Evaluation of the use of serum lathosterol concentration to assess whole-body cholesterol synthesis in rabbits

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Abstract Serum lathosterol concentration in rabbits was assessed as a possible indicator of whole-body cholesterol synthesis. In random-bred New Zealand White (NZW) rabbits fed a control diet or a diet containing either cholesterol, simvastatin, or cholestyramine, neither serum lathosterol concentration nor the serum lathosterol:total cholesterol ratio systematically corresponded with the anticipated rate of cholesterol synthesis. In control rabbits and those fed simvastatin or cholestyramine, whole-body cholesterol synthesis, which was calculated from the sterol balance, was correlated with serum lathosterol concentration when expressed relative to cholesterol in very low, intermediate, and low density lipoproteins (VLDL + IDL + LDL) (r = 0.61; n = 23; P = 0.002). The low correlation coefficient indicates that the predictive value of the lathosterol:(VLDL + IDL + LDL) cholesterol ratio is limited when applied to individual rabbits. Cholesterol and simvastatin feeding reduced the group mean serum lathosterol:(VLDL + IDL + LDL) cholesterol ratio, whereas cholestyramine in the diet raised the group mean ratio in the NZW rabbits. ▬ We conclude that the serum lathosterol:(VLDL + IDL + LDL) cholesterol ratio may be an indicator of group mean rates of whole-body cholesterol synthesis in rabbits but may not yield reliable information on individual rabbits. The latherosterol:(VLDL + IDL + LDL) cholesterol ratio predicted that in hyperresponsive inbred rabbits, showing an excessive hypercholesterolemia after cholesterol feeding, baseline whole-body cholesterol synthesis is lower than in hyporesponsive rabbits. Addition of cholesterol to the diet caused a reduction of predicted cholesterol synthesis in hypo- but not in hyperresponsive rabbits.—**Meijer, G.W., J. G. P. Van der Palen, H. De Vries, H. J. M. Kempen, H. A. Van der Voort, L. F. M. Van Zutphen, and A. C. Beynen.** Evaluation of the use of serum lathosterol concentration to assess whole-body cholesterol synthesis in rabbits. *J. Lipid Res.* 1992. **33:** 281–286.

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The classical methods to assess the rate of whole-body cholesterol synthesis in rabbits have various disadvantages. Measurement of the incorporation of label from radioactive precursors into whole-body cholesterol is laborious and requires large amounts of radioactivity. Measurement of the activity of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme in the cholesterol biosynthetic pathway, can be used to assess cholesterol synthesis in certain organs but not whole-body cholesterol synthesis. Sterol balance studies in which whole-body cholesterol synthesis is calculated as the fecal excretion of acidic and neutral sterols minus cholesterol intake are strenuous and cannot be used in rabbits fed high-cholesterol diets. After cholesterol feeding of rabbits, cholesterol accumulates in the body so that sterol balance studies underestimate whole-body cholesterol synthesis (1). In humans consuming diets with normal amounts of cholesterol, the lathosterol (5α-cholest-7-ene-3β-ol):cholesterol ratio in serum is directly correlated with the rate of cholesterol synthesis as measured by the sterol balance method (2). Thus, the lathosterol:cholesterol ratio can be considered a valid indicator of whole-body cholesterol synthesis. In the present study, we scrutinized serum lathosterol as a possible index of whole-body cholesterol synthesis in the rabbit.

METHODS

The experimental protocols were approved by the ethical committee on animal experimentation of the Veterinary Faculty of the University of Utrecht.

Abbreviations: VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; HMG, 3-hydroxy-3-methylglutaryl; NZW, New Zealand White; IDL, intermediate density lipoprotein.

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Animals and housing

Male, random-bred specified-pathogen-free New Zealand White (NZW) rabbits (Iffa Credo/Broekman Institute, Helmond, The Netherlands) were used. The rabbits were about 10 week old on arrival. We also used rabbits of two inbred strains. The strains were IIIVO/JU and AX/JU and originated from the Jackson Laboratory Colony, Bar Harbor, ME (3). The IIIVO/JU strain has previously been shown to be hyporesponsive and the AX/JU strain to be hyper-responsive to dietary cholesterol (1, 4, 5). All rabbits were housed individually as described (5). Prior to the studies, all rabbits had been fed a nonpurified diet (LKK-20®, Hope Farms, Woerden, The Netherlands). The rabbits were allowed to practice cecotrophy.

Feeding trial to assess serum lathosterol as indicator of whole-body cholesterol synthesis in random-bred rabbits

For a period of 3 weeks, the NZW rabbits were fed a purified control diet without added cholesterol. The composition of this diet (g/100 g diet) was as follows: soybean protein isolate, 15.8; methionine, 0.2; corn starch–dextrose (1:1 w/w), 41.83; cellulose, 15; coconut fat, 9; corn oil, 1; molasses, 10; calcium carbonate, 1; sodium dihydrogen phosphate, 0.5; sodium chloride, 0.5; sodium carbonate, 1.03; potassium bicarbonate, 1.8; magnesium carbonate, 0.14; vitamin premix, 1.2; mineral premix, 1. The composition of the vitamin and mineral premixes has been described

(5). Then, on day 0 of the experiment, the rabbits were divided into four groups, consisting of eight rabbits each. The groups had similar distributions of body weight (average 2.75 kg) and serum cholesterol concentrations (Table 1). One group remained on the cholesterol-free control diet and the other animals were transferred to diets to which, at the expense of cellulose, compounds were added that are known to influence cholesterol synthesis, namely 0.1% (w/w) cholesterol (Duphar, Amsterdam, The Netherlands), 0.03% simvastatin (Merck, Sharpe & Dohme Inc., Rahway, NJ), or 1% cholestyramine (Mead Johnson & Co., Evansville, IN). In rabbits fed a high-cholesterol diet, cholesterol synthesis is depressed (6). Simvastatin (MK-733) is a potent competitive inhibitor of HMG-CoA reductase (7). The bile acid sequestrant cholestyramine induces an increase of hepatic HMG-CoA reductase activity in rabbits (8).

Upon analysis, the control, cholesterol, simvastatin, and cholestyramine diets were found to contain 1, 92, 1, and 2 mg cholesterol/100 g diet, respectively. The diets were in pelleted form. The rabbits were fed 75 g of feed each day; tap water was provided ad libitum. Body weight and feed intake were determined regularly. The experiment lasted 42 days. Feces of individual rabbits were collected during the last 5 days.

Cholesterol feeding trial to determine serum lathosterol in hypo- and hyperresponsive rabbits

Two male and four female rabbits of each of the two inbred rabbit strains were fed the commercial non-

TABLE 1. Serum cholesterol and lathosterol, and liver cholesterol concentrations in random-bred NZW rabbits fed the experimental diets for 42 days.

Parameter	Experimental Diet				
	Control	Cholesterol (0.1%)	Simvastatin (0.03%)	Cholestyramine (1.0%)	ANOVA[1]
Serum cholesterol (mmol/l)					
Whole serum					
Initial	2.57 ± 0.84^a	2.56 ± 0.81^a	2.53 ± 0.64^a	2.55 ± 0.66^a	0.99
Final	1.94 ± 0.43^a	6.94 ± 4.62^b	1.09 ± 0.29^a	1.02 ± 0.19^a	0.00
VLDL + IDL + LDL[2]	1.20 ± 0.34^a	5.80 ± 4.26^b	0.51 ± 0.18^a	0.54 ± 0.20^a	0.00
HDL[2]	0.77 ± 0.22^{ab}	1.02 ± 0.34^a	0.53 ± 0.14^{bc}	0.45 ± 0.18^c	0.00
Liver cholesterol (μmol/g)[2]	10.5 ± 1.9^a	26.6 ± 7.8^b	5.8 ± 0.8^a	5.5 ± 1.2^a	0.00
Serum lathosterol (μmol/l)					
Initial	1.23 ± 0.35^{ab}	1.06 ± 0.32^a	1.04 ± 0.32^a	1.62 ± 0.35^b	0.01
Final	1.07 ± 0.40^a	2.75 ± 1.63^b	0.37 ± 0.16^a	1.28 ± 0.49^a	0.00
Serum lathosterol:total cholesterol (μmol/mmol)					
Initial	0.51 ± 0.15^{ab}	0.44 ± 0.14^a	0.42 ± 0.11^a	0.67 ± 0.21^b	0.02
Final	0.57 ± 0.22^a	0.43 ± 0.09^a	0.36 ± 0.19^a	1.22 ± 0.25^b	0.00
Serum lathosterol:(VLDL + IDL + LDL) cholesterol (μmol/mmol)[2]					
	0.95 ± 0.4	0.54 ± 0.17^a	0.78 ± 0.51^a	2.45 ± 0.87^b	0.00

Results are expressed as means ± SD for eight animals per group. Values in the same row not sharing a common superscript letter are significantly different.
[1]P-value in one-way analysis of variance.
[2]Final values.

purified diet without or with (0.3%, w/w) added cholesterol for 3 weeks. According to the manufacturer the nonpurified diet (gross energy 18,200 kJ/kg) consisted of (g/100 g diet): total protein, 18.8; fat, 3,3; fiber, 15.8; ash, 8.2. The two dietary groups were matched for sex, age (average 27 months), serum total cholesterol concentration (**Table 2**), and mean body weight (3.1 and 2.8 kg in hypo- and hyperresponders, respectively). Upon analysis, the diets without and with added cholesterol were found to contain 2 and 290 mg of cholesterol/100 g diet. The rabbits were fed 80 g of diet per day, which was completely consumed by all animals. Tap water was provided ad libitum.

Chemical analyses

Samples of blood were taken from the marginal ear vein. Sampling was performed just before feeding, between 8:00 and 10:00 AM. To collect serum, blood was allowed to clot for 30 min at room temperature. Serum was separated by low-speed centrifugation and kept at −20°C until analysis.

Serum lipoproteins were isolated from fresh serum by density gradient ultracentrifugation (9) at the following densities (d, g/ml): pooled very low, intermediate, and low density lipoproteins (VLDL + IDL + LDL), d < 1.063; high density lipoproteins (HDL), 1.063 < d < 1.210. Total cholesterol and triglyceride (10) concentrations in serum or lipoprotein fractions were measured enzymatically with test-combinations purchased from Boehringer, Mannheim, Germany.

Activities of alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), and gamma-glutamyl transpeptidase (GGT) in serum were determined with commercial kits (Hoffmann-La Roche, Basel, Switzerland).

The assay of total lathosterol in serum was performed by gas–liquid chromatography as described previously (2). Feces were freeze-dried and homogenized using a coffee grinder. Neutral steroid and bile acid contents of the feces were determined by capillary gas–liquid chromatography (11). Bile acids were methylated and silylated as described (11) and chromatography was performed using an RT-gap column (1 m × 0.53 mm) connected to a CP sil 19 CB (25 m × 0.25 mm) and a CP Sil 5 CB column (5 m × 0.25 mm).

Liver cholesterol was extracted and analyzed colorimetrically as described (12). Cholesterol in feed samples was analyzed by gas–liquid chromatography (13).

Data analyses

Statistics were performed using the SPSS/PC+ computer program. The Kolmogorov-Smirnov one-sample test was used to check for the normal distribution of data. Evaluation of the findings in the experiment with random-bred rabbits was carried out by one-way analysis of variance; differences between treatment means were analyzed with Tukey's multiple comparison test. The data from the experiment with hypo- and hyperresponsive rabbits were subjected to Student's t-test to disclose differences between and within strains. For selected parameters, Pearson correlation coefficient (r) was calculated. Two-sided P values < 0.05 were used as criterion of statistical significance.

TABLE 2. Serum cholesterol and lathosterol concentrations and predicted rates of cholesterol synthesis in inbred hypo- and hyperresponsive rabbits fed diets without or with added cholesterol

Parameter	Diet without Added Cholesterol		Diet with Added (0.3 g/100 g) Cholesterol	
	Hypo	Hyper	Hypo	Hyper
Serum cholesterol (mmol/l)				
Whole serum				
Initial	1.38 ± 0.25	0.83 ± 0.21^a	1.36 ± 0.21	0.81 ± 0.35^a
Final	1.38 ± 0.23	0.98 ± 0.23^a	6.99 ± 1.73^b	$18.76 \pm 3.08^{a,b}$
VLDL + IDL + LDL^c	0.40 ± 0.11	0.54 ± 0.12	4.51 ± 1.34^b	$10.95 \pm 4.05^{a,b}$
Serum lathosterol:(VLDL + IDL + LDL)cholesterol (μmol/mmol)^c				
	1.93 ± 0.58	1.07 ± 0.20^a	0.64 ± 0.20^b	1.42 ± 0.98
Predicted cholesterol synthesis (μmol $\cdot$ kg^{-1} $\cdot$ day^{-1})^d				
	51 ± 6	34 ± 6^a	25 ± 7^b	41 ± 5^a

Results are expressed as means ± SD for six animals per group.
[a]Significant difference between the hypo- and the hyperresponsive rabbit strain fed the same diet.
[b]Significantly different from rabbits of the same strain but fed the diet without added cholesterol.
[c]Final values.
[d]Cholesterol synthesis ± SE, as predicted with the regression equation from the data in Fig. 1. The SE of cholesterol synthesis was estimated using the formula $s. \sqrt{\dfrac{1}{N} + \dfrac{(R - \bar{x})^2}{(N - 1) \cdot s_x^2}}$, in which s is the standard error of the estimate, R is the observed lathosterol:(VLDL + IDL + LDL) cholesterol ratio, and $\bar{x}$ (average lathosterol:(VLDL + IDL + LDL) cholesterol ratio), N (number of animals) and s_x^2 (variance of x) were obtained from the data of the random-bred rabbits.

RESULTS

Experiment with random-bred rabbits

There was a treatment effect on feed intake, which just reached statistical significance ($P = 0.04$; data not shown). The rabbits fed cholesterol consumed slightly less feed (70 ± 2.8 g/day; mean $\pm$ SD, n = 8) than those fed cholestyramine (75 ± 0.1 g/day). Final body and liver weights did not differ significantly among the groups (results not shown).

Inhibition of hepatic HMG-CoA reductase in rabbits by high doses (200 mg $\cdot$ kg^{-1} $\cdot$ day^{-1}) of the specific inhibitor lovastatin, which is structurally related to simvastatin, is associated with pathological changes in the liver and elevated serum ALAT and ASAT activities (14). High intakes of cholesterol may also cause liver damage (15). To check for possible impaired liver function in this study, serum ALAT, ASAT, and GGT activities were determined. There were no significant differences in the activities of these indicators of liver integrity between the four groups (data not shown).

The addition of cholesterol to the diet significantly elevated whole serum cholesterol and cholesterol in VLDL + IDL + LDL (Table 1). Dietary simvastatin and cholestyramine caused a comparable decrease of group mean whole serum and VLDL + IDL + LDL cholesterol, but the decreases were not statistically significant. HDL cholesterol tended to be increased by dietary cholesterol and decreased by the two hypocholesterolemic drugs. Dietary cholesterol significantly elevated liver cholesterol concentration. Dietary simvastatin and cholestyramine lowered group mean liver cholesterol, the effects not being statistically significant. Serum triglyceride level was not affected by the dietary treatments (not shown).

Final serum total lathosterol was significantly increased by the addition of cholesterol to the diet (Table 1). Simvastatin tended to lower serum lathosterol but cholestyramine in the diet had no effect. A significant positive correlation was observed between final serum lathosterol and total cholesterol ($r = 0.89$; n = 32; $P < 0.0005$). To adjust for treatment effects on serum total cholesterol, the lathosterol:cholesterol ratio was calculated (Table 1). This ratio was significantly increased after cholestyramine feeding while simvastatin tended to lower it. However, cholesterol in the diet did not significantly influence this ratio. Treatment effects on serum total cholesterol were reflected by changes in cholesterol in VLDL + IDL + LDL rather than by changes in HDL cholesterol (Table 1). Final cholesterol concentrations in VLDL + IDL + LDL were positively correlated with final serum lathosterol concentrations ($r = 0.85$; n = 32; $P < 0.0005$). Group mean lathosterol:(VLDL + IDL + LDL) cholesterol ratio tended to be reduced by either cholesterol or simvas-

tatin feeding and was significantly increased by dietary cholestyramine (Table 1).

The increased intake of cholesterol did not significantly influence fecal excretion of neutral steroids and bile acids (not shown). In the rabbits fed simvastatin, neutral steroids and bile acids excretion tended to be decreased. As expected (8), fecal bile acid excretion was markedly enhanced by cholestyramine. Whole-body cholesterol synthesis was assessed by the sterol balance method as fecal bile acids and neutral steroids minus cholesterol intake. Cholesterol synthesis in the treatment groups was as follows (μmol $\cdot$ kg body weight^{-1} $\cdot$ day^{-1}; means $\pm$ SD, n = 7 or 8): controls, 25 ± 8; cholesterol-fed group, -20 ± 9; simvastatin group, 19 ± 6 (n = 7); cholestyramine group, 77 ± 27. The cholesterol and cholestyramine effects were statistically significant. The degree of treatment-induced changes corresponded well with that of the changes of the lathosterol:(VLDL + IDL + LDL) cholesterol ratio. To calculate the correlation coefficient between the lathosterol:(VLDL + IDL + LDL) cholesterol ratio and rates of whole-body cholesterol synthesis, the animals fed cholesterol with negative rates had to be excluded. The correlation was statistically significant ($r = 0.61$; n = 23; $P = 0.002$). **Fig. 1** shows the scattergram.

Experiment with inbred hypo- and hyperresponsive rabbits

Mean body weight, which was lower in hyper- than in hyporesponsive rabbits, did not change during the
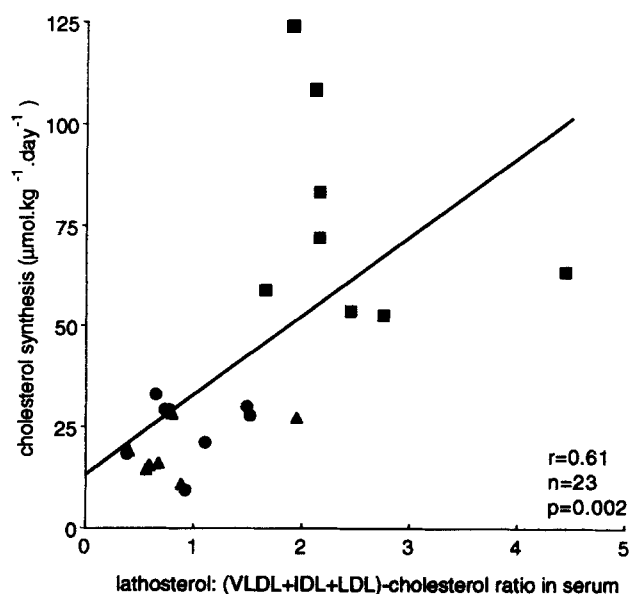

Fig. 1. Relationship between serum lathosterol:(VLDL + IDL + LDL) cholesterol ratio and whole-body cholesterol synthesis in individual, random-bred NZW rabbits fed either a control diet (●) or a diet with simvastatin (▲) or cholestyramine (■). The feces sample of one of the rabbits fed simvastatin was lost during processing. The equation of the regression line is $y = 19.7x + 12.9$.

course of the experiment. Initial serum total cholesterol concentration was lower in hyper- than in hyporesponders (Table 2). As expected (1, 4, 5), the high-cholesterol diet raised serum total cholesterol in the two strains, the effect being significantly greater in the hyperresponders. A substantial part of the increase in serum total cholesterol was located in VLDL + IDL + LDL.

When fed the diet without additional cholesterol, the ratio of lathosterol:(VLDL + IDL + LDL) cholesterol in serum was significantly lower in hyper- than in hyporesponsive rabbits (Table 2). After the addition of cholesterol to the diet, the ratio dropped significantly in hyporesponders but was not decreased in the hyperresponders.

Whole-body cholesterol synthesis in hypo- and hyperresponsive rabbits was predicted with the use of the regression line in Fig. 1. Initial rates of cholesterol synthesis were significantly lower in hyper- than in hyporesponders. Cholesterol feeding depressed cholesterol synthesis significantly in the hyporesponders but not in the hyperresponders.

DISCUSSION

Lathosterol is an intermediate of the cholesterol biosynthetic pathway. The rationale for using serum lathosterol as an indicator of the rate of cholesterol synthesis lies in the assumption that it leaks out of cells and is incorporated into serum lipoproteins at a rate proportional to that of its formation in the cholesterol biosynthetic pathway. In both humans (16) and rats (17), serum lathosterol concentrations are directly associated with hepatic HMG-CoA reductase activity. In the rabbit, serum lathosterol concentration is not likely to be an index of hepatic HMG-CoA reductase activity. In contrast to its inhibitory effect on hepatic HMG-CoA reductase activity (6), cholesterol feeding significantly raised lathosterol concentration in serum. Further, the group mean lathosterol concentration was lowered by cholestyramine in the diet, which contradicts the stimulatory effect of cholestyramine on HMG-CoA reductase activity (8). In the rabbit, unlike in humans and the rat, a major portion of whole-body cholesterol synthesis takes place in extrahepatic tissues (6, 18). Thus, marked changes in hepatic HMG-CoA reductase activity may have only small effects on whole-body cholesterol synthesis. This could imply that in rabbits serum lathosterol concentrations are not correlated with hepatic HMG-CoA reductase activity. However, cholesterol feeding should reduce serum lathosterol concentrations because this treatment dampens whole-body cholesterol synthesis as measured by incorporation of ^{3}H from tritiated water into carcass cholesterol (6, 18).

The lathosterol:total cholesterol ratio in serum has been shown to be an indicator of whole-body cholesterol synthesis in humans (2). However, in the rabbit the serum lathosterol:total cholesterol ratio was neither depressed by cholesterol feeding nor by simvastatin feeding, both of which treatments inhibit cholesterol synthesis (6, 7). In contrast, the serum lathosterol:(VLDL + IDL + LDL) cholesterol ratio was altered by the various treatments in directions that would be anticipated. Cholesterol and simvastatin feeding lowered the group mean values of this ratio, but the changes did not reach statistical significance and the influence of the latter treatment was somewhat small. Cholestyramine in the diet significantly raised the lathosterol:(VLDL + IDL + LDL) cholesterol ratio.

As reported earlier (1), in the cholesterol-fed rabbits cholesterol accumulated in the body, leading to apparent negative rates of cholesterol synthesis. Thus, the serum lathosterol:(VLDL + IDL + LDL)cholesterol ratio was correlated with whole-body cholesterol synthesis as calculated for the control rabbits and those fed either simvastatin or cholestyramine. Fig. 1 shows that the two parameters were directly correlated. However, the predictive value of serum lathosterol:(VLDL + IDL + LDL) cholesterol ratio as an index of whole-body cholesterol synthesis in rabbits may be rather low because it explained only 37% (r^2) of the variance of cholesterol synthesis. Thus, the ratio may not yield reliable information on individual rabbits. Because the various treatments shifted the lathosterol:(VLDL + IDL + LDL) cholesterol ratio into expected directions, the ratio may be considered suitable to predict cholesterol synthesis in groups of rabbits.

The data in Table 2 indicate that hyperresponsive rabbits have lower basal rates of whole-body cholesterol synthesis than hyporesponders. This finding is in line with the higher efficiency of intestinal cholesterol absorption in hyperresponders (1, 19). In hyperresponders more cholesterol from the gut will reach the tissues, and thus cholesterol synthesis will be more depressed. Consistent with this reasoning, a recent report shows that the basal level of hepatic HMG-CoA reductase activity is lower in responsive compared to hypercholesterolemia-resistant rabbits (20). Theoretically, the lower basal rates of cholesterol synthesis in hyperresponders may imply that less extensive depression of cholesterol synthesis can occur after cholesterol consumption, which will provoke hypercholesterolemia. Based on serum lathosterol:(VLDL + IDL + LDL) cholesterol ratios and predicted whole-body cholesterol synthesis rates, it appeared that in hyperresponsive rabbits, in contrast to their hyporesponsive counterparts, cholesterol synthesis is not depressed at all after cholesterol feeding. This agrees with the lower

sensitivity of the feedback inhibition of cholesterol biosynthesis in hyperresponsive rhesus monkeys (21). We conclude that, in addition to the earlier reported (1, 19) higher efficiency of intestinal cholesterol absorption in the hyperresponsive rabbits when compared with the hyporesponders, the lack of depression of cholesterol synthesis after cholesterol challenge also contributes to the marked dietary cholesterol-induced hypercholesterolemia in the hyperresponders. So far, the molecular basis for the two defects in the hyperresponders is unknown. ∎

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