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Differential effects of dietary flaxseed protein and soy protein on plasma triglyceride and uric acid levels in animal models

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Abstract

The effect of dietary soy protein and flaxseed meal on metabolic parameters was studied in two animal models, F344 rats with normal lipid levels and obese SHR/N-cp rats with elevated levels of cholesterol and triglyceride. The rats were fed AIN 93 diet differing only in the source of protein. The rats were fed either 20% casein, 20% soy protein or 20% flaxseed meal. Plasma was analyzed for cholesterol, triglyceride, uric acid, blood urea nitrogen (BUN), creatinine and total protein. In both strains of rats, flaxseed meal significantly decreased plasma cholesterol and triglyceride concentrations. The effect of soy protein on lipids was not as striking as that of flaxseed meal. Flaxseed meal also lowered uric acid in F344 rats and BUN in SHR/N-cp rats. Since cholesterol, triglyceride and uric acid are independent risk factors for cardiovascular disorders, our data show that both flaxseed meal and soy protein may have beneficial effects. Which chemical constituent(s) of flaxseed meal or soybean is (are) responsible for the beneficial effects need to be identified.

Keywords: Cholesterol; Flaxseed; Hypertriglyceridemia; Hyperuricemia; Plasma lipids; Soy protein

1. Introduction

In recent years, there has been a great deal of interest in the effects of soy foods on human health, and especially their potential impact on cardiovascular disease. This was further spurred by the approval by the United States Food and Drug Administration in October 1999 of a health claim by food manufacturers that consumption of 25 g of soy protein, as part of a diet low in saturated fat and cholesterol may help reduce the risk of coronary heart disease [1]. This claim is based on accumulated evidence from numerous studies in humans and animals showing that soy protein reduces serum cholesterol, which is one of the major risk factors of cardiovascular disease [2–8].

Recent studies in normal and hypercholesterolemic human subjects confirm the reduction in plasma total and non-HDL cholesterol without significant effect on HDL cholesterol and triglycerides by soy protein containing isoflavones [9–12]. However, less attention has been paid to the effects of soy protein and other plant-derived proteins on serum triglyceride and uric acid. This is of interest, since both hypertriglyceridemia and hyperuricemia are known to have an independent relationship with other cardiovascular risk factors, such as obesity, type II diabetes mellitus, and hypertension, which are major components of the insulin resistance syndrome [13–16]. Moreover, an elevated serum triglyceride level is not only a common lipid abnormality in free-living individuals but also an independent risk factor for coronary artery disease [17,18].

Besides soy, flaxseed (also known as linseed) has also received increasing attention for its potential role in the prevention of cardiovascular disease because this food ingredient, when used as a supplement in the diet, has been shown to reduce total cholesterol and low-density lipoprotein (LDL) cholesterol in some studies in humans and animals [19–22]. Flaxseed, like soybean, is a rich source of protein. The amino acid pattern of flaxseed protein is closely similar to that of soy protein, which is viewed as one of the

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most nutritious of plant proteins [23–25]. However, data comparing the nutritional effects of these two sources of dietary protein and their impact on serum triglyceride and uric acid are lacking.

We, therefore, compared the effects of dietary flaxseed protein and soy protein on plasma lipids in two different animal models, namely, the Fischer 344 (F344) rat with normal lipid levels and the spontaneously hypertensive/NIH-corpulent (SHR/N-cp) rat with dyslipidemia. The F344 rat is a rodent model for aging research and has been frequently used for studies of nutritional influences on growth and age-related processes [26,27]. The SHR/N-cp rat is a genetic animal model of obesity and type II diabetes mellitus and exhibits many features characteristic of the insulin resistance syndrome, namely glucose intolerance, hyperinsulinemia, hypercholesterolemia, hypertriglyceridemia, and mild hypertension [28–31]. We have previously shown that feeding sucrose as compared to starch as the source of dietary carbohydrate magnifies the glucose intolerance and hypertriglyceridemia in these animals [28,31]. Thus, we specifically sought to determine whether changing the source of protein intake by supplementing either soy protein or flaxseed protein in the diet has a different impact on plasma triglyceride and uric acid levels in this model.

2. Materials and methods

2.1. Animals

Male F344 rats and obese SHR/N-cp rats were used for the experiments. F344 rats were obtained from Harland Laboratories. Obese SHR/N-cp rats were obtained from the National Institutes of Health at approximately 5–6 weeks of age. At this age, obesity is already evident in SHR/N-corpulent (cp/cp) rats as indicated by higher body weight than their lean littermates and increased abdominal girth. The experimental protocol was approved by the Institutional Animal Care and Use Committees of the George Washington University, Washington, D.C., and by the Agricultural Research Service, U.S. Department of Agriculture, Beltsville, Maryland. All animals were housed individually in stainless steel wire cages with controlled temperature (21 to 25°C) and relative humidity (40 to 50%) and maintained on a reverse 12-hr dark (0900 to 2100 hr) and light (2100 to 0900 hr) cycle.

2.2. Diets and experimental protocol

All animals were provided with Purina rat chow and were maintained on this diet for 2 weeks until 8–10 weeks of age. Food and water were consumed ad libitum. The rats were then randomly divided into three groups and fed AIN-93 diet [32] supplemented with flaxseed protein, soy protein, or casein, as the sole source of dietary protein. Group 1 rats received 20% flaxseed meal; group 2 rats received 20% soy protein concentrate; and group 3 rats received 20% casein. Soy protein concentrate contained 234.9 μg/g total isoflavones while flaxseed meal contained 17.0 μg/g secoisolariciresinol diglucoside. With the exception of the protein source, all three diets were identical and contain similar amounts of protein, fat, carbohydrates, minerals, and vitamins. Corn oil was substituted for soybean oil in AN-93 diets as soybean oil may complicate effect of soy protein. The diets were adequate for all macro- and micronutrients. All diets contained (g/kg) cornstarch, 405.692; dextrinized cornstarch, 155; sucrose, 100; corn oil, 40; cellulose, 50; mineral mix (AIN-93M-MX), 35; vitamin mix (AIN-93-VX), 10; L-cystine, 1.8; choline bitartrate, 2.5; and tert-butylhydroquinone, 0.008. Casein and L-cystine were purchased from Sigma Chemicals, St. Louis, MO. Soy protein concentrate and flaxseed meal were obtained gratis from Archer Daniels Midland, Decatur, IL. Tert-butylhydroquinone was purchased from Aldrich Chemical Co., Milwaukee, WI. All other ingredients were purchased from Dyets Inc., Bethlehem, PA. There were eight rats per group for F344 rats and ten rats per group for SHR/N-cp rats. All animals were fed the experimental diets for 6 months and weighed biweekly throughout the study. At the end of the feeding period, animals were sacrificed by decapitation under carbon dioxide anesthesia. Blood samples were collected in EDTA and Trasylol and plasma was separated for subsequent biochemical analyses.

2.3. Analytical measurements

Plasma total cholesterol, triglyceride, creatinine, BUN, and uric acid were measured enzymatically using Alcyon analyzer (Abbott Laboratories) using ATAC reagents and kits (Abbott Laboratories).

2.4. Statistical analysis

Results are expressed as mean $\pm$ standard error of the mean. Comparisons between groups were made using one-way analysis of variance. Differences between mean values in the three groups were tested by Student's t-test. Differences were considered significant when the p value was less than 0.05.

3. Results

3.1. Experiments in F344 rats

Table 1 presents the results of experiments obtained after 26 weeks of feeding the respective diets in F344 rats. All rats gained weight throughout the study and the mean body weight did not differ among the three diet groups. Nature of dietary protein had no significant effect on total plasma protein concentration.

Table 1
Body weight and fasting levels of plasma total protein, total cholesterol, triglyceride, uric acid, BUN and creatinine in F344 rats fed a diet supplemented with either flaxseed meal, soy protein, or casein

Parameter	Flaxseed meal	Soy protein	Casein
Final body weight, g	296 ± 8	317 ± 11	320 ± 5
Total protein, g/L	71.9 ± 0.9	72.7 ± 1.6	73.7 ± 2.2
Total cholesterol, mmol/L	1.52 ± 0.03[a]	1.61 ± 0.07[a]	2.40 ± 0.09[b]
Triglyceride, mmol/L	0.96 ± 0.15[a]	1.43 ± 0.27[a,b]	1.83 ± 0.18[b]
Uric acid, μmol/L	180 ± 13[a]	201 ± 16[a]	238 ± 12[b]
BUN, mmol/L	4.76 ± 0.2	5.15 ± 0.2	5.01 ± 0.2
Creatinine, μmol/L	17.5 ± 1.9[a]	21.8 ± 2.1[a,b]	25.7 ± 2.3[b]

Values are means ± SEM of 8 rats.
Values with different superscripts within a row are significantly different at $P < 0.05$.

Plasma total cholesterol concentrations were significantly lower in rats fed flaxseed meal and soy protein than in those fed casein, while triglyceride concentrations were significantly lower only in rats fed flaxseed meal compared to those fed casein. It is important to note that triglyceride levels were reduced by about 48% in rats fed flaxseed meal and 22% in rats fed soy protein, compared to those fed casein. Plasma uric acid levels were also significantly lower in rats fed flaxseed meal and soy protein than in those fed casein. BUN concentrations were not different among the diet groups. Plasma creatinine was significantly lower in rats fed flaxseed meal compared to rats fed casein. Plasma creatinine in rats fed soy protein was not significantly different from those fed flaxseed meal or casein.

3.2. Experiments in obese SHR/N-cp rats

Table 2 summarizes the results of similar experiments performed in obese SHR/N-cp rats fed diets for 6 months. Obese rats showed distinctly higher plasma concentrations of total cholesterol and triglyceride, when compared to levels observed in F344 rats. After 6 months of feeding, plasma total protein concentrations were significantly lower in obese rats fed flaxseed meal and soy protein, compared to those fed casein. Total plasma cholesterol was lower by 41% ($p < 0.05$) in rats fed flaxseed meal and by 15% in rats fed soy protein; whereas plasma triglyceride was reduced by 37°/o in rats fed flaxseed meal and by 19% in rats fed soy protein. Plasma uric acid was reduced by 12% in rats fed flaxseed meal but was higher in rats fed soy protein compared to those fed casein. BUN was significantly lower in rats fed flaxseed meal but not in rats fed soy protein compared to those fed casein; whereas plasma creatinine was similar among the three diet groups.

Table 2
Body weight and fasting levels of plasma total protein, total cholesterol, triglyceride, uric acid, BUN and creatinine in obese SHR-N-cp rats fed a diet supplemented with either flaxseed meal, soy protein, or casein

Parameter	Flaxseed meal	soy protein	Casein
Final body weight, g	574 ± 17	547 ± 18	558 ± 18
Total protein, g/L	76.4 ± 1.2[a]	77.9 ± 1.6[a]	85.9 ± 2.6[b]
Total cholesterol, mmol/L	3.53 ± 0.05[a]	5.09 ± 0.09[b]	6.01 ± 0.10[b]
Triglyceride, mmol/L	4.09 ± 0.34[a]	5.23 ± 0.92[a,b]	6.48 ± 0.46[b]
Uric acid, μmol/L	182 ± 14	251 ± 40	206 ± 41
BUN, mmol/L	6.60 ± 0.4[a]	7.33 ± 0.4[a,b]	8.20 ± 0.7[b]
Creatinine, μmol/L	28.0 ± 1.9	29.9 ± 2.4	27.5 ± 3.3

Values are means ± SEM of 10 rats.
Values with different superscripts within a row are significantly different at $P < 0.05$.

4. Discussion

The present study demonstrates that substitution of flaxseed meal or soy protein for casein (an animal protein) in the diet significantly reduces both plasma total cholesterol and triglyceride levels in F344 rats with normal lipid levels as well as in obese SHR/N-cp rats with marked hyperlipidemia. However, what is more striking in this study was the pronounced reduction of plasma triglyceride by flaxseed meal, which was observed in both strains of animals. In both strains, flaxseed meal supplementation produced about twice as much reduction in triglyceride concentration compared to soy protein, indicating that flaxseed meal has a greater hypotriglyceridemic effect than soy protein. It should be noted here that the diets used were identical, except for the protein source. The diets contained approximately 20% protein, 65% carbohydrates, 5% fat in the form of corn coil, and 5% fiber with similar amounts of minerals and vitamins. Nevertheless, regardless of the dietary protein source, there were no significant differences in body weight gain among the animals that received the three different protein supplements. This was true for both the F344 rats aud SHR/N-cp rats. Therefore, it seems unlikely that differences in energy or fat intake among the animals can entirely account for the different effects of flaxseed meal and soy protein on plasma triglyceride.

Our results complement a recent study in male Fischer 344 rats made hypertriglyceridemic by feeding them a 13-cis retinoic acid-containing diet with casein as the protein source [33]. In this study, the isonitrogenous replacement of 50% of dietary casein with soy protein isolate resulted in significant reductions in serum concentrations of total cholesterol and triglyceride.

Numerous studies in experimental animals and humans have documented the hypolipidemic effects of soy protein [2–12]. By contrast, there have been only a few studies that have examined the effects of flaxseed on serum lipids. In studies of hypercholesterolemic rabbits, Prasad showed that dietary flaxseed reduced total and LDL cholesterol and prevented hypercholesterolemic atherosclerosis [21]. However, Babu et al. obtained different results in their studies of young female Sprague-Dawley rats [34]. These investigators fed isoenergic modified AIN diet supplemented with either whole ground flaxseed or defatted flaxseed meal in

these animals for 56 days but found no significant effect on total and HDL cholesterol. In this study, plasma triglyceride was increased by defatted flaxseed meal, but was not changed by ground flaxseed. Cunnane et al. showed that consumption of 50 g of flaxseed/day for 4 weeks resulted in a slight but significant reduction in LDL cholesterol in young healthy humans [19]. Bierenbaum et al. showed that flaxseed supplementation in the form of either a flaxseed-containing bread or 15 g of ground flaxseed for 3 months resulted in significant reductions in serum total and LDL cholesterol with no change in HDL cholesterol in human subjects with hyperlipidemia [20]. Similar results were obtained by Jenkins et al. with partially defatted flaxseed in hypercholesterolemic subjects without obesity and diabetes [22]. The results of these studies suggest that the effects of flaxseed may vary according to the models, the age at which flaxseed was initiated, and the pre-existing level of serum lipids. Unfortunately, serum triglyceride levels were not measured in hyperlipidemic subjects. Moreover, some of these studies have used either ground flaxseed or partially defatted flaxseed, which may have confounded the results.

Whole flaxseed contains approximately 41% fat, 28% fiber, 21% protein, 6% other carbohydrates and 4% ash of the seed weight [25]. It is particularly rich in alpha-linolenic acid (e.g., about 57% of the total fatty acid in flaxseed), which has lipid-lowering properties [35–37]. Thus, the reduction of blood cholesterol by dietary flaxseed in these studies may be due in part to alpha-linolenic acid present in the whole seed. However, the flaxseed meal given in the present study has been fully defatted and does not contain alpha-linolenic acid. Therefore, it is highly unlikely this fatty acid was responsible for the observed reduction of plasma cholesterol and triglyceride with flaxseed meal in the present study. This is supported by the observations of Prasad et al. who showed that a diet with a CDC-flaxseed (Type II flaxseed) with very low alpha-linolenic acid content reduces total serum cholesterol and LDL cholesterol in rabbits [38].

An interesting finding in this study is the reduction of plasma uric acid with flaxseed meal, which was observed in both F344 and obese SHR/N-cp rats. To our knowledge this is the first report showing a hypouricemic effect of dietary flaxseed. How flaxseed ingestion lowers plasma uric acid is unknown and needs further investigation. On the other hand, the effect of soy protein on plasma uric acid was variable. In fact, plasma uric acid was even higher in obese SHR/N-cp rats fed soy protein compared to their counterparts fed casein or flax meal. An earlier study by Garrel et al. suggested that soy protein increases serum uric acid [39]. In this study, the investigators compared the acute effects of ingestion of 80 g of soy protein to casein and lactalbumin on serum uric acid concentration in healthy human subjects and observed that serum uric acid decreased after ingestion of casein and lactalbumin but increased after soy protein consumption. The fact that urinary urate excretion increased similarly after ingestion of each of the three proteins suggested that the acute rise in serum uric acid with soy protein was not due to a reduction in urate excretion. In the present study, the increase in uric acid with soy protein was accompanied by a significant decrease in BUN in obese SHR-cp rats and a decrease in plasma creatinine in F344 rats. Therefore, it is also highly unlikely that the increased serum uric acid with soy is related to a decreased urinary urate excretion due to a decrease in renal function. Renal clearance studies are needed to further elucidate the mechanism of the changes in plasma uric acid levels observed with soy protein and flaxseed.

The components present in soy protein or flaxseed meal responsible for their hypolipidemic effects have yet to be identified. It is well known that soy protein is a rich source of isoflavone phytoestrogens [40], which exert hypocholesterolemic effects in animals and humans [2–12,41]. Isoflavones may have contributed to the hypolipidemic effects of soy protein used in the present study. It should also be noted that the soy protein itself, may have direct effects on lipid metabolism. Flaxseed is the richest source of lignans [42], which have also been reported to have antioxidative and hypolipidemic effects [43]. Indeed, secoisolariciresinol diglucoside (SDG), a major lignan isolated from flaxseed, has been shown to reduce serum total cholesterol and LDL in hypercholesterolemic rabbits [44]. Whether SDG has an effect on serum triglyceride and uric acid remains to be determined.

In summary we have shown for the first time that dietary supplementation with flaxseed meal and soy protein reduces both plasma cholesterol and triglycerides in normolipidemic F344 rats and markedly improved both hypercholesterolemia and hypertriglyceridemia in obese SHR/N-cp rats.

Flaxseed meal supplementation appeared to reduce plasma triglyceride levels to a greater extent than soy protein in these two strains of animals. In addition, flaxseed meal also caused a significant reduction in serum uric acid, whereas soy protein appeared to have the opposite effect. Which dietary component(s) present in flaxseed meal or soy protein is (are) responsible for the observed hypolipidemic effects are not clear. The marked hypotriglyceridemic and hypouricemic effects of flaxseed meal may have important therapeutic implications in patients with hypertriglyceridemia and hyperuricemia and deserve a further study in humans with these disorders.

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