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CHARACTERISTICS OF HUMAN HYPO- AND HYPERRESPONDERS TO DIETARY CHOLESTEROL

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The characteristics of people whose serum cholesterol level is unusually susceptible to consumption of cholesterol were investigated. Thirty-two volunteers from the general population of Wageningen, the Netherlands, each participated in three controlled dietary trials in 1982. A low-cholesterol diet was fed during the first half and a high-cholesterol diet during the second half of each trial, and the change (response) of serum cholesterol was measured. The responses in the three trials were averaged to give each subject's mean responsiveness. Fecal excretion of cholesterol and its metabolites were measured in the second trial, and body cholesterol synthesis was calculated. Responsiveness showed a positive correlation with serum high density lipoprotein$_2$ (HDL_2) cholesterol ($r = 0.41$, $p < 0.05$) and with serum total cholesterol level on a high-cholesterol diet ($r = 0.31$, $p = 0.09$). A negative relation was found with habitual cholesterol consumption ($r = -0.62$, $p < 0.01$), with body mass index ($r = -0.50$, $p < 0.01$), and with the rate of endogenous cholesterol synthesis ($r = -0.40$, $p < 0.05$), but not with the reaction of endogenous cholesterol synthesis rate to an increased intake of cholesterol. No relation was found with age, sex, total caloric needs, or the ratio of primary to secondary fecal steroids. Upon multiple regression analysis, only habitual cholesterol intake and serum total and HDL_2 cholesterol levels contributed significantly to the explanation of variance in responsiveness. Thus, a low habitual cholesterol intake, a high serum HDL_2 cholesterol level, or a low body weight do not make one less susceptible to dietary cholesterol-induced hypercholesterolemia.

bile acids and salts; cholesterol; cholesterol, dietary; controlled clinical trials; individuality; lipoproteins, HDL; obesity

Reduction of elevated levels of serum cholesterol by drug (1) or dietary (2) intervention can markedly reduce the risk for coronary heart disease. Not all subjects, however, react to dietary intervention to the same extent. Although much of the apparent variability in the response of serum cholesterol to a cholesterol-lowering diet may be due to chance fluctuations (3, 4), authentic hyper- and hyporesponders to diet do seem to exist. We have established the existence of specific hypo- and

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Abbreviation: HDL, high density lipoprotein.

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hyperresponders to egg yolk cholesterol by employing repeated controlled trials (4).

In the present paper we describe the relation of responsiveness in these trials with various physiologic and dietary characteristics of subjects, and the possible interpretation of these relationships in terms of the mechanisms of cholesterol homeostasis in man. The characteristics of hypo- and hyperresponders to dietary cholesterol are of interest for several reasons. Firstly, knowledge of the hyperresponder profile will help to determine beforehand which patients will benefit most from dietary therapy. Secondly, such characteristics may tell something about underlying mechanisms determining responsiveness, and also about the mechanisms relating diet and serum cholesterol levels in general.

METHODS

Design, subjects, and diets

Twenty-one men and 11 women each participated in three controlled experiments in 1982 spread out over the course of the year (figure 1). Their ages ranged from 19 to 62 years. All were in good health. Eleven were university staff members, 18 were students, and the others were inhabitants of the town of Wageningen, the Netherlands. Out of the original 94 participants in experiment 1, 23 with a response in the highest quartile and 18 with a response in the lowest quintile had been retained. Subjects were not informed about the responsiveness of their serum cholesterol to diet until after experiment 2 had been completed. Nine subjects were lost to follow-up between experiments 2 and 3 because they had moved, were unwilling to continue, or had become pregnant. This left 32 subjects for whom complete data were available. Further characteristics may be found in a previous report (4).

Each experiment consisted of a low-cholesterol period, followed by a high-cholesterol period (figure 1). Natural mixed diets were provided as previously described (4); the diets were formulated so that cholesterol, provided by egg yolk, was the only variable. Cholesterol was fed in a constant proportion to calories. Cholesterol intakes were 12 and 56 mg/megajoule (MJ) (49 and 234 mg/1,000 kcal) in experiment 1; 10 and 57 mg/MJ (40 and 243 mg/1,000 kcal) in experiment 2; and 11 and 84 mg/MJ (48 and 349 mg/1,000 kcal) in experiment 3.

Body weights were checked to the nearest 100 g twice a week and energy intake was adjusted when necessary.

The composition of the habitual free-living diets was estimated one month prior to experiment 1 by having the subjects weigh and record all their food items for two working days plus one weekend day. Prior to experiment 3 the diets were assessed once more, this time using the dietary history method. Intake data were converted into nutrients using the 1982 release of the computerized Dutch food table (5).

Buttock fat tissue biopsies were obtained on the first or second day of experiment 2 and analyzed as described (6).

Blood sampling and analysis

Figure 1 gives the blood sampling scheme, with each vertical dash representing one sample. Total and high density lipoprotein (HDL) cholesterol were determined in fasting blood serum using rigidly standardized techniques (4, 7). Total cholesterol was measured using serum calibrators and direct addition of Liebermann-Burchard reagent; the results met the criteria of the Lipid Standardization Laboratory of the Centers for Disease Control (7). HDL was isolated by precipitating non-HDL lipoproteins with heparin and manganese (92 mmol/liter). Since posture influences serum levels (8), subjects stood and waited until their turn had come for venipuncture. They then sat down and were bled right away.

In experiment 2, serum lipoproteins were separated by density gradient ultracentrifugation (9, 10) at the end of the low-cholesterol diet period (figure 1); to this end, serum samples of days 10 and 11 were pooled per subject. This was repeated at the

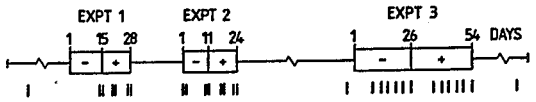

FIGURE 1. Duration of three controlled experiments and timing of blood sampling of 32 subjects in 1982, Wageningen, the Netherlands. Each vertical dash denotes one blood sample. −, low-cholesterol diet period; +, high-cholesterol diet period. Further details are given in reference 4.

end of the high-cholesterol period (experiment 2, days 23 and 24). The following lipoprotein fractions were obtained by aspiration: $d < 1.010$ (very low density lipoproteins); 1.010–1.019 (intermediate density lipoproteins); 1.019–1.055 (low density lipoproteins); 1.055–1.075 (HDL_1, including lipoprotein(a)); 1.075–1.100 (HDL_2); 1.100–1.210 (HDL_3), and $d > 1.210$ (bottom). Cholesterol was determined in the fractions enzymatically as described (10). The mean recovery of serum cholesterol in these fractions (as a percentage of whole serum cholesterol) was 94 per cent in the low- and 95 per cent in the high-cholesterol period.

The concentration of the lipoprotein(a) antigen (11) in whole serum was measured by rocket immunoelectrophoresis (12, 13) using an antiserum provided by Dr. Gerhard M. Kostner (University of Graz, Austria), and human reference standard lipoprotein(a) (Immuno A.G., Industriestrasse 72, Vienna, Austria). For each subject, four sera obtained throughout experiment 2 were analyzed and the results averaged.

Feces

Subjects collected their feces in experiment 2 during the final five days of the low- and of the high-cholesterol period, using 1.35 liter plastic buckets. Buckets were handed in daily at the laboratory and then stored at −20 C. Subjects swallowed 20 small radio-opaque plastic rings with their lunch daily from three days before the start until the end of experiment 2. By the start of the first feces collection period they had thus been swallowing markers for 10 days, so that even subjects with a long intestinal transit time should by then have reached a steady state where on average 20 markers per day were excreted. The mean marker output per five days (± standard deviation (SD)) was 106 ± 19. All individual fecal data were recalculated to a marker output of 100 per five days to correct for irregularities in fecal flow. On the first fecal collection day of both periods, 20 rings of outer diameter 3 mm instead of the usual 4.5 mm were swallowed. In addition to their functioning as the recovery markers mentioned above, these smaller markers allowed calculation of the mean transit time through the gut (14, 15). On average, 90 per cent of these single-dose transit markers were recovered in the feces collected over the following five days; missing markers were arbitrarily assigned a transit time of 175 hours.

Feces were thawed, pooled per person per diet period, weighed, and homogenized with a known and about equal weight of H_2O. The solid matter content was determined on aliquots dried overnight at 105 C. Samples of 150–250 ml were freeze-dried, ground to a fine powder, and stored at −20 C. Neutral steroids were extracted in duplicate from 150 mg of freeze-dried feces essentially as described (15), silylated with trimethylchlorosilane: hexamethyldisilazane:pyridine (1:3:9), and injected into a Varian 2700 gas chromatograph equipped with a 25 m × 0.25 mm capillary CP Sil 5 column (Chrompack, 4330 AA Middelburg, The Netherlands). Thin-layer chromatography showed that this column yields a good separation of cholesterol and its secondary bacterial metabolites from plant steroids and their metabolites, as shown earlier by Miettinen (16). Duplicate samples of a control feces pool were included in each run and used for quality control. The combined within- and between-run coefficients of variation were 5 per cent for coprostanol, 6 per cent for cholesterol, and 28 per cent for coprostanone, which is only a minor metabolite. The total content of cholesterol plus its metabolites in this pool, when averaged over this analysis period, differed less than 3 per cent of that ob-

tained three and four years earlier in the same feces pool in our laboratory (15). Bile acids and their secondary bacterial metabolites were measured in feces by W. G. Brydon (Western General Hospital, Edinburgh, U.K.) as described (15, 17).

Data analysis

The responsiveness to dietary cholesterol was defined as the difference in total serum cholesterol level between the high- and the low-cholesterol diet period in each experiment, averaged over the three experiments. For experiments 1 and 2 we used the final two and for experiment 3 the final four cholesterol values obtained during each dietary period. Thus, the responsiveness was based on 16 cholesterol measurements. In view of the diet-independent within-person variability of cholesterol levels, we regarded this average as the best estimate of each subject's susceptibility to dietary cholesterol (4). The Pearson product-moment correlation coefficient (18) was calculated between this responsiveness and various characteristics. If inspection of scatterplots revealed marked deviations from a normal distribution, the Spearman rank-order correlation coefficient R (18) was also computed. The variables showing the highest correlation with responsiveness were then combined in a multiple linear regression analysis (19).

RESULTS

Serum total and HDL cholesterol

Figure 2 shows the time course of the serum total and HDL cholesterol concentration throughout the three experiments in 15 putative hypo- and 17 hyperresponders. These had been selected at the end of experiment 1 as having a serum cholesterol response to dietary cholesterol appreciably lower or higher than the mean response of the full 94 participants in this experiment (4). On the controlled diets, both serum total and HDL cholesterol concentrations were on average higher in the hyper- than in the hyporesponders throughout the three experiments. The only exception was at the end of the low-cholesterol period of experiment 1, and this was probably caused by the selection procedure that we applied. Serum cholesterol is subject to large chance

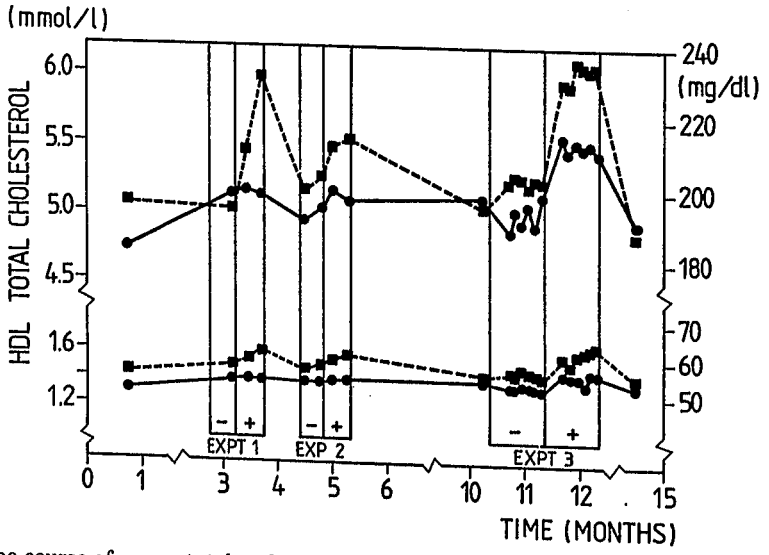

FIGURE 2. Time course of serum total and HDL cholesterol in hypo- and hyperresponders in repeated trials. Subjects were categorized as putative hypo- or hyperresponders according to their total serum cholesterol response in experiment 1, and then retested in experiments 2 and 3. ●, mean of 15 hyporesponders; ■, mean of 17 hyperresponders; –, low-cholesterol diet period; +, high-cholesterol diet period. In experiments 1 and 2, each point represents the mean of two values obtained one day apart. The experiments were performed in Wageningen, the Netherlands, in 1982, with volunteers from the general population of that town.

fluctuations, which are independent of diet (50). As a result, the selection procedure applied after experiment 1 will have caused the inclusion in the hyperresponder group of some subjects whose serum cholesterol through chance fluctuations happened to be depressed at the start of the high-cholesterol diet period of experiment 1 (figure 2). The reverse held for the hyporesponder group. This may explain why the mean serum cholesterol of the group of "hyperresponders" selected in this way was slightly lower than that of the "hyporesponders" just before the start of the high-cholesterol period in experiment 1. Such effects of random within-subject fluctuations show that it is desirable to average results from multiple experiments before classifying a subject as hypo- or hyperresponsive. Figure 3 gives these responses averaged for each of the 32 subjects over the three trials.

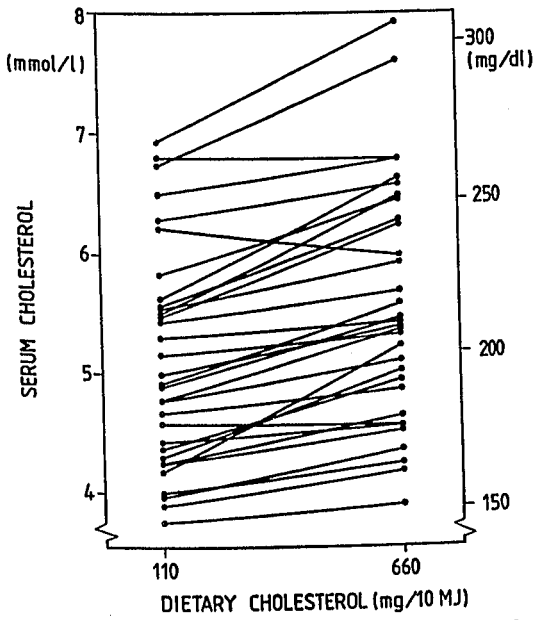

FIGURE 3. Individual responses to dietary cholesterol in 32 volunteers from the general population of Wageningen, the Netherlands, each investigated three times in 1982. Dietary intakes are averages of the respective low- and high-cholesterol periods of the three trials; 10 MJ equals 2,390 kcal. All serum cholesterol values obtained at the end of the three low-cholesterol periods were averaged per subject to yield the serum cholesterol level on a low dietary intake of cholesterol. The values obtained at the end of the three high-cholesterol periods together yielded the final level.

As a measure of the absolute serum cholesterol level on a defined high-cholesterol diet, we took from each experiment the first two cholesterol values obtained on the high-cholesterol diet (figure 1), and averaged these six values per subject. Some of these values had been obtained at a point where serum levels were still adapting to the dietary change. However, we wanted to avoid spurious correlations. None of these six values had been used in constructing the "responsiveness" variable, and the number of days between blood samples was such that the autocorrelation between consecutive serum cholesterol values was negligibly small (4). Thus, these cholesterol levels and the responsiveness calculated as described under "Methods" were based on independent sets of measurements. Dips and peaks in individual serum cholesterol values, caused by, for example, transient subclinical infections or by slight variations in blood sampling and analysis technique, would cause spuriously high correlations if the same serum cholesterol value went into both the x- and the y-variable. By using separate sets of observations for constructing the "absolute level" and the "response" variable we avoided such a statistical artifact.

The Pearson correlation coefficient between the cholesterol level on a controlled diet and the mean response was $r = 0.31$ ($p = 0.09$, $n = 32$). This low value suggests that the differences in absolute cholesterol level observed between subjects on a uniform diet are largely caused by differences in sex, age, and undefined genetic factors not related to dietary responsiveness. The correlation between the absolute individual cholesterol levels on low- and on high-cholesterol diets was $r = 0.95$. Thus, adding cholesterol to the diet had only a small effect on the position of subjects in the serum cholesterol distribution; those that were high stayed high. Figure 3 illustrates this. Thus, responsiveness to dietary cholesterol is not the only nor even the major determinant of a person's serum cholesterol level.

A nonsignificant correlation of 0.19 was found between the mean responsiveness in trials and the free-living serum cholesterol concentration, obtained by averaging the cholesterol values measured before, between, and after the experiments. Figure 2 also shows no consistent elevation of free-living serum cholesterol values in hyperresponders. Differences in free-living dietary habits between hypo- and hyperresponders may play a role here, and are discussed below.

The concentration of cholesterol in manganese-heparin soluble HDL was also higher in those subjects initially selected in experiment 1 as being hyperresponders, both during and outside the trial periods (figure 2). The correlation of the mean responsiveness in the three experiments with the free-living HDL cholesterol level was 0.26 (not significant).

HDL can be divided into fractions of different density, and a high level of the HDL_2 subfraction is associated more strongly than that of total HDL with a low risk for atherosclerosis (20). We therefore subfractionated lipoproteins by density ultracentrifugation at the end of the low- and the high-cholesterol period in experiment 2. The univariate correlation of the responsiveness with the HDL_2 cholesterol concentration in experiment 2 was 0.39 for HDL_2 measured at the end of the low-cholesterol period, 0.42 at the end of the high-cholesterol period, and 0.41 with the mean of these two measurements ($n = 32, p < 0.01$; table 1).

Body mass index

The body mass index (weight/height^2) was negatively correlated with the mean responsiveness ($r = -0.50$, $R = -0.34$, $n = 32$, $p < 0.05$; table 1). The relation was strong for women ($r = -0.77$, $n = 11$, $p < 0.01$), but not significant for men ($r = -0.17$, $n = 21$).

Fecal steroid excretion and cholesterol balance

One hypothesis to explain differences in response between hypo- and hyperresponders is that in hyperresponders the endogenous synthesis of cholesterol does not adapt well to changes in exogenous input of cholesterol with the diet (21). We therefore measured whole body cholesterol synthesis on the low- and high-cholesterol diets in experiment 2 as the balance of fecal excretion of cholesterol and its neutral and acidic metabolites minus the cholesterol intake.

TABLE 1

Correlation coefficients between serum cholesterol response and other variables for 32 subjects participating in three controlled experiments in Wageningen, the Netherlands, in 1982

	Serum cholesterol‡	HDL_2 cholesterol§	Body mass index‖	Cholesterol synthesis¶	Cholesterol consumption††
Response†	0.31	0.41*	−0.50**	−0.40*	−0.62**
Serum cholesterol‡		0.34	−0.08	−0.11	0.10
HDL_2 cholesterol§			−0.09	−0.38*	−0.06
Body mass index‖				−0.01	0.68**
Cholesterol synthesis¶					0.12

* $p < 0.05$, two-tailed; ** $p < 0.01$, two-tailed.

† Responsiveness of serum cholesterol to change in dietary cholesterol intake; mean of three experiments.

‡ Average of six determinations, namely for each experiment the first two serum cholesterol values obtained after the switch to the high-cholesterol diet.

§ Density fraction 1.075–1.100; mean of low- and high-cholesterol period in experiment 2.

‖ Pre-experimental weight (mean of three values) divided by height^2.

¶ Determined as fecal steroid excretion minus cholesterol intake, mmol/24 hours in the last week of the low-cholesterol period of experiment 2.

†† Habitual cholesterol intake (mg) divided by caloric intake (MJ); mean of one assessment prior to experiment 1 and one prior to experiment 3.

The mean balance for the 32 subjects was 2.36 ± 0.73 mmol/24 hours on the low-cholesterol diet, and 1.89 ± 0.66 mmol cholesterol/24 hours on the high-cholesterol diet (913 ± 282 and 731 ± 257 mg/24 hours, respectively). The decrease in balance differed significantly from zero ($p < 0.01$). The mean responsiveness of serum cholesterol to dietary cholesterol in the three experiments showed no correlation with the change in whole body cholesterol synthesis upon going from the low- to the high-cholesterol diet ($r = 0.00$). If the change in synthesis was expressed per kg body weight, no correlation was found, either. However, responsiveness was negatively correlated with the individual cholesterol balance, both on the low-cholesterol diet ($r = -0.40$, $p < 0.05$; table 1), and on the high-cholesterol diet ($r = -0.43$; $p < 0.05$). Thus, subjects with a low absolute synthesis rate proved more responsive to dietary cholesterol. The relation was caused mainly by a negative relation between responsiveness and fecal neutral steroid excretion, the relation with bile acid production being much weaker.

Habitual cholesterol intake

The habitual diet was recorded during the recruitment phase prior to experiment 1, and once more just before the start of experiment 3 (figure 1). Habitual cholesterol intake was expressed as mg/MJ so as to make it independent of absolute energy intake.

Subjects who professed a high cholesterol intake relative to their energy intake turned out to be rather unresponsive to dietary cholesterol in the three controlled trials: the correlation of habitual relative intake of cholesterol with mean responsiveness was $r = -0.43$ for cholesterol intake determined prior to experiment 1, $r = -0.55$ for cholesterol intake in the weeks prior to experiment 3, and $r = -0.62$ ($n = 32$, $p < 0.01$) for the mean of the two intake values (figure 4). The rank order correlation was equally strong ($R = -0.62$), showing that the relation was not due to an occasional outlying value.

There was no correlation of responsiveness with total energy intake, and only a weak negative correlation with the percent-

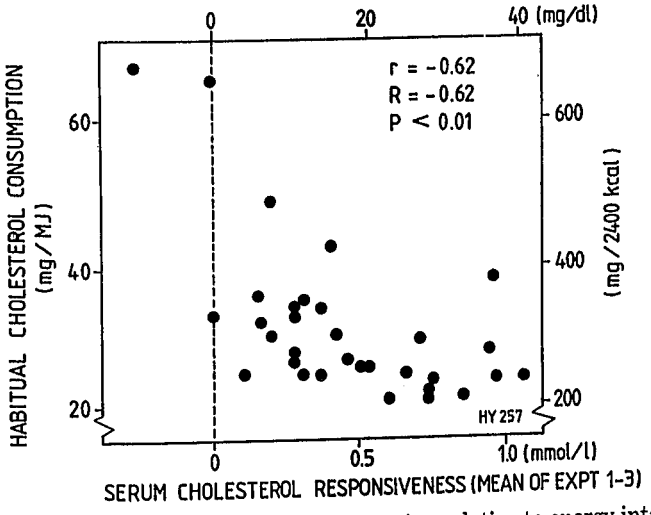

FIGURE 4. Relation between habitual cholesterol consumption relative to energy intake, and responsiveness to dietary cholesterol in controlled trials. The self-selected habitual diet was assessed by a three-day weighing-and-recording method in November or December 1981, prior to experiment 1, and by a dietary history interview in August 1982, prior to experiment 3 (figure 1); the results of the two assessments were averaged. Prior to experiment 1 the mean intake (± standard deviation) of cholesterol was 31 ± 12 mg/MJ or 323 ± 114 mg/day (range: 102 to 618). Prior to experiment 3 the intake was 31 ± 16 mg/MJ or 280 ± 103 mg/day (range: 109 to 476). One megajoule equals 239 kcal. Subjects were healthy volunteers from the general population of Wageningen, the Netherlands.

age of calories provided by saturated fatty acids ($r = -0.27$). The relation between responsiveness and habitual cholesterol intake was therefore due to differences in the intake of foods rich in cholesterol, such as eggs, rather than to foods containing both cholesterol and saturated fat, such as butter and meat. This was in line with the absence of a relation between responsiveness and the polyunsaturated/saturated (P/S) fatty acid ratio in buttock fat tissue. This ratio did show the expected correlation with the P/S ratio in the habitual pre-experimental diet ($r = 0.48$, $p < 0.01$, $n = 40$), which confirms its validity as a measure of the fatty acid composition of the habitual diet (22, 23).

Other variables

We found no relation of responsiveness with age, sex, intestinal transit time, ratio of primary to secondary steroids in the feces, within-subject variability of serum cholesterol while on constant diets, or the serum concentration of the lipoprotein(a) antigen.

Multivariate analysis

As shown in table 1, the variables that correlated with responsiveness in general showed only low correlations among each other. Exceptions were a negative correlation between cholesterol synthesis and serum HDL_2 cholesterol level, and a strong positive correlation between body mass index and habitual cholesterol consumption. The relation of responsiveness with habitual cholesterol consumption, total serum cholesterol level on a high-cholesterol diet, and serum HDL_2 cholesterol concentration persisted upon multiple regression analysis (multiple $r = 0.77$, 28 degrees of freedom; table 2). After these three variables had been taken into account, body mass index and total body cholesterol synthesis no longer contributed significantly to the explanation of variance in responsiveness.

Discussion

Systematic studies of responsiveness of serum cholesterol to dietary cholesterol in man have been rare, and the factors determining responsiveness in animals have not been identified unequivocally (21). Nevertheless, suggestions about possible determinants of responsiveness in man have been put forward, some of them based on metabolic considerations, e.g., down-regulation of cholesterol synthesis (24), and others more intuitively, such as adiposity (25).

Table 2

Multiple regression analysis of determinants of responsiveness of serum cholesterol to dietary cholesterol in 32 subjects participating in three controlled experiments in Wageningen, the Netherlands, in 1982

Independent variable	Unit	Regression coefficient ± standard error*	p
Habitual cholesterol consumption†	mg/MJ‡	−0.019 ± 0.004	0.00
Serum total cholesterol level§	mmol/liter‖	0.10 ± 0.05	0.04
Serum HDL_2 cholesterol¶	mmol/liter‖	0.43 ± 0.20	0.04

* The regression coefficient equals the change in average response of serum cholesterol (in mmol/liter) to the dietary cholesterol loads employed if the independent variable increases by one unit.

† Habitual cholesterol intake (mg) divided by caloric intake (MJ); mean of one assessment prior to experiment 1 and one prior to experiment 3.

‡ 1 MJ equals 239 kcal. Mean ± standard deviation of habitual intake was 31 ± 12 mg/MJ or 323 ± 114 mg/day (range: 102 to 618) prior to experiment 1, and 31 ± 16 mg/MJ (280 ± 103 mg/day, range 109 to 476) prior to experiment 3.

§ Average of six determinations, namely for each experiment the first two serum cholesterol values obtained after the switch to the high-cholesterol diet.

‖ 1 mmol/liter = 39 mg/dl.

¶ Density fraction 1.075–1.100; mean of low- and high-cholesterol period in experiment 2.

The association of various characteristics with responsiveness in our subjects is discussed below. Some of these variables describe known aspects of cholesterol metabolism; for others a link is less clear.

Metabolic differences between hypo- and hyperresponders

Endogenous cholesterol synthesis in man is depressed when cholesterol is ingested (26). Our data confirm this; in experiment 2, mean cholesterol input rose by 1.45 mmol/day (561 mg/day) but fecal steroid output by only 0.98 mmol/day (380 mg/day), the difference presumably being caused by a reduction of 0.47 mmol/day (181 mg/day) in the synthesis of cholesterol by the body itself. Nestel and Poyser (24) found that this *inhibition of body synthesis* after an increase in cholesterol consumption correlated with the rise in plasma concentration of cholesterol; the subjects whose plasma cholesterol rose most on a high-cholesterol diet also showed the smallest decrease of total body synthesis after the increase in exogenous supply. However, we were unable to confirm this. In experiment 2 there was no relation of the change in cholesterol balance from the low- to the high-cholesterol diet period with the serum cholesterol response in this experiment, nor with the mean response in experiments 1 to 3 combined. In univariate analysis, we found that the higher the absolute synthesis level, the lower the responsiveness, and this agrees with findings in several animal species (21). However, the relation did not persist on multivariate analysis (tables 1 and 2).

Cholesterol is not destroyed in the body; it leaves the body via excretion into the intestine, either as such or as bile acids produced from cholesterol in the liver. In the gut, cholesterol and the primary bile acids are largely degraded to secondary steroids by colonic bacteria before being excreted with the feces. Bartizal et al. (27) studied the effect of some of these bacterial strains on cholesterol metabolism in the gerbil. They produced gerbils with an intestinal flora incapable of metabolizing bile acids. These animals showed a greater-than-usual elevation of serum cholesterol when they were challenged with a cholesterol-rich diet. Bartizal et al. (27) speculated that conversion of bile acids hindered their reabsorption, and in that way helped to remove steroids from the body. In our volunteers, the *ratio of unmetabolized to total bile acids* in the feces showed no correlation with responsiveness. However, this ratio was relatively high in all our subjects. It could still be possible that so-called nonconverters, the human equivalent (28) of the nonconverting gerbil of Bartizal et al. (27) are indeed more responsive to dietary cholesterol.

Serum total and HDL cholesterol level

One would expect that, other things being equal, hyperresponders should have higher absolute serum cholesterol levels than hyporesponders. Keys et al. (29) found that in middle-aged men the individual serum cholesterol response to dietary change, ΔX, was strongly correlated with the individual serum cholesterol level, X. The least-squares equation relating the two was $\Delta X/\Delta\bar{X} = 1.84\ X/\bar{X} - 0.84$. Here $\bar{X}$ is the mean population cholesterol level predicted by the formula of Keys et al. (29) for a given diet, and $\Delta\bar{X}$ is the predicted mean response to a given dietary change, all expressed in mg/dl. In our case, the individual cholesterol levels were obtained on diets of a composition that according to their formula should produce a mean population level $\bar{X}$ of 240 mg/dl. Their formula also predicts that the dietary changes in our trials would produce a population mean serum cholesterol change $\Delta\bar{X}$ of 11.6 mg/dl. Substitution of these values in the above equation yields $\Delta X = 0.09\ X - 9.7$. Thus, this equation predicts that in our experiments an increase in absolute serum cholesterol level of one unit would be associated with an increase in responsiveness of 0.09 unit. This is very close to what we found; the absolute serum cholesterol level was significantly and independently asso-

ciated with the response, and the regression coefficient was 0.10 (table 2). This close correspondence is all the more remarkable in view of the differences between the experiments of Keys et al. (29) and our study. Our dietary manipulations involved cholesterol intake alone, while those in the study by Keys et al. (29) dealt predominantly with dietary fatty acids. This suggests that absolute cholesterol level on a given diet, responsiveness to dietary cholesterol, and responsiveness to dietary fatty acids are closely related, and are all determined by a common metabolic pathway.

Still, the proportion of the variance in absolute cholesterol levels that could be explained by variance in responsiveness was quite modest. Figure 3 depicts this graphically: large differences in absolute level were already present on the low-cholesterol diet, and these differences persisted on the high-cholesterol diet. Compared with the range of cholesterol levels found in a normal population, the range of the responses—at least to dietary cholesterol—is quite small. Apparently, age, sex and other constitutional or environmental factors are stronger determinants of absolute cholesterol levels than is dietary cholesterol intake.

The free-living cholesterol levels measured when subjects were eating their habitual self-selected diets showed an even smaller correlation with the extent of the response to dietary cholesterol in the controlled trials. However, the relation may have been disturbed by differences in the self-selected diets; as discussed below, hyperresponders had more prudent dietary habits than hyporesponders. This might override their innate tendency toward higher cholesterol levels. Such prudence might be a specific feature of our sample of hyperresponders and not representative of the population in general. In that case, it would still be possible that part of the variation in serum cholesterol levels found in the normal population is due to differences in sensitivity to the cholesterol-elevating elements in the usual diet.

Hyperresponders not only had higher total, but also higher *HDL cholesterol* levels, and responsiveness correlated especially strongly with the cholesterol concentration in the light subfraction of the high density lipoproteins called HDL_2. This fraction is of special interest because, in contrast to total or low density lipoprotein cholesterol, a high concentration of HDL_2 cholesterol in plasma predicts a lower risk for ischemic heart disease. The positive correlation of responsiveness with HDL_2 cholesterol persisted upon multivariate analysis (table 2). In an independent study of subjects with a high habitual egg consumption, we also found that those volunteers who had the highest HDL levels were most sensitive to a change in cholesterol intake (30). Similar findings were recently reported by Oh and Miller (31). On the other hand, Fisher et al. (32) reported a negative relation between basal HDL levels and cholesterol response to diet. Animal data on response and HDL are also conflicting (21). It could be that HDL is acting as a surrogate variable for an underlying metabolic determinant, e.g., the activity of the low density lipoprotein receptor (33, 34).

Dietary habits

Habitual dietary cholesterol intake (expressed relative to energy intake) was the single variable most strongly related with responsiveness in our subjects, both in univariate and in multivariate analysis (table 2 and figure 4). The relation was negative; thus, volunteers with a high habitual cholesterol consumption were less reactive when cholesterol intake during the experiments was changed from low to high. This association calls into mind the experiments of Reiser and Sidelman (35), who claimed that early cholesterol feeding in rats decreased the response to high-cholesterol diets in later life. However, these results could not be reproduced by others, either in rats (36–38) or in baboons (39). In human infants no indications for such a "programming" effect were found, either (40). An experimental study of the Tarahumara

Indians (41) also showed a normal sensitivity to dietary cholesterol in these people who had lived on a low-cholesterol diet all their life. If long-term intake of cholesterol makes one partly immune to its effects, then a high-cholesterol diet should cause only a transient rise in serum levels which subsides with time. However, such experimental and epidemiologic evidence as we have does not support the concept that one becomes immune to dietary cholesterol if a high-cholesterol diet is maintained over a long enough period.

An alternative explanation for the association of high cholesterol consumption and low responsiveness is that the cause-and-effect relation was the other way around, and that hyporesponders allowed themselves cholesterol-rich food because they knew that they were relatively insensitive to dietary cholesterol. However, the hyporesponders were already eating more cholesterol than the hyperresponders before our experiments started, and it is unlikely that subjects already had information about their sensitivity to cholesterol at that time. Finally, we cannot exclude the possibility that the relation of responsiveness with cholesterol consumption is spurious. Observations in other subjects are obviously needed.

Oh and Miller (31) recently reported that hyporesponders habitually consumed more cholesterol than hyperresponders. However, in their study the baseline value used for calculating the response was not obtained on a uniform, controlled, low-cholesterol diet, but on the subjects' own habitual free-living diet. In such a design it is to be expected that subjects with a lower habitual cholesterol intake show a larger rise in serum cholesterol when eggs are added to their diet. Thus, the study by Oh and Miller neither confirms nor denies our own findings.

Responsiveness did not differ between subjects of different *energy intake*. We fed cholesterol in a constant proportion to energy intake, and thus subjects with higher total food intake received more cholesterol; nevertheless, their serum cholesterol did not rise more than that of subjects with lower food intakes. This implies that subjects who require more food can tolerate higher absolute amounts of cholesterol. Again, this agrees with the results of the classical studies by Keys et al. (42); their formula already expressed cholesterol intake as mg per 1,000 kcal and not as mg per day.

Obesity

Connor and Connor (43) have speculated that the rise in the plasma cholesterol level caused by cholesterol overload perhaps worsens with *adiposity*. However, both in the present study (table 1) and in another population (30) we found that hyperresponders were actually leaner. One could hypothesize that fat tissue provides a receptacle for dietary cholesterol which has to be filled before serum levels start to rise. However, in that case one would expect a more pronounced response in elderly subjects with a high habitual cholesterol consumption, because their receptacles should be overflowing. This is not what we observed: responsiveness did not rise with age, and subjects with a high cholesterol intake showed a lower instead of a higher response. In the present study the relation of obesity with responsiveness was not significant in multivariate analysis, possibly because of the intercorrelation between obesity and HDL cholesterol. It should be stressed that, unlike others (44), we found no relation between obesity and body cholesterol synthesis (table 1).

Variables not related to responsiveness

Contrary to expectation (43), the younger persons in our sample were not less responsive than the older volunteers, the oldest of whom was age 62 years at entry. Serum cholesterol rises with age, and Miller (45) has suggested that this rise is due to a deterioration of the capacity of cellular low density lipoprotein (LDL) receptors to remove cholesterol from the circulation. This would fit with the findings

of Mistry et al. (46) who reported that responsiveness to dietary cholesterol was negatively related with LDL receptor activity in blood lymphocytes. Thus, as people age, their LDL receptor activity might deteriorate, serum levels would rise, and the capacity to deal with a dietary cholesterol load would diminish. Unfortunately, our data provide no support for this attractive hypothesis. Observations on patients with defects in their LDL receptor activity also do not fit this hypothesis, because their response to dietary cholesterol is not unusually high (47–49).

Conclusion

Thus, the pathways that control responsiveness remain to be clarified. Also, the characteristics of hypo- and hyperresponders have as yet not been defined clearly enough to help in deciding which patients will benefit from dietary therapy and which will not: a screening test is not yet in sight. However, our studies do show that a high HDL cholesterol concentration, a low habitual cholesterol intake, and a low body fatness do not make one less susceptible to dietary cholesterol-induced hypercholesterolemia.

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