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Original Contributions

Comparison of rats with mice concerning the response of lipid metabolism to dietary fats

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Summary: Rats and mice were compared regarding their responses to cholesterol-free or high-cholesterol diets containing either corn oil or coconut fat. In rats fed a cholesterol-free diet, but not in the mice, corn oil caused an increase in serum and liver cholesterol when compared with coconut fat. Corn oil reduced serum triglycerides and increased fecal excretion of neutral steroids in the rats, whereas these variables were not affected in the mice. Fecal bile acid excretion was reduced in mice but not altered in rats. When the diets contained 1 % (w/w) of cholesterol, the rats and mice responded rather similar to corn oil. This study suggests that the response to dietary fats can be species-dependent in some respects.

Zusammenfassung: Ratten und Mäuse wurden im Hinblick auf ihre Reaktionen nach Verabreichung von cholesterinfreien oder cholesterinreichen Diäten – die entweder Maisöl oder Kokosöl als Fettträger enthielten – verglichen. Eine cholesterinfreie Diät mit Maisöl verursachte im Vergleich zur cholesterinfreien Kokosöl-Nahrung bei Ratten einen Anstieg der Serum- und Lebercholesterinkonzentration. Bei Mäusen war dies nicht der Fall. Der Serumtriglyceridespiegel wurde bei Ratten durch Maisöl erniedrigt und die Ausscheidung von neutralen Steroiden mit dem Kot erhöht; bei Mäusen wurden diese Parameter nicht beeinflußt. Die Gallensäureausscheidung war bei den Mäusen reduziert, veränderte sich aber nicht bei den Ratten. Wenn die Diäten 1 % Cholesterin enthielten, reagierten Ratten und Mäuse etwa gleich. Dieser Versuch läßt vermuten, daß die Reaktion auf die Art des Nahrungsfettes in vielerlei Hinsicht von der Tierspezies abhängig ist.

Key words: dietary fatty acids; dietary cholesterol; cholesterol metabolism; rats; mice

Schlüsselwörter: diätetische Fettsäuren; diätetisches Cholesterin; Cholesterinstoffwechsel; Ratten; Mäuse

Introduction

The response of serum cholesterol to replacement of dietary saturated by polyunsaturated fatty acids might differ between rats and mice

(2,3,9–11). In order to further investigate the possible species-dependent response to dietary fats, rats and mice were fed the same diets differing in fat type; and various aspects of lipid metabolism were studied. For this purpose we used cholesterol-free and high-cholesterol semipurified diets containing either saturated fatty acids in the form of coconut fat or polyunsaturated fatty acids as corn oil.

Materials and methods

Male, random-bred Wistar rats (Cpb/WU) and mice (NMRI) were used. Until the beginning of the experiment (Day 0) the animals were maintained on a commercial, pelleted diet (RMH-B®, Hope Farms BV, Woerden, The Netherlands). From the age of five weeks the rats were housed individually in cages (24 × 17 × 17 cm) constructed of stainless steel with wire mesh bases. The mice were kept individually in metabolic cages (Tecniplast®, Tecniplast Gazzada, Buguggiate, Italy). All cages were located in a room with controlled lighting (12 h/day), constant temperature (20–22 °C), and relative humidity (55–65 %).

Table 1. Composition of the experimental diets.

	Diet			
	Corn oil	Coconut fat	Corn oil + cholesterol	Coconut fat + cholesterol
Ingredient (g/100 g)				
Constant components[1])	45.0	45.0	45.0	45.0
Corn oil	20	2	20	2
Coconut fat	–	18	–	18
Cholesterol	–	–	1	1
Corn starch	35.0	35.0	34.0	34.0
Chemical analysis (g/100 g)				
Crude fat	19.6	20.0	20.4	21.1
Fatty acids[2]) (g/100 g fatty acids)				
C 12:0	–	39.6	0.2	39.7
C 14:0	–	15.7	0.1	15.3
C 16:0	11.8	10.6	12.2	10.6
C 18:0	2.1	4.7	2.1	4.6
C 18:1	28.1	8.8	27.9	8.7
C 18:2	55.8	7.4	55.4	7.4
Sat. total	14.5	83.7	15.2	83.8
Mono. total	28.5	8.8	28.4	8.7
Poly. total	56.6	7.5	56.2	7.5

[1]) The constant components consisted of (g): casein, 20; sucrose, 10; molasses, 5; sawdust, 2; dicalcium phosphate, 2.9; sodium chloride, 0.6; magnesium carbonate, 0.3; magnesium oxide, 0.2; potassium bicarbonate 1.8; vitamin premix 1.2; mineral premix 1.0. The composition of the mineral and vitamin premixes have been described elsewhere (4).

[2]) Selected fatty acids in shorthand notation: the number before and after the colon represents the number of carbon atoms and of double bounds, respectively.

At Day 0 of the experiment the animals, aged eight weeks, were divided into four dietary groups consisting of six rats and five mice each. Within each animal species, the distributions of serum cholesterol concentrations and body weights were similar for all groups. The compositions of the semipurified diets, which were provided in meal form, are given in Table 1. The amount of cholesterol in the diet (essentially cholesterol-free versus 1 % of cholesterol) and the type of fat (corn oil versus coconut fat) were the only variables. The animals received the experimental diets for 20 days. Food and tap water were provided *ad libitum*.

Blood samples were taken after a 16-h fast by orbital puncture under light diethylether anesthesia. At the end of the experiment the anesthetized animals were killed by decapitation. The livers were removed, weighed and stored at −20 °C until analysis.

Crude fat concentrations and fatty acid composition of the whole diets were determined according to Folch et al. (5) and Metcalfe et al. (8), respectively. Serum total cholesterol was measured enzymatically according to Siedel et al. (12) using a test-combination (Monotest®). Serum triglycerides were measured enzymatically with a test-combination according to Sullivan et al. (13). Both test-combinations were supplied by Boehringer Mannheim GmbH, FRG. During two periods of three days (Days 1 to 3 and 13 to 15), feces of each animal were collected. Neutral steroids were analyzed by gas-liquid chromatography (6) in samples of pooled feces per dietary group per collection period. Bile acids were determined enzymatically (14) in individual samples of feces pooled per collection period. The livers were homogenized in distilled water, and cholesterol was extracted and analyzed according to Abell et al. (1).

Results

Table 2 shows that the body weights and feed intake of the rats and mice were not influenced significantly by the dietary variables. Cholesterol feeding caused a dramatic increase in liver cholesterol concentrations in

Table 2. Body weight and feed intake in rats and mice fed the experimental diets[1]).

Measure	Diet			
	Corn oil	Coconut fat	Corn oil + cholesterol	Coconut fat + cholesterol
Body weight (g)				
Rats				
Day −2	160 ± 21	162 ± 17	155 ± 14	161 ± 16
Day 20	237 ± 30	253 ± 21	247 ± 26	264 ± 31
Mice				
Day −2	32 ± 2	32 ± 2	32 ± 2	32 ± 2
Day 20	35 ± 2	34 ± 3	33 ± 1	33 ± 2
Feed intake (g/20 days)				
Rats	288 ± 36	315 ± 30	302 ± 26	336 ± 37
Mice	75 ± 5	79 ± 5	78 ± 6	77 ± 3

[1]) Results are expressed as means ± SD for five (mice) or six (rats) animals in each group. The experiment lasted 20 days.

both rats and mice (Table 3). On the cholesterol-free diets, corn oil produced significantly higher levels of cholesterol in livers of rats than did coconut fat. Such an effect was not seen in the mice. In both rats and mice fed the high-cholesterol diets, corn oil caused higher concentrations of liver cholesterol than coconut fat.

When incorporated into the cholesterol-free diets, coconut fat caused lower serum cholesterol concentrations in the rats when compared with corn oil (Table 3). In the mice, coconut fat produced somewhat higher levels of serum cholesterol than did corn oil. The addition of cholesterol to the diets tended to reverse the dietary fat type effect after six days in rats, whereas the differential effect of corn oil and coconut fat was not altered in the mice. In the mice, unlike in the rats, dietary cholesterol as the only variable induced higher serum cholesterol concentrations against a dietary background of corn oil.

Table 3. Liver cholesterol, serum cholesterol, and triglycerides in rats and mice fed the experimental diets[1]).

Measure	Diet			
	Corn oil	Coconut fat	Corn oil + cholesterol	Coconut fat + cholesterol
Liver cholesterol (μmol/g)				
Rats	7.8 ± 1.6	5.4 ± 0.3[b]	45.8 ± 6.8[a]	30.8 ± 6.0[a,b]
Mice	6.6 ± 1.2	6.4 ± 0.9	40.5 ± 6.6[a]	15.8 ± 6.1[a,b]
Serum cholesterol (mM)				
Rats				
Day −2	3.08 ± 0.26	3.06 ± 0.26	2.90 ± 0.41	2.92 ± 0.27
Day 6	3.27 ± 0.21	3.09 ± 0.43	3.45 ± 0.27	4.22 ± 0.47[a,b]
Day 20	3.60 ± 0.46	2.65 ± 0.38[b]	3.33 ± 0.19	3.25 ± 0.29[a]
Mice				
Day −2	5.13 ± 1.09	5.00 ± 0.73	5.30 ± 0.72	5.43 ± 0.69
Day 6	5.94 ± 1.16	6.69 ± 0.84	7.57 ± 1.39	9.31 ± 3.40
Day 20	5.64 ± 0.86	6.79 ± 1.20	6.47 ± 0.55	7.57 ± 0.57[b]
Serum triglycerides (mM)				
Rats				
Day −2	1.15 ± 0.29	0.95 ± 0.50	0.94 ± 0.30	1.01 ± 0.52
Day 20	2.59 ± 1.14	4.49 ± 1.52[b]	1.81 ± 0.89	3.15 ± 1.15[b]
Mice				
Day −2	0.78 ± 0.42	0.66 ± 0.09	0.63 ± 0.19	0.63 ± 0.28
Day 20	0.86 ± 0.36	1.16 ± 0.57	0.72 ± 0.26	0.81 ± 0.34

[1]) Results are expressed as means ± SD for five (mice) or six (rats) animals in each group. The experiment lasted 20 days.

[a] Significantly different from comparable group fed the diet without cholesterol and

[b] significantly different from comparable group fed corn oil ($p < 0.05$; two-tailed Student's t-test).

Table 4. Fecal steroid excretion in rats and mice fed the experimental diets[1]).

Measure	Diet			
	Corn oil	Coconut fat	Corn oil + cholesterol	Coconut fat + cholesterol
Neutral steroids (μmol/day)				
Rats				
Days 1–3	20.7	17.0	92.7	133.7
Days 13–15	21.2	12.1	100.7	112.6
Mice				
Days 1–3	2.96	3.64	15.39	33.38
Days 13–15	3.13	3.30	14.23	17.73
Bile acids (μmol/day)				
Rats				
Days 1–3	11.4 ± 0.9	13.3 ± 2.1	40.3 ± 7.5[a]	50.6 ± 6.9[a,b]
Days 13–15	12.8 ± 1.7	10.8 ± 2.5	50.1 ± 6.6[a]	66.3 ± 7.0[a,b]
Mice				
Days 1–3	1.70 ± 0.15	1.68 ± 0.17	4.57 ± 1.43[a]	5.02 ± 0.54[a]
Days 13–15	1.46 ± 0.12	2.04 ± 0.54[b]	4.11 ± 1.36[a]	4.95 ± 1.05

[1]) Results are expressed as means ± SD for five (mice) or six (rats) animals in each group. The experiment lasted 20 days.

[a] Significantly different from comparable group fed the diet without cholesterol and

[b] significantly different from comparable group fed corn oil ($p < 0.05$; two-tailed Student's t-test).

Dietary coconut fat, compared with corn oil, increased serum triglyceride concentrations in the rats, irrespective of whether the diets were cholesterol-free or contained cholesterol (Table 3). In the mice, coconut fat did not significantly influence serum triglycerides.

In rats fed cholesterol-free diets, corn oil increased neutral steroids excretion compared with coconut fat, whereas bile acid excretion was not influenced (Table 4). However, on the high-cholesterol diets corn oil reduced the excretion of both neutral steroids and bile acids. In the mice, the feeding of corn oil instead of coconut fat resulted in lower group mean excretions of neutral steroids and bile acids, this effect being independent of the amount of dietary cholesterol. The addition of cholesterol to the diets led to a marked increase of fecal steroids excretion in rats and mice.

Discussion

Male rats and mice, aged eight weeks, were fed the same semipurified diets with different fat type (corn oil versus coconut fat) and concentration of cholesterol (cholesterol-free versus 1 %, w/w). There were marked differences between rats and mice concerning the responses to corn oil compared with coconut fat. In rats fed a cholesterol-free diet, corn oil

caused an increase in liver and serum cholesterol and fecal excretion of neutral steroids, whereas serum triglycerides were lowered and the excretion of bile acids not affected. In contrast to the rats, mice showed a corn-oil-induced decrease in serum cholesterol and bile acids.

When high-cholesterol diets were fed, the rats and mice responded rather similar to dietary corn oil, although the effects were generally more pronounced in the rats. In the rats, corn oil produced a decrease in serum cholesterol and triglycerides and in the rates of fecal excretion of neutral steroids and bile acids. Liver cholesterol concentrations were drastically elevated by corn oil. Similar effects were seen in the mice, although those on serum triglycerides and bile acid excretion were small, if any.

On the high-cholesterol diets, cholesterol intakes of the rats and mice were about 420 and 100 μmol/day, respectively. Fecal excretions of total steroids by the rats and mice were only about 160 and 25 μmol/day. This suggests that the animals accumulated high amounts of cholesterol and/or excreted steroids through routes other than feces. Daily cholesterol retention in the liver was about 15 μmol/day in rats and 2 μmol/day in mice (cf. Table 3). Thus, it is likely that the animals excreted high amounts of steroids apart from fecal excretion. Possibly, cholesterol was excreted in the form of urinary bile acids (7).

This study clearly shows that animal species as akin as rats and mice may differ markedly in quantitative terms regarding their responses to dietary fats. Qualitative differences between these animal species cannot be excluded. In basic nutrition research, such differences could provide clues as to mechanisms underlying the effects of various dietary fats on lipid metabolism.

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