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Endogenous Cholesterol Synthesis, Fecal Steroid Excretion and Serum Lanosterol in Subjects with High or Low Response of Serum Cholesterol to Dietary Cholesterol

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ABSTRACT In this study we addressed the question whether hypo- and hyper-responders to dietary cholesterol differ with regard to the flexibility of endogenous cholesterol synthesis after changes in cholesterol intake. Whole-body cholesterol synthesis was measured as faecal excretion of neutral steroids and bile acids minus cholesterol intake. In addition, we determined serum concentrations of lanosterol, a precursor of cholesterol and a possible indicator of cholesterol biosynthetic activity. The study was carried out with 2 hyper- and 4 hypo-responders; these subjects had shown a consistently high or low response of serum cholesterol to a decrease in dietary cholesterol in two previous experiments.

The subjects received controlled high- (on average 697 mg of cholesterol per day) and low-cholesterol (109 mg/day) diets for periods of 4 weeks in succession; cholesterol was the only dietary variable. The two hyper-responders again showed a significant decrease in serum cholesterol. There was essentially no decrease in serum cholesterol in three of the four hypo-responders. The decrease in cholesterol intake caused an increase in cholesterol synthesis in five out of the six subjects. There was no association between the individual change in serum cholesterol and the change in cholesterol synthesis. Transfer from the high- to low-cholesterol diet caused an increase in serum lanosterol in all subjects. The increase was 3- to 4-fold higher in three out of the four hypo-responders than in the two hyper-responders.

We tentatively suggest that this study provides some evidence that the flexibility of cholesterol synthesis is involved in the responsiveness to dietary cholesterol.

INTRODUCTION

The response of serum cholesterol to dietary cholesterol differs between subjects, and hypo- and hyper-responders have been defined [1-7]. Hyper-responsiveness to dietary cholesterol in man may be important because genetic disorders, such as familial hypercholesterolemia and combined hyperlipidemia, account for only a small percentage of the hypercholesterolemia found in affluent populations.

The mechanisms underlying hypo- and hyper-responsiveness are unclear [8]. Individual changes in serum cholesterol following cholesterol feeding appear to be unrelated to the efficiency with which dietary cholesterol is absorbed by the gut [3,4,9]. Inter-individual differences in dietary-cholesterol-induced faecal bile acid and neutral steroid excretion do not

appear to be the key to hypo- and hyper-responsiveness to cholesterol in the diet, either [3,4,9].

Nestel and Poyser [4] have presented evidence, on the basis of cholesterol balance studies, that those individuals with the smallest increase in serum cholesterol after cholesterol loading also showed the largest depression of endogenous cholesterol synthesis. This is supported by the observation of Mistry *et al.* [6] that after cholesterol consumption the decrease in the activity of the rate limiting enzyme in cholesterol synthesis, 3-hydroxy-3-methylglutaryl CoA reductase, as measured in freshly isolated blood mononuclear cells, was inversely related ($r = -0.49$, $n = 37$; $p < 0.01$) to the increase in the concentration of low density lipoprotein cholesterol, which is the main carrier of serum cholesterol. In contrast, Quintão and co-workers [3,9,10] were not able to demonstrate a relationship

Table 1 Characteristics of subjects in 1983

	Subject No.					
	1	2	3	4	5	6
Sex	F	F	M	M	F	M
Age (yr)	67	55	51	50	57	35
Weight (kg) ¹	66	83	91	76	63	63
Height (cm) ¹	162	172	178	179	166	173
Serum total cholesterol (mmol/l) ²	5.67	6.46	6.20	5.50	5.21	5.79
HDL cholesterol (mmol/l) ²	1.29	1.08	1.16	1.36	1.90	1.49
Serum cholesterol response to cessation of egg consumption (mmol/l) ^{3,4}						
Expt 1976	+0.51	+0.11	+0.29	+0.17	-0.81	-0.54
Expt 1982	+0.48	+0.47	-0.12	-0.08	-0.59	-0.53

¹Pre-experimental values were measured one or two weeks before the start of the high-cholesterol controlled diet.

²Mean of days -8 and 0 (Figure 1).

³Positive values denote an increase of serum cholesterol after removal of eggs from the diet. Cholesterol: 1 mmol/l = 38.7 mg/dl.

⁴See also reference 16.

between individual decrease in cholesterol synthesis and increase in serum cholesterol after cholesterol feeding.

Part of these discrepancies may be due to the problem of reliably classifying subjects as hypo- and hyper-responsive. A single dietary experiment may not suffice for this purpose [7,8]. We have now measured whole-body cholesterol synthesis on high- and low-cholesterol diets in subjects who had been shown in two previous experiments to be consistently hypo- or hyper-responsive to dietary cholesterol. In addition, we have determined serum levels of a biosynthetic precursor of cholesterol, lanosterol, which has been proposed [11,12] as an indicator for the rate of whole-body cholesterol synthesis. Part of this work has appeared in abstract form [13,14].

PATIENTS AND METHODS

Patients

The volunteers, who habitually ate at least one egg/day, had each participated in two previous experiments during which the effect of cessation of egg consumption for 3 weeks on serum cholesterol levels was determined. The first trial took place in 1976 [15], and the second in 1982 [16]. In 1983 four subjects with consistently high and four subjects with consistently low serum cholesterol responses to withdrawal of eggs from the diet were asked to participate in a third, controlled trial. One subject refused and another subject withdrew about 2 weeks after the beginning of the experiment. The characteristics of the remaining six subjects, four hypo- and two hyper-responders, are given in Table 1. The subjects were all apparently healthy, were normo-cholesterolaemic, and had no anaemia, glucosuria or

proteinuria. The experimental protocol was fully explained to the participants and informed consent was obtained. The study was approved by the Ethical Committee of the Department.

Experimental design and diets

The subjects received controlled high- and low-cholesterol diets for periods of 4 weeks each. After that they returned to their habitual diet, which included at least one egg every day for another 4 weeks. Blood samples were taken four times during each period. Sampling time was between 7.45 to 12.00 a.m., and was fixed per subject. On these days the subjects had a light, low-fat and cholesterol-free breakfast. During the last five days of the controlled high- and low-cholesterol periods feces were collected. The design of the study is illustrated in Figure 1. The subjects noted any illness, drug use and departures from the diets in diaries. Nothing out of the ordinary was noted.

The controlled diets (Table 2) consisted of regular foodstuffs and were identical except for the amount of cholesterol. Dietary cholesterol was provided by whole eggs and egg-yolk. Protein and fat intakes were balanced by adding egg-white, extra milk, meat and margarine and some olive oil to the low-cholesterol diet. Total diets were provided except for 1 MJ (240 kcal) per day, which the subjects were free to choose from a list of foodstuffs not containing cholesterol. All foodstuffs were weighed out for each person in quantities appropriate to his or her energy needs. A typical package consisted of potatoes, other vegetables, salads, meat, sauce, pudding, brown bread high in saturated fat, margarine (P/S ratio = 0.19), meats, cheese, yoghurt and/or milk, marmalade, sugar and/or

Fig. 1 Design of the experiment. Arrows indicate the days on which blood was sampled.

Table 2 Composition of the low- and high-cholesterol controlled diets, and the post-experimental, high-cholesterol habitual diet

	Controlled diets ¹		Self-selected, egg-rich diet ²
	High-cholesterol	Low-cholesterol	
Energy (kcal/day)	2479 ± 483	2489 ± 550	2433 ± 643
(MJ/day)	10.4 ± 2.0	10.4 ± 2.3	10.2 ± 0.3
Total fat (% of energy)	36	39	46 ± 4
Saturated fatty acids	17	19	18 ± 3
Monounsaturated fatty acids	11	12	16 ± 1
Polyunsaturated fatty acids	6	7	7 ± 1
P/S ratio	0.33	0.36	0.39 ± 0.10
Carbohydrates (% of energy)	48	46	38 ± 6
Mono- and disaccharides	23	21	19 ± 5
Polysaccharides	25	25	20 ± 5
Protein (% of energy)	13	12	14 ± 3
Animal proteins	7	6	10 ± 3
Vegetable proteins	6	6	4 ± 1
Alcohol (% of energy)	3	3	3 ± 4
Dietary fiber (g/day)	35	36	22 ± 11
Cholesterol (mg/MJ)	67	10	84 ± 24
(mg/day)	697 ± 148	109 ± 25	823 ± 236

¹The composition represents the total diet consisting of the food supplied and the 1 Megajoule worth of foodstuffs which the subjects were allowed to choose from a list of cholesterol-free, low-fat products. Chemical analysis was performed on the duplicate portions pooled per dietary period; the contents of mono-, di- and polysaccharides, animal and vegetable proteins and dietary fiber were calculated. The composition of the free-choice foodstuffs was also calculated. Nutrient contents were then calculated using the computerised Dutch food table (17).

²Estimated by the dietary-history method; results are expressed as means ± SD ($n = 6$).

honey, apple juice or grapefruit juice, fruit and biscuits. Part of the egg yolk was provided as whole eggs, and the rest was added to salads and puddings. Foodstuffs were delivered (by ACB) to the subjects' homes three times a week. During the study duplicate portions of the high- and low-cholesterol diets were collected for nutrient analysis (Table 2).

During the third period (Fig. 1) the subjects consumed their self-selected diets again including at least one egg per day. Food intakes during this period were estimated in an interview with a dietician using the dietary history method. Nutrient consumption was calculated using the computerised Dutch food table [17].

Serum cholesterol analyses

Total serum cholesterol was measured with the Liebermann-Burchard reagent under rigidly standardised conditions [18]. For high density lipoprotein (HDL) cholesterol the Mn-heparin precipitation method [19] was used. Serum samples of one individual were always analysed within one batch.

Feces collection

The stools were collected in plastic buckets which were immediately placed on dry ice. They were picked up at

the subjects' homes twice weekly and stored at -20°C . Radio-opaque markers were employed as an internal standard to correct for fecal flow variations and stool recovery [20].

Analysis of dietary and fecal neutral steroids

Neutral steroids were analysed in freeze-dried feces pooled per subject per collection period and in duplicate portions of the diets pooled per dietary period. Feces from an individual subject were analysed within one batch.

Neutral steroids were analysed exactly as described [21]. Repeated determinations ($n = 20$) on a control pool of freeze-dried feces revealed a combined within- and between-assay variability of 4.9% (coefficient of variation) for cholesterol, 4.4% for coprostanol and 22.8% for coprostanone over a period of 3 months.

Analysis of fecal bile acids

Bile acids in freeze-dried feces were analysed as described [21]. Analytical reproducibility ($n = 7$) of total bile acids was 3.6% (coefficient of variation).

Analysis of lanosterol in serum

For the lanosterol determination the four sera obtained on each controlled diet and pooled per subject per dietary period (Fig. 1) were used. Lipids were extracted from 2 ml of serum with 40 ml chloroform: methanol (2:1) after the addition of 4 μg of the internal recovery standard, epicoprostanol. The methyl-sterol containing fraction was isolated on 10×20 cm high-performance thin-layer chromatography plates using chloroform as a solvent. The methyl-sterol fraction was scraped off, extracted with chloroform, and dissolved in 50 μl of hexane and separated without derivatisation by gas-liquid chromatography on a capillary column coated with CP Sil 5 (Chrompack, Middelburg, The Netherlands). The analytical variability was 10–15% (coefficient of variation).

RESULTS

Table 2 shows the composition of the high- and low-cholesterol diets. The diets were formulated so that cholesterol was the only variable. All subjects consumed the same amounts of cholesterol when expressed relative to the amount of dietary energy. As the subjects required different amounts of energy the daily intakes of cholesterol varied between subjects; they ranged from 495–859 mg/day during the high-, and from 73 to

140 mg/day during the low-cholesterol period (Table 4). The egg-rich, self-selected diet consumed in the third period contained more fat and less carbohydrates than the controlled high- and low-cholesterol diets consumed in periods 1 and 2. The cholesterol contents of the high-cholesterol controlled diet and the self-selected diet were similar (Table 2).

During the high-cholesterol period the mean body weight of the six subjects changed by 0.0 kg (range, -1.7 – 1.0 kg); during the low-cholesterol period there was an increase of $+0.3$ kg (range -0.9 – 1.0 kg). Body weight changes over the 8 weeks when controlled diets were given ranged from -2.2 (subject no. 4) to $+1.9$ kg (subject no. 2).

Of the six subjects, two had been hyper-responsive and four had been hypo-responsive to cessation of egg consumption in both 1976 and 1982 (Table 1). Table 3 documents that the two hyper-responders (nos. 5 and 6) again showed a significant decrease (by 8–9%) in the level of serum total cholesterol when cholesterol consumption was reduced under strictly controlled conditions. There was essentially no decrease in serum total cholesterol in three of the four hypo-responders (Table 3). One subject (no. 1) who was hypo-responsive in the trials in 1976 and 1982 (Table 1) now showed a decrease in serum cholesterol after the reduction in cholesterol intake (Table 3). As would be anticipated, serum cholesterol increased again after the switch from the low-cholesterol controlled diet to the self-selected egg-rich diet in the two hyper-responders (nos. 5 and 6; Table 3). No change in serum total cholesterol was seen in three out of four putative hypo-responders (nos. 1, 2 and 3).

HDL cholesterol levels were also affected by dietary cholesterol. The average decrease in HDL cholesterol was -0.06 mmol/l after replacement of the high-cholesterol by the low-cholesterol diet, which amounts to 24% of the decrease in serum total cholesterol (Table 3). The subsequent consumption of the habitual diet caused an average increase in HDL cholesterol of 0.07 mmol/l, corresponding to 34% of the total change in serum cholesterol. The changes in the concentration of HDL cholesterol were more pronounced in the hyper- than in the hypo-responders (Table 3).

During the last 5 days of the high- and low-cholesterol periods (Fig. 1) the subjects collected their feces. To correct for variations in daily fecal flow, the subjects ingested 20 radio-opaque markers per day during the collection period and the 11 days preceding it. Recovery of the markers in the feces was 93% (range, 88–104%) for the high-, and 100% (range, 72–143%) for the low-cholesterol period.

Plant sterols in the diet are not absorbed, and on a mixed natural diet they are not destroyed in the gut to an appreciable extent (22). Thus the mean intake of

Table 3 Serum cholesterol levels on the controlled and habitual diets

	Subject No.					
	1	2	3	4	5	6
Serum total cholesterol (mmol/l)						
High-cholesterol, controlled diet	6.44 ± 0.14	6.34 ± 0.07	5.40 ± 0.52	5.39 ± 0.14	5.85 ± 0.16	6.03 ± 0.18
Low-cholesterol, controlled diet	5.68 ± 0.10	6.31 ± 0.22	5.82 ± 0.26	5.31 ± 0.06	5.39 ± 0.17	5.47 ± 0.15
Response	-0.76	-0.03	+0.42	-0.08	-0.46	-0.56
Self selected, egg-rich diet	5.52 ± 0.43	6.22 ± 0.18	5.89 ± 0.29	5.91 ± 0.10	5.91 ± 0.33	5.78 ± 0.17
Response	-0.16	-0.09	+0.07	+0.61	+0.52	+0.31
HDL cholesterol (mmol/l)						
High-cholesterol, controlled diet	1.19 ± 0.05	1.08 ± 0.04	1.01 ± 0.07	1.63 ± 0.12	2.06 ± 0.08	1.62 ± 0.04
Low-cholesterol, controlled diet	1.12 ± 0.06	1.04 ± 0.02	1.08 ± 0.06	1.56 ± 0.05	1.94 ± 0.05	1.44 ± 0.04
Response	-0.07	-0.04	+0.07	-0.07	-0.12	-0.18
Self selected, egg-rich diet	1.11 ± 0.12	1.09 ± 0.01	1.04 ± 0.02	1.68 ± 0.03	2.13 ± 0.15	1.56 ± 0.11
Response	-0.01	+0.05	-0.04	+0.12	+0.19	+0.12

Results are expressed as means ± SD of four determinations per dietary period. Cholesterol: 1 mmol/l = 38.7 mg/dl. The mean serum total cholesterol levels are also given in reference 16.

Table 4 Steroid balance data on the high- and low-cholesterol controlled diets

	Subject No.					
	1	2	3	4	5	6
(mmol/day)						
<i>High cholesterol diet</i>						
Cholesterol intake	1.28	2.22	1.82	2.03	1.40	2.07
Neutral steroid excretion with feces	2.06	4.89	2.62	3.44	2.45	2.37
Bile acid excretion with feces	0.34	2.09	0.54	0.71	0.30	0.58
Cholesterol synthesis (excretion minus intake)	1.12	4.76	1.44	2.07	1.32	0.90
<i>Changes on low-cholesterol diet</i>						
△ Cholesterol intake	-1.09	-1.93	-1.56	-1.67	-1.19	-1.76
△ Neutral steroid excretion with feces	-0.65	-2.10	-1.02	-1.39	-0.98	-1.06
△ Bile acid excretion with feces	0.00	-0.51	+0.02	-0.08	-0.01	-0.23
△ Cholesterol synthesis (excretion minus intake)	+0.44	-0.68	+0.56	+0.20	+0.20	+0.47

All subjects consumed 0.174 mmol of cholesterol per MJ on the high- and 0.026 mmol/MJ on the low-cholesterol diet. The steroid balance data refer to the last five days of the high- and low-cholesterol periods (see Fig. 1). Cholesterol: 1 mmol = 387 mg; neutral steroids (as coprostanol): 1 mmol = 389 mg; bile acids (as deoxycholic acid): 1 mmol = 393 mg.

plant sterols should equal excretion. The intakes of plant sterols were similar during both dietary periods. When expressed relative to the intake, the fecal excretion of β -sitosterol, campesterol and stigmasterol and their respective bacterial conversion products was 116% (range, 65–185%), 90% (range, 45–143%) and 156% (range, 86–193%), respectively, during the high-cholesterol period. For the low-cholesterol period these values were 122 (range, 59–215%), 93 (range, 52–145%) and 120% (range, 47–133%).

Table 4 shows the sterol balance data for the six volunteers; whole-body cholesterol synthesis was calculated as excretion minus intake. The decrease in cholesterol intake reduced the fecal excretion of neutral

steroids in all subjects, whereas the excretion of bile acids was essentially unchanged. Withdrawal of cholesterol from the diet increased whole-body cholesterol synthesis in five subjects (not in subject no. 2). There were no clear differences between the two hyper-responders (nos. 5 and 6) and four hypo-responders with regard to the response of steroids excretion and/or cholesterol synthesis to dietary cholesterol loading.

Lanosterol is an intermediate in the biosynthesis of cholesterol from squalene in man, and the lanosterol level in serum has been proposed to be a valid index of whole-body cholesterol synthesis [11,12]. Table 5 shows that lanosterol levels on the high-cholesterol diet were similar in all subjects. Transfer to the low-choles-

Table 5 Serum lanosterol levels on the high- and low-cholesterol controlled diets

	1	2	Subject No.		5	6
			3	4		
	Lanosterol in serum ($\mu\text{mol/l}$)					
High-cholesterol diet	0.36	0.44	0.44	0.47	0.44	0.39
Low-cholesterol diet	0.67	0.75	0.67	0.52	0.52	0.47
Difference	+0.31	+0.31	+0.23	+0.05	+0.08	+0.08

Lanosterol was measured in pools of four serum samples per diet period per subject (see Fig. 1).

terol diet caused an increase in serum lanosterol in all subjects, which would be in line with the increase in cholesterol synthesis as illustrated in Table 4. The increase in lanosterol levels tended to be higher in the hypo- than in the hyper-responders.

DISCUSSION

The two subjects who were hyper-responsive in both 1976 and 1982 to cessation of egg consumption (Table 1) again showed a marked decrease in serum cholesterol under conditions where cholesterol was the only dietary variable (Table 3). When these two subjects (nos. 5 and 6) consumed their self-selected cholesterol-rich diet, their serum cholesterol rose again (Table 3). Thus the hyper-responders were consistently hyper-responsive. For the four putative hypo-responders (nos. 1–4, Table 1), the situation was not as clear. Nevertheless, with the controlled diets we found no decrease in serum cholesterol in three of the four hypo-responders (Table 3).

The decrease in cholesterol intake significantly lowered the group mean fecal excretion of neutral steroids by 40% (range 32–45%). This effect of dietary cholesterol has been reported by various other workers [3,4,10,23]. The excretion of bile acids with the feces remained virtually constant (change, $-12 \pm 17\%$). This has also been demonstrated earlier [3,4,10], although Lin and Connor [23] observed that re-excretion of cholesterol as bile acids occurred after cholesterol feeding. Group mean whole-body cholesterol synthesis was increased by 24% (range -14 –52%) after the reduction of cholesterol intake (Table 4), an effect which is well-known [3,4,23,24]. In one subject (no. 2) cholesterol synthesis appeared to decrease when cholesterol consumption was lowered (Table 4).

In several animal species the individual variability in serum cholesterol response to dietary cholesterol is determined by their capacity to stimulate excretion of bile acids with the feces. Hypo-responsive squirrel monkeys [25], pigeons [26] and rabbits [27] have been shown to enhance their bile acid excretion after cholesterol feeding to a higher extent than their hypo-responsive counterparts. However, there is no evidence from

the present or other [3,4,9] studies that this also holds for humans. Fecal excretion of neutral steroids is increased by cholesterol feeding, but most of the increase is due to non-absorbed cholesterol [4,23]. Bile acid synthesis and excretion is little affected [3,4,10] and excretion of endogenously produced cholesterol goes down [4] because total body synthesis is depressed in response to cholesterol consumption in man. Nestel and Poyser [4] found that after cholesterol loading excretion of endogenous steroids decreased most in those subjects who showed the smallest increase in serum cholesterol levels, i.e. the hypo-responders. Our data neither refute nor confirm this; there was an inverse relation between the change in excretion of total neutral steroids and the change in serum cholesterol ($r = -0.45$), but with six subjects this relation does not reach statistical significance.

In another controlled dietary trial (Katan *et al.*, unpublished) we found that whole-body cholesterol synthesis on a low-cholesterol diet, when calculated from steroid balance data, was negatively associated with the subsequent increase in serum cholesterol on a high-cholesterol diet ($r = -0.44$, $n = 32$). In the present study three of the hypo-responders (nos. 2, 3 and 4) also had higher rates of cholesterol synthesis on the low-cholesterol diet than the two hyper-responders (Table 4). This was associated with higher levels of lanosterol in 3 out of the 4 hypo-responders during the low-cholesterol period (Table 5). The data in Table 5 would suggest that higher lanosterol levels are associated with higher rates of whole-body cholesterol synthesis: on the low-cholesterol diet there was a weak positive association ($r = 0.65$), but this was not as clearly seen on the high-cholesterol diet ($r = 0.42$). In a controlled experiment [30] with another group of subjects ($n = 41$) we were unable to demonstrate a significant relationship between the rate of whole-body cholesterol synthesis and serum lanosterol. The correlation coefficients were $r = 0.30$ on a low- and $r = 0.11$ on a high-cholesterol diet (unpublished). Thus, in contrast to previous work [11,12], we ourselves have found only weak positive associations between serum lanosterol and cholesterol synthesis.

We thus have some evidence that hypo-responders

have higher rates of cholesterol synthesis on low-cholesterol diets than hyper-responders. This difference between hypo- and hyper-responders has also been shown in rhesus monkeys [28], rats [22] and pigeons [29]. After cholesterol feeding hypo-responders should theoretically be able to suppress cholesterol synthesis over a wider range than hyper-responders. This has indeed been shown by Nestel and Poyser [4] but not by Quintão and co-workers [3,8,9]. Like the latter investigators we did not observe a relationship between the individual change in serum cholesterol and change in whole-body cholesterol synthesis ($r = -0.16$). The change in cholesterol synthesis in the two hyper-responders was comparable with that in three hypo-responders (nos. 1, 3 and 4). However, with regard to the concentration of lanosterol in the serum there were differences. The increase in serum lanosterol was 3- to 4-fold lower in the two hyper-responders than in three hypo-responders (nos. 1, 2 and 3), which suggests a difference in the rate of some step in cholesterol biosynthesis. However, this is not substantiated by the overall rates of whole-body cholesterol synthesis calculated as steroids excretion minus cholesterol consumption. Likewise, in an independent group of subjects fed successively a low- and high-cholesterol diet, no relationship was found between the change in whole-body cholesterol synthesis (sterol balance method) and the change in serum lanosterol concentration ($r = -0.06$; $n = 41$) (unpublished). Further work is necessary to prove or disprove the hypothesis that the ability to adjust the rate of cholesterol synthesis is a major determinant of the responsiveness to dietary cholesterol.

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