INTERACTION OF DIETARY CHOLESTEROL WITH CHOLATE IN RATS: EFFECT ON SERUM CHOLESTEROL, LIVER CHOLESTEROL AND LIVER FUNCTION

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ABSTRACT

Female rats were fed diets to which cholesterol (2.0%, w/w) or cholate (0.5%, w/w) or both cholesterol and cholate had been added. Dietary cholesterol alone caused an increase in serum and liver cholesterol, and so did cholate although the increases were much smaller. Cholate markedly enhanced the cholesterol-induced increase in serum and liver cholesterol. Both cholesterol and cholate alone caused hepatomegaly, and there was a significant interaction of both dietary components as to the increase in liver wet weight. The activities in serum of the indicator enzymes for liver function, alkaline phosphatase and alanine amino transferase were significantly increased after feeding diets containing either cholesterol or cholate. Concerning the activities of these serum enzymes there was no significant interaction of dietary cholesterol with cholate. We therefore tentatively suggest that cholesterol and cholate act on liver function enzymes via a common pathway. In contrast, different pathways may be involved as to the effects of cholesterol and cholate on serum and liver cholesterol and the degree of hepatomegaly, because there was a significant interaction of cholesterol with cholate regarding these parameters.

INTRODUCTION

The feeding of diets containing high amounts of both cholesterol and cholate is common practice when rats are used for cholesterol and atherosclerosis research. Such diets cause high degrees of hypercholesterolemia, massive accumulation of cholesterol in the liver, and also increased activities in the serum of the indicators for liver diseases, alanine amino transferase, aspartate amino transferase and alkaline phosphatase (1). Thus high-cholesterol, high-cholate diets probably damage the liver, which in turn may lead to biased interpretation of the data. This study addresses the question whether either the cholesterol or the cholate component of the diet, or the combination of both components, is responsible for the increased activities of liver function enzymes in the serum of rats.

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MATERIALS AND METHODS

Animals, diets and housing. Female rats, aged 7 weeks, were derived from our random-bred colony (Small Animal Center, CKP, Agricultural University). The rats are descendants of the Wistar CPB/WU strain, purchased about three years ago from the Central Institute for the Breeding of Laboratory Animals, CPB-TNO, Zeist, The Netherlands. Until Day 0 of the experiment the animals were fed a commercial pelleted rat diet (RMH-B®, Hope Farms, Woerden, The Netherlands). According to chemical analysis (Weende method) the composition of the commercial diet was as follows (g/100 g): moisture, 12.5; ash, 4.8; crude protein, 24.1; crude fat, 5.9; and crude fiber, 4.1.

At Day 0 of the experiment, the rats were divided into 5 groups, each consisting of 6 animals. The groups had similar distributions of serum cholesterol concentration and body weight. Group A received the low-cholesterol, commercial diet (analysed cholesterol content, 23 mg/100 g). Group B received the commercial diet to which 7.5% (w/w) olive oil had been added. For groups C, D and E part of the olive oil was replaced by 2% of cholesterol, 0.5% of cholate, and 2% of cholesterol plus 0.5% of cholate, respectively. Table 1 shows the composition of the diets. The diets were offered in powdered form, and fed for 28 days. Food and water were provided ad libitum.

TABLE 1. *Composition of the diets*

Ingredient	Diet				
	A	B	C	D	E
	(g/100 g)				
Commercial rat diet[1]	100	92.5	92.5	92.5	92.5
Olive oil[2]	–	7.5	5.5	7.0	5.0
Cholesterol[3]	–	–	2.0	–	2.0
Sodium cholate[4]	–	–	–	0.5	0.5

[1]*RMH-B®, Hope Farms, Woerden, The Netherlands (gross energy, approximately 4.6 kcal/g on as fed basis)*
[2]*Levant®, Huilerie l'Abeille, Marseille, France*
[3]*Duphar BV, Veenendaal, The Netherlands*
[4]*Sigma®, Chemical Co., St. Louis, MO, U.S.A.*

During the experiment the animals were kept in groups of 6 animals in cages (120 x 42 x 19 cm) constructed of stainless steel with wire mesh bases. The cages were placed in a room with air conditioning (18-20 °C), controlled lighting (light: 0600-1800 hours; dark: 1800-0600 hours) and humidity (55 to 65%).

Analytical methods. Blood samples were taken after an 18-hour fast by orbital puncture under light diethyl-ether anesthesia between 0900 and 1100 hours. Serum total cholesterol was measured enzymatically using the

kit (Monotest®) supplied by Boehringer-Mannheim GmbH, F.R.G.

The determinations of the activities in serum of alanine amino transferase (ALAT), aspartate amino transferase (ASAT) and alkaline phosphatase (AP) were performed according to the recommendations of the German Society of Clinical Chemistry (2). Reagents were purchased from Boehringer-Mannheim GmbH, F.R.G.

Total arylesterase activities in serum were determined at pH 8 using β-naphthylpropionate as substrate according to Pilz (3). Reaction conditions were chosen so that the amount of product formed was linear with time and enzyme concentration. Enzyme activity was corrected for spontaneous hydrolysis of the substrate.

At the end of the experiment the anesthetized rats were killed by decapitation, and the livers were removed. Liver cholesterol was extracted and analysed according to Abell et al. (4).

Statistics. The significance of the effect of replacement of 7.5% (w/w) of the commercial diet by olive oil (dietary group B versus dietary group A) was calculated using two-tailed Student's t test. The significance of the effect of replacement of olive oil by cholesterol (dietary group C versus B) or by cholate (dietary group D versus B) or the interaction between cholesterol and cholate was calculated by the analysis of variance.

RESULTS

Body weight at the end of the experiment was similar for all dietary groups (Table 2). Body-weight gain however, was significantly decreased

TABLE 2. *Body weight, body-weight gain and feed intake*

	Diet				
	A	B	C	D	E
Body weight (g)					
Day -1	112±15	118±12	114±10	115±10	115±12
Day 28	153±19	163± 9	154±16	150±10	146± 9
Body-weight gain (g/day)					
Days -1 to 28	42± 7	45± 9	40± 9	35± 4^d	32± 5
Feed intake (g/day)					
Days 0 to 28	13.3	12.5	12.7	12.3	12.5

Results, expressed as means ± SD for 6 animals per dietary group. Feed intake is given as mean value only, since the animals were housed in groups. ^{d}Statistically significant effect of cholate (P<0.05).

by the addition of cholate to the diet (diet D). This cholate effect on body-weight gain was also seen when both cholate and cholesterol had been added to the diet (diet E). The addition of olive oil caused an increase in energy density of the diets (cf. Table 1). This may explain why feed

intake was decreased after the replacement of part of the commercial diet by olive oil (diet A versus other diets).

TABLE 3. *Time course of serum total cholesterol concentration*

	Diet				
	A	B	C	D	E
			(mmol/l)		
Day -2	2.67±0.58	2.72±0.52	2.72±0.51	2.72±0.50	2.77±0.38
Day 7	2.28±0.25	2.17±0.56	3.03±0.63^c	2.27±0.23^d	7.07±2.16^e
Day 14	2.29±0.41	2.14±0.48	4.08±1.30^c	2.45±0.26^d	12.32±4.19^e
Day 28	2.06±0.71	1.87±0.30	5.42±1.28^c	1.65±0.29	7.47±4.50

Means ± SD for six animals per dietary group.
^{c}*Statistically significant effect of cholesterol (P<0.05).*
^{d}*Statistically significant effect of cholate (P<0.05).*
^{e}*Statistically significant interaction between cholesterol and cholate (P<0.05).*

Table 3 shows the time course of serum cholesterol concentration in the rats. The addition of olive oil to the commercial diet did not affect serum cholesterol concentrations (diet B versus A). The analysis of variance showed statistically significant effects of treatments (diets C, D and E versus diet B) at all time points. The addition of cholesterol to the diet (diet C) caused a two-fold increase in serum cholesterol after 28 days. Cholate also increased serum cholesterol (diet D versus B) but the increase was only 5 to 15%, and it was not seen at all at the end of the experiment. A statistically, significant interaction of dietary cholesterol with cholate was only observed at days 7 and 14 of the experiment.

Cholesterol feeding for 28 days caused an increase in liver weight, irrespective of whether it was expressed in absolute terms or relative to body weight (diet C versus B). Table 4 further documents that cholate had a similar effect (diet D versus B), but it was not very pronounced. As to liver weight there was a significant interaction of cholesterol with cholate. The absolute amount and concentration of cholesterol in liver was drastically increased by cholesterol in the diet (diet C versus B); both parameters showed an approximate 18-fold increase. Dietary cholate caused liver cholesterol to increase by about 100%. A clear interaction amongst dietary cholesterol and cholate was seen with regard to their effect on liver cholesterol (Table 4).

Table 4 also shows the activities in serum of arylesterases and of indicator enzymes for liver function. Dietary cholesterol as well as cholate increased the activities of AP, ALAT and ASAT, but the effect of cholate on AP did not reach statistical significance. Concerning these enzyme activities, no interaction of cholesterol with cholate was found. The activity of total serum esterases was not significantly affected by any of the treatments (Table 4).

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TABLE 4. *Liver weight, liver cholesterol and activities in serum of liver-function-indicator enzymes and of arylesterases*

	Diet				
	A	B	C	D	E
Liver weight					
(g)	4.8± 0.7	5.0± 0.3	6.5± 0.7c	5.4± 0.5d	9.2± 0.8e
(g/100 g body weight)	3.1± 0.2	3.1± 0.1	4.2± 0.1c	3.6± 0.3d	6.3± 0.8e
Liver cholesterol					
amount/liver (µmol)	39 ± 7	45 ± 7	882 ±232c	100 ±30d	2018 ±532e
concentration (µmol/g)	8 ± 1	9 ± 1	136 ± 34c	18 ± 5d	218 ± 46e
AP (U/l)	218 ±33	215 ±22	398 ± 98c	306 ±53d	377 ± 80
ALAT (U/l)	30 ±10	31 ± 3	56 ± 20c	37 ± 6d	98 ± 49
ASAT (U/l)	90 ±19	65 ±12	96 ± 13c	90 ±18d	116 ± 29
Esterases (µmol/min/ml)	11.4± 1.6	11.1± 1.2	11.0± 1.2	12.1± 0.9	12.9± 1.0

Means ± SD for six animals per dietary group. Data refer to Day 28 of the experiment.
c Statistically significant effect of cholesterol (P<0.05).
d Statistically significant effect of cholate (P<0.05).
e Statistically significant interaction between cholesterol and cholate (P<0.05).

DISCUSSION

Cholesterol feeding alone caused a marked increase in serum and liver cholesterol of the rats. These effects are well-known. The addition of cholate to the diet had similar effects, but when compared to cholesterol the effects of cholate were small. However, cholate markedly enhanced the effects of cholesterol (cf. ref. 5). There were statistically significant interactions of cholesterol with cholate regarding serum and liver cholesterol. It is possible that cholate improves cholesterol absorption by its emulsifying properties. On the other hand, it could be suggested that cholate after being absorbed and taken up by the liver inhibits the conversion of cholesterol into bile acids: cholate may inhibit hepatic cholesterol 7α-hydroxylase (6), which catalyses the rate limiting step in bile acid synthesis. Thus cholate may either stimulate cholesterol uptake by the body or inhibit cholesterol clearance from it, or both.

It is interesting to note that the serum cholesterol elevating effect of cholate per se had disappeared after 28 days (Table 3). Likewise, at Day 28 of the experiment no interaction between dietary cholesterol and cholate as to the concentration of serum cholesterol was seen. It could be suggested that the rats develop a certain form of adaptation to high cholate intakes. This may then explain the fall in serum cholesterol after 14 days in the rats fed cholate either in the absence or presence of cholesterol. We have suggested earlier (1) that the time-dependent decrease in serum cholesterol on high-cholesterol, high-cholate diets is related to a change in the distribution of cholesterol between serum and liver. In any case, at Day 28 of the experiment liver cholesterol was significantly increased by cholate alone, and cholate also drastically enhanced the effect of cholesterol (Table 4).

Both cholesterol and cholate in the diet caused hepatomegaly: the increase in liver wet weight was 30 and 8%, respectively (Table 4). The combination of cholesterol and cholate increased liver weight by 84%. Thus there was a clear interaction of cholesterol with cholate. Such interaction was not observed with respect to the activities in serum of the indicator enzymes for liver function, AP, ALAT and ASAT. However, cholesterol and cholate alone caused an increase in the activities of these enzymes. The lack of interaction suggests that cholesterol and cholate act on liver function enzymes via a common pathway.

The cholesterol- and cholate-induced increase in the activity of ASAT should be interpreted with caution. The activity measured in the control group (diet B) may be spuriously low because it would not be expected that the addition of olive oil to the diet (diet B versus A) causes a decrease in ASAT activity. In an earlier study we did not find a clear increase of ASAT on a high-cholesterol, high-cholate diet either (1).

In agreement with earlier studies (7) the high-cholesterol, high-cholate diet (diet E) caused an increase in the serum total activity of esterases (Table 4). However, the increase did not reach a level of statistical significance. The absolute activity of esterases in the present study was higher than that reported earlier, which may be related to differences in the procedure of emulsifying the substrate,

i.e. β-naphthylpropionate. The data presented in Table 4 suggest that cholate, but not cholesterol, increases the activity of serum esterases. Clearly, further studies are required on this point.

To summarize, we have observed a significant interaction of dietary cholesterol with cholate in determining the levels of cholesterol in serum and liver. Cholate drastically enhanced the cholesterol-induced increase in liver weight; there was a significant interaction between the two compounds. Both cholesterol and cholate increased serum activities of AP and ALAT, but there was no interaction between the effects of cholesterol and cholate.

ACKNOWLEDGEMENTS

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