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PREVIOUS HIGH-FAT OR HIGH-CARBOHYDRATE INTAKE AND THE SUBSEQUENT CHOLESTEROLEMIC RESPONSE TO A HIGH-CHOLESTEROL DIET IN RATS

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ABSTRACT

Groups of 10 male plus 10 female rats aged 10 weeks were fed either a high-fat (60%; w,w) diet or a commercial diet together with drinking water fortified with sucrose (32 g/100 ml) for 79 days. Both groups were then fed a commercial diet for 48 days and a hypercholesterolemic diet (2% cholesterol; 0.5% cholic acid) for another 22 days. A control group received the commercial diet for 127 days followed by the hypercholesterolemic diet for another 22 days. The high-fat and high-carbohydrate diet did not significantly affect body weight. The high-fat diet raised serum cholesterol levels in both sexes. The high-carbohydrate diet significantly lowered serum cholesterol concentrations in the males, but not in the females. When compared with the control group, previous high-fat or high-carbohydrate intake did not influence the serum cholesterol response to the hypercholesterolemic diet.

INTRODUCTION

It has been reported that homeostatic mechanisms for the control of serum cholesterol can be permanently altered by the composition of the diet during early life. It has been suggested (1, 2) that high, early cholesterol intakes by rats reduced the serum cholesterol response to dietary cholesterol in later life. However, other workers (3-5) did not provide supportive evidence for such an inprinting effect on cholesterol metabolism. In guinea pigs, cholestyramine feeding for four weeks has been found to lower significantly the response of serum cholesterol to cholesterol loading four to six weeks later (6, 7). Evidence was presented (7) that such short-term cholestyramine feeding caused a persistent, enhanced excretion of bile acids with the feces.

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The present study was undertaken to see whether early dietary manipulations other than cholesterol or cholestyramine feeding can affect the later response to a cholesterol challenge. For this purpose rats aged 10 weeks were fed high-fat or high-carbohydrate diets (but no added dietary cholesterol) for 11 weeks followed by a low-cholesterol commercial diet for a "wash-out" period of about 7 weeks. Then their cholesterolemic response to a high-cholesterol diet was determined. As the response to a hypercholesterolemic diet in rats depends on sex (8) the experiment was performed both in males and in females.

MATERIALS AND METHODS

Animals, experimental design and diets

Male and female rats, aged 10 weeks, were used. The animals were derived from our random-bred Wistar colony (Small Animal Center, CKP, Agricultural University). These rats are descendants of the Wistar CPB/WU strain, purchased about 3 years ago from the Central Institute for the Breeding of Laboratory Animals, CPB-TNO, Zeist, The Netherlands. Before and after weaning, at the age of 3 weeks, up until the age of about 10 weeks (Day 0 of the experiment) the animals were fed a commercial, pelleted rat diet (RMH-B®, Hope-Farms, Woerden, The Netherlands). The rats were kept in groups of 10 animals of the same sex in cages (120 x 42 x 19 cm) constructed of stainless steel with wire mesh bases. They were housed in a room with air conditioning (20 °C) and controlled lighting (12 hours light/dark cycle).

At Day 0 of the experiment, the rats were divided into three groups each consisting of 10 males and 10 females. The animals were divided in such a way that within each sex the diet groups had similar distributions of serum cholesterol concentration, body weight and litter origin. The control group received the commercial diet for 127 days. The other groups first received either the commercial diet together with sucrose in the drinking water (32 g/100 ml) or the semipurified, high-fat diet for 79 days (period A). Under these conditions rats consume about 60% of sucrose (supplied through drinking water) on the basis of dry matter intake (9). Subsequently, these groups were transferred to the commercial diet for another 48 days (period B). During the last period of 22 days (period C) all animals were challenged with the high-cholesterol diet. The design of the experiment is illustrated in Fig. 1.

According to chemical analysis (Weende method) the composition of the commercial diet was as follows (g/100 g): moisture, 12.4; ash, 4.8; crude protein, 23.0, crude fat, 6.2 and crude fiber, 4.3. The semi-purified, high-fat diet contained the following ingredients (g/100 g): casein, 21; sucrose, 38; molasses, 5; corn oil, 6; coconut fat, 54; sawdust, 2; dicalcium phosphate, 2.9; sodium chloride, 0.8; magnesium carbonate, 0.3; magnesium oxide, 0.2; potassium bicarbonate, 1.8; vitamin premix, 1.2 and mineral premix, 1.0. The composition of the vitamin and mineral premixes have been described elsewhere (10). The high-cholesterol diet fed to the rats during period C consisted of

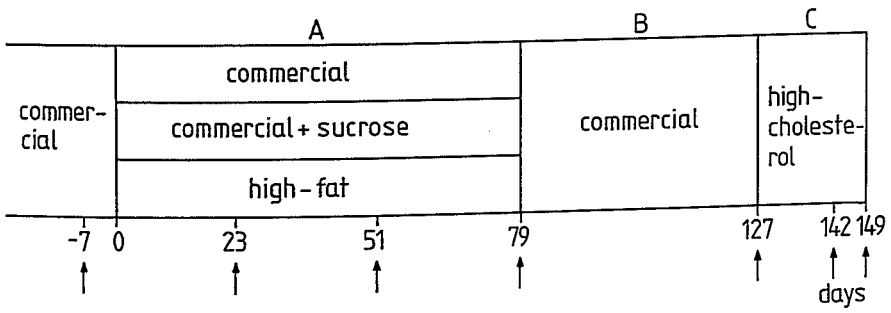

Fig. 1. Experimental design. All animals were fed the commercial diet up until Day 0 of the experiment, when they were aged about 10 weeks. Period A provided different diets during early life, B is a wash-out period, and C the later challenge with a high-cholesterol diet. Each of the three dietary groups consisted of 10 males and 10 females. Arrows indicate the days on which blood was sampled.

(g/100 g): commercial diet, 92.5; olive oil (Levant, Huilerie l'Abeille, Marseille, France), 5; cholesterol (Duphar BV, Veenendaal, The Netherlands), 2 and cholic acid (Merck, Darmstadt, FRG), 0.5. The diets were offered as meal. Diets and water were provided ad libitum. The individual body weights were measured. Feed consumption was only determined during period C (cf. Fig. 1).

Blood sampling and cholesterol analysis

Blood samples were taken in the non-fasting state on the days indicated in Fig. 1, by orbital puncture under light diethyl-ether anesthesia between 08.00 and 10.00 hours. Serum total cholesterol was measured enzymatically according to Röschlau et al. (11) using the kit (Monotest) supplied by Boehringer Mannheim GmbH, FRG.

RESULTS

Body weight

Initial body weight of the male rats was about 40% higher than that of the females (Table 1). The high-fat diet did not affect body weight and body-weight gain when compared with the commercial diet. The addition of sucrose to the drinking water increased mean final body weight in both male and female animals by about 5% over the control group. This difference did not reach statistical significance. However, body-weight gain in both sexes was significantly higher in the group receiving sucrose than in the control group.

During the wash-out period (period B), females in the high-fat and control group caught up with those in the sucrose group and the difference in body weight between the groups of females disappeared. The males fed the control diet tended to grow somewhat slower than

TABLE 1. Body weight (g) and body-weight gain (g/day) of rats fed different diets during period A

	Commercial diet		Commercial diet plus sucrose in drinking water		High-fat diet	
	Males	Females	Males	Females	Males	Females
Pre-experimental body weight; Day -5	229 ±22	161 ± 9	229 ±18	160 ±13	230 ±24	162 ±12
Period A (start; Day 0)						
Initial body weight; Day 1	248 ±21	169 ±10	245 ±16	166 ±13	240 ±20	167 ±11
Final body weight; Day 78	358 ±38	218 ±13	379 ±26	228 ±12	358 ±29	215 ±14
Body weight gain; Days 1 to 78	1.4± 0.2	0.6± 0.1	1.7± 0.2*	0.8± 0.1*	1.5± 0.2	0.6± 0.2
Period B (start; Day 79)						
Body weight gain; Days 78 to 126	0.3± 0.4	0.2± 0.1	0.5± 0.3	0.0± 0.2	0.8± 0.5	0.4± 0.1
Final body weight; Day 126	370 ±40	227 ±14	400 ±31	229 ±15	395 ±39	234 ±15
Period C (start; Day 127)						
Body weight gain; Days 126 to 148	0.2± 0.4	0.2± 0.2	0.1± 0.3	0.1± 0.2	0.3± 0.3	0.1± 0.2
Final body weight; Day 148	375 ±43[3]	232 ±16	402 ±33	231 ±15	400 ±40	238 ±15
Feed intake[1]; Days 127 to 149	14.5[4]	11.2	16.1	11.1	16.4	11.7

Results are expressed as means ± SD for 10 animals. [1]*, Only mean feed intakes (g/day) are given because the animals were housed in groups.* [3]*Results for 8 animals; one animal died at Day 135 and another animal was ill.* [4]*Results for 9 animals, including the sick rat.*
For experimental design and explanation of the dietary periods, see Fig. 1.
*, *Versus rats fed the commercial diet: $P < 0.05$ (two-tailed Student's t test).*

the experimental groups during period B and their mean final body weight was about 7% lower (Table 1).

During the period of cholesterol loading (period C) there was essentially no further increase in body weight in any group. Feed intake in the males was higher than in the females.

Serum cholesterol

Pre-experimental serum cholesterol values were lower in the female than in the male rats (Table 2). Sucrose in the drinking water caused a significant additional fall in serum total cholesterol in the male rats. No such effect was seen in the female rats. The high-fat diet significantly elevated concentrations of cholesterol in the serum of both male and female rats (Table 2). The diet-induced changes in serum cholesterol disappeared completely during the wash-out period (period B).

Feeding the diet containing cholesterol and cholic acid (period C) drastically increased serum cholesterol concentrations in the female rats, the maximum effect being seen within 15 days (cf. Fig. 1). In the male rats the effect on serum cholesterol was less pronounced (Table 2). The diet given during period A had no significant effect in either sex on the serum cholesterol response to the high-cholesterol diet given during period C.

DISCUSSION

The question we originally wanted to address in this study was whether diet-induced obesity would affect the cholesterolemic response to a high-cholesterol diet. However, in contrast to other investigators (9, 12, 13) who found increases in body weight by 20 to 50%, we were not able to induce obesity in our rats by either sucrose in the drinking water or a high-fat diet (Table 1). Our strain of Wistar rats may have been unsuitable: inter-strain differences in the production of obesity by dietary means have been reported (13).

Nevertheless, our study still addresses the difficult question whether early dietary history affects the later response of serum cholesterol to dietary challenge. Some studies with rats have shown a negative correlation between early cholesterol or fat feeding and subsequent response of serum cholesterol to a cholesterol-rich diet (1, 2); others have shown a positive association (3, 14) or no effect at all (4, 5). Our study presents evidence that an extreme high-fat or high-carbohydrate intake by rats in early life does not influence the later cholesterolemic sensitivity to a high-cholesterol diet. Several explanations can be offered for the conflicting results obtained so far. Differences in experimental design, composition of diets and rat strains may be involved. Results obtained with baboons (15) and humans (16) challenged with high-cholesterol diets also suggest that cholesterol homeostatic mechanisms are not permanently altered by early diet. On the other hand, we found in rabbits that a hypercholesterolemic diet (a diet containing casein or cholesterol) fed for 25 days would increase the serum cholesterol response to such a hypercholesterolemic diet being fed 36 days later (17).

TABLE 2. Serum cholesterol concentrations (mmol/l) of rats fed different diets during period A.

	Commercial diet		Commercial diet plus sucrose in drinking water		High-fat diet	
	Males	Females	Males	Females	Males	Females
Pre-experimental (Day -7)	2.35 ± 0.29	2.02 ± 0.36	2.44 ± 0.29	2.03 ± 0.26	2.39 ± 0.20	2.00 ± 0.15
Period A (start, Day 0)						
Day 23	2.14 ± 0.21	1.84 ± 0.25	1.96 ± 0.09*	1.95 ± 0.19	3.03 ± 0.32*	2.44 ± 0.09*
Day 51	2.13 ± 0.23	1.83 ± 0.24	1.86 ± 0.13*	1.73 ± 0.18	2.96 ± 0.32*	2.57 ± 0.12*
Day 79	2.18 ± 0.24	1.96 ± 0.26	1.94 ± 0.14*	1.90 ± 0.20	2.92 ± 0.23*	2.72 ± 0.28*
Change	-0.17 ± 0.21	-0.06 ± 0.21	-0.50 ± 0.27*	-0.13 ± 0.15	+0.53 ± 0.26*	+0.72 ± 0.26*
End period B (Day 127)	2.27 ± 0.36	2.05 ± 0.29	2.24 ± 0.18	2.20 ± 0.27	2.31 ± 0.39	2.09 ± 0.16
Period C (start, Day 127)						
Day 142	5.78 ± 1.71[1]	15.06 ± 5.16	4.18 ± 0.97	13.89 ± 3.55	4.68 ± 1.08	17.49 ± 3.92
Day 149	5.99 ± 2.56[2]	17.60 ± 6.98	4.32 ± 0.78	15.30 ± 4.22	4.91 ± 1.56	17.39 ± 3.14
Change	+3.72 ± 2.49[2]	+15.55 ± 7.08	+2.08 ± 0.70	+13.10 ± 4.15	+2.60 ± 1.59	+15.30 ± 3.15

Results are expressed as means ± SD for 10 animals. [1], *n=9;* [2]*n=8.*

For experimental design and explanation of the dietary periods, see Fig. 1.

*, *Versus rats fed the commercial diet: P < 0.05 (Student's t test).*

The results obtained so far with cholestyramine feeding suggest that cholesterol metabolism can be permanently influenced. In postweaning (7) and neonatal (6) guinea pigs cholestyramine feeding caused relative insensitivity to high cholesterol intakes during later life. In rats born to dams fed cholestyramine during gestation and early lactation a significantly increased response of plasma cholesterol to a high-fat, high-cholesterol diet at the age of about 13 weeks was observed (18). Further studies are required to establish the conditions under which permanent alteration of cholesterol metabolism by dietary means is possible.

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