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Hypo- and Hyperresponse of Serum Cholesterol Level and Low Density Lipoprotein Production and Degradation to Dietary Cholesterol in Man[a]

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INTRODUCTION

The effect of dietary cholesterol on the level of serum cholesterol varies among individuals. In animals, these differences in response are marked and appear to have a genetic basis.[1] Strains of hypo- and hyperresponding monkeys, rabbits, and rats have been bred. Using repeated experiments with large numbers of subjects we were able to show that modest but reproducible inter-individual differences in the serum cholesterol response to egg yolk cholesterol exist in humans, too.[2–4] However, most of the variability in apparent response in single trials, and even more so the extremes of response seen in clinical

[a] This study was financially supported by the Netherlands Heart Foundation, grants no. 26.003, 32.015 and 86.117, and by grant No. 13-33-35/900-562-035 of the Netherlands Organisation for Scientific Research ZWO.

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practice, are due to random diet-independent fluctuations in serum lipid levels and are not reproducible within subjects or patients. The source of such apparent hypo- and hyperresponsiveness is illustrated in FIGURE 1.

The physiological mechanism determining responsiveness to dietary cho-

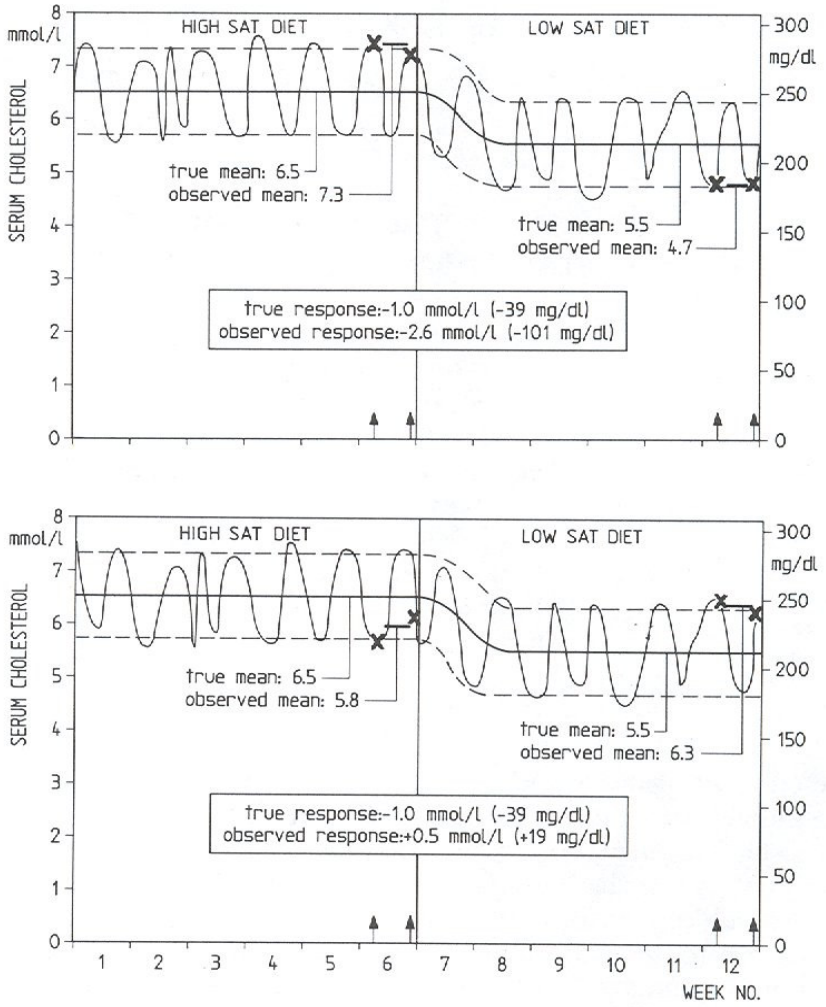

FIGURE 1. Hypothetical continuous recording of serum cholesterol (oscillating line) in an ostensible "hyperresponder" (*top*) and "hyporesponder" to diet (*bottom*). The graphs assume a within subject coefficient of variation for total cholesterol of 0.4 mmol/L or 6%, and a frequency of one oscillation per 3–4 days.[23] Crosses (X) represent cholesterol values as they might actually be measured. The actual decline in cholesterol is 1.0 mmol/L (38.7 mg/dL) in both cases. However, because of the points in time where he happens to be bled, subject A appears to be hyperresponsive while cholesterol in subject B appears to go up when saturated fat intake is reduced. Such phenomena are common in clinical practice, and they lead to mistaken impressions about a particular patient's sensitivity to diet.

lesterol is not known. We have found that hyperresponders to dietary cholesterol are characterized by higher initial total and HDL cholesterol levels, a lower habitual cholesterol intake, and, surprisingly, a low body mass index.[4] In our sample, neither gender nor age nor apo E phenotype[5] were associated with responsiveness.

Several authors have suggested that the individual variability in the cholesterolemic response relates to differences among individuals in their capacity to depress whole-body cholesterol synthesis.[6,7] Mistry *et al.* presented evidence that blood lymphocytes of hyperresponders, when compared with hyporesponders, have a reduced capacity to catabolize low density lipoproteins (LDL).[8] In monkeys and in several other species the primary cause of variation in responsiveness appears to be differences in the intestinal absorption of cholesterol.[9]

In order to study more directly the metabolic basis of responsiveness, we have measured the rate of turnover of LDL apolipoprotein apo B in healthy volunteers on low- and high-cholesterol diets, and the catabolism of LDL by blood mononuclear cells isolated from these subjects. For all subjects entering this study, the responsiveness of serum cholesterol had already been defined in three earlier dietary trials. The present results show that responsiveness of serum levels is directly related to the responsiveness of LDL apo B production rate to dietary cholesterol.

SUBJECTS AND METHODS

Subjects

Altogether eighteen men and eleven women participated during one or more dietary periods of the present experiment. All were healthy normolipidemic volunteers from the general population living in or near Wageningen, the Netherlands. They had previously participated in three controlled trials on the effect of dietary cholesterol on serum lipids,[3,4] and most of them had also participated in an experiment on responsiveness to saturated fat.[10] The present trial was thus experiment no. 5, and by the end of it subjects had been in close contact with the Wageningen group for a period spanning almost five years. The original recruitment and selection of the subjects have been described in detail previously.[3] Characteristics at entry are given in TABLE 1. The experimental protocol was thoroughly explained to the subjects, and informed consent was obtained. The study was approved by the Medical-Ethical Committee of the Department of Human Nutrition, Wageningen and by the radiation protection officer of Nijmegen University, School of Medicine.

TABLE 1. Baseline Characteristics of All Subjects Entering the Study

Age at entry in 1982 (years)	32 ± 13
Height (cm)	177 ± 9
Weight (kg)	70 ± 10
Body mass index (kg/m2)	22.4 ± 2.5
Serum total cholesterol (mmol/l)	4.97 ± 0.77
Serum HDL cholesterol (mmol/l)	1.41 ± 0.31
Serum triacylglycerols (mmol/l)	0.86 ± 0.28

Values are means ± SD for 29 subjects.

Study Design and Diets

The experimental design is depicted in FIGURE 2. The study consisted of three dietary periods: a high-cholesterol period ("High-1"), a low-cholesterol period ("Low") and another high-cholesterol period ("High-2"). Eight subjects participated for the full duration of all three periods. The others missed one or two periods, or did not take part in the LDL-apo B turnover test at the end of the low-cholesterol period. The periods were separated by several months during which subjects followed their habitual diet. On day 18 or 19 of each period, blood mononuclear cells were isolated to study LDL uptake and degradation *in vitro*. The turnover of labeled autologous LDL apo B was measured *in vivo* in nine volunteers at the end of the low-cholesterol period, and in eight of these plus five additional volunteers at the end of period High-2.

During period High-1, which lasted 18 days, subjects consumed their habitual diet,[3] but added about 2 extra eggs/day, so that their daily cholesterol

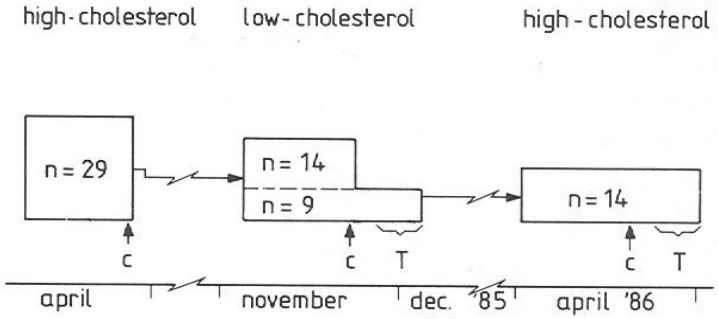

FIGURE 2. Design of the present study on LDL metabolism in human hypo- and hyper-responders to dietary cholesterol, showing periods High-1, Low and High-2, and the numbers of participants in each. C, blood mononuclear cell study; T, measurement of turnover of ^{125}I-labeled autologous LDL apolipoprotein B. This study constituted No. 5 out of a series of repeated experiments on these same subjects. Experiments 1–3[3,4] and 4[9] have been described elsewhere.

TABLE 2. Mean Nutrient Intakes in the First High-, the Low-, and the Second High-Cholesterol Period of the Study

Dietary Component	Period High-1[a] (n = 29)	Period Low[b] (n = 23)	Period High-2[c] (n = 14)
Energy (MJ/day)	10.1 ± 2.8	9.8 ± 2.4	10.7
(kcal/day)	2420 ± 680	2350 ± 590	2560
Protein (% of energy)	15.5 ± 3.2	13.2 ± 1.0	14.4
Fat, total (% of energy)	37.3 ± 6.3	30.1 ± 2.6	32.8
Saturated fatty acids	14.8 ± 2.9	12.8 ± 1.3	14.4
Monounsaturated fatty acids	13.5 ± 3.0	9.5 ± 1.1	11.1
Polyunsaturated fatty acids	6.4 ± 2.7	6.9 ± 0.9	6.0
Carbohydrates (% of energy)	43.7 ± 8.0	53.9 ± 3.5	50.9
Alcohol (% of energy)	3.4 ± 5.2	2.8 ± 3.2	2.0
Cholesterol (mg/MJ)	91 ± 22	14 ± 2	82
(mg/day)	898 ± 257	138 ± 19	870

Results for periods High-1 and Low are means ± SD for the indicated number of subjects.
[a] Estimated from a single 24 h recall during the third week.
[b] Estimated from weekly 24 h recalls.
[c] Chemically determined in duplicate portions collected throughout the experiment; alcohol use was calculated from records kept by subjects in diaries, as described.[3]

consumption amounted to about 90 mg/MJ (376 mg/1000 kcal). Nutrient intake was estimated in the third week by a 24 h recall (TABLE 2). Blood samples for routine lipid analysis were drawn on days 14 or 15 after an overnight fast. On day 18 the subjects received a fat-free breakfast of toast and jelly in Wageningen. They were then flown to London, England, where in the early afternoon 60 ml of blood was drawn at St. Thomas' Hospital for isolation of mononuclear cells and for lipid analysis.

During the second period of the study the subjects received a controlled diet low in cholesterol and composed of mixed natural foodstuffs. Subjects prepared their meals at home, but collected individual packages containing ingredients for the hot meals and most of the fat-containing items for the other meals at the Department in Wageningen two or three times a week, as they had done in experiment 3, three years earlier.[3] They were carefully instructed by the dieticians about the selection and preparation of other items. Food intake was measured by weekly 24 h recalls evenly distributed over the days of the week (TABLE 2). Body weights were checked weekly. Blood was sampled on day 14 or 15 for lipid analysis. On day 19 a 60-ml blood sample was drawn in London from all 23 participants for the isolation of mononuclear cells, exactly as described above for period High-1. Nine subjects continued on the diet; they were injected with autologous ^{125}I-LDL on day 23 for a study on the *in vivo* turnover of LDL apo B (see below), and they were bled on days 23 and 30 of the Low period.

During the third period the diet was again high in cholesterol; almost all

food was weighed out and prepared for each subject. On week days hot meals were prepared and served at noon at the Department in Wageningen, and other meals and food for the weekends were given as packages, as described.[3] The composition of the diet (TABLE 2) was assessed by chemical analysis of duplicate portions for one imaginary person of average energy intake, as described.[3] Blood was sampled for lipid analysis on days 15, 19, 23 and 30, mononuclear cells were obtained on day 19, and labeled LDL was injected in all 14 participants on day 23 of this period. Cell and turnover studies were performed by the same investigators with essentially the same materials as in the Low period, but the measurements were now done at the Department of Internal Medicine of the University of Nijmegen, 20 miles from Wageningen.

Throughout the experiment, scrupulous attention was paid to maximizing understanding and motivation by the participants.

Mononuclear Cell Studies

Degradation of LDL by freshly isolated and by derepressed mononuclear cells (approximately 90% lymphocytes) was measured, essentially as previously described.[8,11,12] In brief, cells were isolated from 50 ml heparinized (10 U/ml) blood with the aid of Ficoll-Paque (Pharmacia Fine Chemicals, Uppsala, Sweden), yielding between 40 and 60 $\times$ 10^6 cells. A portion of the cells were immediately incubated at a concentration of 2 $\times$ 10^6 cells/ml with ^{125}I-labeled human LDL apo B, 20 μg protein/ml, at 37°C for 8 h (fresh cells), in the presence or absence of a 10-fold excess of unlabeled human LDL. The remaining cells were depressed for 72 h in lipoprotein-deficient medium, and then incubated under the same conditions but now for 6 h. All incubations and assays were carried out in triplicate. The rate of degradation of LDL apo B was calculated from the amount of non-trichloroacetic-acid-precipitable ^{125}I in the medium after incubation, and expressed as ng LDL protein degraded per 8 h per mg cell protein.

In order to compare the LDL apo B degradation capacity of the isolated cells from one period to another, cells were also isolated in all three periods from fasting blood obtained from one of us (PRT) on an average Western diet. The total and receptor-mediated degradation activity of both fresh and derepressed cells were found to be reasonably constant over the periods (data not shown).

LDL Turnover Studies

The turnover of ^{125}I-labeled autologous LDL apo B was measured according to the method of Turner *et al.*,[12–15] in which the LDL apo B fractional catabolic rate (FCR) is derived from the urine/plasma radioactivity ratio seven days after injection rather than from serial plasma radioactivity measurements.

In brief, blood was drawn on day 19 of the low-cholesterol and of the second high-cholesterol period, autologous LDL (d = 1.019–1.063 g/ml) was isolated by preparative ultracentifugation, labeled with ^{125}I under sterile conditions, and on the morning of day 23 of periods Low and High-2 5 μCi were injected intravenously. Blood samples were obtained at 10 min and at 7 days after injection for measurement of LDL apo B mass and radioactivity. The percentage of radioactivity not associated with LDL[6] amounted to 3.9 ± 2.0% for the 10-min sample and to 3.2 ± 2.1% for the 7-day sample (means ± SD for 14 subjects). A 24-h urine pool for radioactivity measurement was collected on the seventh day following the injection; the first voiding on the morning of day 29 was rejected and that on the morning of day 30 included. Quantitative collection of the urine was assessed by measuring the recovery of *p*-aminobenzoic acid after oral administration of three 80-mg capsules on day 7.[16] Recovery amounted to 98.6 ± 6.0% on the low-cholesterol and to 98.0 ± 4.5% on the second high-cholesterol diet (means ± SD for 9 and 14 subjects, respectively). The pool size of LDL apo B was calculated from the product of its serum concentration and the plasma volume.[17] The latter was estimated from the dilution of injected radioactivity and was found to be equal to 4.1 ± 0.2% of body weight on the low- and to 4.3 ± 0.3% of body weight on the high-cholesterol diet (means ± SD for 9 and 14 subjects, respectively). The LDL apo B fractional catabolic rate (FCR) was calculated as the total radioactivity of the 24-hr urine collection from day 6 to 7 divided by the total plasma radioactivity at day 7; for convenience we calculated FCR as 24-h urine counts on day 7 (expressed as % of injected counts) divided by plasma counts on day 7 (expressed as % of plasma counts at 10 min). The LDL apo B production rate was calculated as FCR times LDL apo B pool size.

The response of serum total cholesterol to dietary cholesterol was calculated as the difference between the level of serum cholesterol during the low-cholesterol period (mean of 2 or 4 blood samples) and the high-cholesterol periods (mean of 2 blood samples in period High-1 for 15 subjects or of 6 samples in periods High-1 and High-2 combined for the other 14). The response of serum LDL cholesterol to dietary cholesterol was defined as the difference in LDL cholesterol, as measured after sequential ultracentifugation, between day 19 of the low- and day 19 of the second high-cholesterol period.

RESULTS

Dietary adherence, as evaluated from frequent contact with the dieticians and the authors, was excellent. The dietary regime was most stringent during the High-2 period, but even then mean weight loss (± SD) from start to end was limited to 0.1 ± 0.6 kg (range = −1.1 to +0.9, n = 14). Apart from cholesterol, the nutrient composition was similar in the three trials, with the exception of a somewhat higher total fat intake in the first high-cholesterol period (TABLE 2).

Response of Serum Cholesterol to Dietary Cholesterol

For the 23 subjects who participated in the low- plus at least one of the high-cholesterol periods the average response (± SD) amounted to 0.84 ± 0.52 mmol/L (n = 23). This value is similar to their average response of 0.65 mmol/L in experiment 3 in 1982[3] in which the differences in cholesterol intake were similar. The individual responses in the present experiment were significantly correlated with the mean individual responses in the three previous trials ($r = 0.41, p < 0.05$; FIG. 3). This finding confirms the reproducibility of differences in responsiveness over a period of several years.[2] In view of the variability of the cholesterol response from one experiment to another,[3,4] we defined the responsiveness to dietary cholesterol as the mean over all four cholesterol feeding trials. This mean is the best available estimate of each subject's susceptibility to dietary cholesterol.

The changes in total serum cholesterol concentration induced by the dietary interventions were 80–90% accounted for by changes in LDL cholesterol, with the remaining 10–20% of the changes being observed in HDL cholesterol (TABLE 3). The LDL apo B concentration in serum was also increased (TABLE 4). These findings are consistent with those of several other investigations.[7,18,19] The cause of the slight differences in average cholesterol

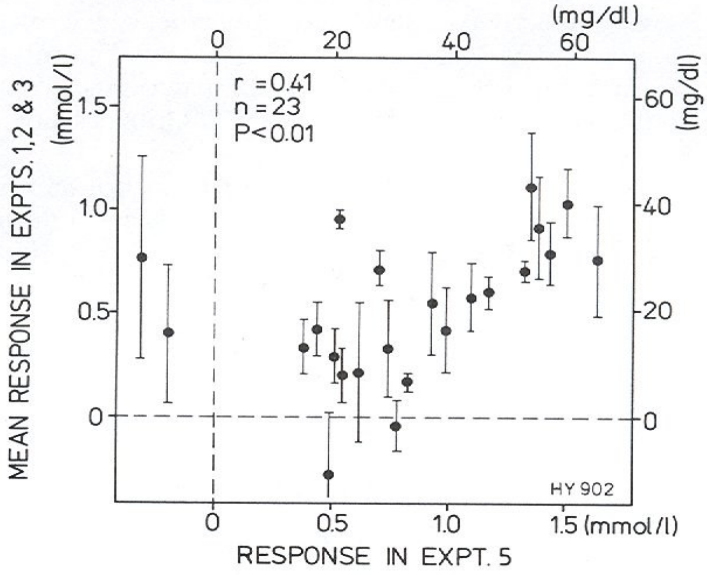

FIGURE 3. Correlation of the mean individual response ± SE of total serum cholesterol to increased dietary cholesterol in three previous trials with that in the present study. Calculation of the responses in the previous experiments were based on a total of 16 blood samples obtained over the course of 3 experiments;[3] and those in the present experiment on 4 to 10 blood samples per subject.

TABLE 3. Average Serum Lipid Values (± SD) by the End of Each Diet Period

Serum Lipid	Period High-1 (High-cholesterol diet)	Period Low (Low-cholesterol diet)	Period High-2 (High-cholesterol diet)
		(mmol/L)	
Cholesterol			
Total	5.54 ± 0.74	4.75 ± 0.79	5.88 ± 0.90
VLDL + IDL	0.47 ± 0.24	0.46 ± 0.30	0.45 ± 0.28
LDL	3.73 ± 0.75	3.12 ± 0.67	4.11 ± 0.78
HDL	1.34 ± 0.21	1.17 ± 0.24	1.32 ± 0.25
Triglycerides	1.06 ± 0.46	1.02 ± 0.40	1.06 ± 0.54

Results are means ± SD for the 13 subjects for whom lipid values were available in all three periods. The lipid parameters shown are the mean of 2 or 4 blood samples obtained between 14 and 30 days after the start of an assigned diet. Total and HDL cholesterol and total triglycerides were measured enzymatically, and used for calculation of (VLDL + IDL) cholesterol.[22] The concentration of LDL cholesterol was then taken as the difference between total cholesterol and the sum of (VLDL + IDL) and HDL cholesterol.

level between periods High-1 and High-2 is unknown; it does not differ significantly from 0, and may thus be due to random fluctuations. Aging of the subjects or slight differences in dietary fatty acid composition could also contribute.

LDL apo B Turnover

In the eight subjects for whom turnover data were available on both diets, adding cholesterol to the diet caused a significant ($p < 0.01$) increase in the average fractional catabolic rate of LDL protein, from 0.24 on the low- to 0.31 pools/day on the high-cholesterol diet. The amount of LDL protein replaced per day per kg of body mass increased on average (± SD) from 4.8 ± 1.2 on the low- to 8.0 ± 1.4 mg/kg per day on the high-cholesterol diet ($p < 0.01$).

Within the group, the extent of this increase in LDL turnover rate, expressed either as pools per day or as mg/kg per day, was strongly correlated with the change in total or LDL cholesterol concentration in serum (FIG. 4). TABLE 5 gives the relevant correlation coefficients. Even the serum cholesterol response determined in these subjects in previous experiments, 3 to 4 years earlier, was significantly correlated with the increase in LDL turnover or production rate in the present experiment (TABLE 5).

Mononuclear Cell Studies

The capacity of freshly isolated and of derepressed blood mononuclear cells to catabolize LDL was assayed in all participants in each period of the study. Increased cholesterol consumption caused a marked reduction by 41% of

TABLE 4. Responsiveness to Dietary Cholesterol in Previous plus Present Experiment and LDL Apolipoprotein B Metabolism in Present Experiment in Healthy Subjects on Low- and High-Cholesterol Diets

		Low-Cholesterol Diet (Period Low)				High-Cholesterol Diet (Period High-2)			
Subject ID No.	Responsiveness to Dietary Cholesterol[a] (mmol/l)	Serum LDL Cholesterol (mmol/l)	Serum, LDL apoB (mg/dl)	FCR (pools/day)	Production Rate (mg/kg·day)	Serum LDL Cholesterol (mmol/l)	Serum, LDL apoB (mg/dl)	FCR (pools/day)	Production Rate (mg/kg·day)
Subjects with Complete Data									
16	−0.09 ± 0.59	4.02	64	0.24	6.9	4.39	75	0.30	8.9
34	0.29 ± 0.27	2.75	43	0.24	4.2	2.90	46	0.28	5.6
128	0.30 ± 0.52	3.88	58	0.23	5.3	4.22	66	0.29	7.7
151	0.52 ± 0.42	4.54	63	0.21	5.4	5.52	79	0.30	9.4
98	0.67 ± 0.36	1.92	30	0.28	3.6	3.34	45	0.33	6.0
111	0.85 ± 0.47	2.52	38	0.24	4.2	4.12	61	0.35	8.6
142	1.00 ± 0.43	3.62	53	0.25	5.7	4.52	69	0.30	9.3
118	1.12 ± 0.39	3.03	40	0.21	3.3	4.16	61	0.33	8.2
Mean ± SD (n = 8)		3.29 ± .88	49 ± 13	0.24 ± 0.02	4.8 ± 1.2	4.15 ± .78	63 ± 12	0.31 ± .02	8.0 ± 1.4
Other Subjects									
62	−0.01 ± 0.24	—	—	—	—	3.09	48	0.28	6.0
17	0.18 ± 0.45	—	—	—	—	4.23	64	0.35	8.7
32	0.21 ± 0.70	3.16	46	0.26	4.8	—	—	—	—
117	0.32 ± 0.35	—	—	—	—	3.09	51	0.40	9.4
85	0.32 ± 0.17	—	—	—	—	2.68	42	0.35	6.8
80	0.87 ± 0.32	—	—	—	—	2.28	32	0.54	8.1
52	1.17 ± 0.42	—	—	—	—	3.61	57	0.39	10.4
Mean ± SD, all 15 subjects		3.27 ± 0.82	48 ± 12	0.24 ± 0.03	4.8 ± 1.1	3.73 ± 0.88	57 ± 13	0.34 ± 0.07	8.1 ± 1.5

The fractional catabolic rate (FCR) and production rate of ^{125}I-labeled autologous LDL apolipoprotein B were measured as described under MATERIALS AND METHODS. Serum LDL cholesterol and LDL apo B mass were determined after sequential ultracentrifugation of the blood sample obtained on day 18 or 19 of the assigned diet.

[a] Each subject had participated in 4 cholesterol feeding experiments, each comprising a low-cholesterol and one (experiments 1–3, ref. 3) or two (present experiment; cf. FIG. 2) high cholesterol periods. Values are the averages of the individual cholesterol changes in these 4 experiments, ± SD.

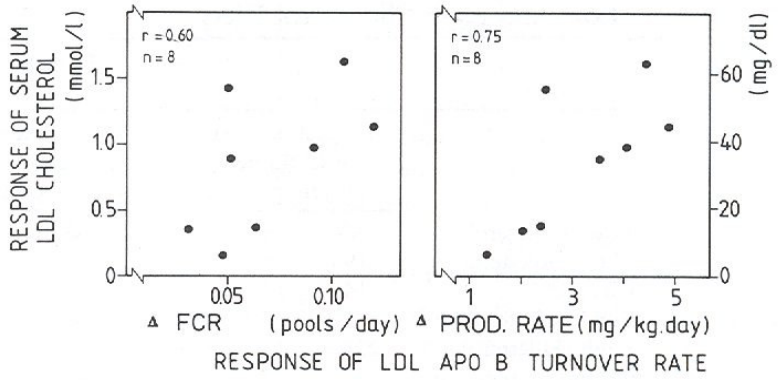

FIGURE 4. Change in serum LDL cholesterol concentration from period Low to period High-2 as a function of the change in LDL apo B fractional catabolic rate (*left*) and in production and turnover rate (*right*). Turnover data were obtained by injection of autologous ^{125}I-labeled LDL into subjects while on a low- and on a high-cholesterol diet (cf. FIG. 2).

the total LDL degradation activity of fresh cells; the reduction in receptor-mediated and receptor-independent activities were of similar magnitude (TABLE 6). When LDL degradation by the mononuclear cells was studied after derepression by incubation for 72 h in lipoprotein-deficient medium, the mean activities were not significantly different (TABLE 6), indicating that the maximum LDL receptor activities that can be induced per mg cell protein was not influenced by the cholesterol content of the diet.

The LDL apo B degradation activity of mononuclear cells showed considerable variation between subjects. We calculated correlation coefficients between various measures of cellular catabolic activity (TABLE 6) and responsiveness of the serum concentrations of total or LDL cholesterol to dietary

TABLE 5. Correlation Coefficients between the Change in LDL Turnover Rate and the Change in Serum Lipid or apo B Concentrations in Response to Increased Cholesterol Intake, from Period Low to Period High-2

Serum Lipid Change	Δ FCR (pools/day) Correlation Coefficient	Δ apo B Turnover Correlation Coefficient
LDL apo B response in present experiment	0.70	0.90
LDL cholesterol response in present experiment	0.60	0.75
Average serum cholesterol response in previous 3 experiments[3]	0.54	0.74
Average serum cholesterol response in all 4 experiments	0.66	0.79

TABLE 6. LDL Metabolism by Blood Mononuclear Cells Isolated from Healthy Subjects on Low- and High-Cholesterol Diets

Condition	Diet	LDL Degradation Activity	
		Total Activity (ng LDL/mg cell protein per 8 h)	Receptor-mediated Activity (% of total)
Fresh cells	Low cholesterol	336 ± 166 (23)	55.7 ± 12.6 (22)
	High cholesterol	199 ± 86 (28)[a]	52.6 ± 11.6 (13)
	Change[a]	−147 ± 180 (23)[b]	−1.6 ± 19.8 (12)
Derepressed cells	Low cholesterol	2072 ± 570 (18)	64.7 ± 9.4 (18)
	High cholesterol	2266 ± 1032 (10)	56.0 ± 9.7 (8)
	Change[a]	+276 ± 1208 (6)[b]	−9.0 ± 8.1 (4)

Values represent means ± SD for the number of subjects given in parentheses. For some subjects an insufficient number of cells was available, and only LDL degradation activity by freshly isolated cells was measured.

[a] Value measured in period High-1 for 14, and mean of values for High-1 and High-2 for another 14 subjects.

[b] Significantly different from zero, $p < 0.01$.

cholesterol. No significant correlations were found, except for a negative correlation between the LDL apo B degradation activity of derepressed cells on the low-cholesterol diet and responsiveness of total cholesterol ($r = -0.45$, $n = 18$, $p < 0.10$) or LDL cholesterol ($r = -0.57$, $n = 18$, $p < 0.05$).

For those subjects for whom the LDL apo B fractional catabolic rate had been measured *in vivo* after injection of labeled LDL apo B, we found a positive (although not significant) correlation of this rate with the rate of LDL apo B degradation by the mononuclear cells *in vitro*, both on the low-cholesterol diet ($r = 0.61$, $n = 7$) and on the high-cholesterol diet ($r = 0.49$, $n = 9$).

DISCUSSION

Effect of Diet on LDL Turnover

The present study was carried out with subjects who had already been tested for their susceptibility to consumption of cholesterol; we were able to confirm that the differences in cholesterolemic response in man are reproducible over a period of at least 4 years (FIG. 3). Kinetic analysis of LDL apo B metabolism revealed that an increased intake of cholesterol increased both the serum LDL pool size and the proportion of the pool replaced per day (TABLE 4). As a result, the mass of LDL protein produced and removed per day

increased by 67%, from 4.8 to 8.0 mg per day per kg body weight. Nestel and Billington reported an increased formation of intermediate density lipoprotein after cholesterol feeding.[20] The same may have occurred in our subjects, because intermediate density lipoprotein is a rapidly metabolized precursor of LDL. If LDL production is stimulated by cholesterol feeding, then the absolute amount of LDL catabolized per day must also rise to achieve a new steady state in which outflow equals input. The observed enlargement of the fraction of LDL turning over per day (TABLE 4) is more difficult to explain. In a model with a fixed number of non-saturated catabolic sites following first-order kinetics, the rate of LDL clearance from plasma is proportional to its concentration, and the fractional turnover rate cannot vary. In reality, fractional catabolism via the LDL receptor pathway would be expected to decrease, since dietary cholesterol supplementation will expand the liver cholesterol pool, and this, in turn, will decrease the number of hepatic LDL receptors through down-regulation. A remarkably large decrease in the LDL receptor activity of peripheral lymphocytes was indeed observed in previous[8,18] as well as in the present egg yolk feeding study (TABLE 6). If the LDL receptor activity in the liver also decreases then the FCR for LDL should lessen on cholesterol feeding, not increase. The underlying paradigm is that of familial hypercholesterolemia, where impairment of LDL receptor function indeed causes a reduction in FCR. However, there does not need to be a fixed relation between FCR and LDL receptor function in general. The crucial variable might be the activity of other routes for LDL removal. Indeed, in a study of the effect of egg supplementation on LDL metabolism in seven volunteers, Packard *et al.* found that most of the extra plasma cholesterol was channelled into the receptor-independent route.[19] At the same time, these investigators observed a slight fall in FCR. The discrepancy with our findings remains unexplained. In our hands, at least, the rise in plasma LDL upon egg yolk feeding appears to be due exclusively to increased production and not to impaired removal of LDL.

Hypo- and Hyperresponders to Dietary Cholesterol

The individual change of serum cholesterol concentration in response to an increase in dietary cholesterol intake was correlated with the individual changes in the proportion and in the absolute amounts of LDL protein turning over per day (FIG. 4). The correlation (FIG. 4) between the change in plasma LDL cholesterol concentration and in turnover of LDL protein (as mg apo B/kg per day) is probably somewhat inflated because mass turnover equals FCR times LDL apo B concentration; as LDL cholesterol and apo B concentrations are strongly correlated, LDL apo B is partly being correlated with itself. However, the increase in LDL protein turnover rate in the present

experiment was also strongly correlated ($r = 0.74$) with the responsiveness of total serum cholesterol as measured in other trials several years previously, which appears to exclude a statistical artifact. For the study of Packard *et al.* we ourselves calculated that the increment in LDL production was also higher in subjects with a high response of serum LDL (but not total) cholesterol or LDL apo B if compared with those with a low response.[19] Nestel and Billington also found a significant correlation between the responses of plasma total cholesterol and the change in LDL apo B production rate.[20] The statistical caveat mentioned above does apply to both studies,[19,20] because response and turnover were not measured in independent experiments. Still, dietary cholesterol does appear to stimulate LDL production in hyperresponders more than it does in hyporesponders.

All the same, this does not tell us which is the primary mechanism that determines responsiveness. A multitude of processes could be responsible for individual variations in the responsiveness of LDL production and turnover to dietary cholesterol.[1] Two deserve closer attention.

Firstly, individuals may differ in the extent to which they can compensate for an increased cholesterol load by suppression of cholesterol synthesis in the liver and/or stimulation of the excretion of excess sterol into the bile, as suggested by studies of Nestel and Poyser.[7] Both processes affect the amount of extra cholesterol available for hepatic lipoprotein synthesis after cholesterol feeding. In previous studies[4] we were not able to demonstrate a relationship between the decrease in whole body cholesterol synthesis and the increase in serum cholesterol after cholesterol feeding, but our whole body balance method may not have been sufficiently sensitive and precise for this purpose.

Secondly, differences among subjects may exist in the fraction of intestinal cholesterol absorption, producing differences in the flux of cholesterol into the liver which may result in differences in lipoprotein production. Indeed, an association between increased cholesterol absorption and increased susceptibility to diet-induced hypercholesterolemia has been documented in several monkey species.[9] Inbred strains of hyperresponder rabbits also absorb a significantly higher percentage of dietary cholesterol than hyporesponder rabbits.[21] The relative contribution of these two mechanisms to the observed variability in the response of serum cholesterol remains to be established.

SUMMARY

Serum cholesterol in man rises when cholesterol intake increases, but the extent of the elevation varies between subjects. Part of the variation between subjects is spurious and not reproducible; it is caused by random diet-independent fluctuations of serum lipid levels. Part is due to consistent metabolic differences between subjects. We have earlier found that responsiveness was

associated with higher initial total and HDL cholesterol, lower habitual cholesterol consumption, and lower body mass index, and unrelated to gender, age, or apo E phenotype. We have now investigated the metabolic basis of variability by measuring turnover rates of low density lipoprotein (LDL) apolipoprotein B (apo B) on a low-cholesterol diet (140 mg/day) and a high-cholesterol diet (900 mg/day) in 8 volunteers with well-defined differences in the responsiveness of their serum cholesterol to diet.

Autologous ^{125}I-LDL was injected on day 23 of each diet period. Its fractional catabolic rate (FCR) was estimated from the ratio of ^{125}I in urine over that in plasma, seven days after injection. FCR (mean ± SD) increased from 0.24 ± 0.02 pools/day on the low- to 0.31 ± 0.20 on the high-cholesterol diet. LDL-apo B concentration rose from 49 ± 13 to 63 ± 12 mg/dl, and LDL-apo B production rate, calculated as FCR × concentration/body weight, from 4.8 ± 1.2 to 8.0 ± 1.4 mg/kg/day. The individual rise in production rate was significantly correlated with the rise in the serum concentration of LDL-apo B ($r = 0.90$) or LDL-cholesterol ($r = 0.75$), and also with the rise in total serum cholesterol measured in these same subjects in similar experiments 3–4 years earlier ($r = 0.74$). Degradation of LDL by freshly isolated blood mononuclear cells and by mononuclear cells incubated for 72 h in lipoprotein-deficient medium (derepressed cells) was measured on both diets in these and in additional volunteers. The rate of degradation (mean ± SD) of standard human LDL by fresh cells was 336 ± 166 ng LDL protein/mg cell protein per 8 h on the low-cholesterol diet, and decreased by 147 ± 180 ng/mg per 8 h or 44% on the high-cholesterol diet ($n = 23$, $p < 0.01$). The catabolic activity of derepressed cells obtained when subjects were on the low-cholesterol diet was negatively related to the LDL cholesterol response ($r = -0.57$, $n = 18$, $p < 0.05$), and to the total cholesterol response in earlier experiments ($r = -0.45$, $n = 18$, $p < 0.10$). Fresh cell activity was not related to these responses. It is concluded that variability in the response of serum cholesterol is related to variability in the stimulation of LDL production by dietary cholesterol.

ACKNOWLEDGMENTS

The authors are greatly indebted to the volunteers for their excellent cooperation and interest in the study. We also thank our dietician A. Severijnen-Nobels for her expert assistance, Mr. P. van de Bovenkamp for the analysis of the duplicate diets, and Drs. P.N.M. Demacker and M.J.T.M. Mol (Dept. Internal Medicine, University of Nijmegen, the Netherlands) for expert advice and help with the LDL turnover experiment.

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