Comparison of the Mechanisms Proposed to Explain the Hypocholesterolemic Effect of Soybean Protein versus Casein in Experimental Animals

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Summary Diets containing soybean protein generally induce low levels of serum cholesterol in experimental animals, when compared with diets containing casein. The hypocholesterolemia in animals fed soybean protein is associated with increased rates of fecal excretion of bile acids and neutral steroids, low liver cholesterol concentrations, increased numbers of hepatic apo B/E receptors, increased rates of hepatic cholesterol synthesis, increased rates of bile acid synthesis and decreased rates of lipoprotein cholesterol output by the liver. In this communication the development of the hypocholesterolemia induced by soybean protein is described. The various mechanisms that have been proposed to explain the hypocholesterolemic effect of soybean protein are scrutinized, compared and contrasted.

Key Words dietary proteins, soybean protein, casein, cholesterol metabolism

Introduction

It has been published repeatedly by various groups of investigators that the type of protein in the diet of experimental animals affects serum cholesterol concentrations. In most studies, the effects of casein and soybean protein are compared. Rabbits are extremely susceptible to the type of dietary protein and show marked increases in serum cholesterol concentrations after feeding diets containing casein, whereas in rabbits fed soybean protein serum cholesterol concentrations remain low. As to the metabolic basis for the hypocholesterolemic effect of soybean protein versus casein, various concepts have been advanced. In this communication, these concepts will be scrutinized.

The type of dietary protein may influence various metabolic parameters, including hormonal status. Such parameters are not necessarily related to cholesterol metabolism, and if they are, they may even be the result of altered serum cholesterol concentrations. In order to establish cause–protein-induced changes in metabolic parameters and effect–protein-induced changes in serum cholesterol concentrations–relationships convincingly, there are certain requirements to be met. Causative metabolic changes should occur prior to changes in serum cholesterol concentrations. This is an experimentally feasible prerequisite as for serum cholesterol concentrations to reach a new steady state after replacement of dietary casein by soybean protein, it generally takes at least 14 days.

It should be possible to link putative causative metabolic changes to changes in serum cholesterol concentrations, at least within our current knowledge of the pathways of cholesterol metabolism and their regulation. As to causative metabolic changes, one should distinguish between primary and secondary features. The cholesterol lowering induced by soybean protein can be the result of a cascade of different effects.

Non-protein components and serum cholesterol

Even the purest preparations of casein and isolated soybean protein contain up to 20% of non-protein material. Various non-protein components have been suggested to be responsible for the differential cholesterolemic effect of dietary casein and soybean protein. It is essential to exclude such effects prior to studying possible mechanisms by which the protein component of protein preparations could influence serum cholesterol concentrations. Otherwise, thinking about possible mechanisms would not be opportune.

The hypocholesterolemic effect of soybean protein has been attributed to substances in the preparation such as fiber, phytosterols, phytic acid...
and saponins. However, there is no experimental evidence that these substances indeed play a major role, if any. Samman and Roberts (1) have put forward that casein-induced hypercholesterolemia in rabbits is due to low zinc intakes as casein contains less zinc than soybean protein. However, inspection of the data revealed that after increasing dietary zinc in the casein diet equal to the zinc content of the soybean diet, group mean serum cholesterol concentrations still clearly increased in rabbits fed casein when compared with those fed soybean protein. The casein-induced hypercholesterolemia did not reach statistical significance which can be explained by the small numbers of animals used and large between-animal variation in cholesterolemic response.

The amount and type of fat in the protein preparations could be important. The amount of fat in the cholesterol-free diet of rats must be as little as 1% (w/w) in order to demonstrate a hypocholesterolemic effect of soybean protein versus casein (2). It could be suggested (3) that this actually represents a fat effect. Since soybean protein isolate contains up to 5% of fat, and casein about 1%, animals fed the former protein received more fat, this soybean fat being rich in polyunsaturated fatty acids. Perhaps, the animals fed the low-fat casein diet had a marginal deficiency of polyunsaturated fatty acids, which amplified the protein effect on serum cholesterol. This would explain why the addition of extra corn oil to the diets counteracted the differential cholesterolemic effect to feeding soybean protein and casein (2). After feeding rabbits on semipurified diets containing either casein or soybean protein, but carefully balanced for residual fat and cholesterol in the protein preparations, the differential cholesterolemic effect of the proteins became very small (4). However, when cholesterol was added to the diets to a final concentration of 0.15%, a marked hypocholesterolemic effect of soybean protein could be demonstrated (4). This indicates that the protein component of the protein preparations specifically influences serum cholesterol concentrations.

**Fecal excretion of bile acids and neutral steroids**

In rabbits, the fecal excretion of cholesterol and its bacterial metabolites (5, 6) and of bile acids (6, 7) is decreased almost immediately after soybean protein is replaced by casein, and before the concentration of serum cholesterol is increased. This suggests that effects of the dietary proteins on the enterohepatic circulation of bile acids and cholesterol is at least one of the primary features of protein-induced changes in serum cholesterol concentrations. Dietary proteins do not influence the biliary efflux of bile acids and neutral steroids (8, 9). This would imply that soybean protein versus casein affects intestinal absorption of cholesterol and bile acids.

There is direct evidence that soybean protein impairs cholesterol absorption (10–12). There is only indirect evidence that soybean protein inhibits the absorption of bile acids: as mentioned above, in animals fed soybean protein, bile acid excretion in feces is increased. Furthermore, in pigs fitted with re-entrant cannulas at the end of the ileum and in the cecum, the ileal output of bile acids is higher and the uptake of bile acids by cecum and/or colon is lower in animals fed soybean protein compared with their counterparts fed casein (13). Thus, it would appear that soybean protein inhibits the (re-)absorption of cholesterol and bile acids, which in turn leads to increased excretion of neutral steroids and bile acids in feces.

**Hepatic bile acid and cholesterol metabolism**

A decreased absorption of bile acids induced by soybean protein will cause lower concentrations of bile acids in the portal vein, and consequently in the liver. Decreased hepatic concentrations of bile acids in animals fed soybean protein have not been demonstrated directly. However, by diminishing feed-back inhibition they should lead to stimulation of 7α-hydroxylase, the enzyme catalysing the first step in the conversion of cholesterol to bile acids. Indeed, this has been demonstrated experimentally, though only indirectly. In rabbits fed soybean protein the oxidation of intravenously administered [26-14C]cholesterol is increased (10), and in rats fed soybean protein there is increased conversion of radioactive serum cholesterol into radioactive fecal bile acids (11). Stimulated bile acid synthesis in animals fed soybean protein would result in the decreased amounts of cholesterol in liver that have been observed frequently (10, 14). The decreased absorption of cholesterol in animals fed soybean protein, may cause a decreased flux of cholesterol carried by chylomicron remnants to the liver. This effect will also lead to decreased liver cholesterol.
HYPOCHOLESTEROLEMIC ACTION OF SOYBEAN PROTEIN

The decreased liver cholesterol concentrations in animals fed soybean protein may elicit several reactions: stimulation of de novo cholesterol synthesis, up regulation of apo B/E receptor activity and decreased output of lipoprotein cholesterol. Stimulated hepatic cholesterol synthesis in animals fed soybean protein has been shown directly by increased activities of liver microsomal 3-hydroxy-3-methylglutarylcoenzyme A reductase (11, 15, 16) and indirectly by increased turnover of injected radioactive cholesterol (10, 11), which reflects increased whole-body cholesterol synthesis. Increased hepatic lipoprotein receptor activity in animals fed soybean protein has been demonstrated directly by decreased binding of beta-very low density lipoproteins (β-VLDL) to liver membranes (15), and indirectly by increased fractional catabolic rates of VLDL apoprotein B (17), whereas VLDL apoprotein B production was not affected (17). Decreased hepatic lipoprotein cholesterol output in animals fed soybean protein has been shown both directly and indirectly. Perfused livers from donor rats fed soybean protein secreted less cholesterol into the perfusate than did livers from rats fed casein (8). Indirect evidence comes from a study showing that in rats fed soybean protein and injected with Triton X-100 so as to block extrahepatic breakdown of lipoproteins, there was less accumulation of cholesterol in serum than in rats fed casein (18).

Development of hypocholesterolemia in animals fed soybean protein and attainment of a new steady state

The development of hypocholesterolemia induced by dietary soybean protein can be summarized as follows. After substitution of soybean protein for casein there is a decreased influx of cholesterol and bile acids from the intestine to the liver, causing a decrease in the amount of cholesterol in liver. The liver responds by increasing the number of apo B/E receptors, decreasing lipoprotein cholesterol output and stimulating cholesterol and bile acid biosynthesis. The first two effects account for the decrease in serum cholesterol concentration until a new steady state is reached. In this new steady state, the decreased absorption of bile acids and increased loss with feces is compensated for by increased conversion of cholesterol to bile acids. The decreased absorption of cholesterol and resulting increased loss with feces as well as the increased conversion of cholesterol into bile acids are compensated for by the enhanced rates of cholesterol biosynthesis. Thus, in animals fed soybean protein, low serum cholesterol concentrations are associated with high rates of cholesterol turnover.

Interaction between dietary soybean protein and intestinal steroids

The reasoning above implies that the first step in cholesterol lowering induced by soybean protein versus casein involves inhibition of cholesterol and bile acid absorption. Thus, the primary effect of soybean protein is at the level of the intestine. However, there is no direct evidence that soybean protein depresses bile acid absorption. It could be argued that the primary effect of soybean protein is stimulation of hepatic bile acid synthesis which then leads to increased fecal excretion of bile acids and decreased liver cholesterol concentrations. However, it is difficult to see how increased biliary output of bile acids would lead to the observed inhibition of cholesterol absorption in animals fed soybean protein.

Saeki et al. (19) have shown that neither jejunectomy nor ileectomy in rats affects the response of serum cholesterol to soybean protein versus casein. This would exclude that interactions between dietary proteins and intestinal steroids are responsible for their cholesterolemic effects. Two comments should be made here. First, in the studies of Saeki et al. (19), only 1% of corn oil as the sole source of fat was added to the base diet. Using such diets containing either 20% of soybean protein or casein entails a considerable risk of the fat type in the protein preparations determining the cholesterolemic response to the diets. This problem has been addressed above. Secondly, the studies with jejunectomized or ileectomized rats do not exclude interactions between dietary proteins and bile acids at the level of the large intestine. Studies with cannulated pigs have shown that soybean protein depresses bile acid absorption in cecum and/or colon (13).

Mechanisms with primary features at the intestinal level

Two concepts have been proposed to explain inhibition of intestinal steroid absorption in animals fed soybean protein. One concept is based on the
fact that soybean protein is less digestible than casein and purports that undigested protein binds bile acids and neutral steroids and thereby reduces steroid (re-)absorption. This concept is supported by in vitro experiments (20), and by the observation that mice fed soybean protein had increased amounts of nitrogen and bile acids in their intestine compared with animals fed casein (21). Furthermore, Sugano et al. (22) have isolated a high-molecular weight fraction from soybean protein that stimulates fecal bile acid and neutral steroid excretion and is more hypocholesterolemic than equal amounts of intact soybean protein when fed to rats. There is indirect evidence that the high-molecular weight fraction of soybean protein, which is a hydrophobic peptide, may bind bile acids in the intestine (23). However, the high molecular weight fraction of soybean protein is rich in saponins (22) which may have contributed to the cholesterol lowering activity of this fraction. The digestibility theory is not supported by the observation that formaldehyde treatment of casein, which lowers its digestibility, does not lower serum cholesterol concentrations in cholesterol-fed rats (24) and rabbits (25).

The second concept to explain inhibition of intestinal steroid absorption in animals fed soybean protein versus casein, relates to the differential phosphorylation state of the two proteins (26). Casein, when compared with soybean protein, is a highly phosphorylated protein as about 40% of the serine residues in casein are esterified with phosphate. The phosphorylation concept can be described as follows. Dietary casein and phosphopeptides derived from it remove calcium from the calcium phosphate sediment in the intestine and thereby dissolves the sediment and solubilizes phosphate. This reduces the number of binding sites on the sediment for bile acids and increases the concentration of free bile acids. As a result, bile acid absorption increases. Put in another way, in animals fed soybean protein, more intestinal bile acids are bound to the calcium phosphate sediment (27). As would be anticipated, dietary casein stimulated the absorption of phosphate (28). Moreover, the hypercholesterolemia in rabbits induced by feeding casein was partly counteracted by addition of calcium carbonate to the diet (28). Addition of calcium carbonate to the diet will prevent dissolution of the calcium phosphate sediment as induced by phosphopeptides.

The phosphorylation concept would explain why cholesterol absorption is decreased in animals fed soybean protein, and it also explains species differences in the sensitivity to dietary proteins. As to the former, it is reasonable to assume that changes in the efficiency of absorption of cholesterol and bile acids go hand in hand: the two steroids are absorbed in the form of mixed biliary micelles. As to species-differences, rabbits are more sensitive to the type of dietary protein than rats. Rabbits, when compared with rats, have low intestinal phosphatase activity, and their bile acids are almost exclusively conjugated with glycine (29). Thus, in rabbits, but not in rats, there is substantial bile acid binding to the intestinal calcium phosphate sediment which can be diminished by the feeding of casein.

The weak point of the phosphorylation concept is that it may be restricted to the comparison of casein and soybean protein, and may not extend to other animal proteins that are not highly phospho-
ylated but do increase serum cholesterol concentrations. Furthermore, it is difficult to see that casein and its phosphopeptides could influence bile acid absorption in the large intestine since proteins are completely digested at this stage. Dietary soybean protein versus casein may inhibit bile acid absorption in cecum and/or colon (13).
ratio being a causative factor in protein-induced cholesterolemia.

Single amino acids (33, 34), including the lysine: arginine ratio (35, 36) in dietary proteins have been proposed to be responsible for the effects of the intact proteins. The involvement of single amino acids is based on studies showing that the addition of amino acids to the diets of experimental animals perturbs serum cholesterol concentrations (33-35). However, there is no metabolic basis for the action of amino acids. Furthermore, it is questionable whether the experimental feeding of amino acids can provide information about the mechanism of action of intact proteins. Digestion and absorption of intact proteins differs from that of single amino acids. Nagata et al. (11) have shown that the feeding of amino acid mixtures simulating soybean protein or casein produced serum cholesterol concentrations in rats that were identical to those seen after feeding the intact proteins. However, the amino acid mixtures and intact proteins had different effects on other aspects of cholesterol metabolism, such as fecal steroid excretion (11).

The lysine: arginine ratio of soybean protein is 0.88 (on a weight basis) and that of casein 2.04. Addition of lysine to soybean protein so as to increase the lysine: arginine ratio to that of casein caused an increase of serum cholesterol concentrations but the concentrations were lower than those seen with casein (35). The addition of arginine to casein lowered serum cholesterol levels but not to values seen with soybean protein (35). Thus, it is unlikely that the lysine: arginine ratio fully explains the differential cholesterolemic effects of soybean protein and casein. Furthermore, increasing the amount of casein in the diet of rabbits further increased serum cholesterol levels (37), while the lysine: arginine ratio obviously remained constant.

It has been shown that the type of dietary protein affects metabolism of polyunsaturated fatty acids in rats. Dietary soybean protein, when compared with casein, increased the ratio of 20:4n-6 to 20:3n-6 fatty acids in liver phospholipids of cholesterol-fed rats (38). Possibly, soybean protein increases the activity of the Δ-5-desaturase enzyme. It is not known whether this effect is related, either as cause or effect, to changes in serum cholesterol concentrations.

Dietary soybean protein, when compared to casein, causes increased serum concentrations of thyroxine (32, 39). Increased concentrations of thyroxine could theoretically explain various characteristics of animals fed soybean protein such as increased hepatic cholesterol synthesis, decreased hepatic lipoprotein cholesterol output, increased number of hepatic apo B/E receptors and increased hepatic bile acid synthesis and consequently increased fecal bile acid excretion (39). However, various questions remain to be settled before changes in serum thyroxine concentrations can be considered a primary feature, rather than a non-causative, associated metabolic change in the hypocholesterolemia induced by soybean protein. How can altered thyroxine levels explain the depressed efficiency of cholesterol absorption in animals fed soybean protein? Can the changes in the various aspects of cholesterol metabolism in animals fed soybean protein be mimicked by administration of thyroxine at concentrations seen after feeding of soybean protein? Do changes in serum thyroxine precede those in serum cholesterol concentrations after replacement of dietary casein by soybean protein?

Conclusion
Many studies have been carried out to explain the hypocholesterolemic effect of soybean protein when compared with casein. There is solid evidence that the increased excretion of bile acids and neutral steroids in feces by animals fed soybean protein is the key to the hypocholesterolemic activity of this protein. Two types of concepts have been advanced so as to describe the increased fecal bile acid excretion in animals fed soybean protein: those with primary features at the intestinal level and those with primary features at the postabsorptive level. Future work should concentrate on both proving and disproving one of the two concepts.

REFERENCES


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