Cholesterol Metabolism in Two Strains of Rats with High or Low Response of Serum Cholesterol to a Cholesterol-Rich Diet[1]

A. C. BEYNEN,*† A. BOOGAARD,* H. L. J. M. VAN LAACK* AND M. B. KATAN*

*Department of Human Nutrition, Agricultural University, De Dreijen 12, 6703 BC Wageningen and †Department of Laboratory Animal Science, State University, Yalelaan 1, 3508 TD Utrecht, The Netherlands

ABSTRACT Transfer from a low cholesterol commercial diet to a high cholesterol diet, containing 2% (wt/wt) cholesterol and 0.5% cholate, caused an increase in serum cholesterol from about 2.5 mmol/L in two inbred rat strains to 5 mmol/L in the hyporesponsive strain and to 20 mmol/L in the hyperresponsive strain. In both strains the excess of cholesterol in the serum was exclusively located in the very low density lipoproteins. Cholesterol feeding caused a sevenfold increase in the amount of cholesterol in the liver, the increase tending to be greater in the hyporesponders. On the commercial diet, the decay of specific radioactivity of serum cholesterol after the intravenous administration of labeled cholesterol was faster in the hyporesponsive rats. The rate of fecal excretion of radioactive bile acids on this diet was higher in the hyporesponders when compared with the hyperresponders, whereas there was no strain difference with regard to the output of fecal neutral steroids. Sterol balance data showed that whole-body cholesterol synthesis on the low cholesterol diet was about twofold higher in the hypo- than in the hyperresponders. When fed the high cholesterol diet the half-life in the serum of injected radioactive cholesterol was about six times shorter in the hyporesponders. In absolute amounts, the hypo- and hyperresponders excreted similar amounts of endogenous (radioactive) bile acids and fecal steroids with the feces on this diet. J. Nutr. *114*: 1640–1651, 1984.

INDEXING KEY WORDS dietary cholesterol • hyperresponders • steroid excretion • cholesterol turnover • rat

When animals are fed cholesterol-rich diets, certain individuals respond with only a small increase in serum cholesterol (hyporesponders), whereas others develop a marked hypercholesterolemia (hyperresponders). This has been well-established in monkeys (1, 2), rabbits (3, 4), rats (5, 6) and pigeons (7). The individuality of the cholesterolemic response to dietary cholesterol in these animal species appears to be under genetic control (1, 3, 5, 7, 8). The phenomenon of hyper- and hyporesponsiveness to dietary cholesterol is also seen in humans (9–12).

The individual variability in cholesterolemic response to dietary cholesterol must lie in differences in absorption and/or the efficiency of compensatory mechanisms. Hyporesponsive squirrel monkeys (13), pigeons (14) and rabbits (15) have been shown to enhance their bile acid excretion after cholesterol feeding to a higher extent than their hyperresponsive counterparts. In humans, hyporesponsive individuals have a greater degree of feedback inhibition of cholesterol

 Received for publication 20 February 1984.

[1]This work was supported by The Netherlands Heart Foundation, Grant 31.013 and an established investigatorship to M. B. K.

biosynthesis than hyperresponders (10). In rhesus monkeys the opposite has been reported (16), but hyperresponsive animals may absorb more cholesterol than hyporesponders (16).

Thus it appears that a variety of mechanisms are involved. The present study was carried out in an attempt to reveal the mechanisms underlying the difference in serum cholesterol response in two strains of inbred rats. Part of these studies have appeared in abstract form (17).

MATERIALS AND METHODS

Animals and diets. In these experiments we used two fully inbred strains of male rats (SD/CPB and SHR/CPB). The rats were purchased from the Central Institute for the Breeding of Laboratory Animals (CPB/TNO), Zeist, The Netherlands. It has been shown earlier that the SHR/CPB strain is hyporesponsive and the SD/CPB strain is hyperresponsive to dietary cholesterol (6).

On arrival the animals, aged 4 weeks, were maintained on commercial rat pellets (Hope Farms BV, Woerden, The Netherlands). The composition of this diet is given in footnote 1 of table 1. Food and water were provided ad libitum. After 2 weeks the animals were allowed to eat between 0900 and 1200 hours only; the diet was now provided as meal. Water was provided ad libitum. After two further weeks (day 0; fig. 1) the animals were divided into three groups per strain each consisting of six rats, so that the group mean serum cholesterol concentrations and body weights were similar.

The experimental design is given in figure 1. In experiment A one group of each strain was fed the commercial diet throughout the entire experiment. Two other groups (one of each strain) were transferred to the high cholesterol diet (table 1) at day 0, and continued to receive this diet for 24 days. In experiment B six animals of each strain were successively fed the commercial diet for 24 days and the high cholesterol diet for 41 days.

The rats were either housed per group of six animals (expt A) or individually (expt B) in cages with wire-mesh bases constructed of galvanized steel in a room with controlled lighting (light: 1800–0600 hours, dark: 0600–1800 hours), constant temperature (18 °C) and constant humidity. Body weight and feed intake were recorded.

TABLE 1

Composition of the experimental diets

	Commercial diet	High cholesterol diet
	g/100 g	
Ingredient		
Commercial diet[1]	100	92.5
Olive oil[2]	—	5.0
Sodium cholate[3]	—	0.5
Cholesterol[4]	—	2.0
Chemical analysis		
Moisture	12.6	11.8
Ash	4.7	4.5
Crude protein	25.3	23.2
Crude fat	5.5	12.0
Crude fiber	3.8	3.6
Carbohydrates[5]	48.1	44.9
Cholesterol	0.02	1.96

[1]Hope Farms, Woerden, The Netherlands. The composition of the commercial diet (according to the manufacturer) was as follows: *in percent*: protein, 23.9; total fat, 6.2; linoleic acid, 2.2; fiber, 4.3; carbohydrates, 58.5; calcium, 0.85; phosphorus, 0.60; potassium, 0.65; magnesium, 0.20; sodium, 0.46; chlorine, 0.67; *in milligrams/kilogram*: iron, 195.0; manganese, 72.0; zinc, 63.0; copper, 22.4; cobalt, 0.2; iodine, 0.42; selenium, 0.24; tin, 0.70; chromium, 1.70; nickel, 2.5; vanadium, 0.095; *in milligrams/kilogram (except as noted)*: vitamin A, 20,900 IU/kg; cholecalciferol, 2200 IU/kg; α-tocopherol, 96.0; vitamin K, 10.5; thiamin, 15.6; riboflavin, 13.5; niacin, 84.4; pantothenic acid, 23.4; folic acid, 6.8; pyridoxine, 12.3; choline, 1670; vitamin B-12, 53.2 μg/kg; biotin, 200 μg/kg; and vitamin C, 100. [2]Puget Marseille, France. [3]Merck, Darmstadt, West Germany. [4]Duphar BV, Veenendaal, The Netherlands. [5]By difference.

Analysis of diets. Nitrogen, crude fat, crude fiber and ash in the diets were determined by the Weende method. Protein was calculated as N × 6.25. Cholesterol in the diet was determined by gas–liquid chromatography (18) of the nonsaponifiable fraction.

Analysis of serum cholesterol. Blood samples were taken by orbital puncture under light anesthesia between 1300 and 1400 hours. Serum total cholesterol was measured enzymatically according to Röschlau et al.

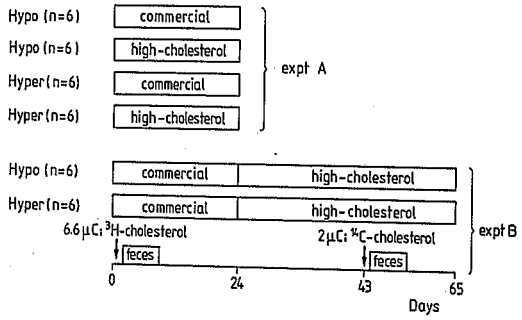

Fig. 1 Design of the experiments. Days are indicated on which radioactive cholesterol was injected and feces were collected in experiment B. All rats were fed the commercial diet from weaning until day 0. On day 0 all animals were 8 weeks of age. For composition of diets, see table 1.

(19), by using the kit (Monotest) supplied by Boehringer-Mannheim GmbH, West Germany. As cholesterol standards, three calibration sera with low, medium and high cholesterol concentrations were used; the cholesterol concentrations of these sera were determined by the method of Abell et al. (20).

At the end of experiment A the animals were killed by decapitation after a fast of 30 hours. Blood was collected, and lipoproteins in pooled sera were isolated by density gradient ultracentrifugation as described by Terpstra et al. (21). The various lipoprotein fractions were collected by aspiration on the basis of the known density gradient in the centrifuge tubes (21). Lipoprotein density classes are based on the pattern observed in humans. Lipoprotein cholesterol was determined according to Röschlau et al. (19), by using the kit (catalase method) supplied by Boehringer-Mannheim GmbH.

Analysis of liver and carcass cholesterol. At the end of experiment A the livers were removed and homogenized in distilled water (about 0.1 g/ml). After determination of the volume of the liver homogenate, samples (triplicates) were taken and extracted according to Folch et al. (22). The lipid extracts were dissolved in the detergent-containing mixture of isopropanol:polyoxyethylene 9-lauryl ether:water (12.5:10:77.5, by vol) prior to the determination of total cholesterol according to Röschlau et al. (19), with the kit (Monotest) supplied by Boehringer-Mannheim GmbH.

The carcasses minus liver were kept in 30% (wt/vol) sodium hydroxide (about 0.5 g/ml) for 1 week at room temperature. After homogenization with a Polytron tissue homogenizer (Brinkmann Instruments, Westbury, NY) and determination of the volume, samples were taken, which were extracted and analyzed for cholesterol exactly as described above.

Cholesterol turnover. On the days indicated in figure 1, the rats in experiment B intravenously received 6.6 μCi of [1α,2α(*n*)-^{3}H] cholesterol and 2 μCi of [4-^{14}C]cholesterol (Amersham International Ltd., Amersham, U.K.). The labeled cholesterol was dispersed in 0.1 ml of a mixture containing physiological saline:ethanol:Tween 20 (95:4.9:0.1) and was injected into the tail vein.

The radioactive serum was extracted according to Folch et al. (22); the lipid extract was mixed with a xylene-based scintillation fluid (Packard, Brussels, Belgium) and counted for its radioactivity. The cholesterol specific radioactivity in the serum was calculated; cholesterol was determined in whole serum as described above.

Analysis of fecal neutral steroids. Feces of each rat were collected daily for 7 days after the injection of radioactive cholesterol (expt B, fig. 1). Radioactivity in fecal neutral steroids was determined in all samples per day; the samples of 1 week were then pooled per animal for analysis by gas–liquid chromatography.

An aliquot (150 mg) of freeze-dried, ground feces was saponified by refluxing in 1.09 *N* NaOH in 76% methanol for 2 hours at 80°C. After the addition of acetic acid and sodium chloride at concentrations of 8.3% and 0.03 g/ml, respectively, the neutral steroids were extracted with three portions of petroleum ether (b.p. 60–80°C). The aqueous phase served for bile acid analysis (see below). The petroleum ether fractions were combined, and the solvent was evaporated under a stream of N_2. The residue was dissolved in 2 ml of petroleum ether and washed with a mixture of 1.4 ml methanol, 0.6 ml H_2O and a drop of acetic acid. The petroleum ether phase was quantitatively removed and the solvent evaporated under N_2. The residue was mixed with scintillation fluid and counted for its radioactivity. The recovery of [^{14}C]cholesterol added to feces was found to be 97 ± 1% (± SD, n = 3).

For the pooled samples, 2.5 μmol of the

internal recovery standard, 5α-cholestane, was added prior to saponification; otherwise the extraction procedure was identical. Trimethylsilyl ethers of the steroids were formed by incubating the residue with a mixture of pyridine:hexamethyl disalazane (Me_6DS): trimethyl chlorosilane (Me_3ClSi) (9:3:1, by vol). The neutral steroid derivatives were analyzed on a Varian Aerograph Series 2700 equipped with a hydrogen flame ionization detector (Varian Associates, Palo Alto, CA). Separation was performed on a fused silica capillary CP Sil 5 column (Chrompack, Middelburg, The Netherlands) with length, 25 m and inner diameter, 0.25 mm. Helium was used as the carrier gas; column pressure was 100 kPa. The temperature of the oven was 260 °C, of the injector 300 °C and of the detector 310 °C. The peaks were integrated by a Spectra-Physics Integrator SP 4100 (Spectra-Physics, Mountain View, CA). Appropriate detector response corrections were made.

Analysis of fecal bile acids. The lower aqueous phases, obtained after extraction of the neutral steroids, were combined, and the methanol component was evaporated under a stream of nitrogen at 80 °C. The water phase was brought to pH 1 with HCl. The bile acids were extracted with three portions of diethyl ether. The ether fractions were combined, and the solvent was evaporated under a stream of nitrogen. The residue was mixed with scintillation fluid and counted for its radioactivity. The recovery of a mixture of radioactive (^{14}C-labeled) bile acids added to feces was found to be 100 ± 3% (± SD, n = 3). These radioactive bile acids had been obtained from rat feces by thin-layer chromatography according to Goswami and Frey (23).

In feces pooled per strain and dietary group, bile acids were also determined (in triplicate) by gas–liquid chromatography. 7-Keto deoxycholic acid (0.75 μmol per sample) was added as an internal recovery standard prior to saponification. After extraction as described above, bile acid methyl esters were prepared by incubating with a mixture of 2,2-dimethoxypropane:methanol:4.5 M HCl (1:1:0.1, by vol). The solvent was evaporated under nitrogen, and trimethylsilyl derivatives were formed by incubating with a solution of pyridine:Me_6DS:Me_3ClSi (9:3:1, by vol) for 30 minutes at 80 °C. The solvent was evaporated, and the residue was dissolved in hexane. The derivatives were separated on a Packard Model 439 gas–liquid chromatograph equipped with a hydrogen flame ionization detector (Packard). A fused silica capillary CP Sil 19 CB column (Chrompack) was used (length, 25 mm; inner diameter, 0.22 mm). Helium (column pressure, 100 kPa) was used as the carrier gas. The oven temperature was programmed to increase from 160 to 275 °C in 34 minutes and then held at 275 °C for 26 minutes; the injector was kept at 275 °C and the detector at 310 °C. The peaks were integrated by a Spectra Physics Integrator SP 4100. Appropriate detector response corrections were made for lithocholic acid, deoxycholic acid, cholic acid and chenodeoxycholic acid; for other bile acids a response factor of 1.0 was assumed.

RESULTS

Serum cholesterol and growth (expts A and B). Figure 2 shows that the two inbred strains differed significantly in their serum cholesterol response to a high cholesterol diet. The SHR/CPB strain was hyporesponsive and the SD/CPB strain was hyperresponsive. This confirms earlier observations (6). Figure 2 also suggests that in both strains the sensitivity to the high cholesterol diet decreases with aging (compare expts A and B). Furthermore, it appeared that serum cholesterol concentrations decreased in the hyperresponsive strain when the high cholesterol diet was fed for longer periods (expt B, fig. 2).

In experiment A body weight gain of animals fed the high cholesterol diet was lower in the hyperresponsive than in the hyporesponsive rats (table 2). This difference was not seen on the commercial diet. Similar results were obtained in experiment B (table 3).

Cholesterol in serum lipoproteins (expt A). The lipoprotein density profiles of the rats can be seen from the photograph after density gradient ultracentrifugation of the serum prestained with Sudan black (fig. 3). In humans, four major lipoprotein classes are separated: very low density lipoproteins (VLDL) at the top of the tube, low density lipoproteins (LDL) and the two high density

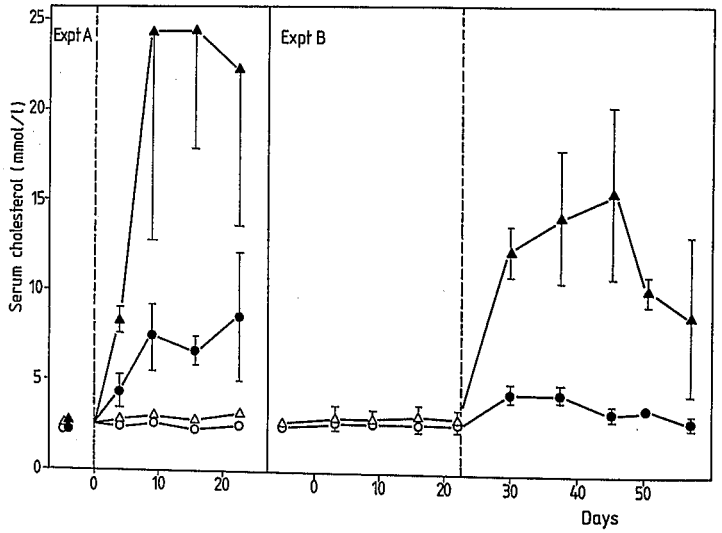

Fig. 2 Time course of serum cholesterol concentrations in hypo- (circles) and hyperresponsive (triangles) rats fed a low cholesterol commercial (open symbols) or high cholesterol (closed symbols) diet. For experimental design, see fig. 1. Results are expressed as means ± SD for six animals per group.

lipoprotein fractions (HDL_2 and HDL_3) further down, with residual stain at the base (fig. 3). In the hyporesponsive rats fed the commercial diet a VLDL band and a very pronounced, single lipoprotein band in the density region of 1.040 to 1.125 g/ml were observed. In the hyperresponders the latter band was somewhat broader. After feeding the high cholesterol diet for 24 days the band with density 1.040 to 1.125 g/ml had completely disappeared in the hyperresponders; in the hyporesponders the band was partly gone (fig. 3). Cholesterol feeding induced accumulation of light particles in the density range <1.040 g/ml. In the hyporesponsive animals, the density range of these particles was greater than in the hyperresponders (fig. 3).

As would be anticipated based on the intensity of the Sudan black staining, the hyperresponders fed the commercial diet had somewhat higher levels of cholesterol in the density range 1.019 to 1.063 g/ml (table 4). After cholesterol feeding there was a dramatic increase in VLDL and intermediate density lipoprotein (IDL) cholesterol, the effect being most pronounced in the hyperresponders. The concentration of HDL_2-cholesterol was decreased to a larger extent in the hyper- than the hyporesponders. In both inbred rat strains the diet-induced increase in serum cholesterol was almost exclusively located in the VLDL fraction (table 4).

Tissue cholesterol (expt A). Table 5 documents that the amounts of cholesterol in

TABLE 2

Body weight, body weight gain and feed intake of hypo- and hyperresponsive rats in experiment A[1]

Measure	Commercial diet		High cholesterol diet	
	Hyporesponder	Hyperresponder	Hyporesponder	Hyperresponder
Body wt, *g*				
Initial[2]	82 ± 12	81 ± 10	81 ± 13	80 ± 12
Final[3]	166 ± 11	169 ± 27	171 ± 15	153 ± 6
Body wt gain,[4] *g/day*	3.3 ± 0.5	3.4 ± 0.8	3.4 ± 0.4	2.8 ± 0.5*
Feed intake,[4] *g/day*	11.3	9.4	11.3	8.1

[1]Results are expressed as means ± SD for six animals in each group. [2]Day −5. [3]Day 23. [4]Days 2–23; for feed intake only mean values are given because the animals were housed in groups. For experimental design, see fig. 1. *Significantly different from hyporesponders, $P < 0.05$ (two-tailed Student's *t*-test).

TABLE 3

Body weight, body weight gain and feed intake of hypo- and hyperresponsive rats fed the low cholesterol commercial diet and high cholesterol diet in succession (expt B)[1]

Diet and measure	Hyporesponder	Hyperresponder
Commercial diet		
Body wt, g		
Initial[2]	95 ± 6	93 ± 7
Final[3]	165 ± 12	156 ± 17
Body wt gain,[4] *g/day*	2.7 ± 0.3	2.7 ± 0.6
Feed intake,[5] *g/day*	10.0 ± 1.0	7.9 ± 0.6*
High cholesterol diet		
Body wt, g		
Final[6]	254 ± 18	242 ± 16
Body wt gain,[7] *g/day*	2.4 ± 0.2	2.0 ± 0.3*
Feed intake,[5] *g/day*	15.7 ± 1.3	12.0 ± 0.9*

[1]Results are expressed as means ± SD for six animals in each group. [2]Day −5. [3]Day 23. [4]Days 2–23. [5]Measured during feces collection period (expt B, fig. 1). [6]Day 65. [7]Days 23–65. For experimental design, see fig. 1. *Significantly different from hyporesponders, $P < 0.05$ (two-tailed Student's *t*-test).

liver and carcass of the hypo- and hyperresponsive rats fed the commercial diet did not show a strain difference. In both strains about 80% of total tissue cholesterol was localized in the carcass minus liver. After cholesterol feeding the amount of cholesterol in the liver increased drastically; the increase tended to be more pronounced in the hyporesponders. The increase in liver wet weight of 2–4 g can only partly be attributed to the increase in cholesterol; the latter represented about 800 mg, assuming that the extra cholesterol was esterified.

Turnover of serum cholesterol (expt B). The specific radioactivity of serum cholesterol as a function of time is shown in figure 4. In rats fed the low cholesterol commercial diet the specific activity decreased more rapidly in the hyporesponders. A reduction to 50% of the calculated (24) initial specific activity was seen after 1.4 days in the hyporesponders, and after 2.7 days in the hyperresponsive rats.

The kinetic experiments were repeated for the rats fed the high cholesterol diet. In the hyperresponders fed cholesterol the calculated (24) initial specific activity of serum cholesterol was lower than in the hyporesponders (0.24×10^4 versus 0.57×10^4 dpm/μmol), which was caused by the higher level of serum cholesterol. The time required to reach 50% of the calculated (24) initial specific activity was 2.1 for the hypo- and 13.0 days for the hyperresponders fed the high cholesterol diet. Because the growing rats were not in a steady state (table 6), no attempt was made to analyze the turnover curves in terms of the two-pool model described by Goodman and Noble (24).

Fecal excretion of radioactive steroids (expt B). As shown in figure 5 the fecal excretion rate of radioactive neutral steroids was similar in the two inbred rat strains, irrespective of the type of diet. In contrast, when they were fed the commercial diet the hyporesponders excreted significantly more radioactive bile acids per day than the hyperresponders (fig. 6); this difference was less pronounced in rats fed the high cholesterol diet. In both rat strains dietary cholesterol caused a decrease in the output rate of radioactive cholesterol in the form of neutral steroids (fig. 5) and bile acids (fig. 6).

Sterol balance (expt B). As shown in table 6, after being fed the low cholesterol commercial diet the total amount of neutral and acidic steroids excreted per day with the feces was higher in the hypo- than in the hyperresponsive animals. This strain difference was also seen for the excretion rate of endogenous bile acids, but not for endogenous neutral steroids. In both rat strains the total output of bile acids measured in the feces by gas chromatography was very close to the output of endogenous bile acids calculated from the amount of radioactivity in the feces and the specific activity of cholesterol in serum. This was to be expected, since the low cholesterol commercial diet did not provide bile acids. The total amount of cholesterol plus its bacterial metabolites excreted per day with the feces was higher than the amount of endogenous origin calculated from the radioactivity in neutral steroids in the feces. Whole-body cholesterol synthesis on the commercial diet was almost twofold higher in the hyporesponsive strain than in the hyperresponders.

The hyporesponders fed the high cholesterol diet excreted significantly higher amounts per day of total neutral steroids with the feces than the hyperresponders, but

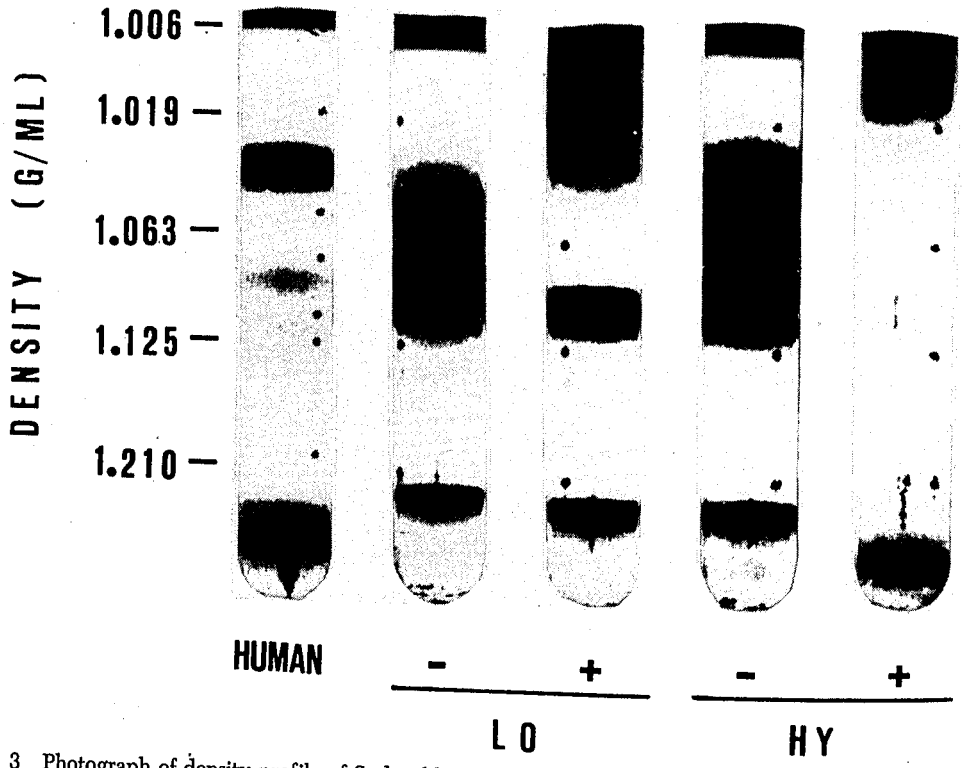

Fig. 3 Photograph of density profile of Sudan black–stained lipoproteins observed after ultracentrifugation of pooled serum of hypo- (LO) and hyperresponsive (HY) rats fed the low cholesterol commercial (−) or high cholesterol (+) diet. Blood was taken from the rats at the end of experiment A (fig. 1).

they also consumed more cholesterol (table 6). The excretion rate of total bile acids was similar in both rat strains, despite the higher intake of cholate by the hyporesponders. The hyperresponders excreted significantly greater amounts per day of endogenous neutral steroids than the hyporesponders (table 6). There was no clear difference between the strains in the excretion of endogenous bile acids with the feces expressed as absolute amounts per day. Cholesterol synthesis of rats fed the high cholesterol diet

TABLE 4

Cholesterol concentrations of serum lipoproteins in hypo- and hyperresponsive rats at the end of experiment A[1]

Fraction	Cholesterol			
	Commercial diet		High cholesterol diet	
	Hyporesponder	Hyperresponder	Hyporesponder	Hyperresponder
	mmol/L of whole serum			
VLDL ($d < 1.006$)	0.54	0.39	5.51	16.28
IDL ($1.006 < d < 1.019$)	0.02	0.13	0.66	1.18
LDL ($1.019 < d < 1.063$)	0.56	0.91	0.63	0.47
HDL_2 ($1.063 < d < 1.125$)	1.40	1.15	0.61	0.22
HDL_3 ($d > 1.125$)	0.08	0.09	0.08	0.04
Sum	2.60	2.67	7.49	18.19
Whole serum	2.55	3.27	8.56	22.28
Recovery, %	102	82	88	82

[1]Blood samples were taken at the end of expt A (day 24). Lipoproteins were isolated from pooled sera of six animals in each group. *d*, density in grams/milliliter.

TABLE 5

Amount of cholesterol in liver and in carcass minus liver of hypo- and hyperresponsive rats at the end of experiment A[1]

Measure	Commercial diet		High cholesterol diet	
	Hyporesponder	Hyperresponder	Hyporesponder	Hyperresponder
Liver				
Wet wt, g	5.1 ± 0.7	5.7 ± 1.1	9.1 ± 0.6	7.8 ± 0.6*
Cholesterol mass, *mmol*	0.17 ± 0.18	0.18 ± 0.03	1.51 ± 0.17	1.14 ± 0.42
Carcass minus liver				
Wt, g	157 ± 11	159 ± 26	158 ± 14	142 ± 6*
Cholesterol mass, *mmol*	0.75 ± 0.08	0.68 ± 0.07	0.81 ± 0.11	0.91 ± 0.09

[1]Results are expressed as means ± SD for six animals per group. All rats were fed the commercial diet up until day 0 (expt A); then half of the animals were allocated to the high cholesterol diet. Values refer to day 24 of the experiment. *Significantly different from hyporesponders, $P < 0.05$ (two-tailed Student's *t*-test).

could not be estimated because the sum of retention and excretion (neutral steroids plus bile acids) minus intake was negative.

DISCUSSION

Serum cholesterol response to dietary cholesterol. It is clear from the present study that the two inbred strains of rats differ markedly in their cholesterolemic response to the diet containing added cholesterol and cholate. The hyperresponders are also more responsive to an increased intake of cholesterol alone when incorporated into a semi-purified diet (25). The increase in serum cholesterol after consumption of the high cholesterol diet was exclusively located in the VLDL fraction, irrespective of the rat strain. It is possible that these lipoproteins are similar to the so-called β-VLDL particles, which have been isolated from the serum of cholesterol-fed rabbits, dogs and monkeys (26). These lipoproteins contain large amounts of cholesteryl esters and arginine-rich protein (apoprotein E) and migrate with β-lipoprotein mobility on electrophoresis (26).

The main objective of this study was to gain some insight into the mechanisms underlying the differential serum cholesterol response in the two rat strains. The variation in the response must be due to differences in cholesterol absorption and/or the efficiency of compensatory mechanisms.

Cholesterol distribution in tissues. Hyporesponsive rabbits stored more cholesterol in their liver after cholesterol feeding than hyperresponders in one study (15), but this was not seen in another study (27). Our hyporesponsive rats tended to accumulate somewhat more cholesterol in the liver than hyperresponders (table 5). The capacity to store cholesterol in the liver may be an important mechanism in the regulation of serum cholesterol levels. After cholesterol feeding the difference in the amounts of liver cholesterol between the hypo- and hyperresponders (1510 vs. 1140 μmol) was greater than the difference in the amounts of cholesterol in the serum (17 vs. 45 μmol; if one assumes that the rats contained 2 ml of serum).

Whole-body cholesterol synthesis after eating the commercial diet. Table 6 shows

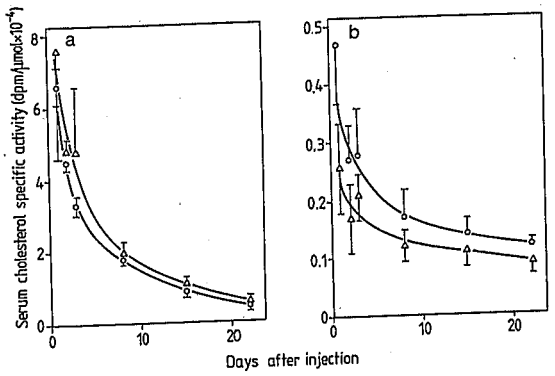

Fig. 4 Turnover of radioactive cholesterol in serum of hypo- (○) and hyperresponsive (△) rats fed a low cholesterol commercial (*4a*) or high cholesterol (*4b*) diet. Results are expressed as means ± SD for three rats; the individual rats (six per group) were sampled alternately. For experimental design, see fig. 1 (expt B).

TABLE 6

Sterol balance data in hypo- and hyperresponsive rats (expt B) successively fed the commercial and the high cholesterol diet[1]

Measure	Rate			
	Commercial diet		High cholesterol diet	
	Hypo-responder	Hyper-responder	Hypo-responder	Hyper-responder
	μmol/day			
Cholesterol intake[2]	5.2 ± 0.5	4.1 ± 0.3*	795 ± 66	608 ± 46*
Cholate intake[3]	—	—	182 ± 15	139 ± 10*
Total fecal excretion of neutral steroids[4]	12.4 ± 2.1	7.6 ± 2.0*	504 ± 61	383 ± 53*
Total fecal excretion of bile acids[5]	14.0	8.6	208	196
Cholesterol retention[6]	11	5	61	49
Cholesterol synthesis[7]	32	17		
Fecal excretion of endogenous neutral steroids[8]	3.7 ± 0.6	3.3 ± 0.3	12.8 ± 2.7	22.3 ± 3.0*
Fecal excretion of endogenous bile acids[9]	13.3 ± 1.6	7.1 ± 1.4*	75 ± 21	97 ± 14

[1]Results are expressed as means (± SD) for six animals per group. Data refer to the periods during which feces was collected in expt B (fig. 1). Body weights (g) at the last days of the feces collection periods were: commercial diet: hyporesponder, 125 ± 10; hyperresponder, 109 ± 8; high cholesterol diet: hyporesponder, 240 ± 18; hyperresponder, 200 ± 30. [2]Calculated by using the analyzed cholesterol contents of the diets (table 1) and feed intakes (table 3). [3]Calculated by using the amount of cholate added to the diet (table 1) and feed intakes (table 3). [4]Measured by gas–liquid chromatography (sum of cholesterol, coprostanone and coprostanol). [5]Measured by gas–liquid chromatography (sum of isolithocholic acid, lithocholic acid, isodeoxycholic acid, deoxycholic acid, cholic acid, chenodeoxycholic acid, ursodeoxycholic acid, 12-keto lithocholic acid and 12-keto chenodeoxycholic acid). [6]Assessed on the basis of cholesterol data for carcass minus liver (table 5) and growth during the fecal collection periods (days 2–8 and days 44–50); for rats fed the high cholesterol diet retention of cholesterol in the liver (table 5) was also taken into account, assuming that cholesterol accumulation in the liver was linear with time. [7]Cholesterol synthesis equals fecal total neutral steroids plus total bile acids plus cholesterol retention minus cholesterol intake. [8]Mean of the total radioactivity of the neutral steroid fraction for days 3–7 of the collection period divided by the serum specific activity of the previous day (derived from fig. 4). [9]Mean of the total radioactivity of the bile acid fraction for days 3–7 of the collection period divided by the serum specific activity of the previous day (derived from fig. 4). *Significantly different from hyporesponders, $P < 0.05$ (two-tailed Student's *t*-test).

that the hyporesponsive rats fed the low cholesterol commercial diet synthesized about twice as much cholesterol per day as their hyperresponsive counterparts. This is also evident from the higher rate of replacement of injected, radioactive cholesterol by unlabeled cholesterol in the hyporesponders (fig. 4), and the more rapid excretion of endogenous (radioactive) bile acids with the feces (fig. 6). The difference in the rate of cholesterol turnover on the commercial diet between the rat strains is accompanied by almost identical levels of serum cholesterol (fig. 2).

After consumption of the low cholesterol commercial diet, the amount of fecal total neutral steroids excreted per day by the hyporesponders was significantly higher than that excreted by the hyperresponders, while the intake of cholesterol and the excretion rate of endogenous neutral steroids did not differ much (table 6). Part of the higher excretion rate of neutral steroids by the hyporesponders could be caused by a lower cholesterol absorption, as has been shown for rhesus monkeys (16). However, it is more likely that when fed the commercial diet the hyporesponders synthesized greater amounts of cholesterol in their intestines. Such cholesterol does not exchange with the serum cholesterol pool (28) and consequently is not measured as endogenous neutral steroids or bile acids. According to Chevalier (29) two-thirds of the total neutral steroids in the feces of rats fed cholesterol-free diets originate from intestinal cholesterol synthesis. This would imply that the hyporesponders synthesize more cholesterol in their intestines than the hyperresponders.

The liver probably also synthesized more

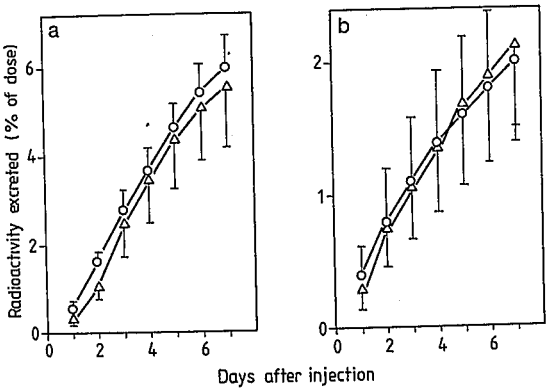

Fig. 5 Cumulative fecal excretion in neutral steroids of intravenously administered radioactive cholesterol by hypo- (○) and hyperresponsive (△) rats fed a low cholesterol commercial (5*a*) or high cholesterol (5*b*) diet. Results are given as means ± SD for six animals. For experimental design, see fig. 1 (expt B).

cholesterol per day in the hyporesponsive rats fed the commercial diet. This is suggested by the higher output of endogenous bile acids (fig. 6) and the larger cholesterol retention (table 6) in the hyporesponsive rats.

High rates of cholesterol synthesis on low cholesterol diets have also been found in hyporesponsive rhesus monkeys (16), pigeons (7) and humans (Katan, M. B. and Beynen, A. C., unpublished results) when compared with hyperresponders. Thus after cholesterol feeding hyporesponders should theoretically be able to suppress cholesterol synthesis over a wider range than hyperresponders. Indeed, Nestel and Poyser (10) have presented evidence that individuals capable of reducing whole-body cholesterol synthesis most markedly, showed the smallest increase in plasma cholesterol from cholesterol feeding. It should be realized, however, that such a mechanism can only partly explain the low cholesterolemic response in the hyporesponsive rats in this study since the extra amount of cholesterol absorbed per day in rats fed the high cholesterol diet was about 10 times the baseline rate of whole-body cholesterol synthesis (see below).

Whole-body cholesterol synthesis after eating the high cholesterol diet. Cholesterol synthesis on the high cholesterol diet could not be assessed as the sterol balance (excretion plus retention minus intake) was negative in both strains. We have seen this earlier in cholesterol-fed calves (30). Considerable amounts of cholesterol and cholate must have been removed from the body via routes other than the feces or perhaps cleaved by bacteria in the intestine. Possibly, cholesterol-feeding of rats enhances the output of bile acids in the urine. Rabbits have been shown to excrete about 10% of total bile acids in the urine (31).

Cholesterol absorption. Cholesterol absorption in rats fed the high cholesterol diet can be calculated if one assumes that the intestinal synthesis of cholesterol is negligible when compared with the intake (32). Cholesterol absorption (intake plus fecal endogenous neutral steroids minus fecal total neutral steroids) was found to be 304 ± 14 (± SD) and 247 ± 47 μmol/day in the hypo- and hyperresponders, respectively. This suggests that the differential cholesterolemic responses to dietary cholesterol in the two strains cannot be explained by less efficient absorption of dietary cholesterol in the hyporesponders.

Neutral steroid and bile acid excretion. Hyporesponsive squirrel monkeys (13), pigeons (14) and rabbits (15) have been shown to enhance their bile acid excretion after cholesterol feeding to a higher extent than their hyperresponsive counterparts. There is no evidence that this holds for humans (9, 10) or for the rat strains that we studied. Fecal total neutral steroid excretion in the hyporesponders was higher, but this can be due to the higher intake of cholesterol, as cholesterol absorption was similar in hypo- and

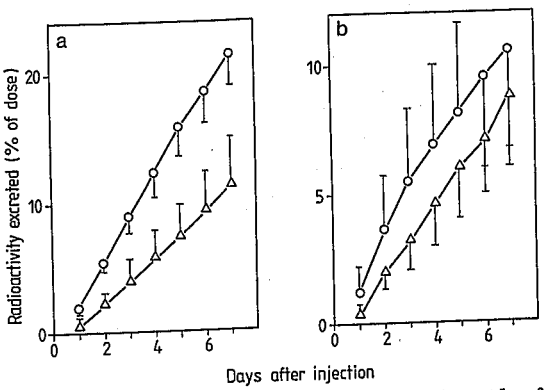

Fig. 6 Cumulative fecal excretion in bile acids of intravenously administered radioactive cholesterol by hypo- (○) and hyperresponsive (△) rats fed a low cholesterol commercial (6*a*) or high cholesterol (6*b*) diet. Results are expressed as means ± SD for six animals. For experimental design, see fig. 1 (expt B).

hyperresponders. The higher endogenous neutral steroid excretion in the hyperresponsive rats fed the high cholesterol diet may be secondary to their higher serum cholesterol levels. In humans after cholesterol feeding the increases in plasma cholesterol and endogenous neutral steroid excretion were also positively associated (10). Thus, changes in steroid excretion apparently are not the key to hyper- and hyporesponsiveness to dietary cholesterol in humans and rats.

Turnover of serum cholesterol. The calculated mean half-life of radioactive cholesterol in the serum of animals fed the commercial diet was lower in the hyporesponsive than in the hyperresponsive rats (1.4 vs. 2.7 days). Such a difference between hypo- and hyperresponders has also been demonstrated in Wistar-King strain rats (33), pigeons (7) and squirrel monkeys (13) fed low cholesterol diets. The faster turnover of serum cholesterol in the hyporesponsive rats is in agreement with the higher rates of cholesterol synthesis (table 6) and the higher output rate of bile acids (fig. 6).

Cholesterol feeding increased the half-life in the serum of intravenously administered cholesterol, the effect being most pronounced in the hyperresponsive strain. In contrast, Takeuchi et al. (33) found that dietary cholesterol decreased the half-life of radioactive serum cholesterol in rats. The clearance of cholesterol remained faster in the hyporesponders than in the hyperresponders (mean half lives: 2.1 vs. 13.0 days). The difference in turnover of serum cholesterol in rats fed the high cholesterol diet in the two strains can be explained by differences in endogenous fecal steroid excretion (table 6). Although the hyperresponders excreted somewhat more endogenous bile acids and neutral steroids in absolute amounts per day, they excreted less than the hyporesponders when expressed relative to the level of serum cholesterol (compare table 6 and fig. 2). This would suggest that the hyporesponsive rats excrete steroids more effectively with the feces than the hyperresponders. However, the opposite would hold if fecal steroid excretion is expressed relative to the rapidly miscible cholesterol pool, i.e., serum cholesterol plus liver cholesterol (24). This cholesterol pool tended to be larger in the hypo- than in the hyperresponders (table 5). It is possible that in the hyporesponders fecal steroid excretion is increased earlier after the commencement of cholesterol feeding than in the hyperresponders. Such a difference between hypo- and hyperresponders has been shown in squirrel monkeys (13).

Conclusions. In sum, we have observed that in two inbred strains of rats the differential cholesterolemic response to a high cholesterol diet is related to a number of differences in cholesterol metabolism. The hyporesponsive rats have higher rates of whole-body cholesterol synthesis when fed the low cholesterol commercial diet, and thus they have more room for down regulation after cholesterol feeding. The hyporesponsive rats also tended to store more cholesterol in their livers after cholesterol feeding than the hyperresponders. With the high cholesterol diet the turnover of serum cholesterol was faster in the hyporesponsive animals, but this could not be explained by a higher absorption and fecal excretion of steroids, when expressed in absolute amounts. However, expressed relative to the level of serum cholesterol, the hyporesponders excreted more steroids with the feces than the hyperresponders.

ACKNOWLEDGMENTS

We thank A. W. Goldsmid and G. den Engelsman for expert analytical assistance.

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