Species-Dependent Responsiveness of Serum Cholesterol to Dietary Proteins

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Substitution of casein for soybean protein in the diet causes high degrees of hypercholesterolemia in rabbits. When rats or humans were fed exactly the same diets, no response of the concentration of serum cholesterol to the type of protein was observed. The hypothesis is put forward that, in rabbits, dietary casein and peptides derived from it—because of their high degree of phosphorylation—inhibit the binding of glycine-conjugated bile acids to insoluble calcium phosphate in the intestinal lumen. As a result, feeding of casein causes an increase in the availability of bile acids, which leads to enhanced absorption of bile acids and cholesterol. Eventually, the concentration of serum cholesterol will be increased. In rabbits this cascade of events occurs because these animals have a relatively low activity of intestinal alkaline phosphatase, and a high ratio of glycine to taurine in conjugated bile acids. Unlike glycine conjugates, taurine-conjugated bile acids do not effectively bind to the intestinal calcium-phosphate sediment. The low activity of intestinal alkaline phosphatase in rabbits secures the high degree of phosphorylation of casein and its peptide products in the small intestine. In contrast with rabbits, rats and humans have high activities of intestinal alkaline phosphatase and a low glycine-to-taurine ratio in conjugated bile acids. Thus the hypothesis presented would explain why rabbits, but not rats and humans, are susceptible to dietary casein with respect to the concentration of serum cholesterol. The relevance of the hypothesis as to the mechanisms underlying the hypercholesterolemic effect of some other dietary proteins is discussed.

Experiments with rabbits fed cholesterol-free, semipurified diets, in which the protein was the only variable, have shown that casein is more atherogenic than soybean protein (1-3). The induction of atherosclerosis in rabbits fed casein was associated with a marked increase in the concentration of cholesterol in the serum, whereas serum cholesterol concentrations of animals fed diets containing soybean protein remained low (4). The response of serum cholesterol to a variety of dietary proteins has been investigated (3,5). These experiments often are interpreted as showing that proteins from animal sources produce hypercholesterolemia in rabbits, whereas diets containing plant proteins result in relatively low concentrations of serum cholesterol. Actually, such a generalization is an oversimplification. First, the distribution of cholesterolemic responses to animal and plant proteins does not show evidence of two discrete subgroups; rather, it has a continuous shape (3). Second, diets containing mixtures of various animal proteins produce markedly lower cholesterol values than do diets containing only one type of animal protein (6).

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In most studies on dietary proteins and serum cholesterol the effects of casein and soybean protein are compared. Both proteins can be obtained in a relatively pure form, and in rabbits they have a significantly different cholesterolemic effect. Figure 1 shows that rabbits may be most susceptible in this respect. Guinea pigs, rats, pigs, hamsters and monkeys also respond to the type of dietary protein, but not as markedly as rabbits do, and only if the diet contains cholesterol. Chickens, calves and mice appear to be rather insensitive to this dietary manipulation.

It should be emphasized that Figure 1 gives only a general impression of the species-dependent susceptibility to dietary proteins. Other components of the diet, such as type of fat and fiber, and certain characteristics of the experimental animals such as age, sex and strain, may also determine susceptibility to proteins in the diet (11). It is clear from Figure 1 that the differential effect of casein and soybean protein becomes more pronounced when background diets enriched with cholesterol are used.

In rats, the amount of fat in the diet also plays a role. When rats were fed cholesterol-free, low-fat diets containing either casein or soybean protein, an elevation of serum cholesterol was observed in animals fed the casein diets when compared with those fed soybean protein diets (12,13). No such effect was seen when the rats were fed similar high-fat diets (12). The situation in rats is even more complicated, because sex and strain may also determine susceptibility to type of dietary

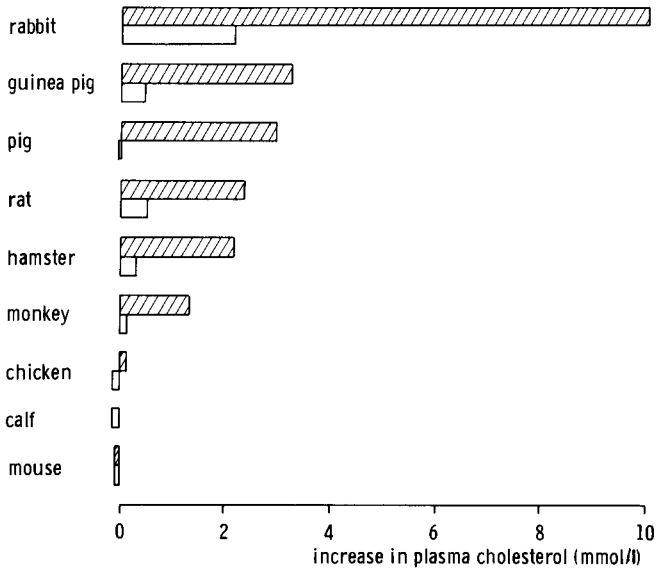

FIG. 1. Increase in plasma total cholesterol of animals fed casein diets versus that of animals fed diets containing soybean protein. Diets with (hatched bars) or without (open bars) cholesterol base were compared. Data compiled by Terpstra et al. (7), and further completed using data taken from Cho et al. (8), Mahfouz-Cercone et al. (9) and Barth et al. (10).

protein. With cholesterol-enriched semipurified diets, a hypercholesterolemic effect of casein was observed in female lean Zucker rats, but not in the males (14). Furthermore, rats fed low-fat cholesterol-free diets showed marked strain differences in the response of serum cholesterol to casein (12,13).

In a number of studies with humans, the cholesterolemic effects of casein and soybean protein have been compared (15-20). It was reported in these studies that casein had either a very small or no effect at all on serum cholesterol, in comparison with that of soybean protein. Thus, at least in short-term studies lasting four to six weeks, adult humans appear to be significantly less susceptible to dietary casein with respect to serum cholesterol concentration than various animal species, especially the rabbit.

It is the aim of this paper to describe, in molecular terms, possible mechanisms underlying the species-dependent susceptibility to dietary casein in comparison with that to soybean protein. First, we present evidence that the differences between species are genuine, and not caused by the different diets fed the various animals.

COMPARATIVE STUDIES USING IDENTICAL DIETS

It could be argued that substances other than protein in the test diets were not the same as those in the diets used in the studies with various animal species, including man. These dietary components may have overridden a possible protein effect in those species showing no differential response. This reasoning is supported by the observation that, in rabbits, an increase in the proportion of essential fatty acids at the expense of saturated fatty acids in the diet causes complete disappearance of casein-induced hypercholesterolemia (21). Thus, only comparative studies in which identical diets are used may give conclusive results as to species-dependent effects.

Figure 2 shows the results of experiments in which rabbits were fed the same diets as either humans or rats. In contrast to the human subjects and the rats, the rabbits displayed a hypercholesterolemic response to casein, when compared to soybean protein. This indicates that there are indeed true differences between species in response of serum cholesterol to dietary casein. The rabbit appears to be extremely susceptible in this respect.

POSSIBLE UNDERLYING MECHANISMS

The intriguing species-dependence of casein-induced hypercholesterolemia must be due to inter-species differences in biochemical parameters. These parameters may only be identified when the cause-and-effect relationships underlying casein-induced hypercholesterolemia are known.

There is evidence that effects of the dietary proteins on the enterohepatic circulation of bile acids and cholesterol are primarily responsible for serum cholesterol concentrations. In rabbits, the fecal excretion of cholesterol and its bacterial metabolites (22) and of bile acids (23) was found to have decreased almost immediately (within two days) after casein had been substituted for soybean protein, and before the concentration of serum cholesterol increased. Thus, in casein-fed animals either the biliary efflux of bile acids and cholesterol is decreased or the absorption of these steroids in the gut is increased. Since casein does not inhibit the biliary efflux of steroids (24), it is most likely that an increase in absorption is the main effect of casein. Indeed, it has been directly demonstrated that casein enhances the absorption of cholesterol, when compared to soybean protein (25,26). In cholesterol-fed pigs, casein has also been shown to stimulate the absorption of bile acids (27).

Thus, casein-induced enhanced absorption of cholesterol and bile acids appears to be a contributing factor in the development of hypercholesterolemia. This implies that the mechanisms underlying the species-dependent susceptibility to casein may be disclosed by focussing on the enterohepatic cycle of cholesterol and bile acids. Various hypotheses have been put forward to explain the hypercholesterolemic effect of casein. We feel that such hypotheses would be most valuable if they also explain the species-dependent susceptibility to dietary casein. Keeping this in mind, we shall discuss the hypotheses advanced.

Kritchevsky (2), and later Park and Liepa (28), have proposed that the cholesterolemic potential of a dietary protein is determined by its lysine-to-arginine ratio. This ratio is more than twice as high in casein as in soybean protein. The lysine-to-arginine ratio has been suggested to influence cholesterol metabolism by affecting the synthesis of apoprotein E (29) or by affecting the ratio of insulin to glucagon in plasma (30,31). However, as yet no evidence for a cause-and-effect relationship has been presented. Furthermore, at least in rats this hypothesis could not be substantiated, as the addition of lysine to the soybean protein diet and of arginine to the casein diet did not affect serum cholesterol levels in two out of three studies (28,30,31). As was to be anticipated, the addition of these amino acids did not affect the fecal excretion of neutral steroids and bile

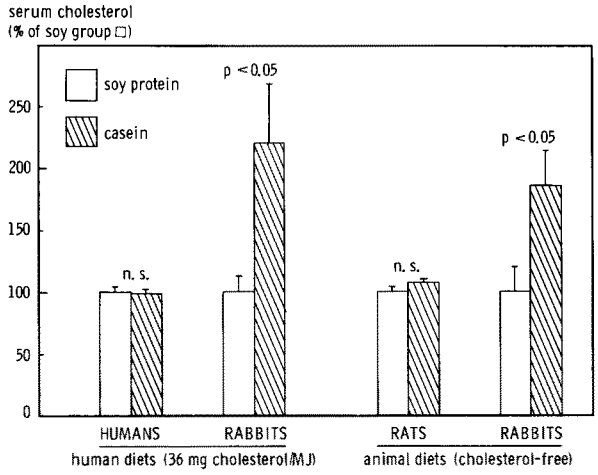

FIG. 2. Effect of casein versus that of soybean protein on serum cholesterol concentrations in humans and rabbits fed similar human diets, and in rats and rabbits fed similar animal diets. The type of protein was the only variable in these diets. Based on data taken from Van Raaij et al. (15) and Van der Meer et al. (23).

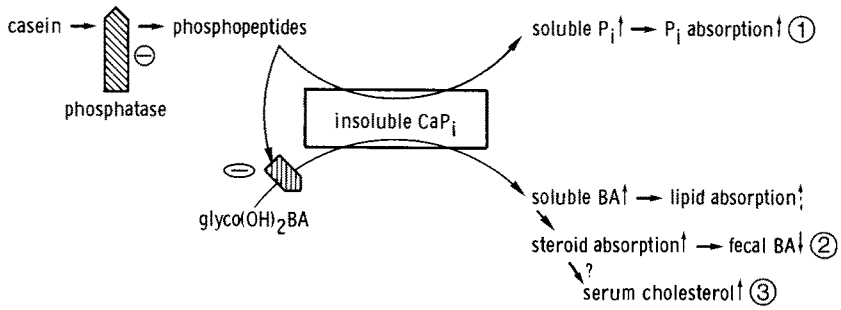

FIG. 3. Hypothetical steps for the interaction of dietary casein with bile acid metabolism in the small intestine.

acids either (31). It is important to note that in these studies with rats low-fat, cholesterol-free diets were used, which is the condition in which a differential effect of casein and soybean protein will be observed in rats (12,13,30,31). When these findings are taken together, it is difficult to see how the lysine-to-arginine hypothesis may explain the differences between species in their response to dietary casein. However, the effects of single amino acids or amino acid combinations may not provide information about the intact proteins, because the order in which amino acids or peptides are released during digestion may not be identical for amino acids and intact proteins. There is some evidence that the ratio of lysine-to-arginine within intact proteins rather than amino acid mixtures is a more powerful determinant of serum cholesterol (32,33).

Another hypothesis purports that the cholesterolemic effect of a dietary protein is related to its digestibility (3,34). Incompletely hydrolyzed proteins would bind bile acids and thus interrupt the enterohepatic cycle of steroids, which ultimately would result in a lower serum cholesterol concentration. With regard to casein-induced hypercholesterolemia, this hypothesis implies that casein is more readily digested than soybean protein. Theoretically, this is difficult to reconcile with the strong concentration dependency of casein-induced hypercholesterolemia (35), because a concentration-dependent increase in digestibility of casein is not very likely to occur. Cross-linking of casein with formaldehyde reduces protein digestibility, but studies in which formaldehyde-treated casein was incorporated into rabbit and rat diets give equivocal results as to serum cholesterol levels (34,36,37). Furthermore, in the small intestine of the rabbit no differences between digestibility of casein and soybean protein could be observed (36). Likewise, in cholesterol-fed pigs the ileal digestibility of casein did not differ from that of soybean protein, whereas casein-fed pigs excreted significantly lower amounts of neutral steroids and bile acids (27). In conclusion, we feel that it is difficult to explain the species dependence of casein-induced hypercholesterolemia by the digestibility hypothesis.

In order to explain the mechanism underlying the differential effect of dietary casein in rabbits, rats and humans, an alternative hypothesis has been formulated (38). According to this hypothesis, which is visualized in Figure 3, the hypercholesterolemic potential of casein is related to its phosphorylation state (about 40% of the serine residues in casein are esterified with phosphate). In the hypothesis it is assumed that in the lumen of the small intestine bile acids and biliary micelles may bind to insoluble calcium phosphate and that this binding is inhibited by dietary casein and phosphopeptides derived from this protein. Consequently, casein would increase the availability of bile acids, which would result in enhanced lipid, cholesterol and bile acid absorption. Eventually, this might cause an increase in serum cholesterol concentration. The following cascade of events may occur when casein in the diet is substituted for soybean protein. First, there is an increase in the influx of cholesterol and bile acids from the intestine to the liver, which causes an increase in the amount of cholesterol in the liver stores. The liver responds by inhibition of the cholesterol biosynthetic pathway, and by suppression of the number of plasma lipoprotein receptors. Both compensatory mechanisms protect the hepatocyte against further accumulation of cholesterol. The suppression of hepatic receptors accounts for an increased lipoprotein cholesterol concentration in the serum until a new steady state is reached in which lipoprotein clearance again equals lipoprotein production. The increased absorption of bile acids, which depresses conversion of cholesterol into bile acids by feedback inhibition, and the increased absorption of cholesterol from the intestine are then compensated for by the diminished rate of cholesterol biosynthesis. However, this regulatory device only protects the animal against further development of hypercholesterolemia.

First, we shall present evidence for the phosphorylation-state hypothesis, as obtained in in vitro experiments. Subsequently, we shall attempt to explain the

TABLE 1

Approximate Values of Intestinal Alkaline Phosphatase Activity and of the Glycine:Taurine Ratio in Conjugated Bile Acids in Various Species

Species	Intestinal alkaline phosphatase (U/g protein)	Glycine:taurine ratio in conjugated bile acids
Rabbit	80	100
Rat	700	0.03
Man	350	3

Based on references 43 to 47.

species-dependent responsiveness to casein on the basis of this hypothesis.

The results of experiments made to put the hypothesis to the test in in vitro systems can be summarized as follows (Fig. 3):

i. Insoluble calcium phosphate binds glycine-conjugated dihydroxy bile acids at pH values above 5.5, that is at pH values found in the small intestine. Similar binding does not occur when the bile acids are conjugated with taurine (39). This would imply that the availability for absorption of taurine-conjugated bile acids is higher than that of the glycine-conjugated ones. Indeed, it has been shown that the efflux from the enterohepatic cycle of taurine conjugates is slower than that of glycine conjugates (40).
ii. Casein also binds the calcium in the calcium-phosphate sediment and thus solubilizes phosphate. The binding of casein prevents the binding of the glycine-conjugated bile acids, and thus would render more bile acids available for absorption. With dephosphorylated casein these effects were not observed (23).
iii. The intestinal enzyme alkaline phosphatase is capable of dephosphorylating casein (41). Thus, a high activity of alkaline phosphatase would prevent the casein-mediated cascade of effects presented in Figure 3.

The in vivo relevance of this model can be tested by studying the time course of its deduced determinants (1 to 3 in Fig. 3) in controlled feeding experiments using different animal species. In rabbits, casein caused stimulation of phosphate and lipid absorption and inhibition of fecal excretion of bile acids (23,42). Almost instantaneously, these intestinal effects of casein reached a new steady state, whereas serum cholesterol continued to increase, which indicates a cause-and-effect relationship. These results are consistent with the hypothetical model because casein-fed rabbits have a low intestinal phosphatase activity, and their bile acids are almost exclusively conjugated with glycine (Table 1). After feeding rats identical cholesterol-free diets, these casein effects were not observed (23). This is in accordance with the high intestinal phosphatase activity and the predominant taurine conjugation of bile acids in this species (Table 1).

In a strictly controlled experiment with humans, Grundy and Abrams (19) observed that casein, when compared to soybean protein, did not affect fecal excretion of bile acids. As was to be anticipated, serum cholesterol concentrations in the normo- and hypertriglyceridemic patients were not affected either (19). In addition, Schuette and Linkswiler (48) reported results suggesting that casein does not influence phosphate absorption in humans.

The model presented in Figure 3 may also explain why casein-induced hypercholesterolemia in rabbits is aggravated when the amount of casein in the diet is increased (35). It may be expected that such a dietary change will decrease the number of binding sites for bile acids on the intestinal calcium-phosphate sediment. On the other hand, it could be suggested that an increased intake of calcium would cause the number of binding sites for bile acids to increase. In agreement with this suggestion, it has been found that dietary calcium counteracts casein-induced hypercholesterolemia in rabbits (42).

Thus, it would appear that the hypercholesterolemic potential of dietary casein will be expressed only in species with a low intestinal phosphatase activity, and with a high ratio of glycine-conjugated to taurine-conjugated bile acids. This explains, in molecular terms, why rabbits are extremely susceptible to dietary casein, whereas rats and humans are rather insensitive. Moreover, the proposed mechanism is consistent with the observation that casein-induced hypercholesterolemia is caused primarily by the effect of casein on the enterohepatic cycle of steroids. It should be stressed that this model may be restricted to the effect of dietary casein and may not be extended to giving a general explanation for the cholesterolemic effects of other dietary proteins. Nevertheless, our description of the mechanism of casein-induced hypercholesterolemia may provide clues as to the mode of action of other hypercholesterolemic proteins.

MODIFICATION OF CASEIN EFFECT BY CHOLESTEROL AND FAT CONTENT OF THE BACKGROUND DIET

We have adduced evidence that both a low activity of intestinal alkaline phosphatase and a high ratio of glycine to taurine in conjugated bile acids are prerequisites for a difference in cholesterolemic effect between dietary soybean protein and casein. We have also discussed that if this difference, in effect, is absent in a given animal model, it may be elicited in certain cases by changing dietary components other than proteins or by using another strain of the same animal species. We propose that under such conditions the activity of intestinal alkaline phosphatase is decreased and/or that the ratio of glycine-conjugated to taurine-conjugated bile acids is increased. In any case, a situation is created in which the animal model fulfills both prerequisites for a protein effect of serum cholesterol to be observed. Experimental evidence is needed to prove or disprove this proposal. Some preliminary evidence is discussed below.

Casein-induced hypercholesterolemia is also dependent on other components of the diet. Figure 1 illustrates that in various animal species a hypercholesterolemic effect of casein can be elicited by the use of diets rich in cholesterol. This hypercholesterolemic effect may be related to an effect of dietary cholesterol on the conjugation of bile acids with either taurine or glycine. La Font et al. (46), who used rats, have shown that dietary cholesterol drastically increases the glycine-to-taurine ratio in conjugated bile acids. Thus a high cholesterol intake would make rats react in the same way as rabbits with respect to conjugated bile acids (Table 1).

In rats a small cholesterol-elevating effect of casein can also be observed when cholesterol-free diets are used. However, the diet should then also be low in other fats (12,13,30,31). There is suggestive evidence that low-fat diets lower the activity of intestinal alkaline phosphatase (43,49). Thus, rats fed low-fat casein diets may have higher intestinal concentrations of phosphopeptides (Fig. 3) than rats fed high-fat diets. As a result,

a small hypercholesterolemic effect of casein, in comparison with that of soybean protein, is obtained with low-fat background diets but not with high-fat ones (12).

SPECULATIONS ON THE MODE OF ACTION OF HYPERCHOLESTEROLEMIC PROTEINS OTHER THAN CASEIN

In rabbits fed cholesterol-free, semipurified background diets, proteins other than casein may also have a hypercholesterolemic effect. Examples are egg yolk protein, lactalbumin and beef protein (3,5). Unfortunately, only the cholesterolemic effects of these proteins have been studied, and not their effects on the fecal excretion of bile acids. We feel that it is not justified to assume a priori that similar effects must have similar underlying mechanisms. In other words, the hypercholesterolemic effects of the proteins mentioned above may be caused by a mechanism which is different from the mode of action of casein. Nevertheless, in an attempt to unravel this mechanism, the above-mentioned combined biochemical and nutritional approach might be promising.

For example, egg yolk protein, which is hypercholesterolemic in rabbits (3), contains phosvitin, which is a highly phosphorylated protein (50). As regards beef protein it may be relevant that myofibrilar proteins are phosphorylated and have a high affinity for calcium (51). With some animal proteins, conjugation of bile acids with either glycine or taurine may be considered to have an effect. Vegetable proteins do not contain taurine, but animal proteins, such as beef protein, have a high taurine content (52), and thus it is tempting to speculate that animal proteins may increase taurine conjugation of bile acids. Taurine conjugates do not effectively bind to the calcium-phosphate sediment (39). If beef protein indeed enhances taurine conjugation, it may cause a decrease in the fecal excretion of bile acids (Fig. 3), which in turn results in the hypercholesterolemia observed (3,5).

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