DIETARY CASEIN, SOYBEAN PROTEIN AND SERUM CHOLESTEROL IN EXPERIMENTAL ANIMALS AND MAN

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INTRODUCTION

During the last 15 years there has been a revival of interest in the effects of dietary proteins on atherosclerosis and cholesterol metabolism. This work builds on the observations made 75 years ago by Ignatowski [1]. Serendipitously, he found that atherosclerosis could be induced by dietary means. Rabbits fed diets containing meat, milk and eggs developed arterial lesions. Ignatowski [1] ascribed this to the animal protein components of the diets. Only 50 years later, this idea was supported by experiments with cholesterol-free, semipurified diets in which the protein was the only variable. Lambert et al. [2] and Wigand [3] found in rabits that casein was more atherogenic than soybean protein. The induction of atherosclerosis in rabbits fed casein was associated with a marked increase in the concentration of cholesterol in the serum, whereas on diets containing soybean protein serum cholesterol remained low [2-5]. The serum cholesterol response to a series of proteins has been investigated [4], but in most studies the effects of casein and soybean protein are compared. Both proteins can be obtained commercially in a relatively pure form, and in rabbits they have a significant, differential cholesterolemic effect.

Dietary protein and serum cholesterol in experimental animals

The differential effect of casein and soybean protein on the level of serum cholesterol is dependent on other components of the diet and on the animal model employed. The hypercholes-

terolemic effect of casein in a cholesterol-free diet was ob-
served in young growing rabbits but not in their mature coun-
terparts [6].   In mature rabbits a differential effect of
casein and soy protein was seen only when cholesterol was added
to both semipurified diets [7]. An increase in the proportion
of essential fatty acids in the diet by the addition of corn
oil causes disappearance of the casein-induced hypercholes-
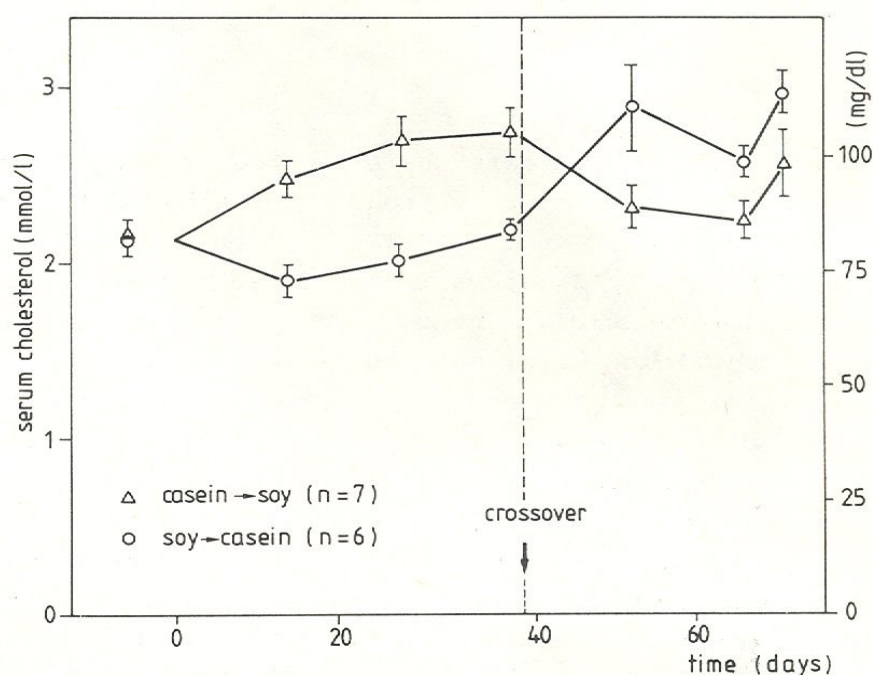terolemia in rabbits [3,8].

Fig. 1.   Effects of casein and soybean protein on serum
           cholesterol concentrations in rats. Male Wistar
           rats, aged about 6 weeks, were fed the semi-
           purified diet containing soybean protein isolate
           for 23 days. Then on day 0 one group (casein→soy)
           was allocated to the casein diet, and the other
           group (soy→casein) remained on the soybean protein
           diet. After 39 days the diets were crossed over.
           The diets were cholesterol-free and contained 21%
           (w/w) protein. Values expressed as means + SE.
           Based on data taken from Beynen et al. [17].

The hypercholesterolemic effect of dietary casein has been
clearly established in pigs [9] and rhesus monkeys [10] fed

cholesterol containing diets. On the other hand, in pigs [9], mice [11], guinea pigs [12], chickens [13] and calves [14] fed essentially cholesterol-free diets, casein did not significantly affect serum cholesterol levels when compared with soybean protein. In rats, the situation is complicated; sex and strain may determine the susceptibility to the type of dietary protein. With cholesterol-enriched semipurified diets, a hypercholesterolemic effect of casein was observed in female lean Zucker rats [15, 16] but not in the males [15]. When rats were fed low-fat, cholesterol-free diets containing either casein or soy protein, we observed an elevation of serum cholesterol with casein when compared with soybean protein in male Wistar rats (Fig. 1), but no clear effect was found in female Wistar rats or in male and female lean Zucker rats [17].

Amino acid composition of casein and soybean protein and serum cholesterol

Huff et al. [18] fed rabbits semipurified diets containing 25% (w/w) of an amino acid mixture resembling the composition of either casein or soy protein. It was found that the amino acid mixture equivalent to casein produced concentrations of serum cholesterol similar to those obtained with casein, whereas the mixture imitating soybean protein induced higher levels of serum cholesterol than the intact protein, but the levels were still lower than those seen with casein (Fig. 2). This would suggest that at least part of the differential effect of casein and soybean protein on serum cholesterol is related to differences in the amino acid composition of these proteins.

Further support for the idea that the amino acid composition of casein is at least partly responsible for its hypercholesterolemic effect comes from the work of Hermus et al. [19]. These workers found that the hypercholesterolemic effect of dietary casein in rabbits was greatly reduced if part of the casein was replaced by gelatin plus fish protein. The amino acid composition of this protein mixture resembled that of commercial rabbit feed. Supplementation of casein with amino acids so as to simulate the composition of the protein mixture partly abolished the hypercholesterolemic effect of casein, while
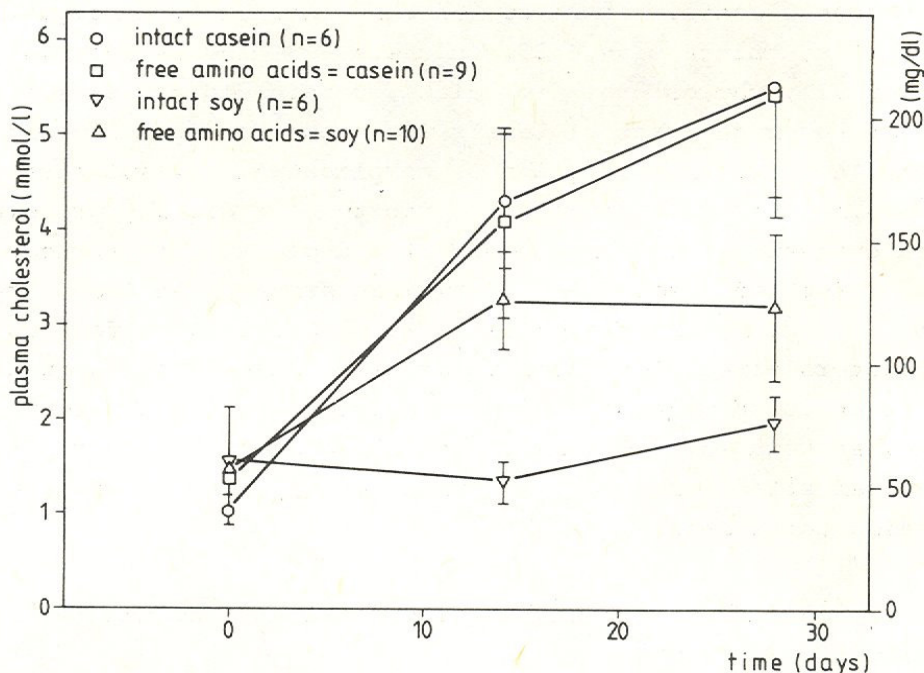
Fig. 2. Concentration of plasma cholesterol in rabbits fed semipurified diets containing 25% (w/w) soybean protein isolate, casein or amino acid mixtures corresponding to these proteins. The rabbits were aged about 10 weeks at the beginning of the experiment. Values are expressed as means ± SE. Based on data taken from Huff et al. [18].

supplementation of the protein mixture so as to simulate the amino acid composition of casein caused marked hypercholesterolemia [19]. Addition to casein of an amino acid mixture that did not change the overall amino acid composition, aggravated the casein-induced hypercholesterolemia [19].

We have carried out experiments with rabbits fed semipurified diets containing 42% (w/w) casein. When half of the protein was replaced by an amino acid mixture imitating either casein or soybean protein, serum cholesterol levels were somewhat lower, but no differential cholesterolemic effect of the amino acid mixtures was found [20]. Replacement of half of the casein by intact soybean protein drastically reduced serum cholesterol concentrations (Fig. 3). It can be concluded that intact soybean protein is more effective in lowering serum
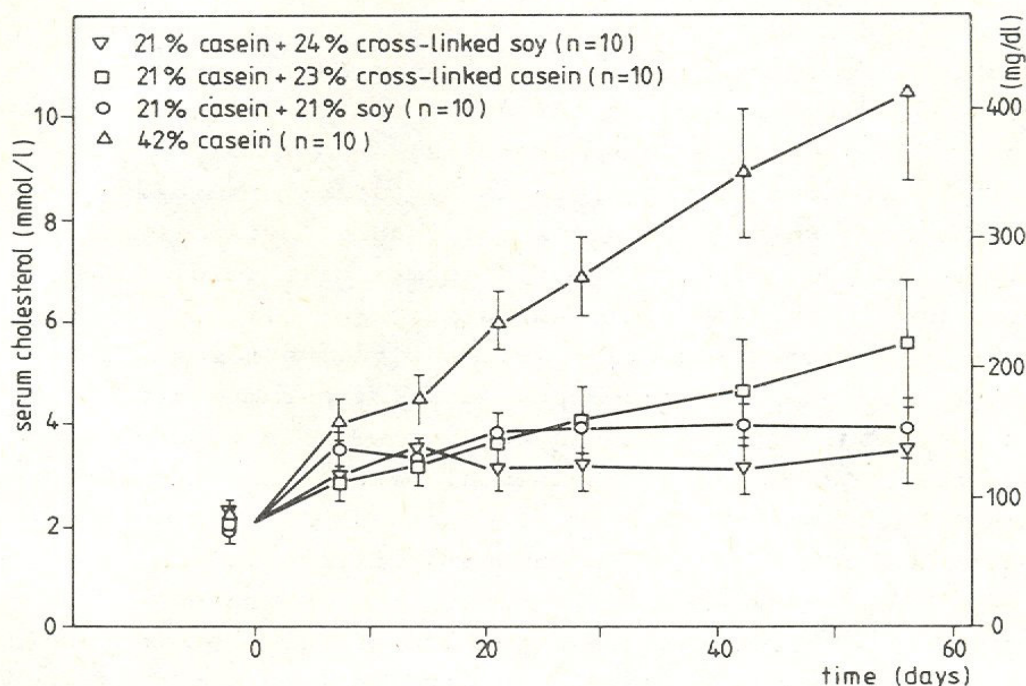
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Fig. 3.   Serum total cholesterol levels in rabbits fed
semipurified diets containing formaldehyde-treated
(cross-linked) casein or soybean protein isolate.
The rabbits were aged about 12 weeks at the begin-
ning of the experiment. Values expressed as means
± SE. Based on data taken from West et al. [20].

cholesterol levels than an amino acid mixture equivalent in
composition. This suggests that the hypocholesterolemic effect
of intact soybean protein is at least in part due to its struc-
ture and/or the order in which amino acids are released during
digestion.

The importance of the structure of casein

We have carried out an experiment in which the structure of
both casein and soybean protein was changed by formaldehyde
treatment [20]. The modified proteins with cross-linked chains
were incorporated into semipurified diets and fed to rabbits.
We observed that replacement of "native" casein by formaldehyde-
treated casein results in a significant reduction of hyperchol-
esterolemia, and that this preparation was almost as effective
as soybean protein (Fig. 3). Treatment of soybean protein with

formaldehyde did not further increase its hypocholesterolemic effect. It follows that the differences in the structure of casein and soybean protein influence the cholesterolemic responses in rabbits to these proteins.

The structure of the proteins may affect their digestibility, and this in turn may influence cholesterol metabolism. Proteins that are not completely digested adsorb bile acids [21] and may interrupt the enterohepatic circulation of bile acids, resulting in an enhanced loss of steroids with the feces and a subsequent lowering of the concentration of serum cholesterol. This idea would imply that soybean protein is less digestible than casein, at least in the distal part of the small intestine where the absorption of bile acids takes place. Mice fed soybean protein had indeed increased amounts of material in their intestine compared with their counterparts fed casein [22], suggesting a slower rate of digestion of soy protein when compared with casein. One should realize, however, that soy protein preparations generally contain some poorly digestible carbohydrates.

If rabbits fed casein have higher re-absorption of bile acids than animals fed soybean protein, this would have several consequences with respect to cholesterol metabolism. The fecal excretion of bile acids would be decreased in the short term, and the enhanced amount of re-absorbed bile acids would inhibit hepatic cholesterol conversion into bile acids by a feed-back mechanism, which would also diminish fecal bile acid output in the long term. The reduced cholesterol clearance from the body, including the blood stream, could then cause hypercholesterolemia.

A number of observations are in line with such a cascade of events. The bile acid sequestrant cholestyramine prevents and counteracts casein-induced hypercholesterolemia in rabbits [23]. Rabbits fed casein excrete less bile acids than animals fed soybean protein [24]. An increased re-absorption of bile acids may reduce the number of hepatic low density lipoprotein (LDL) receptor sites, as has been demonstrated in dogs [25]. This would result in a decreased clearance of the major cholesterol transporting vehicle, LDL, from the circulation. Indeed, in

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casein-fed rabbits much of the excess of cholesterol in the serum is carried in the LDL particles [26]. In addition, casein-fed rabbits have a reduced number of LDL receptors on liver membranes [23]. When compared to rabbits fed soybean protein, casein-fed animals display slower rates of removal of injected, radioactive cholesterol [24]. Thus the accumulation of serum cholesterol in the casein-fed rabbit appears to be the result of a decreased clearance of circulating cholesterol.

Total body cholesterol synthesis in rabbits fed casein has been found to be reduced when compared to animals on a diet containing soybean protein [24]. The depressed synthesis of cholesterol in casein-fed rabbits is most likely the result of feed-back inhibition effected by the increased level of serum cholesterol. This regulatory device, however, only protects the animals against further development of hypercholesterolemia. The reduced cholesterol synthesis together with the diminished hepatic uptake of serum lipoproteins will lower biliary cholesterol output and results in a decreased fecal excretion of neutral steroids, as has been demonstrated [27].

Although the mechanism described above reconciles most observations obtained thus far, several important points remain to be settled. For instance, it is not clear why free amino acids in the diet influence serum cholesterol levels.

Dietary protein and serum cholesterol in man

Strictly controlled studies in our laboratory with healthy volunteers [28, 29] have demonstrated that casein, when compared to soybean protein isolate, does not significantly affect the level of serum total cholesterol. In these studies diets were used in which 60 or 65% of the protein in the diet consisted of either casein or soybean protein. The diets were essentially identical with respect to the contents of cholesterol and fat, and the polyunsaturated:saturated fatty acid (P/S) ratio [28, 29]. Although there was no differential effect on serum total cholesterol, the diet containing soybean protein isolate produced a shift in cholesterol from the LDL to the high density lipoproteins (HDL), resulting in a 7 [29] to 14% [28] increase in the HDL/LDL-cholesterol ratio. This effect of soybean protein

versus casein may be favorable with regard to the risk for atherosclerotic diseases. It was remarkable that this effect was found only with the purest soy protein preparation (soy isolate), and not with a cruder concentrate [29]. This seems to exclude favorable effects of dietary fiber in soy protein concentrate on lipoprotein concentration in man.

Sirtori and co-workers [30, 31] have shown reductions by up to 25% in the level of serum total cholesterol in hypercholesterolemic patients after the transfer from a mixed protein diet to a diet containing predominantly soybean protein. However, consumption of the soybean protein diet also involved complete elimination of cholesterol in the diet and an increase in the P/S ratio. It is thus not possible to assess the effect of the dietary protein independently. The work of investigators who used experimental diets which differed only in their protein constituents, suggests that soybean protein, when compared to animal proteins, lowers cholesterol levels in hypercholesterolemic patients by no more than 4% [32, 33].

In short-term studies, adult humans appear to be much less sensitive to the type of dietary protein with respect to the level of serum cholesterol than young rabbits. When young rabbits were fed duplicate portions of the same diets fed to volunteers, the casein diet caused 50% higher serum cholesterol levels than the soybean protein diet within 2.5 weeks [28]. This confirms the species-dependent sensitivity to dietary protein referred to above. However, it is also possible that 4 to 6 weeks is not long enough to observe a protein effect in humans. Furthermore, it is conceivable that in young, rapidly growing infants clear effects of the type of dietary protein could be seen, because the hypercholesterolemic effect of casein was only observed in young growing rabbits and not in their mature counterparts [6].

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