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HYPERRESPONSIVENESS OF PLASMA CHOLESTEROL TO DIETARY CHOLESTEROL, SATURATED FATTY ACIDS AND CASEIN IN INBRED RABBITS

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ABSTRACT

We have investigated whether inbred rabbits that are either hyper- or hyporesponsive to dietary cholesterol are also hyper- and hyporesponsive to the type of fat and protein in the diet. Two strains of rabbits were fed cholesterol-free, semipurified diets differing only in their fat source (coconut fat versus corn oil) or protein source (casein versus soy protein). The inbred rabbits hyperresponsive to dietary cholesterol were also found to display an increased cholesterolemic response to dietary coconut fat and to casein. Thus in our two inbred strains responsiveness of plasma cholesterol to dietary cholesterol, to saturated fatty acids and to animal protein coincides.

INTRODUCTION

An increased intake of cholesterol has been found to elicit marked differences in the response of plasma cholesterol between inbred strains of rabbits (1, 2). Certain strains exhibit a relatively high response (hyperresponders), whereas others show only small changes in the level of plasma cholesterol (hyporesponders). It is important to know whether hyperresponders to dietary cholesterol are also hyperresponsive to other dietary components, as such information may provide further clues to the mechanisms underlying the differences in cholesterolemic response to cholesterol. It is well-known that cholesterol-free, semipurified diets containing casein as a protein source produce hypercholesterolemia in rabbits, and that no such effect is observed with soy protein. The replacement of polyunsaturated fat (corn oil) by saturated fat (coconut fat) also elevates serum cholesterol levels in rabbits. In the present study we have measured the plasma cholesterol response to the type of dietary fat and protein in two inbred strains of rabbits, one of which is hypo- and the other hyperresponsive to dietary cholesterol. Part of this work has appeared in a preliminary form (3).

MATERIALS AND METHODS

Animals and diets

Male rabbits of two inbred strains, maintained at the Department of Laboratory Animal Science, were used. These strains (IIIVO/Ju and AX/Ju) originated from the Jackson Laboratory, Bar Harbor, ME, and had

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previously shown to be hypo- and hyperresponsive to dietary cholesterol (1, 2). 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) without and with (0.3%, w/w) added cholesterol were used. The analysed cholesterol contents of the commercial diets were found to be 11 and 313 mg/100 g. The semipurified diets (Table 1) differed either in their protein component (coconut fat-soy protein diet versus coconut fat-casein diet) or fat component (corn oil-casein diet versus coconut fat-casein diet). Food and water were provided ad libitum.

TABLE 1
Composition of semipurified diets

	Amount for diet (g/100 g)		
Ingredients	Coconut fat-soy protein diet	Coconut fat-casein diet	Corn oil-casein diet
Soybean protein	20.8	-	-
Methionine	0.2	-	-
Casein	-	21	21
Corn oil	1	1	10
Coconut fat	9	9	-
Sawdust	18	18	18
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.*

Experimental design

The experiment consisted of 7 periods each of 4 weeks. The order in which the diets were offered is illustrated in Fig. 1. The effect of casein was studied by comparing periods II and III. Replacement of polyunsaturated fat (corn oil) by saturated fat (coconut fat) took place when the animals went from period IV to V. The last two periods served to check the difference in responsiveness to dietary cholesterol in the two strains. At the beginning of the experiment (day 0, Fig. 1), the

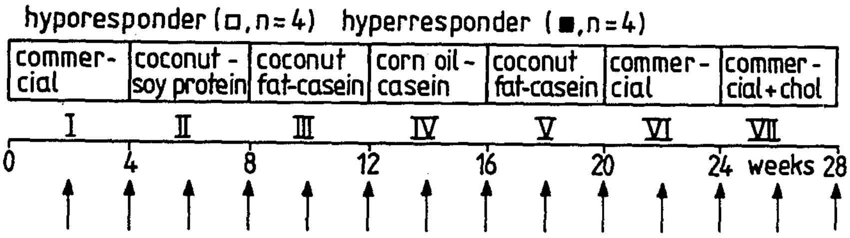

Fig. 1. Experimental design. Arrows indicate the days on which blood samples were taken. Composition of the semipurified diets is given in Table 1.

rabbits were aged 12 to 16 weeks. Four animals per strain were used; there were no deaths.

Analysis of serum cholesterol

Samples of blood were taken between 0800 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 with a drop of heparin. Cholesterol in plasma was measured enzymatically using the kit (Mono test) supplied by Boehringer-Mannheim GmbH (West Germany). Serum calibrators were used as described (4).

RESULTS

Body weight

Table 2 shows the increase in body weight during the experiment. The

TABLE 2
Body weight and feed intake

	Body weight, g		Feed intake, g/day	
	Hypo-responder	Hyper-responder	Hypo-responder	Hyper-responder
Period I	2284 ± 68	1826 ± 217*	n.d.	n.d.
Period II	2243 ± 152	2610 ± 162*	112 ± 10	92 ± 4*
Period III	2701 ± 160	2387 ± 98*	81 ± 12	75 ± 7
Period IV	2852 ± 142	2548 ± 9*	81 ± 14	74 ± 5
Period V	2806 ± 162	2478 ± 88*	61 ± 8	53 ± 12
Period VI	3002 ± 200	2725 ± 103*	99 ± 11	120 ± 11
Period VII	2996 ± 93	2852 ± 100	114 ± 20	139 ± 12

*Body weights refer to the end of the dietary periods. Results are expressed as means ± SD for 4 animals per strain. For experimental design, see Fig. 1. The animals were fed ad libitum. *, Statistically different (P<0.05; two-tailed Student's t test) from hyporesponders. n.d. = not determined.*

body weight of the hyperresponsive inbred rabbits measured just before feeding the semipurified diet containing soy protein was lower than that of their hyporesponsive counterparts. During the experiment body weight of the hyperresponders remained lower, except for the last measurement.

Plasma cholesterol

The time course of plasma cholesterol in the inbred rabbits is shown in Fig. 2. At the end of period I, plasma cholesterol concentrations were 1.00 ± 0.30 and 1.02 ± 0.19 mmol/l in the hyper- and hyporesponders, respectively. Feeding the semipurified diet containing coconut fat-soy protein caused an increase in plasma cholesterol in both strains. The

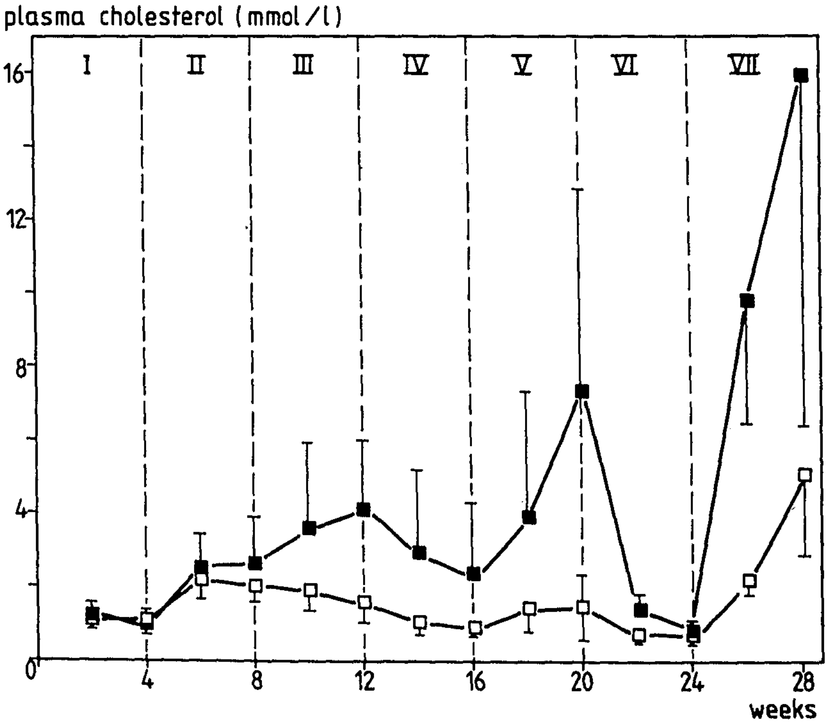

Fig. 2. Plasma cholesterol concentrations (means ± SD) of the inbred rabbits. □, Hyporesponders, IIIVO/Ju strain (n=4); ■, Hyperresponders, AX/Ju strain (n=4). The experimental design is shown in Fig. 1.

subsequent replacement of soy protein by casein produced an elevation in plasma cholesterol in the hyperresponders, but not in the hyporesponders. The cholesterolemic response to coconut fat versus corn oil (compare periods IV and V) was also more pronounced in the hyperresponders. At the end of the 4 weeks of feeding the commercial diet (period VI), plasma cholesterol levels in the hyper- and hyporesponders were 0.77 ± 0.14 and 0.75 ± 0.12 mmol/liter, respectively. Successively, the animals received the commercial diet plus cholesterol, and Fig. 2

confirms (1, 2) that the AX/Ju and IIIVO/Ju strains are hyper- and hypo-responsive, respectively, to dietary cholesterol.

Table 3 documents the responses of plasma cholesterol throughout the entire experiment. The hyperresponders were both more sensitive to dietary cholesterol and to the type of protein and to the type of dietary fat than the hyporesponders.

TABLE 3
Plasma cholesterol responses to dietary variables in the inbred rabbits

	Change in plasma cholesterol, mmol/l	
	Hyporesponder (n=4)	Hyperresponder (n=4)
Casein vs soy protein (III-II)	-0.36 ± 0.08	1.28 ± 0.96*
Coconut fat vs corn oil (V-IV)	0.64 ± 0.77	4.93 ± 3.70*
Cholesterol vs no cholesterol (VII-VI)	4.39 ± 2.25	15.20 ± 9.73*

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

DISCUSSION

In a previous study (5) we showed that in random-bred New Zealand White rabbits there was a statistically significant correlation between the cholesterolemic responses to dietary cholesterol and to casein. The present study extends this observation to inbred strains of rabbits differing in their response of plasma cholesterol to dietary cholesterol. The hyperresponsive strain was also more sensitive to casein than to soy protein with a cholesterol-free background. It is also clear that responsiveness to dietary cholesterol and to saturated fat coincided in our inbred rabbits.

Individuals, hypo- or hyperresponsive to dietary cholesterol, have been identified among animal species such as monkeys, rabbits, rats and pigeons (6). 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 (7). The present 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. The same may hold for the casein-induced hypercholesterolemia. More experimental work is needed to shed more light on the relations between dietary components and cholesterol metabolism. The use of inbred strains of animals with defined, but differential responsiveness to diet, may be of great importance in this respect.

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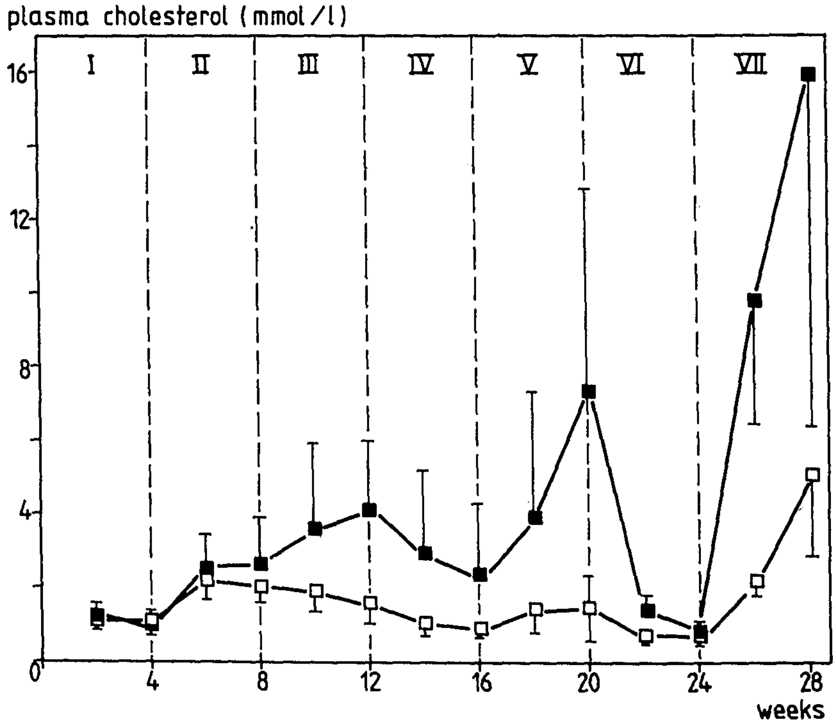

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