FECAL STEROID EXCRETION BY RABBITS HYPO- OR HYPERRESPONSIVE TO DIETARY CHOLESTEROL

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ABSTRACT

In two inbred strains of rabbits with high or low response of plasma cholesterol to dietary cholesterol, the excretion of steroids with feces was determined. Cholesterol synthesis, measured as fecal excretion of bile acids and neutral steroids, was similar in hypo- and hyperresponders fed a low-cholesterol diet. Rates of fecal bile acid excretion tended to be higher in hypo- than in hyperresponders. Dietary cholesterol stimulated fecal excretion of neutral steroids, the increase being more pronounced in hypo- than hyperresponders. There were strain differences in the relative composition of fecal bile acids but not in that of neutral steroids. After cholesterol feeding, hyperresponders accumulated significantly more cholesterol in their body than did hyporesponders.

INTRODUCTION

We are studying the metabolic basis for the difference in cholesterolemic response between two inbred strains of rabbits (1,2). In this study we have focussed on fecal excretion of bile acids and neutral steroids in the two strains. Part of this work has appeared in a preliminary form (3).

MATERIALS AND METHODS

Animals and housing. Adult rabbits of two inbred strains, aged 1 to 3.5 years, were used. The strains were IIIVO/JU and AX/JU and originated from the Jackson Laboratory colony, Bar Harbor, ME, USA (4). The IIIVO/JU strain has previously been shown to be hypo-, and the AX/JU strain to be hyperresponsive to dietary cholesterol (1,2). The animals were maintained at the Department of Laboratory Animal Science and kept individually in cages with wire-mesh bases constructed of galvanized steel, in a room with controlled lighting (light, 05.00-19.00 hours), temperature (16-19 °C) and relative humidity (55-65%). Throughout the experiment the hyporesponsive rabbits weighed about 3.5 kg, and the hyperresponders about 3.0 kg.

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Experimental design. Of each strain three male and three female animals were used. They were fed a commercial (LK-04^{R}, Hope Farms, Woerden, The Netherlands), low-cholesterol (0.01%, w/w) diet up until Day 0 of the experiment. Subsequently, they received the commercial diet fortified with 0.3% (w/w) of cholesterol. Analyses of the low- and high-cholesterol, commercial diets by gas-liquid chromatography showed that they contained 26 and 944 umol cholesterol/100 g of diet. Food and water were provided ad libitum. Body weight and plasma total cholesterol were measured weekly. During three periods (Days -7 to -4, 0 to 3 and 28 to 31) feces of each rabbit were collected daily. During these collection periods food intake was recorded daily.

Analytical methods. Samples of blood were taken from the marginal ear vein of the rabbits into heparinized tubes. Sampling and measurement of the body weights were performed between 08.00 and 10.00 hours after the removal of any remaining food, at 16.00 hours the previous day. Total cholesterol in plasma was measured enzymatically using a test-combination (Monotest^{R}) supplied by Boehringer-Mannheim GmbH, FRG.

Steroids were analysed by gas-liquid chromatography (5) in samples of feces pooled per rabbit and per collection peroid. 5α-Cholestane was used as internal standard for neutral steroid determination, and 23-nordeoxycholic acid for that of bile acids. The combined within- and between-run coefficients of variation for the contents of neutral steroids and total bile acids in a control pool of rabbit feces were 6.2% and 9.0% (n=4).

Calculation of sterol balance. Cholesterol balance was calculated as cholesterol intake minus excretion of neutral steroids and bile acids. Negative values refer to net whole-body cholesterol synthesis. Positive values should be interpreted as accumulation of cholesterol in the body.

Statistical methods. Comparisons between the hypo- and hyperresponsive strains were evaluated by Student's two-tailed t-test.

TABLE 1. PLASMA TOTAL CHOLESTEROL CONCENTRATIONS IN HYPO- AND HYPERRESPONSIVE RABBITS FED A LOW- AND A HIGH-CHOLESTEROL COMMERCIAL DIET

Day	Hyporesponders	Hyperresponders
	(mmol/l)	
- 4	0.9 ± 0.3	0.7 ± 0.5
3	2.0 ± 0.5	3.0 ± 1.6
10	3.7 ± 1.7	9.3 ± 5.0*
17	5.4 ± 2.2	14.4 ± 5.4*
31	6.7 ± 3.2	29.0 ± 3.6*

*On Day 0 the rabbits were transferred from the low- to the high-cholesterol, commercial diet. Results are expressed as means ± SD for 5 or 6 animals. Significant strain difference: *, p <0.05.*

RESULTS

Since there were no significant differences between males and females, all values were pooled per strain. As would be anticipated (1,2), the two rabbit strains differed greatly in their cholesterolemic responses to dietary cholesterol (Table 1). Body weights and feed intake (data not shown) of the rabbits did not change during the course of the experiment. One male rabbit of the hyper- (Day 22) and one of the hyporesponsive strain died (Day 24). The cause of death could not be identified clearly, but the plasma was found to be icteric.

Table 2 shows that on the low-cholesterol, commercial diet the hyporesponders excreted more bile acids with their feces than the hyperresponders. The amounts of excreted neutral steroids were similar in both strains. Fecal excretion of neutral steroids was enhanced immediately after transfer of the animals to the high-cholesterol diet.

TABLE 2. CHOLESTEROL INTAKE AND STEROID EXCRETION IN HYPO- AND HYPERRESPONSIVE RABBITS FED A LOW- AND A HIGH-CHOLESTEROL COMMERCIAL DIET

	Steroid intake, excretion or balance		
	LCD[1] Days -7 to -4	HCD Days 0 to 3	HCD Days 28 to 31
	(μmol/kg body weight/day)		
Cholesterol intake			
Hyporesponders	8 ± 1	270 ± 30	279 ± 27
Hyperresponders	8 ± 1	304 ± 15*	304 ± 29
Fecal neutral steroids			
Hyporesponders	29 ± 5	97 ± 25	191 ± 64
Hyperresponders	34 ± 3	98 ± 9	147 ± 41
Fecal bile acids			
Hyporesponders	47 ± 10	41 ± 11	56 ± 22
Hyperresponders	32 ± 8*	35 ± 3	36 ± 15
Total steroid excretion			
Hyporesponders	76 ± 13	137 ± 35	246 ± 85
Hyperresponders	66 ± 6	133 ± 8	183 ± 46
Cholesterol balance			
Hyporesponders	-68 ± 13	+133 ± 28	+ 33 ± 60
Hyperresponders	-57 ± 6	+170 ± 12*	+121 ± 30*

[1] *LCD, low-cholesterol diet; HCD, high-cholesterol diet. Results, expressed as means ± SD for 5 or 6 animals. Significant strain difference: *, $p < 0.05$.*

After feeding the high-cholesterol diet for 28 days, there was a more pronounced increase in neutral steroids excretion in the hyporesponders than in the hyperresponders. This strain difference did not reach statistical significance. Cholesterol feeding did not clearly affect bile acids excretion in either strain. On the low-cholesterol diet,

cholesterol synthesis tended to be higher in the hyporesponders. After cholesterol challenge the hyperresponders accumulated significantly more cholesterol in their body than the hyporesponders (Table 2). During the course of the experiment cholesterol accumulation decreased, with the hyporesponders being able to compensate more efficiently than the hyperresponders.

Table 3 shows the composition of neutral steroids and bile acids in terms of individual compounds. Coprostanol and cholesterol represented the major neutral steroids. Cholesterol feeding caused an increase in the percentage of coprostanol at the expense of cholestanol. There were no strain differences. Feces of hyporesponders contained relatively more deoxycholic acid and 12-keto lithocholic acid and relatively less

TABLE 3 PERCENTAGE DISTRIBUTION OF FECAL NEUTRAL STEROIDS AND BILE ACIDS IN HYPO- AND HYPERRESPONSIVE RABBITS FED A LOW- AND A HIGH-CHOLESTEROL COMMERCIAL DIET

	Composition of fecal steroids					
	LCD[1] Days -7 to -4		HCD Days 0 to 3		HCD Days 28 to 31	
	Hypo	Hyper	Hypo	Hyper	Hypo	Hyper
	(mol/100 mol)					
Neutral steroids						
Cholesterol	56±14	43±12	53±14	54±14	45±21	46±10
Coprostanol	26± 7	34±13	35± 9	35±16	44±31	44±25
Cholestanol	16± 2	22± 5*	9± 1	10± 2	10± 3	9± 2
Coprostanone	3± 1	1± 2	2± 1	1± 1	2± 1	1± 0
Bile acids						
Cholic acid	0	3± 1	0	4± 0	0	2± 1
Chenodeoxycholic acid	3± 1	4± 2	4± 1	4± 1	2± 0	3± 1
Iso-deoxycholic acid	3± 1	6± 1*	3± 1	6± 1*	3± 1	4± 2
Deoxycholic acid	38±17	18± 3*	37±12	17± 4*	42±16	24±15
Hyodeoxycholic acid	19± 2	27± 7*	13± 1	24± 4*	8± 1	17± 8*
Hyocholic acid	1± 1	2± 2	1± 1	2± 2	-	2± 0
Iso-lithocholic acid	2± 1	2± 0	2± 1	2± 0	2± 0	3± 1
Lithocholic acid	7± 1	17± 9*	10± 3	19± 7*	14± 2	21± 5*
12-keto iso-litho-cholic acid	3± 2	3± 1	3± 1	2± 0	3± 1	2± 1
12-keto lithocholic acid	20± 6	11± 3*	22± 9	12± 2*	22±11	14± 7
Other bile acids[2]	5± 3	7± 2	6± 3	8± 2	4± 2	7± 4

[1] *LCD, low-cholesterol diet; HCD, high-cholesterol diet. On Day 0 the rabbits were transferred from the low- to the high-cholesterol, commercial diet. Results, expressed as means ± SD for 5 or 6 animals.* [2] *Iso-chenodeoxycholic acid and 3α, 12β-deoxycholic acid. Significant strain difference: *, p <0.05.*

hyodeoxycholic acid and lithocholic acid than feces of hyperresponders. These differences were not affected by the amount of cholesterol in the diet.

DISCUSSION

In keeping with earlier investigations (1,2), the two rabbit strains differed markedly in their cholesterolemic response to extremely high cholesterol loads (Table 1). Feeding the commercial diet containing 0.3% (w/w) of cholesterol resulted in massive accumulation of cholesterol in the bodies of the rabbits, especially in the hyperresponders (Table 2). Apparently, compensatory mechanisms such as enhanced steroid excretion and depressed cholesterol synthesis (6) were overwhelmed by the cholesterol challenge.

Whole body synthesis of cholesterol was similar in the hypo- and hyperresponsive rabbits when fed the low-cholesterol diet (Table 2). In contrast, faster cholesterol turnover in hyporesponders fed low-cholesterol diets, when compared with hyperresponders, has been reported for species other than rabbits (6), including man (7).

Bile acid excretion in the hyporesponders was consistently higher than in the hyperresponders (Table 2). Hyporesponsive squirrel monkeys have been shown to accelerate bile acid excretion after cholesterol feeding when compared with hyperresponders (8). In this study with rabbits such an effect was not seen convincingly. Fecal excretion of neutral steroids was similar in hypo- and hyperresponders fed the low-cholesterol diet (Table 2). The high cholesterol loads caused an increase in excretion of neutral steroids with feces, the increase being somewhat more pronounced in hypo- than hyperresponders.

The relative composition of fecal bile acids rather than that of neutral steroids showed strain differences (Table 3). This difference could reflect differences in bacterial activity in the colon and/or cecum. It remains to be established whether such differences can determine differences in cholesterolemic response to dietary cholesterol.

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