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Dietary glycine and cholesterol metabolism in rats

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Summary: Generally, diets containing casein induce higher concentrations of serum cholesterol in rats than diets containing soybean protein. It has been suggested that at least part of this effect is due to the relatively low level of glycine in casein, when compared to soybean protein. We have tested this suggestion by adding glycine to a semipurified casein diet, so that the level of glycine was similar to that in a diet containing soy protein. The addition of glycine did not affect the casein-induced concentrations of cholesterol in serum and liver of rats. We conclude that the low proportion of glycine in casein does not play a role in casein-induced hypercholesterolemia.

Zusammenfassung: Im allgemeinen verursachen Diäten, die Kasein enthalten, höhere Serumcholesterinkonzentrationen bei Ratten als Diäten mit Sojaeiweiß. Es wurde postuliert, daß dieser Effekt zumindest teilweise auf den relativ niedrigen Glycinanteil des Kaseins im Vergleich zu Sojaeiweiß zurückzuführen ist. Diese Hypothese wurde getestet, indem einer halbgereinigten Diät mit Kasein Glycin zugefügt wurde, so daß die Glycinkonzentration hier ähnlich war wie in der Diät mit Sojaeiweiß. Die Zugabe von Glycin hatte keinen Effekt auf die durch Kasein induzierten Serum- und Lebercholesterinkonzentrationen bei Ratten. Als Schlußfolgerung ergibt sich, daß der niedrige Anteil an Glycin im Kasein für den hypercholesterinämischen Effekt dieses Eiweißes keine Rolle spielt.

Key words: dietary glycine, dietary casein, serum cholesterol, liver cholesterol, rats

Introduction

It has been repeatedly shown that diets containing casein as the sole source of protein produce higher concentrations of serum cholesterol in rats than do diets containing soybean protein (3, 5–8). Casein and soybean protein differ markedly in their amino acid compositions. One difference is that the proportions of glycine in casein is very low when compared to soybean protein. Casein and soybean protein contain about 1.8 % and 3.6 % (w/w) of glycine, respectively. It has been suggested that the low level of glycine in casein is responsible for the hypercholesterolemia in rats which is observed after feeding casein diets. Fortification of diets containing 10 % to 20 % of casein with 2 % (w/w) of glycine, reduced the hypercholesterolemic effect (5, 9). However, if diets containing 20 % of protein are used, the difference in glycine content between casein and soybean protein diets is only about 0.4% of the whole diet. Thus, the formulation of casein diets containing higher levels of added glycine may not provide conclusive evidence as to the role of glycine in casein-induced hypercholesterolemia when compared to the cholesterolemia produced by soy protein. The present study with rats was carried out to see whether glycine at relatively low levels would counteract the hypercholesterolemia induced by dietary casein.

Materials and Methods

Male rats from a random-bred Wistar Cpb/WU colony were used. At the age of 5 weeks, the animals were transferred from a commercial, pelleted rat diet (RMH-B, Hope Farms, Woerden, The Netherlands) to the semipurified diet containing soybean protein isolate (Table 1).

After another 14 days, on day 0 of the experiment, the rats were divided into six groups consisting of six animals each, so that the groups had similar serum cholesterol concentrations and body weights. One group remained on the soybean protein diet. The other groups received a casein diet to which either no or various amounts of glycine (Degussa AG, Hanau, FRG) had been added (Table 1). All diets contained 1.2% (w/w) of cholesterol. The casein and soy protein diets were balanced for the sodium and fat contents of the protein preparations. The experimental period lasted 28 days.

The rats were housed individually in cages (24 × 17 × 17 cm) constructed of stainless steel with wire mesh bases. The cages were placed in a room with air conditioning (20 °C), controlled lighting (light: 06.00–18.00 h; dark: 18.00–06.00 h) and humidity (55–65%).

Blood samples were taken in the non-fasting state by orbital puncture under light diethyl-ether anesthesia between 08.00 and 10.00 h. Serum total cholesterol was

Table 1. Composition of the experimental diets.

Ingredient	Dietary protein					
	Soy Protein	Casein	Casein + 0.2% glycine	Casein + 0.4% glycine	Casein + 0.8% glycine	Casein + 1.6% glycine
	(g/100 g)					
Soy protein	20.8	–	–	–	–	–
Methionine	0.2	–	–	–	–	–
Casein	–	21.0	21.0	21.0	21.0	21.0
Glycine	–	–	0.2	0.4	0.8	1.6
Corn starch	58.5	57.6	57.4	57.2	56.8	56.0
Corn oil	2.0	3.0	3.0	3.0	3.0	3.0
Coconut fat	7.3	7.0	7.0	7.0	7.0	7.0
Cholesterol	1.2	1.2	1.2	1.2	1.2	1.2
Constant components	10.0	10.2	10.2	10.2	10.2	10.2

The constant components consisted of (g/100 g): cellulose, 2.0; dicalciumphosphate, 2.9; sodium chloride, 0.6 (0.8 in the casein diets); magnesium carbonate, 0.3; magnesium oxide, 0.2; potassium bicarbonate, 1.8; vitamin premix, 1.2; mineral premix, 1.0. The composition of the vitamin and mineral premixes has been described (4).

Table 2. Performance and cholesterol metabolism in rats fed the experimental diets.

	Dietary protein					
	Soy protein	Casein	Casein + 0.2 % glycine	Casein + 0.4 % glycine	Casein + 0.8 % glycine	Casein + 1.6 % glycine
Body weight (g)						
day 0	124 ± 3	121 ± 2	127 ± 1	125 ± 2	126 ± 3	120 ± 4
day 28	165 ± 7	164 ± 4	175 ± 2[a]	175 ± 4[a]	189 ± 4[a]	171 ± 8
Feed intake (g/day)	12.1 ± 0.5	11.9 ± 0.4	13.0 ± 0.2[a]	12.7 ± 0.3	13.9 ± 0.4[a]	12.2 ± 0.5
Serum cholesterol (mmol/l)						
day −2	3.28 ± 0.12	3.28 ± 0.11	3.30 ± 0.09	3.26 ± 0.10	3.27 ± 0.10	3.26 ± 0.09
day 15	2.99 ± 0.09	3.51 ± 0.14[b]	3.38 ± 0.21	3.53 ± 0.12	3.86 ± 0.18	3.43 ± 0.12
day 28	3.02 ± 0.07	3.80 ± 0.09[b]	3.60 ± 0.35	3.85 ± 0.19	4.14 ± 0.19	3.69 ± 0.23
Liver weight (g)	6.3 ± 0.4	7.4 ± 0.3	8.1 ± 0.1	7.8 ± 0.2	8.7 ± 0.3	7.4 ± 0.4
Liver cholesterol (μmol/g)	14.0 ± 1.0	32.3 ± 3.8[b]	43.8 ± 4.0	28.1 ± 3.8	42.8 ± 6.5	29.8 ± 3.3

Results, expressed as means ± SE for 6 rats per group. [a]Significantly different from casein diet without added glycine. [b]Significantly different from soy protein diet ($P < 0.05$; two-tailed Student's t test).

measured enzymatically using the kit (Monotest) supplied by Boehringer-Mannheim GmbH, FRG. At the end of the experiment, immediately after blood sampling, the anesthetized rats were sacrificed by decapitation. The livers were removed and stored at −20 °C until analysis. Extraction and determination of liver cholesterol was performed as described by Abell et al. (1).

Results and Discussion

Feed intake and body-weight gain were similar in the rats fed the diets containing soy protein or casein without added glycine (Table 2). The addition of glycine to the casein diet at levels of 0.2 % to 0.8 % caused an increase in both feed intake and body weight. This glycine effect tended to have disappeared at a level of 1.6 % of the diet.

In keeping with previous studies (3, 5–8) the casein diet (without added glycine) produced higher concentrations of cholesterol in serum and liver than the diet containing soybean protein (Table 2). The addition of glycine to the casein diet at levels ranging from 0.2 % to 1.6 % of diet, did not affect the degree of casein-induced hypercholesterolemia. Likewise, glycine did not systematically influence liver cholesterol.

The present study suggests that the low level of glycine in casein, when compared to soy protein, does not play a role in casein-induced hypercholesterolemia. When added up to levels of 1.6 % of the diet, glycine did not influence serum and liver cholesterol. Glycine might only reduce the casein-induced hypercholesterolemia when added to the diet at levels as high as 2 % (5, 9). This level is much higher than that in a diet containing soy protein as the sole source of protein. The mechanism of action of this glycine effect is not clear. It is interesting to note that acetate, which resembles glycine in that it is also a carboxylic compound consisting of two carbon atoms, has been found to lower liver cholesterol concentrations in rats fed a high-cholesterol diet (2).

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