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Originalarbeiten

Dietary acetate and cholesterol metabolism in rats

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Summary: Rats were fed either cholesterol-free or high-cholesterol (1 %, w/w) semipurified diets containing acetate (0.5 %) or cholestyramine (0.44 %) or both compounds for 29 days. The bile-acid binding resin, cholestyramine, did not affect serum and liver cholesterol, irrespective of whether the diet was cholesterol-free or contained cholesterol. In the cholesterol-free diets, acetate tended to lower the concentration of serum cholesterol, but did not influence liver cholesterol. When the diets contained cholesterol, acetate lowered liver cholesterol concentrations by about 20 % both in the presence and absence of cholestyramine. Acetate did not affect the excretion of bile acids in feces. The mechanism by which acetate may partly counteract the increase in liver cholesterol seen after cholesterol feeding of rats, remains to be established.

Zusammenfassung: Während 29 Tagen wurden an Ratten entweder cholesterinfreie oder cholesterinreiche (1 g/100 g) halbgereinigte Diäten, die Acetat (0,5 g/100 g) oder Cholestyramin (0,44 g/100 g) oder beides enthielten, verabreicht. Der Gallensäurebinder Cholestyramin hatte keinen Einfluß auf Serum- und Lebercholesterin, unabhängig davon, ob die Diät cholesterinfrei oder cholesterinreich war. Bei den cholesterinfreien Diäten tendierte das Acetat dazu, die Serumcholesterinkonzentration zu erniedrigen, aber beeinflußte nicht das Lebercholesterin. Bei den cholesterinreichen Diäten erniedrigte das Acetat die Lebercholesterinkonzentration um etwa 20 % sowohl in Anwesenheit als Abwesenheit von Cholestyramin. Acetat hatte keinen Einfluß auf die Ausscheidung von Gallensäuren im Kot. Der Mechanismus, womit Acetat der cholesterininduzierten Erhöhung der Lebercholesterinkonzentration bei Ratten teilweise entgegenwirkt, sollte noch erforscht werden.

Key words: dietary acetate, cholestyramine, serum cholesterol, liver cholesterol, fecal bile acids, rats

Introduction

The feeding of the gel-forming fiber, pectin, to rats prevents the dietary-cholesterol-induced rise in serum and liver cholesterol when compared to the feeding of cellulose (10, 15). The observed increase in fecal bile acid excretion after pectin feeding (11) may be the key to the cholesterol-lowering activity of this fiber. It is unlikely however, that the enhanced loss of steroids is fully responsible, at least in the rat. This notion is derived from the observation that the bile-acid binding resin, cholestyramine, increases bile acid excretion in the feces (8), but does not lower serum cholesterol in rats on either cholesterol-free (2) or high-cholesterol diets

(13). The lack of effect of cholestyramine on serum cholesterol probably lies in the dramatic increase in hepatic cholesterol synthesis which has been reported for rats fed this resin (12). Possibly, in rats fed pectin such a compensatory mechanism does not gather momentum.

Pectin, unlike cellulose, is fermented almost completely by colonic bacteria to acetate, propionate and butyrate. Indeed, the concentrations of these fatty acids in hepatic portal venous blood of rats fed pectin were higher than those fed cellulose-rich wheat bran (9). In isolated rat hepatocytes acetate has been shown to suppress de novo cholesterol synthesis (3). Perhaps the generation of short-chain fatty acids prevents the rise in hepatic cholesterol synthesis which would be anticipated to occur upon an increased loss of steroids with the feces after pectin feeding. This reasoning implies that the feeding of cholestyramine together with acetate would produce a decrease in serum and liver cholesterol when compared to the feeding of cholestyramine alone. In addition, acetate per se might also have cholesterol-lowering activity. In the present study we have tested these possibilities.

Materials and Methods

Male rats from a random-bred Wistar Cpb/WU colony were used. Until day 0 of the experiment the animals were fed a commercial, pelleted rat diet (RMH-B®, Hope Farms, Woerden, The Netherlands). From the age of about 10 weeks, 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 %).

At day 0 of the experiment, when the rats were aged 12 weeks, they were divided into eight groups consisting of five animals each. The groups had similar distributions of serum cholesterol concentration and body weight. The groups were fed the diets shown in Table 1. The diets were either essentially cholesterol-free or contained 1 % (w/w) of cholesterol. Cholestyramine was added to the diets, as indicated in table 1, in the form of Questran® (Mead Johnson & Co., Evansville, IN, USA); this

Table 1. Composition of the low- and high-cholesterol diets.

Ingredient	Control	Questran®	Acetate	Questran® + acetate
		(g/100 g)		
Questran®	–	1.0	–	1.0
Sodium acetate	–	–	0.5	0.5
Sodium carbonate	0.5	0.5	–	–
Cholesterol	– / 1.0	– / 1.0	– / 1.0	– / 1.0
Corn starch	38.5/37.5	37.5/36.5	38.5/37.5	37.5/36.5
Constant components	61.0	61.0	61.0	61.0

The constant components consisted of (g/100 g diet): casein, 21; sucrose, 10; corn oil, 5; coconut fat, 15; sawdust, 2; dicalcium phosphate, 2.9; sodium chloride, 0.6; magnesium carbonate, 0.3; magnesium oxide, 0.2; potassium carbonate, 1.8; vitamin premix, 1.2, and mineral premix, 1.0. The compositions of the vitamin and mineral premixes have been described elsewhere (5).

product consists of 44.4 % anhydrous cholestyramine resin and 55.6 % carrier. Sodium acetate (J. T. Baker Chemicals BV, Deventer, Holland) was added at a level of 0.5 % of diet, as shown in Table 1. The acetate-free diets were balanced for sodium acetate by the addition of sodium carbonate on a gram for gram basis. The diets were fed for 29 days. All diets were in powdered form. Diets and tap water were provided ad libitum.

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 measured enzymatically using the kit (Monotest®) supplied by Boehringer-Mannheim GmbH, FRG. Feces of each rat were collected daily during the last 3 days of the experiment. Bile acids in freeze-dried feces were extracted and measured according to Van der Meer et al. (14). 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

Table 2 shows that body-weight gain and feed intake were similar for all dietary groups. During the course of the experiment the level of serum cholesterol displayed an increment in all groups. The addition of

Table 2. Performance and cholesterol metabolism in rats fed the experimental diets for 29 days.

	Dietary variables			
	Control	Questran®	Acetate	Questran® + acetate
Cholesterol-free background				
Body weight (g)				
initial (day –1)	280 ±15	285 ±13	284 ±12	278 ± 8
final	355 ±17	363 ±14	357 ±11	348 ±16
Feed intake (g/day)	16.5± 0.3	16.8± 0.6	16.4± 0.4	16.2± 0.6
Serum cholesterol (mmol/l)				
initial (day –6)	2.42± 0.06	2.44± 0.07	2.44± 0.12	2.49± 0.13
final	3.37± 0.14	3.32± 0.15	3.11± 0.18	3.09± 0.15
Liver weight (g)	13.3 ± 0.6	14.1 ± 0.4	13.4 ± 0.7	12.5 ± 0.8
Liver cholesterol (μmol/g)	5.4 ± 0.3	5.9 ± 0.4	5.2 ± 0.1	5.2 ± 0.3
Fecal bile acids (μmol/day)	12.8 ± 2.4	28.0 ± 1.9	14.7 ± 2.4	24.6 ± 1.4
High-cholesterol background				
Body weight (g)				
initial (day –1)	279 ± 8	284 ±10	271 ±10	271 ±13
final	369 ± 9	373 ±16	362 ±16	353 ±17
Feed intake (g/day)	17.0± 0.5	17.0± 0.6	16.7± 0.7	16.5± 0.5
Serum cholesterol (mmol/l)				
initial (day –6)	2.51± 0.11	2.51± 0.13	2.49± 0.13	2.43± 0.11
final	3.08± 0.11	3.04± 0.15	3.03± 0.19	2.77± 0.05
Liver weight (g)	16.0 ± 0.5	16.7 ± 0.9	16.9 ± 1.3	15.4 ± 1.0
Liver cholesterol (μmol/g)	35.5 ± 1.8	35.2 ± 5.0	27.0 ± 3.3	28.2 ± 6.0
Fecal bile acids (μmol/day)	39.9 ± 6.3	71.7 ± 6.0	37.9 ± 3.8	65.4 ± 4.4

Results expressed as means ± S.E. for five rats per group

cholesterol (1 %, w/w) to the diet did not affect this increase. This is compatible with earlier work showing only a small (2, 4) or no (13) increase at all of serum cholesterol in cholesterol-fed rats. In keeping with previous studies, cholestyramine did not significantly lower serum and liver cholesterol, irrespective of whether the diet was cholesterol-free or contained high amounts of cholesterol (2, 13). Cholestyramine however, did cause an increase in the fecal output of bile acids (Table 2), which confirms its well-known bile-acid binding property.

On the cholesterol-free diets, the ingestion of acetate tended to lower serum cholesterol both in the absence and presence of cholestyramine (Table 2). However, the difference did not reach statistical significance. When the diets contained cholesterol, acetate tended to lower serum cholesterol only when cholestyramine was also added to the diet. Acetate did not affect the concentration of cholesterol in liver when the diet was cholesterol-free. However, acetate partly prevented the rise in liver cholesterol seen after cholesterol feeding. Both with and without cholestyramine in the diet, acetate lowered liver cholesterol by about 20 % (Table 2), but statistical significance ($p < 0.05$) was only reached in the absence of the resin. Dietary acetate did not influence the excretion of bile acids in feces.

The data presented here suggest that dietary acetate partly counteracts the increment in liver cholesterol concentration which is observed after feeding cholesterol to rats. Chen et al. (7) have shown that dietary propionate also has cholesterol-lowering activity in cholesterol-fed rats. Thus short-chain fatty acids such as acetate and propionate appear to affect liver cholesterol concentrations. This may be related to inhibition of hepatic cholesterol synthesis imposed by these compounds (3, 6). However, in the present study there was no interaction of acetate with cholestyramine concerning the concentration of liver cholesterol, which suggests that acetate may not counteract the observed (12) cholestyramine-mediated stimulation of hepatic cholesterol synthesis.

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