# Cholesterol Metabolism in Swine Fed Diets Containing Either Casein or Soybean Protein

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**Diets containing casein produce higher concentrations of serum cholesterol in swine than those containing soybean protein, but only when such diets contain high levels of cholesterol. We suggest that dietary casein causes an increase in the absorption of cholesterol and bile acids, thus explaining the observed decrease in fecal excretion of neutral steroids and bile acids when compared to soybean protein. The mechanism in molecular terms by which dietary proteins influence steroid absorption remains to be established.**

Most studies on the relationship between diet, cholesterol metabolism and atherosclerosis using animal models involve either the rat or the rabbit. However, unlike these models, swine usually are considered an excellent model for man. Swine and man share similarities in their omnivorous food habits, metabolism of cholesterol and serum lipoproteins (1) and cardiovascular physiology (2). Furthermore, lesions in the aorta and coronary arteries of swine resemble atherosclerotic lesions in man (3).

Proteins are among the dietary components that affect serum cholesterol concentrations and atherosclerosis in rabbits. The milk protein casein causes hypercholesterolemia and atherosclerosis in this species. In contrast, rabbits fed diets containing soybean protein maintain low levels of serum cholesterol and do not develop atherosclerotic lesions (4,5). The objective of this paper is to describe the effects of dietary soybean protein and casein on cholesterol metabolism in swine. An attempt will also be made to disclose the mechanisms underlying the differential cholesterolemic response to these proteins.

## THE RESPONSE OF SERUM CHOLESTEROL CONCENTRATION

When young, male Yorkshire swine on a semipurified high-fat, high-cholesterol diet containing soybean protein were transferred to a similar diet containing casein instead of soybean protein, the concentration of serum cholesterol increased rapidly (Fig. 1). Conversely, when casein was replaced by soybean protein, there was a decrease in serum total cholesterol. Components in the diet other than the protein would appear to be essential in order to observe the differential cholesterolemic effects of soybean protein and casein.

When Kim et al. (6,7) added either soybean protein or casein to a commercial hog mash, they noted no differences in the level of serum cholesterol produced. The amount of cholesterol in the diet may be a determining factor. Cho et al. (8) found that the hypercholesterolemic effect of casein with respect to soybean protein is seen only with a diet containing much cholesterol (Table 1).

Evidence from the work of Cho et al. (8) also suggests

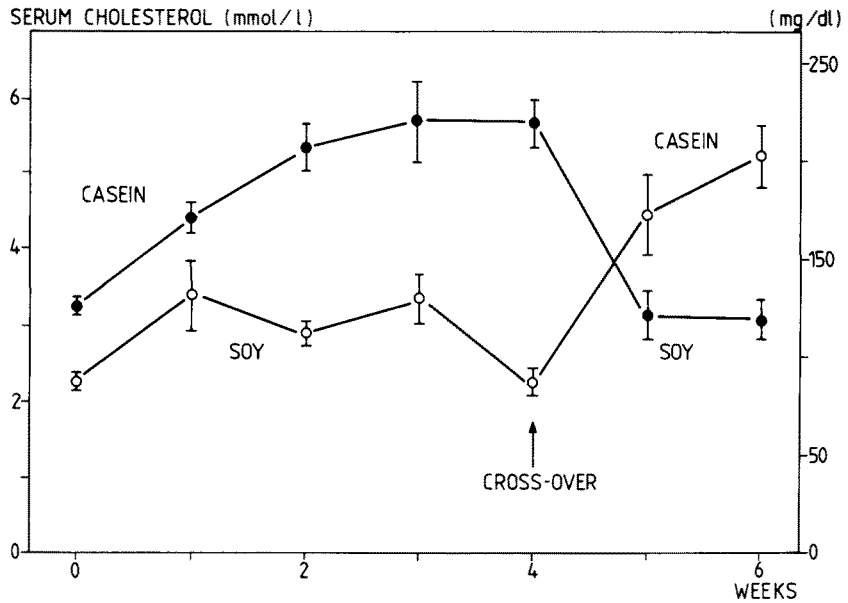


**FIG. 1. Serum cholesterol concentrations in young, male Yorkshire swine fed high-fat, high-cholesterol diets containing either 21% (w/w) casein or soybean protein. Open circles, soybean protein→casein; closed circles, casein→soybean protein. Each point denotes the mean from 5 pigs; the bars represent the SE. Based on Kim et al. (6).**

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that the amount and type of fiber in the diet may override a possible protein effect. We feel, however, that the amount of cholesterol in the diet is the most crucial factor. The lack of effect of soybean protein compared with casein, when added to a commercial hog mash as background (6,7), may then be explained by the low cholesterol content of such a mash. Richard et al. (9) also did not observe a difference in serum cholesterol levels between swine fed either casein or soy protein isolate as constituents of low-cholesterol, semipurified diets.

At least part of the differences in serum cholesterol levels between swine fed diets containing casein or soybean protein (Fig. 1, Table 1) may be caused by factors other than the protein component of the diets. The protein preparations used are not completely pure (casein, about 90% protein; soybean protein, 65 to 92% protein), and thus other substances could have played a role.

In the experiments of Kim et al. (6,7, and Fig. 1), swine fed casein-containing diets did not grow as well as those fed diets containing soybean protein. The authors fed the swine a constant daily amount (1055 mg) of crystalline cholesterol, and consequently cholesterol intake on a body weight basis was higher in the casein-fed swine. This may have contributed to the greater cholesterolemic response on the diet containing casein, at least during the period before the cross-over (Fig. 1).

Cho et al. (8), on the other hand, reported a somewhat better efficiency of feed conversion in the swine receiving casein (Table 1). In this study the proportion of cholesterol in the diet was constant, and thus the swine on the casein diet actually consumed less cholesterol on a body weight basis. It can be concluded that under the conditions of the studies described (Fig. 1, Table 1) the slight differential cholesterol intakes of the swine fed casein or soybean protein may not have had an important impact on the outcome with respect to serum cholesterol concentrations. However, if the techniques of pair-feeding or restricted feeding were applied the interpretation of these studies would have been more straightforward.

TABLE 1

**Plasma Total Cholesterol Levels in Male Piglets Fed Semipurified Diets Containing Either Casein or Soybean Protein**

| | Dietary protein | |
|---|---|---|
| | Soybean protein | Casein |
| | (mmol/l) | |
| Cholesterol-free diet | 3.72 ± 0.14 | 3.68 ± 0.15 |
| High-cholesterol diet | 5.25 ± 0.23 | 7.96 ± 0.36 |

Results expressed as means ± SE for 5 animals/group. The diets were isonitrogenous and contained 19% (w/w) of crude protein. Corrections were made for the fiber components of the soy protein preparation. The high-cholesterol diet contained 0.5% of cholesterol. Data taken from Cho et al. (8).

## CHOLESTEROL DISTRIBUTION BETWEEN SERUM LIPOPROTEINS

The cholesterol concentrations in the different serum-lipoprotein fractions of swine fed either casein or soybean protein cholesterol-rich (0.2%, w/w) diets are given in Table 2. The increase in serum cholesterol level of the casein-fed animals was reflected in the low density lipoprotein fraction with lower density ($LDL_1$). The cholesterol content of the other lipoproteins was barely affected by the replacement of soybean protein by casein. The results presented in Table 2 agree well with those found by Cho et al. (8), who used diets containing 0.5% (w/w) cholesterol (Table 1).

## MODE OF DIFFERENTIAL CHOLESTEROLEMIC EFFECT OF CASEIN AND SOYBEAN PROTEIN

The amount of cholesterol in the body is regulated by the balance of input and output. In essence, this can occur by cholesterol absorption and synthesis and in

TABLE 2

**Cholesterol Concentration in Lipoprotein Fractions of Swine Fed High-Cholesterol, Semipurified Diets Containing Either Soybean Protein or Casein**

| | Baseline (Day –2) | | Change (Day –2 to 19) | |
|---|---|---|---|---|
| Lipoprotein fraction | Soybean protein | Casein | Soybean protein | Casein |
| | (mmol/l of whole serum) | | | |
| VLDL (d<1.006) | 0.07 ± 0.04 | 0.06 ± 0.03 | –0.03 ± 0.03 | –0.02 ± 0.03 |
| IDL (1.006<d<1.019) | 0.03 ± 0.02 | 0.02 ± 0.01 | –0.02 ± 0.03 | 0.02 ± 0.02 |
| $LDL_1$ (1.019<d<1.040) | 0.57 ± 0.23 | 0.51 ± 0.12 | 0.18 ± 0.14 | 0.63 ± 0.34* |
| $LDL_2$ (1.040<d<1.063) | 0.85 ± 0.21 | 0.73 ± 0.08 | –0.07 ± 0.04 | 0.11 ± 0.11 |
| $HDL_2$ (1.063<d<1.125) | 1.15 ± 0.23 | 1.00 ± 0.23 | –0.05 ± 0.18 | 0.24 ± 0.28 |
| $HDL_3$ (1.125<d<1.210) | 0.74 ± 0.05 | 0.76 ± 0.16 | –0.03 ± 0.08 | –0.11 ± 0.15 |
| Bottom (d>1.210) | 0.01 ± 0.01 | 0.00 ± 0.00 | –0.01 ± 0.01 | 0.00 ± 0.00 |
| Whole serum | 3.42 ± 0.41 | 3.15 ± 0.37 | 0.07 ± .021 | 0.61 ± 0.47* |

Results expressed as mean ± SD for 6 swine/group. All swine were fed the diet containing soybean protein (24%) for 12 days when at Day 0 six swine weighing about 56 kg at that time were allocated to the diet containing casein for another 19 days. See also legend to Table 4. *, $P<0.05$; two-tailed Student's $t$ test. Unpublished data of Beynen and West.

the fecal excretion of neutral steroids and bile acids. Obviously, the amount of cholesterol in the serum also is regulated by these processes; in addition, it can be influenced by cholesterol uptake and release by tissues.

Theoretically, the cholesterol-elevating effect of casein must be due to differences in one or more of these aspects of cholesterol metabolism. In the paragraphs following, we review the effects of casein and soybean protein on cholesterol metabolism in swine, attempting to unravel the mode of action of this hypercholesterolemic dietary component.

## CHOLESTEROL CONTENT OF TISSUES

Sooner or later, swine fed casein-containing diets should reach a steady state in which the efflux of cholesterol and its metabolites from the body equals the rate of influx from the diet and from biosynthesis. However, in most experiments this steady state often is not reached. Either the studies do not last long enough to allow the serum cholesterol concentrations to reach a plateau, or the swine continue to grow during the study. Thus, casein-induced hypercholesterolemia might reflect a change in the distribution of cholesterol between serum and tissues. However, this possibility can be ruled out. In casein-fed pigs the accumulation of cholesterol in serum occurs pari passue with that in tissues such as liver, ileum, skin, muscle and adipose tissue (7).

## CHOLESTEROL SYNTHESIS

As the steady state is not reached in studies on casein feeding of swine, the steroid balance method can be used only if the retention of cholesterol in the tissues of the growing animals can be assessed. Using this approach, Kim et al. (10) conclude that cholesterol synthesis was inhibited maximally in both casein- and soybean-protein fed swine. This conclusion was supported by the direct measurement of the activity of microsomal hydroxymethyl-glutaryl-Coenzyme A reductase in the liver (6,11). Thus swine fed either casein- or soybean-protein containing diets may not differ with respect to de novo cholesterol synthesis. Possible differences may be overridden by the high cholesterol load, which essentially suppresses all cholesterol biosynthetic activity.

## FECAL EXCRETION OF STEROIDS

Table 3 shows that swine fed casein excrete lower amounts of bile acids and neutral steroids than their counterparts fed soybean protein. When the swine on the casein diet were transferred to the diet containing soybean protein, an increase in the fecal excretion of bile acids and neutral steroids occurred within one week (Table 3). Conversely, changing the swine from the diet containing soybean protein to that containing casein resulted in a decrease in excretion of bile acids and neutral steroids with the feces, but this effect was seen only during the second week after the cross-over. In contrast, the level of serum cholesterol increased almost immediately after the replacement of soybean protein by casein (Fig. 1). Thus, there may be a cause-and-effect relationship between fecal steroid excretion and serum cholesterol when swine are transferred from a diet containing casein to one containing soybean protein, but when the reverse is done such a relationship is less clear.

## CHOLESTEROL ABSORPTION

The information on cholesterol absorption in swine fed diets containing casein or soybean protein is sparse. The data available suggest that casein enhances cholesterol absorption when compared to soybean protein. Using the ratio of cholesterol to β-sitosterol in feces as

TABLE 3

**Excretion of Bile Acids and Neutral Steroids With the Feces by Swine Fed Diets Containing Either Casein or Soybean Protein**

| | Dietary protein | |
|---|---|---|
| Before cross-over: | Soybean protein | Casein |
| After cross-over: | Casein | Soybean protein |
| | (mmol/day) | |
| Excretion during week before cross-over | | |
| Bile acids | 0.9 ± 0.1 | 0.5 ± 0.1 |
| Neutral steroids | 1.6 ± 0.1 | 1.2 ± 0.03 |
| Excretion during week after cross-over | | |
| Bile acids | 1.0 ± 0.3 | 0.8 ± 0.2 |
| Neutral steroids | 1.4 ± 0.1 | 1.7 ± 0.1 |
| Excretion during week after cross-over | | |
| Bile acids | 0.7 ± 0.1 | 0.8 ± 0.1 |
| Neutral steroids | 1.1 ± 0.1 | 1.7 ± 0.2 |

Results expressed as mean ± SE for 5 swine/group. See also legend to Fig. 1. Based on Kim et al. (6). Body weight and feed intake were not reported; thus corrections of the absolute amounts in the table, if necessary, could not be made.

a measure of absorption, Kim et al. (7) found cholesterol absorption to be 56 ± 3% and 42 ± 5% (means ± SE, n = 3) for swine fed casein and soybean protein-containing diets, respectively. These authors (10) also have presented evidence that the absorption of total dietary fat is somewhat more efficient in casein-fed swine.

We have used swine provided with re-entrant cannulae in order to gain more insight into the absorption of steroids in various regions of the gut. The experimental details were as follows. Castrated swine were used; the animals were offspring of a cross between Dutch Landrace and Large White pigs. Housing conditions have been described (12).

The swine were fed a semipurified diet containing soybean protein. On Day 0 of the experiment, six animals were transferred to the semipurified diet containing casein, while six other animals remained on the soy protein diet. The diets contained 24% (w/w) of cholesterol. The composition of the diets is reported elsewhere (12). Two to three mo before Day 0 of the experiment, the swine were surgically fitted with a re-entrant cannula at the distal end of the ileum, just before the ileocecal sphincter. Feces (Day 11 to Day 18) and ileal chyme (Day 19 to Day 21 and Day 24 to Day 26; the samples of both intervals were pooled) were collected quantitatively. During ileal chyme collection the animals were infused with saline directly into the colon via the distal part of the fistula. Analytical methods have been described in detail (12).

Table 4 shows the ileal and fecal excretion of bile acids and neutral steroids in the cannulated swine fed diets containing either casein or soybean protein. Transferring the swine from the soybean protein diet to the casein diet resulted in a 50% decrease in the ileal excretion of neutral steroids, whereas the ileal output of bile acids may be only slightly decreased. Our data may not be fully in agreement with observations by Scholz et al. (13) using miniature swine fed high-cholesterol (1%, w/w) semipurified diets containing either 22% (w/w) casein or soybean protein isolate. These workers present evidence which suggests that the intestinal content of bile acids is markedly higher in swine fed a diet containing soybean protein, when compared with swine on a diet containing casein. On the other hand, the intestinal content of cholesterol in these swine was similar (13).

Regarding steroid excretion with the feces, dietary casein significantly reduced both that of bile acids and of neutral steroids when compared to soybean protein. This observation agrees well with that of Kim et al. (6) presented in Table 3. From Table 4 it can also be concluded that in swine the large intestine plays a quantitatively important role in the absorption of bile acids but not of neutral steroids. The data given in Table 4 suggest that absorption of bile acids in the cecum and/or colon is enhanced in swine fed diets containing casein compared to soybean protein. Furthermore, casein may increase the absorption of cholesterol in the small intestine. The possibility of decreased biliary cholesterol and bile acid efflux in casein-fed swine cannot be excluded on the basis of the information in Table 4. However, when Hagemeister et al. (14) collected bile directly from the ductus choledochus, they did not observe a difference in bile acid and cholesterol excretion between mini-swine fed either casein or soybean protein in a diet containing 1% (w/w) of cholesterol. This would imply that the ileal output of steroids (Table 4) reflects the efficiency of their absorption rather than their excretion with the bile fluid.

It is clear that casein in a high-cholesterol diet elevates the level of serum cholesterol in swine when compared to soybean protein. We suggest that this effect is due to an increase in the absorption of cholesterol in the small intestine and of bile acids, especially in the large intestine. Both effects may be causative factors in the hypercholesterolemic action of casein (15). In the long term, the increased absorption of bile acids and cholesterol would be compensated for by changes in metabolism of other tissues of the body. Theoretically, a diminished rate of cholesterol biosynthesis is the only change which can lead to the development of a new steady state. However, this contemporary device may not gather momentum because cholesterol synthesis is already fully depressed by the high load of dietary cholesterol. Thus, swine fed casein together with cholesterol may accumulate cholesterol continuously. The lack of effect on serum cholesterol of casein in a low-cholesterol diet, when compared with soybean protein, may be explained by the ability of swine to compensate for the enhanced absorption of cholesterol and bile acids by depressing cholesterol biosynthesis. However, at present other possibilities cannot be excluded (16).

In most studies with swine the effects of casein and soybean protein have been compared. Both proteins can be obtained commercially in a relatively pure form, and they have a significant, differential cholesterolemic effect. In rabbits some protein sources, such as extracted whole egg, lactalbumin and beef protein concentrate, produced rather high concentrations of serum cholesterol, whereas other sources of protein, such as wheat gluten, peanut meal and cottonseed protein, were able to maintain low levels of serum cholesterol (4). Such data are not available for swine, and it would be interesting to know whether these proteins have similar effects in this animal species. It may be even more important to ascertain whether our description of the possible mechanism involved in the hypercholesterolemic effect of casein versus soybean protein also extends to other proteins with differential cholesterolemic effects.

TABLE 4

**Excretion of Bile Acids and Neutral Steroids by Cannulated Pigs Fed Either Casein or Soybean Protein**

| | Dietary protein | |
|---|---|---|
| | Soybean protein | Casein |
| | (mmol/day) | |
| Ileal excretion | | |
| Bile acids | 3.74 ± 0.56 | 3.17 ± 0.91 |
| Neutral steroids | 3.41 ± 0.73 | 1.70 ± 0.70* |
| Fecal excretion | | |
| Bile acids | 2.71 ± 1.08 | 1.77 ± 0.68* |
| Neutral steroids | 3.19 ± 0.43 | 1.95 ± 0.68* |

Results expressed as means ± SD for 6 swine/group. The data are given in absolute amounts as body weights and feed intake did not differ between the dietary groups. *, $P<0.05$, two-tailed Student's t test.

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