in some individuals in response to dietary soy protein as they did in most subjects. The aim of the present study was therefore to examine in detail the individual lipemic response of normolipemic men to dietary soy protein.

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SUBJECTS AND METHODS

Subjects

Nine healthy men aged 21–64 y (mean 37 y) were recruited among friends and family of the laboratory staff. All men were nonobese with a body mass index (BMI) between 20.1–26.0 kg/m². At the initial screening, they reported no personal or family history of hyperlipidemia, diabetes, hypertension, or cardiovascular diseases. They were not using any medication and had normal fasting plasma glucose concentrations. The protocol was approved by the regional committee on human experimentation.

Experimental design

The subjects were studied during 3 dietary periods: in the first, most subjects (n = 5) ate their usual, self-selected, solid-food diet; in the second and third, subjects consumed liquid-formula diets of identical composition except for the protein component, which was either casein or soy protein. The liquid formulas were consumed for 33 d (n = 2) or 45 d (n = 7), and the dietary periods were separated by an interval of 53 ± 33 days (n = 7) in which the subjects ate self-selected, solid-food diets. The subjects alternately started on the casein or the soy-protein diet. Fasting blood samples were drawn throughout the study.

Diets

The liquid-formula diets, described previously in detail (8), contained 20% of energy as protein, 55% as carbohydrate, and 25% as fat. The protein preparations, both ≥90% pure, were calcium caseinate (Casec; Mead Johnson Laboratories, Evansville, IN) and a soy-protein isolate (Supro 660; Protein Technologies International, St Louis). The mean (±SD) daily intake (n = 7) of soy protein was 154 ± 7.9 g and that of casein was 154 ± 33 g. In both diets the carbohydrate was a cornstarch hydrolysate (Maltodextrin 01915; Cerestar, Haubourdin, France) and the fat component was the high-oleate variant of safflower oil (Oleinate 181; Pacific Vegetable Oil Corporation, San Francisco). Slightly more cholesterol was added to the soy-protein diet than to the casein diet to compensate for the small amount of cholesterol in the casein preparation. The cholesterol intake was 56.4 mg/MJ (236 mg/1000 kcal). Calcium lactate was added to the soy-protein diet to compensate for the high calcium content of the casein preparation; to compensate for the lactate, an equivalent amount was added to the casein diet in the form of sodium lactate. With additional supplements of vitamins, other micronutrients, and salts, the diets fulfilled recommended dietary allowances (12).

The subjects were asked to weigh themselves daily and to increase or decrease the intake of formula to maintain their body weight. Overall, they lost (x ± SD) 1.8 ± 1.44 and 2.3 ± 1.41 kg after the soy and casein diets, respectively (P > 0.6). They were allowed energy-free beverages and were specifically asked not to drink alcohol.

Lipids, lipoproteins, and apolipoproteins

Blood samples were drawn after the subjects had been fasting for ≥12 h and had spent ≥15 min in a recumbent position. Blood was collected in tubes containing potassium EDTA and plasma was immediately separated by low-speed centrifugation at 2000 × g at 4°C for 30 min. Plasma lipids and lipoprotein concentrations were measured according to the Lipid Research Clinics protocol (13), with the modification that HDL was isolated for cholesterol measurement after precipitation of apolipoprotein (apo) B-containing lipoproteins with magnesium chloride and dextran sulfate (14). In addition, the apo B-containing lipoproteins VLDL (Svedberg flotation unit (S) 400–600) and LDL (S < 200) were prepared by cumulative density gradient ultracentrifugation (15). Percentage recoveries of lipoprotein cholesterol from the density gradients were determined by comparison with total plasma cholesterol, and correction for losses was done by using the same percentage recovery for all fractions. Correction for losses of LDL apoB were done by using the same correction factor as for LDL apoC. Cholesterol and triacylglycerols were analyzed by enzymatic methodology (Boehringer, Mannheim, Germany). Plasma apo A-I and apo B were measured by radioimmun assay (Pharmacia Diagnostics AB, Uppsala, Sweden). The procedure used for apo A-I was a competitive assay in which a radiolabeled apo A-I competes with the A-I in the plasma sample for binding to a monoclonal anti-apo A-I antibody attached to sepharose microbeads; after incubation, centrifugation, and decanting, the radioactivity associated with the pelleted microbeads was inversely proportional to the amount of apo A-I in the sample. The plasma apo B assay used 2 different monoclonal anti-apo B antibodies, 1 as a trapping antibody bound to sepharose microbeads and the other reporting antibody radioiodinated; after incubation the microbeads were pelleted and counted and the amount of radioactivity was directly proportional to the amount of apo B in the plasma sample. Plasma apo A-II was measured by immunoturbidimetry by using an antihuman apo A-II antibody (Boehringer). The concentration of LDL apoB was determined as the isopropanol-precipitated protein, measured as the difference between total protein and protein soluble in the presence of isopropanol (16), analyzed by a modified Lowry procedure (17); the apo B value was subsequently corrected for preparative losses as mentioned above.

Statistical analysis

Comparison of variations among the 3 different diets in all 9 subjects was done by one-way repeated analysis of variance (ANOVA), whereas comparisons of pairs of diets were based on the Bonferroni multiple comparisons test. Comparison of the effects of soy-protein and casein diets in individual subjects and in subgroups of subjects were done by paired comparisons using Student’s t test, with P < 0.05 as an indicator of significant difference (GraphPad INSTAT version 2.0; GraphPad Software, San Diego).

RESULTS

When a change is made from self-selected, solid-food diets to the liquid formulas, it takes ≥2–3 wk before plasma lipoproteins stabilize at concentrations characteristic of the particular diet (8). The lipid, lipoprotein cholesterol, and apolipoprotein concentrations after >30 d of the self-selected and liquid-formula diets are shown in Table 1. Consumption of both liquid diets caused all plasma concentrations to decrease, particularly those of total and LDL-C (P < 0.001), plasma apo B (P < 0.001), HDL-C (P < 0.001), and plasma apo A-I (P < 0.01). Although the ratio of LDL-C to HDL-C remained unaffected by the casein diet, it...
TABLE 1
Plasma concentrations of lipids and apolipoproteins of subjects after > 30 days on each diet

<table>
<thead>
<tr>
<th></th>
<th>Self-selected</th>
<th>Casein</th>
<th>Soy protein</th>
<th>Percentage difference (Soy–Cas)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>4.56 ± 1.15</td>
<td>3.50 ± 0.82</td>
<td>3.46 ± 0.63</td>
<td>0 ± 14</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/L)</td>
<td>2.62 ± 0.82</td>
<td>2.01 ± 0.77</td>
<td>1.72 ± 0.57</td>
<td>−11 ± 23</td>
</tr>
<tr>
<td>Apolipoprotein B (g/L)</td>
<td>0.80 ± 0.27</td>
<td>0.67 ± 0.24</td>
<td>0.61 ± 0.20</td>
<td>−9 ± 11</td>
</tr>
<tr>
<td>LDL2 apolipoprotein B (g/L)</td>
<td>0.43 ± 0.19</td>
<td>0.36 ± 0.17</td>
<td>0.30 ± 0.13</td>
<td>−15 ± 19</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>1.39 ± 0.20</td>
<td>1.08 ± 0.15</td>
<td>1.21 ± 0.17</td>
<td>11 ± 7</td>
</tr>
<tr>
<td>Apolipoprotein A-I (g/L)</td>
<td>1.18 ± 0.07</td>
<td>0.93 ± 0.09</td>
<td>1.03 ± 0.10</td>
<td>10 ± 8</td>
</tr>
<tr>
<td>Apolipoprotein A-II (g/L)</td>
<td>0.35 ± 0.05</td>
<td>0.31 ± 0.05</td>
<td>0.30 ± 0.04</td>
<td>−3 ± 6</td>
</tr>
<tr>
<td>Triacylglycerol (mmol/L)</td>
<td>0.87 ± 0.36</td>
<td>0.71 ± 0.22</td>
<td>0.77 ± 0.31</td>
<td>−2 ± 40</td>
</tr>
<tr>
<td>VLDL cholesterol (mmol/L)</td>
<td>0.67 ± 0.52</td>
<td>0.49 ± 0.22</td>
<td>0.56 ± 0.27</td>
<td>12 ± 26</td>
</tr>
<tr>
<td>LDL: HDL cholesterol</td>
<td>1.94 ± 0.77</td>
<td>1.96 ± 0.99</td>
<td>1.49 ± 0.65</td>
<td>−22 ± 21</td>
</tr>
</tbody>
</table>

1± SD; n = 9. Mean percentage differences ± SD between the soy protein and casein diets were calculated from the natural logarithms of the plasma concentrations.

2,3,4: Variation among diets significant at: 2 P < 0.0001, 3 P < 0.002 and 4 P < 0.0035 by repeated measures ANOVA.

5,6,7: Self-selected diet versus casein and soy protein diets were different: 5 P < 0.001, 6 P < 0.01 and 7 P < 0.05 by Bonferroni multiple comparisons test.

5,7: Soy protein versus casein diet different: 5 P < 0.01 and 7 P < 0.05 by Bonferroni multiple comparisons test.

was significantly (P < 0.01) reduced by the soy-protein diet. The marked changes observed in all subjects indicated excellent dietary compliance.

Plasma concentrations measured after the subjects had consumed the casein and soy-protein diets for > 30 d (Table 1) showed that neither total nor LDL-C was significantly reduced by the soy diet. Only HDL-C (P < 0.01), and plasma apo A-I (P < 0.05) were significantly increased by the soy-protein diet.

The individual lipid response to dietary soy protein varied considerably. Because of the importance of the relation of LDL and HDL to vascular disease, the subjects were classified into 3 groups according to their response of LDL-C and HDL-C: those in whom 1) LDL decreased and HDL increased significantly, 2) HDL increased significantly without reduction in LDL, and 3) neither LDL decreased nor HDL increased. Although this meant that all subjects within each group reacted similarly with regard to LDL and HDL concentrations, they did not necessarily have similar responses for other lipids, lipoproteins, and apoproteins.

Shown in Table 2 are data from the largest group, 5 of 9 subjects, all of whom had decreased LDL-C and increased HDL-C in response to the soy-protein diet. The mean 26% reduction in LDL-C was due to a lowering of both LDL and LDL2; because the ratio of cholesterol to apo B in LDL2 remained unchanged, the reduction of LDL2 cholesterol was due to a reduced number of lipoprotein particles rather than a change in particle composition. The mean increase of both HDL-C and plasma apo A-I was 10%, but whereas 5 individuals in this subgroup showed significant increases in HDL (in accordance with the selection criteria), only 4 of them had significant increases in apo A-I. The response of triacylglycerol and of triacylglycerol-rich lipoproteins was variable.

Data from 3 subjects who did not have reduced LDL-C in response to the soy diet are provided in Table 3. The major effect was a marked increase in HDL-C (17%) and in plasma apo A-I (12%). The increase in total cholesterol was due in part to the increase in HDL-C but also in part to a 40% increase in LDL-C in one subject. This increase in LDL-C may be explained by the difficulty he had consuming the casein diet, which led to a weight loss of 3.5 kg during this period, and to his extraordinarily low LDL-C; during the soy-diet period, he experienced no difficulty consuming the diet and did not lose weight.

The third subgroup consisted of one individual who responded differently from all the others by showing neither a decrease in LDL-C nor an increase in HDL-C with the soy diet. In fact, the mean LDL-C (1.85 mmol/L) and HDL-C (1.97 mmol/L) concentrations were identical with the 2 liquid diets. This subject did, however, respond to the soy-protein diet. Only HDL-C (17%) and in plasma apo A-I were significantly (P < 0.01) reduced by the soy-protein diet. The increase in HDL-C but also in part to a 40% increase in LDL-C in one subject. This increase in LDL-C may be explained by the difficulty he had consuming the casein diet, which led to a weight loss of 3.5 kg during this period, and to his extraordinarily low LDL-C; during the soy-diet period, he experienced no difficulty consuming the diet and did not lose weight.
DISCUSSION
This study showed marked individual differences in the effects of dietary soy protein on the lipid transport system in plasma. Despite the variability in lipemic response among individuals, soy protein generally affected the major lipid risk factors favorably, as indicated by the reduction in the ratio of LDL-C to HDL-C (Table 1), whereas the casein diet had no such effect. In this respect the casein diet resembled the NCEP Step II diet (1), which similarly was without beneficial effect on the ratio of LDL-C to HDL-C (3). The most common effects of soy protein were increases in HDL-C and apo A-I, which occurred in 8 and 7, respectively, of 9 subjects. Almost as common as the increase in antiatherogenic lipoproteins was the decrease in apo E and apo A-II, observed in 4 individuals, soy protein generally affected the major lipid risk factor 16. The effect on cardiovascular risk was particularly favorable when both HDL and LDL were thus affected, and this was observed in 5 subjects. Less favorable effects were the increases in plasma triacylglycerol concentrations observed in 4 subjects and an increase of LDL-C in 1 subject, although the latter may have been due to extraordinary weight loss during the casein diet period rather than to a paradoxic response to soy protein. Whether the decrease in apo A-II, observed in 4 individuals, reduced cardiovascular risk remains uncertain, although studies in transgenic mice suggest that it might (18–20).

Variability in responsiveness to dietary soy protein has so far received scant attention and little is known about its causes. Because more is known about conditions influencing the individual total and HDL-C response to dietary fat and cholesterol, the relevance of those factors to the present observations are examined below. Varying dietary compliance (21), random fluctuations in lipoprotein concentrations in studies using single blood samples to determine dietary effects (22), differences in adiposity among subjects (23), and differences in the composition of the baseline diet (24) all seem irrelevant to the present study for the following reasons: 1) all the men had excellent dietary compliance as indicated by marked lipemic effects when changing from the self-selected to the formula diets, 2) repeated blood samplings eliminated the effects of random fluctuations of plasma concentrations, 3) all subjects were nonobese, and 4) the casein diet, which served as the baseline diet for determination of the effects of soy protein, was the same for all subjects. Whether differences in composition of the men’s self-selected diets might have affected the response to soy protein remains uncertain because our information on this point is inadequate for firm conclusions.

The degree of LDL reduction with low-fat diets has been found to correlate with the LDL-C concentration at baseline (3). In this study we likewise found a significant correlation between the decrease in LDL-C with the soy-protein diet and the LDL concentration with the casein diet (r = 0.76, P < 0.02), in agreement with previous studies of the effects of soyprotein (6). The change in HDL-C with a low-fat diet was negatively correlated with the HDL concentration with the basal diet (3), but here we found no correlation between the HDL concentration on the casein diet and the increase of HDL on the soy diet. Weight changes have been known to modify the LDL responsiveness to low-fat diets: weight gain reduced the LDL-lowering effect whereas weight loss magnified it (25). In this study, the overall weight changes were modest and similar with both the casein and soy-protein diets and so presumably did not contribute to the variable response of LDL-C to soy protein. There was one exception, however, because one subject lost 3.5 kg with the casein diet and maintained his body weight with the soy diet. Because of the known effect of weight loss on LDL, we favor the interpretation that the casein diet plus weight loss had a greater LDL-lowering effect than did the soy diet without weight changes, rather than the alternative interpretation that the soy diet in this subject had a paradoxical LDL-lowering effect. Thus, the above-mentioned conditions affecting individual LDL responsiveness to low-fat diets do not explain much of the variable responsiveness to soy protein in the present study. We therefore tend to believe that the individual responsiveness to soy protein was determined largely by intrinsic and probably genetic factors.

One intrinsic factor that might be important in the heterogeneous response to dietary soy protein is the variability in the lipemic response to dietary cholesterol, because some individuals respond to a high intake of cholesterol with an elevated plasma cholesterol concentration, whereas others respond to a much lesser extent or not at all (24). We found previously that the LDL-lowering and HDL-lowering effects of soy protein depended on a certain cholesterol intake, because diets otherwise identical in composition but virtually free of cholesterol showed no difference in the lipemic effects of soy protein and casein (8, 26). Because marked individual differences exist in sensitivity to dietary cholesterol in terms of total and HDL-C response (24), it appears plausible that differences in sensitivity to dietary cholesterol might explain parts or all of the variability in response to dietary soy protein. Of the several hypotheses proposed to explain the variable individual sensitivity to dietary cholesterol, one is based on the role of apo E and its different isoforms that result from allelic variation of the APOE gene. Although some investigators have found that the different apo E isoforms explain variations in the lipemic response to NCEP diets (3), others have not (2). We examined the APOE genotypes of our subjects (5 had E3/4, 3 had E3/3, and 1 had E2/3) and found no significant difference in the LDL reduction due to the soy-protein diet between the different genotypes by ANOVA.
The protocol of this study had the advantages of precise control of composition and intake of the liquid-formula diets, a detailed study of the lipemic response of each individual subject, and a homogeneous study population of normolipemic, nonobese men. The study also had the following limitations if the aim was to apply the findings to the general use of soy protein in preventive diets: 1) the small number of subjects and the absence of women made the study population highly selected and not representative of the general population, 2) the duration of the dietary periods may have been too brief to allow for adaptation to the diets that might take place over extended periods of time, 3) the use of liquid formulas containing only one kind of protein together with the total absence of fibers could have modified the lipemic response compared with a solid-food diet, and 4) the cholesterol content of the diet, although not different from that consumed by a considerable portion of the background population, was higher than that recommended for cholesterol-lowering diets.

Despite the limitations of our present knowledge about the usefulness and safety of dietary soy protein in reducing cardiovascular lipid risk factors, the potentially beneficial effects clearly indicate both the need and justification for more clinical and experimental studies.

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REFERENCES