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RELATION BETWEEN THE RESPONSES OF SERUM CHOLESTEROL TO DIETARY CHOLESTEROL AND TO THE TYPE OF DIETARY FAT IN RANDOM-BRED RABBITS

A.C. Beynen[1, *], C.E. West[2], L.F.M. van Zutphen[1] and M.B. Katan[2]

[1]Department of Laboratory Animal Science, State University, P.O. Box 80.166, 3508 TD Utrecht, [2]Department of Human Nutrition, Agricultural University, De Dreijen 12, 6703 BC Wageningen (The Netherlands).

ABSTRACT

We have investigated whether rabbits that are hyperresponsive to dietary cholesterol are also hyperresponsive to saturated fatty acids in the diet. One group of 26 random-bred rabbits was fed successively a semipurified diet containing corn oil plus cholesterol (4 weeks), the corn-oil diet without cholesterol (8 weeks) and a coconut-fat diet (4 weeks). Another group of 26 random-bred animals received consecutively the diet containing coconut fat, corn oil, and corn oil supplemented with cholesterol. Compared with corn oil, both coconut fat and cholesterol increased the group mean levels of serum cholesterol; there were no time or sequence effects. The individual variation in cholesterolemic responses to the type of fat and cholesterol was large, the coefficients of variation being about 200 and 60%. For the individual responses to coconut fat and cholesterol a linear correlation coefficient of 0.41 (n=52) was calculated, but this coefficient was inflated by the results in two animals. Exclusion of these animals resulted in a correlation coefficient of 0.11 (n=50, P>0.05). Thus this study does not provide solid evidence that rabbits hyperresponsive to dietary cholesterol are also hyperresponsive to saturated fatty acids.

INTRODUCTION

In random-bred rabbits, the individual response of serum cholesterol to dietary cholesterol can vary markedly; some animals show only small changes in serum cholesterol, whereas others show pronounced increases (1, 2). We have recently presented evidence that animals who are hypo- and hyperresponsive to dietary cholesterol, are also hypo- and hyperresponsive, respectively, to a cholesterol-free, semipurified diet containing casein as the sole source of protein (2). Replacement of polyunsaturated fatty acids in a cholesterol-free diet by saturated fatty acids causes an increase in group mean serum cholesterol in rabbits, but individual variation in the response has been found (3, 4). This prompted us to address the question whether animals hypo- and hyperresponsive to dietary cholesterol are also hypo- and hyperresponsive, respectively, to saturated fat. The information obtained may contribute to the further understanding of the mechanism of the hypercholesterolemic action of dietary cholesterol and saturated fatty acids.

*To whom correspondence should be addressed.

MATERIALS AND METHODS

Animals and diets
Random-bred, male rabbits of the New Zealand White strain were obtained from the Broekman Institute, Helmond, The Netherlands. All rabbits were kept individually in cages with mesh bases constructed of galvanized steel in a room with controlled lighting (14 h/day), constant temperature (16 to 20 °C) and humidity (55 to 65%).

Commercial rabbit pellets (Hope Farms, Woerden, The Netherlands) were used during the pre-experimental period. The semipurified diets (Table 1) differed either in their fat component (corn oil-casein diet versus coconut fat-casein diet) or cholesterol content (corn oil-casein diet versus diet containing corn oil-casein plus cholesterol).

TABLE 1
Composition of semipurified diets

Ingredients	Amount for diet (g/100 g)		
	Coconut fat--casein diet	Corn oil--casein diet	Corn oil-casein plus cholesterol diet
Casein	21	21	21
Corn oil	1	10	10
Coconut fat	9	-	-
Cholesterol	-	-	0.15
Sawdust	18	18	17.85
Constant components[1]	51	51	51

[1]The constant components consisted of (grams): corn starch, 17; dextrose, 21; molasses, 5; 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. The vitamin premix contained (in milligrams, except as noted): thiamin, 6; riboflavin, 2.25; niacin, 15.2; Ca-pantothenate, 5.6; choline chloride, 200; inositol, 100; folic acid, 0.85; biotin, 0.06; pyridoxine . HCl, 2.25; p-aminobenzoic acid, 50; vitamin B-12, 1.5 µg; dl-α-tocopheryl acetate, 5; menadione, 0.4; vitamin A, 1500 iu; cholecalciferol, 300 iu; vitamin C, 40. The mineral premix contained (in milligrams): Na citrate . $2H_2O$, 153.5; $FeSO_4 . 7H_2O$, 90; MnO_2, 14; $KAl(SO_4)_2 . 12H_2O$, 10; $ZnSO_4 . 7H_2O$, 20; KBr, 2; $NiSO_4 . 6H_2O$, 0.85; $CuSO_4 . 5H_2O$, 10; $CoSO_4 . 7H_2O$, 0.5; $Na_2MoO_4 . 2H_2O$, 0.5; KI, 0.5; As_2O_3, 0.02; NaF, 0.85; $Na_2B_4O_7 . 10H_2O$, 0.5.

Food was provided to the animals each day at 0900 hours on a restricted basis, the animals receiving 70 g/day. Most rabbits consumed all their food within 4 hours. Water was provided ad libitum.

Experimental design
On arrival in the animal house the random-bred animals, which were aged about 9 weeks, were maintained on the commercial diet for 2 weeks. They were then transferred to the cholesterol-free corn oil-casein diet for 4.5 weeks. Subsequently, two groups of 26 animals each were allocated to either the coconut fat-casein diet or the corn oil-casein diet fortified with cholesterol. The groups had similar distributions of body weight and serum cholesterol. After another 4.5 weeks all rabbits were transferred to the cholesterol-free diet containing corn oil-casein for 8 weeks. Then, the hypercholesterolemic diets were fed again so that the group fed cholesterol in the first part of the experiment received the coconut fat-casein diet (referred to as Chol-Coconut fat group) and vice versa (Coconut fat-Chol group). The diets were fed for 4 weeks. Four animals were fed the cholesterol-free semipurified diet containing corn oil-casein throughout the whole experiment. During the experiment no animals died. The design of the experiment is given in Fig. 1.

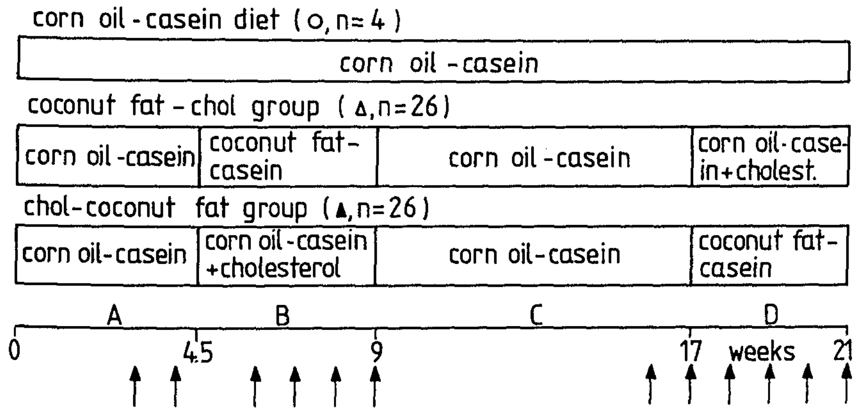

Fig. 1. Experimental design. Arrows indicate the days on which blood samples were taken. Composition of the semipurified diets is given in Table 1.

Analysis of serum cholesterol
Samples of blood were taken between 0800 hours and 1000 hours; any remaining food had been removed at 1600 hours the previous day. The samples were taken from a marginal ear vein into tubes without anticoagulant. Cholesterol in serum was measured enzymatically using the kit (Monotest) supplied by Boehringer-Mannheim GmbH (West Germany). Serum calibrators were used as described (5).

RESULTS

Body weight
Table 2 shows the increase in body weight during the experiment. There was no difference in growth performance between the dietary groups.

TABLE 2
Body weight of the rabbits

	Body weight, g		
	Corn oil-casein diet (n=4)	Coconut fat-Chol group (n=26)	Chol-Coconut fat group (n=26)
End of period A	2633 ± 126	2582 ± 138	2600 ± 162
End of period B	2843 ± 140	2825 ± 131	2867 ± 161
End of period C	3148 ± 146	3159 ± 163	3195 ± 174
End of period D	3221 ± 134	3288 ± 172	3284 ± 171

Results are expressed as means ± SD. For experimental design, see Fig. 1. The animals were fed on a restricted basis.

Serum cholesterol
The concentration of cholesterol in the serum of the random-bred rabbits throughout the experiment is shown in Fig. 2. In the rabbits that remained on the corn oil-casein diet during the entire experimental period, the level of serum cholesterol gradually decreased from 2.14 ± 0.90 mmol/liter at the beginning (mean ± SD) to 0.69 ± 0.32 mmol/liter at the end of the experiment. The rabbits transferred to the corn oil--casein diet enriched with cholesterol (Chol-Coconut fat group) reached a cholesterol level of 7.13 ± 3.18 mmol/l at the end of period B; individual values ranged from 2.35 to 13.82 mmol/l. At the end of period B

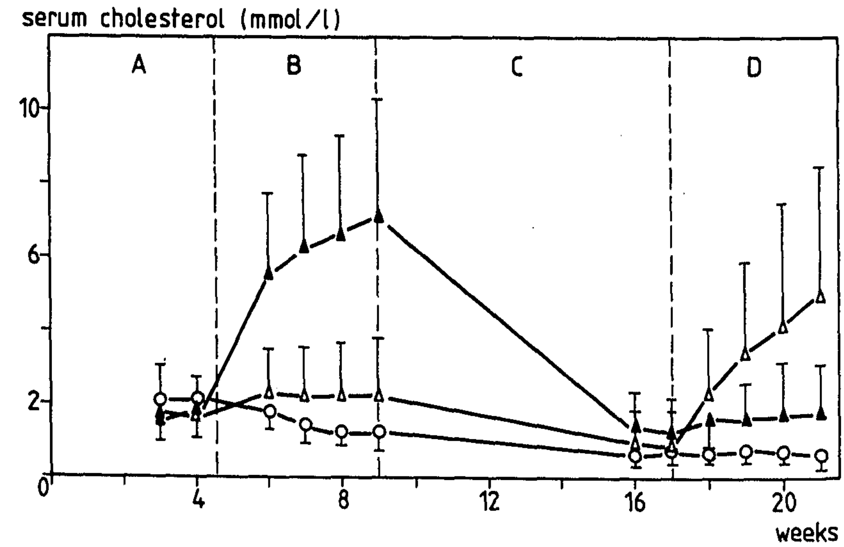

Fig. 2. Serum cholesterol concentrations (means ± SD) of the random-bred rabbits fed semipurified diets. O, Corn oil-casein fed animals (n=4); Δ, Coconut fat-Chol group (n=26); ▲, Chol-Coconut fat group (n=26). Fig. 1 shows the experimental design.

the serum cholesterol level of the Coconut fat-Chol group was 2.23 ± 1.46 mmol/l, with individual values ranging from 0.74 to 6.52 mmol/l.

From week 9 of the experiment all rabbits received the cholesterol-free semipurified diet containing corn oil-casein. After 8 weeks on this diet cholesterol values of the Coconut fat-Chol and Chol-Coconut fat group were 0.90 ± 0.84 and 1.25 ± 0.70 mmol/liter, respectively. These values did not differ significantly from the value (0.89 ± 0.33 mmol/liter) in the animals fed corn oil-casein during the entire experiment.

From week 17 of the experiment the rabbits were challenged with the other hypercholesterolemic diet, containing corn oil-casein plus cholesterol (Coconut fat-Chol group) or coconut fat-casein (Chol-Coconut fat group) for 4 weeks. At the end of this period (period D) the cholesterol-enriched diet had induced a cholesterol level of 5.01 ± 3.46 mmol/liter, with the individual values ranging from 1.84 to 17.72 mmol/l. In the group fed coconut fat-casein (Chol-Coconut fat group) the level of serum cholesterol was 1.73 ± 1.26 mmol/l, with a range of 0.63 to 6.59 mmol/l.

The responses of serum cholesterol to dietary cholesterol and to coconut fat in the random-bred rabbits are given in Table 3. Coconut fat versus corn oil caused a mean increase in serum cholesterol of about 0.4 mmol/l. The addition of 0.15% (w/w) cholesterol to the corn oil diet produced an increase of about 4.5 mmol/l. There was no clear time and/or sequence effect on the response of serum cholesterol to dietary cholesterol and to saturated fat.

TABLE 3
Serum cholesterol responses to dietary cholesterol and to coconut fat versus corn oil

	Change in serum cholesterol, mmol/l	
	Coconut fat-Chol group (n=26)	Chol-Coconut fat group (n=26)
Coconut fat <u>vs</u> corn oil	0.39 ± 1.11	0.47 ± 0.88*
Cholesterol <u>vs</u> no cholesterol	4.12 ± 2.77*	5.26 ± 2.82*

*Results are expressed as means ± SD. Change in serum cholesterol is the difference between values at the end of dietary periods indicated; see Fig. 1 for experimental design. *, Significantly different from zero: P<0.05 (two-tailed Student's t test).*

<u>Correlation between responses to cholesterol and coconut fat</u>
The individual cholesterolemic responses to cholesterol and to coconut fat are plotted in Fig. 3. As the mean responses and their standard deviations were similar, irrespective of the order in which the diets were fed (Table 3), the data of the two dietary groups (Chol-Coconut fat group and Coconut fat-Chol group) were pooled. For all animals (n=52) the correlation coefficient was 0.41 (P<0.01). Two animals

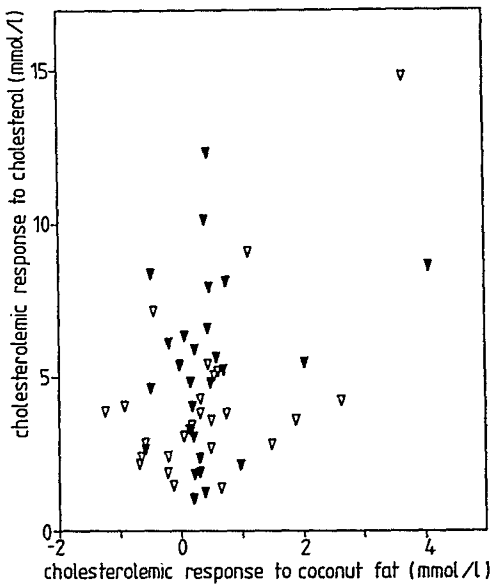

Fig. 3. Relationship between the cholesterolemic responses to cholesterol and to coconut fat (versus corn oil) in the random-bred rabbits. ▽, Coconut fat-Chol group (n=26); ▼, Chol-Coconut fat group (n=26). The cholesterolemic responses were calculated as the differences between values at the end and beginning of dietary periods. Fig. 1 illustrates the experimental design.

(one of each dietary group) were extremely responsive to coconut fat (Fig. 3). The cholesterol values of the two animals were: initial, 2.80 and 2.49; after 1 week of coconut fat feeding, 3.70 and 4.02; after 2 weeks, 4.77 and 5.13; after 3 weeks, 5.32 and 7.35; after 4 weeks, 6.46 and 6.59 mmol/liter. If these two animals are excluded, a linear correlation coefficient of 0.11 (n=50, P>0.05) between the responses to cholesterol and coconut fat was computed.

DISCUSSION

In the random-bred rabbits the responses of serum cholesterol to dietary cholesterol and to coconut fat tended to be positively associated (Fig. 3). However, the correlation was weak. In another study we have found that inbred strains of rabbits either hypo- or hyperresponsive to dietary cholesterol were also hypo- and hyperresponsive, respectively, to dietary saturated versus polyunsaturated fatty acids (6, 7). Thus in rabbits responsiveness to dietary cholesterol and saturated fatty acids appears to coincide, and this phenomenon is most clearly seen in inbred animals.

The mean response of serum cholesterol to coconut fat versus corn oil was about 5-fold lower than observed earlier for animals from the same breeder and using diets of the same composition (8, 9). The low response to coconut fat in these rabbits may have contributed to the observed weak correlation between the responses to cholesterol and coconut fat.

Individuals, hypo- or hyperresponsive to dietary cholesterol, have been identified among animal species such as monkeys, rabbits, rats and pigeons (10). In these animal species the difference in response to dietary cholesterol has a strong genetic basis. We have presented evidence that the phenomenon of hypo- and hyperresponsiveness to dietary cholesterol also exists in humans (11). The observation that rabbits hyperresponsive to dietary cholesterol are also hyperresponsive to saturated fat in a cholesterol-free diet, may be important. It could be suggested that the cholesterol-elevating effect of saturated fat has pathways in common with the hypercholesterolemic effect of dietary cholesterol.

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