

EXISTENCE OF CONSISTENT HYPO- AND HYPERRESPONDERS TO DIETARY CHOLESTEROL IN MAN¹

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Hyper- and hyporesponsiveness of serum cholesterol to dietary cholesterol is an established concept in animals but not in man. The authors studied the stability of the individual response of serum cholesterol to dietary cholesterol in three controlled experiments in 1982. The subjects were volunteers from the general population living in or near Wageningen, the Netherlands. Each experiment had a low-cholesterol baseline period (121, 106, and 129 mg/day in experiments 1, 2, and 3, respectively) and a high-cholesterol test period (625, 673, and 989 mg/day). Duplicate portion analysis showed that dietary cholesterol was the only variable. The 94 healthy men and women who completed experiment 1 showed an increase (mean \pm standard deviation (SD)) in serum cholesterol of 0.50 ± 0.39 mmol/liter (19 ± 15 mg/dl). Seventeen putative hyperresponders, defined by their response in experiment 1, were retested in experiments 2 and 3; they showed responses of 0.28 ± 0.38 mmol/liter (11 ± 15 mg/dl) and 0.82 ± 0.35 mmol/liter (32 ± 14 mg/dl), respectively. Fifteen hyporesponders, selected in experiment 1, showed responses in experiments 2 and 3 of 0.06 ± 0.35 mmol/liter (2 ± 14 mg/dl) and 0.47 ± 0.26 mmol/liter (18 ± 10 mg/dl), significantly lower than the corresponding values for hyperresponders. The standardized regression coefficient for individual responses in experiment 2 on those in experiment 1 was $\beta = 0.34$ ($p = 0.03$, $n = 32$); the corresponding regression coefficient for experiment 3 and experiment 1 was 0.53 ($p < 0.01$). After correction for intraindividual fluctuations the true responsiveness distribution was found to have a between-subject standard deviation of about 0.29 mmol/liter (11 mg/dl). This implies that if the mean response to a certain dietary cholesterol load amounts to e.g., 0.58 mmol/liter (22 mg/dl), then the 16% of subjects least susceptible to diet will experience a rise of only 0.29 mmol/liter (11 mg/dl) or less, while in the 16% of subjects most susceptible to diet, serum cholesterol will rise by 0.87 mmol/liter (34 mg/dl) or more. The authors conclude that modest differences in responsiveness of serum cholesterol to dietary cholesterol do exist in man, and that the wide scatter of responses observed in single experiments is largely due to chance fluctuations.

cholesterol, dietary; cholesterol; controlled clinical trial; egg yolk; individuality; lipoproteins, HDL

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Abbreviation: HDL, high density lipoprotein.

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Numerous studies have dealt with the effect of dietary cholesterol on serum cholesterol in man (for a review of these studies, see reference 1). A striking variability in the response of serum cholesterol to diet between subjects was already noted many years ago (2). In several animal species individual hyper- and hyporesponsiveness of serum cholesterol to hypercholesterolemic diets have been well established (3-7) and appear to have a genetic basis. Although it has frequently been suggested that human hyper- and hyporesponders also exist (1, 8, 9), the reproducibility of individual differences in response has never been properly established in man. Studies with up to six subjects revealed that a subject who shows a marked response to addition of egg yolk to the diet in one experiment may appear completely nonresponsive on another occasion and vice versa (10, 11). Thus, the observed differences in response between individuals in a single short-term experiment might be due to random diet-independent fluctuations, and the existence of human hypo- and hyperresponders remains uncertain.

It is important to know whether individuals with a consistently low or high response of serum cholesterol to dietary cholesterol do exist. Although severe hypercholesterolemia is often due to genetic disorders, the majority of subjects with mild hypercholesterolemia have no clearly defined metabolic defect. This category might contain many persons with an increased sensitivity to dietary cholesterol and/or saturated fatty acids.

We now report on three thoroughly controlled dietary trials with a large number of subjects, which were set up to answer the following questions: 1) Are differences in responsiveness of serum cholesterol to dietary cholesterol a stable, reproducible trait in man, when cholesterol is the only dietary variable? 2) If they are, then what are the characteristics of human hypo- and hyperresponders, and what underlying mechanisms determine responsiveness?

The results show for the first time that

reproducible differences in responsiveness to dietary cholesterol as the only variable do exist. Some of these results have been described previously in preliminary form (11, 12).

METHODS

Design and statistics

Our null hypothesis was that a subject's position in the distribution of serum cholesterol responses in one cholesterol feeding experiment is not related to his position in the distribution of responses in another experiment. The model employed was $R_2 = \beta \times R_1 + e$; R_2 and R_1 are the responses in consecutive experiments adjusted to a common scale, e is a random term, and β is the regression coefficient of standardized responses; β is numerically equal to the Pearson correlation coefficient r . Under the null hypothesis β equals zero, and a person's response in one experiment bears no relation to his response in another experiment. The statistical power for rejecting this hypothesis was optimized by doing the first experiment with a large number of subjects and retesting only those subjects with the highest or lowest responses (figure 1A). Dismissing subjects with responses close to the mean reduced costs while the statistical power was hardly affected and the estimate of the slope did not become biased (M. A. J. Van Montfort, unpublished data).

Figure 1A describes the sequence of the experiments and the type and number of participants in each. Figure 1B shows the general design of each experiment: a low-cholesterol baseline period of 14 days in experiment 1, 11 days in experiment 2, and 25 days in experiment 3, followed by a high-cholesterol period lasting 13, 13, and 28 days, respectively. Thus a subject was always measured on both diets, and the response was defined as the serum cholesterol level at the end of the high-cholesterol period minus the level at the end of the baseline low-cholesterol period.

Regression coefficients and their significance were calculated using SPSS New

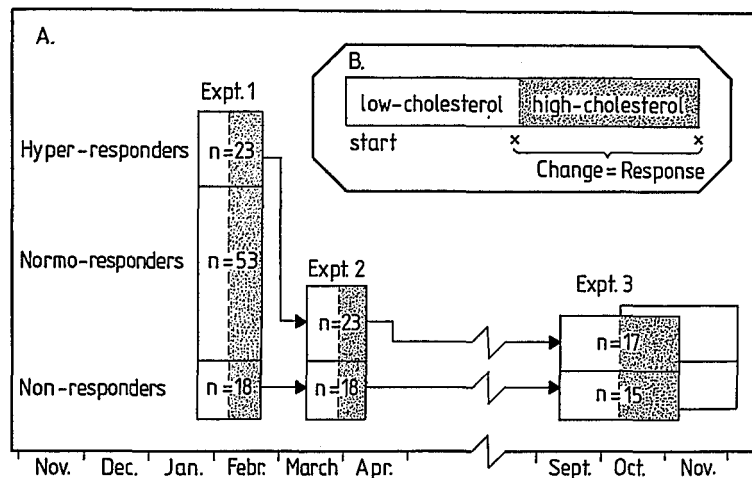


FIGURE 1. A. Sequence of trials and number of participants in a study on hypo- and hyperresponders to cholesterol. All subjects were volunteers from the general population of Wageningen and surroundings, the Netherlands. B. General design of each trial. The experiments were performed in 1982.

Regression (13). The significance of regression coefficients, and of differences in response between putative hypo- and hyperresponders (table 3), was calculated using one-tailed tests, because responses in successive experiments should either be unrelated or positively related. A negative relationship is biologically implausible.

Analysis of variance of total cholesterol in experiment 3 was performed with GLIM routines (14) using one classification by response group, a classification by diet period for each person, and six measurements for each diet period.

Subjects

The subjects were volunteers from the general population living in or near Wageningen, a college town of 30,000 inhabitants in the east of the Netherlands, 80 km from Amsterdam. Subjects were recruited through local newspapers and through posters in university buildings. Out of about 400 applicants, mostly students, we accepted all those aged 30 years or over, so as to obtain a fair representation of older age groups. Most of these were university staff members; others were regular town inhabitants. We then added 57 younger persons, mostly students, by lottery.

Medical examination (15) revealed no se-

rious pathology except for two cases with a history of cardiovascular disease and one with familial type IIa hypercholesterolemia. After consultation with the subjects' physicians and the Ethical Committee all three were allowed to participate. All three turned out to be normoresponders and were dismissed after experiment 1. Table 1 provides baseline characteristics of the subjects, including their dietary habits.

The design and execution of the experiments were thoroughly explained to the subjects, and informed consent was obtained. Prior approval was obtained from the Medical-Ethical Committee of the Department.

No reward was given in experiment 1, apart from the food; 100 Dutch florins (\$35 US) was given after experiment 2, and 200 Dutch florins after experiment 3.

Diets

Each experiment consisted of a low-cholesterol period followed by a high-cholesterol period (figure 1). In experiments 1 and 2, natural mixed diets were provided daily as previously described (15, 16). Total diets were provided, except for free-choice items described below. All foodstuffs were weighed out for each person in quantities appropriate to his or her energy needs. On

TABLE 1

Baseline characteristics for all subjects who completed experiment 1 (mean \pm standard deviation) in a study on hypo- and hyperresponders to cholesterol. Participants were volunteers from the general population of Wageningen and surroundings, the Netherlands. Baseline data were obtained in November and December, 1981

| Characteristic* | Men (n = 46) | Women (n = 48) | All (n = 94) |
|--|-----------------|-------------------|-----------------|
| Age at entry (years) | 32 \pm 13 | 34 \pm 15 | 33 \pm 14 |
| Height (cm) | 182 \pm 7 | 169 \pm 5 | 175 \pm 9 |
| Weight (kg) | 73 \pm 8 | 65 \pm 9 | 69 \pm 10 |
| Body mass index (kg/m ²) | 22.0 \pm 2.2 | 22.6 \pm 2.8 | 22.3 \pm 2.5 |
| Blood pressure (mmHg) | | | |
| Systolic | 134 \pm 17 | 126 \pm 15 | 130 \pm 16 |
| Diastolic | 82 \pm 10 | 81 \pm 9 | 82 \pm 10 |
| Serum total cholesterol† (mmol/liter) | 4.95 \pm 0.79 | 5.30 \pm 1.33 | 5.12 \pm 1.11 |
| Serum HDL cholesterol† (mmol/liter) | 1.31 \pm 0.24 | 1.59 \pm 0.36 | 1.45 \pm 0.34 |
| Serum triglycerides‡ (mmol/liter) | 1.00 \pm 0.43 | 0.85 \pm 0.37 | 0.92 \pm 0.40 |
| Habitual diet | | | |
| Energy | | | |
| (MJ/day) | 11.8 \pm 2.5 | 8.1 \pm 2.1 | 9.9 \pm 3.0 |
| (kcal/day) | 2,817 \pm 604 | 1,943 \pm 502 | 2,370 \pm 705 |
| Protein (% of energy) | 14 \pm 2 | 15 \pm 3 | 14 \pm 2 |
| Fat (% of energy) | 35 \pm 5 | 35 \pm 7 | 35 \pm 6 |
| Polyunsaturated fatty acids (% of energy) | 6 \pm 2 | 6 \pm 2 | 6 \pm 2 |
| Polyunsaturated:saturated ratio | 0.4 \pm 0.2 | 0.4 \pm 0.2 | 0.4 \pm 0.2 |
| Carbohydrates (% of energy) | 48 \pm 6 | 49 \pm 8 | 49 \pm 7 |
| Mono- and disaccharides (% of energy) | 23 \pm 4 | 24 \pm 5 | 24 \pm 5 |
| Alcohol (% of energy) | 4 \pm 4 | 2 \pm 3 | 3 \pm 4 |
| Dietary fiber (g/day) | 37 \pm 10 | 30 \pm 10 | 33 \pm 10 |
| Cholesterol (mg/day) | 340 \pm 128 | 271 \pm 136 | 305 \pm 136 |

* Standing height was measured without shoes. Weights were measured to the nearest 0.1 kg, after breakfast, and without shoes, sweaters, jackets, key rings, etc. The determination of serum triglycerides has been described (16). About eight weeks before the start of experiment 1, prospective subjects weighed and recorded their habitual diet for two working days plus one weekend day. The records were elaborated using the 1980 edition of the computerized Dutch food table (40).

† Cholesterol: 1 mmol/liter = 39 mg/dl.

‡ Triglycerides: 1 mmol/liter = 89 mg/liter.

week days, subjects came in at noon for their dinner, which was served in a special dining room at the Department. Evening bread, breakfast and other foods were distributed as packages each week day at noon and consumed at home. Food for the weekend including ingredients for the hot meals was provided each Friday. Subjects followed their normal daily routines, except that for some the rhythm of fixed midday meals and early-morning blood samples

and the restrictions on convivial eating and drinking made for a somewhat more regular life-style than was their habit. The diets were formulated so that cholesterol, provided by egg yolk, was the only variable. 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. In order to allow a con-

stant high intake of saturated fat and a variable intake of cholesterol, we provided special bread containing 9 g/100 g hydrogenated palm kernel oil, Iodine value 0-2 (Crock-Laan, Wormerveer, The Netherlands) and a special margarine (van den Bergh & Jurgens B.V., Rotterdam, The Netherlands) high in saturated fat. Diets were formulated at 17 levels of energy intake, ranging from 6.5 to 16.2 MJ/day. A day's menu providing 10 MJ (2,390 kcal) consisted of six 30-g slices of high-fat bread, 25 g of margarine, two 15-g slices of cheese, one 15-g slice of medium-fat meat, 325 g of milk or yoghurt (1.5 per cent fat), one piece of fruit, two 10-g cookies, 42 g of sugar or its equivalent in fruit juice, jam or honey, 200 g of potatoes, 200 g of cooked vegetables, 30 g of salad garnished with 19 g of freshly cooked egg yolk, 65 g of margarine-based gravy, and 175 g of a milk-based dessert containing 23 g of fresh egg yolk. In the low-cholesterol diet, egg yolk was omitted and the fat and protein of the egg yolk were balanced by some olive oil plus slight adjustments in other items. Each subject was allowed to consume each day 1 MJ (240 kcal) worth of self-selected food-stuffs low in fat and/or cholesterol; these were specified in a list. Typical selections were alcoholic drinks, fruit and candy. Subjects recorded these free-choice items daily in a diary. Departures from the diet were also recorded, as were illness and drug use.

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

In experiment 3 the subjects prepared their hot meals at home, except for a communal lunch at the Department every Tuesday. Subjects collected individual packages containing high-fat bread, margarine, cookies, salads, salad dressing, egg yolk and desserts at the Department two or three times a week, and they were carefully instructed by the dieticians (J. H. M. de V. and A. N.) about the selection and preparation of other items. Weekly 24-hour recalls evenly distributed over the days of the week provided further control. Body

weights were also checked weekly. The diets were designed to be identical with those provided in experiments 1 and 2 except that the high-cholesterol diet now contained more cholesterol (table 2).

Every effort was made to meet the subject's individual preferences and problems and to keep up morale and adherence. A weekly magazine provided information, tips and entertainment. The authors shared lunch with the subjects on working days during experiments 1 and 2; during experiment 3, participants were seen several times a week at the blood sampling and the breakfast following it, at the 24-hour recall, at the weekly lunch, and when they collected food. Throughout the experiment a cordial and friendly relationship was in evidence between subjects and investigators, and attendance at meals and blood sampling was 100 per cent.

Blood sampling and analysis

Blood was sampled after an overnight fast, serum was stored at -80°C , and total and high density lipoprotein cholesterol were determined in a rigidly standardized laboratory as earlier described (15, 17, 18). Since posture influences serum levels (41), subjects stood and waited until their turn had come to sit down for venipuncture.

Experiment 1 started on January 21, 1982. Blood was sampled at the end of the low-cholesterol period (days 14 and 15) and during the high-cholesterol period (days 20, 21, 27, and 28). Diets were changed at day 15, after blood sampling (figure 1). The response was calculated as the mean of days 27 and 28 minus the mean of days 14 and 15.

Experiment 2 started on March 15, 1982. Blood was sampled on days 1, 2, 10, 11, 17, 18, 23, and 24, and the response calculated as the means of days 23 and 24 minus days 10 and 11. Replacement of the low-cholesterol diet by the high-cholesterol diet took place on day 11 after blood sampling.

Experiment 3 had an overlapping design, with 17 subjects (eight hypo- and nine hyperresponders in experiment 1) starting on

TABLE 2

Mean nutrient intakes in the low- and high-cholesterol periods of the three experiments in a study on hypo- and hyperresponders to cholesterol.* Subjects in experiment 1 were volunteers from the general population; putative hypo- and hyperresponders were selected in experiment 1 and then participated in experiments 2 and 3. All experiments took place in Wageningen, the Netherlands, in 1982

| Nutrient | Mean nutrient intakes in low- and high-cholesterol periods | | | | | |
|---------------------------------|--|-------|--------------|-------|--------------|-------|
| | Experiment 1 | | Experiment 2 | | Experiment 3 | |
| | Low | High | Low | High | Low | High |
| Energy† | | | | | | |
| MJ/day | 10.4 | 11.1 | 11.2 | 11.6 | 11.3 | 11.8 |
| kcal/day | 2,490 | 2,670 | 2,674 | 2,768 | 2,700 | 2,830 |
| Protein (% of energy) | 11 | 12 | 11 | 12 | 10 | 12 |
| Fat (% of energy) | 42 | 41 | 41 | 41 | 41 | 42 |
| Polyunsaturated fatty acids | | | | | | |
| (% of energy) | 3.6 | 3.3 | 3.4 | 3.7 | 4.0 | 4.5 |
| Polyunsaturated:saturated ratio | 0.16 | 0.16 | 0.16 | 0.18 | 0.19 | 0.22 |
| Carbohydrates (% of energy) | 44 | 44 | 45 | 44 | 45 | 43 |
| Sugars (% of energy) | 22 | 22 | 22 | 22 | 22 | 22 |
| Alcohol (% of energy) | 2 | 2 | 3 | 3 | 1 | 1 |
| Dietary fiber (g/day) | 30 | 34 | 31 | 31 | 37 | 38 |
| Cholesterol (mg/day) | 121 | 625 | 106 | 673 | 129 | 989 |

* Protein, fat, fatty acids, total carbohydrates and cholesterol were determined in duplicate portions collected throughout the experiments, as described (39). For experiments 1 and 2 the contribution of free choice items including alcohol and the consumption of fiber and sugars were calculated using the 1982 edition of the computerized Dutch food table (40). Intakes in experiment 3 were calculated from the 24-hour recalls using the food table (40) supplemented with analyzed values for foods supplied by us (see Methods).

† Including 1 MJ (240 kcal) as free-choice items. Individual intakes ranged from 5.8 to 16.2 MJ/day (1,386 to 3,872 kcal) in experiment 1, from 7.6 to 16.2 MJ/day in experiment 2, and from 7.6 to 16.0 MJ/day in experiment 3.

September 13, 1982, and the remaining 15 subjects (eight of them putative hyperresponders) starting four weeks later. Both groups reacted to the diets in very similar ways, so their results were pooled. Blood samples were obtained on days 8, 12, 15, 19, 22, and 26, and again on days 36, 40, 43, 47, 50, and 54. Diets were changed at day 26. The response was defined as the mean of days 43, 47, 50, and 54 minus the mean of days 15, 19, 22, and 26. However, all 12 measurements were used in calculating the within- and between-subject components of the variance of the response (table 5).

RESULTS

Three controlled trials on the effect of dietary cholesterol on serum cholesterol were performed. The first trial served to identify putative hyper- and hyporesponders. The second and third experiments were performed so as to measure the reproducibility of the response of serum chole-

sterol to dietary cholesterol in participants selected in the first experiment. Figure 1 is a schedule of the trials and table 1 gives the baseline characteristics of the subjects.

The results of each trial and the reproducibility of the individual response from one trial to another are discussed in detail below.

Experiment 1

Ninety-eight subjects entered experiment 1 and 94 of them completed it successfully. From the diaries no serious disease or other confounding events were in evidence.

Diets. The composition of the experimental diets is given in table 2. The diets were made high in saturated fat because several studies (19-21) have shown this to cause a more pronounced response to dietary cholesterol. The diets were defined in terms of per cent of energy or of (m)g per MJ, and were then elaborated in g per day for a range of energy intakes. Thus a higher en-

ergy intake involved a higher intake of all nutrients, including cholesterol. During the low-cholesterol period, cholesterol intake was set as low as practicable because the response to changes in cholesterol intake is highest in the lower regions of intake (22). The amount and composition of nutrients other than cholesterol were the same in the high- and low-cholesterol diets (table 2).

Body weight. Although individual energy intakes were adjusted at the first sign of weight loss, there was an average weight change of -0.8 kg (range: -2.5 to $+1.1$, $n = 94$) over the four weeks of experiment 1. There was no correlation between the changes in weight and changes in serum total ($r = 0.01$, nonsignificant) or high density lipoprotein (HDL) cholesterol ($r = 0.03$, nonsignificant).

Serum cholesterol. In comparison with pre-experimental values, the high-saturated fat, low-cholesterol baseline diet given during the first 14 days caused a very slight increase in serum total cholesterol ($+0.12 \pm 0.53$ mmol/liter, $+5 \pm 20$ mg/dl) and HDL cholesterol ($+0.05 \pm 0.16$ mmol/liter, $+2 \pm 6$ mg/dl). Obviously, the reduction in cholesterol intake was more than offset by the increase in saturated fatty acid consumption compared with the baseline diet. One may thus assume that for the group as a whole, serum lipids were in a steady state by the end of the baseline period.

The high-cholesterol diet provided on average an extra 500 mg of cholesterol per day. As a result, serum total cholesterol rose by 4 per cent after six days and by 10 per cent after 13 days (table 3). The concentration of Mn-heparin soluble HDL cholesterol was on average unchanged after six days and had risen by 4 per cent after 13 days (table 3). The total cholesterol response showed a normal Gaussian distribution with no evidence for subgroups of different responsiveness.

Experiment 2

Subjects with responses of total serum cholesterol in the upper quartile and lower quintile of the response distribution in ex-

periment 1 were selected for participation in experiment 2. We selected an excess of subjects with a high response because we expected that in the long run hyperresponders might be more inclined to refuse participation in new experiments. The selection yielded 18 putative hyporesponders (10 men and eight women), with responses in experiment 1 ranging from -11 to $+4$ per cent (mean: 0 per cent), and 23 putative hyperresponders (12 men and 11 women) with responses ranging from $+11$ to $+42$ per cent (mean: $+20$ per cent). All accepted the invitation to participate in experiment 2. They were not told their response to diet in experiment 1 until after they had completed experiment 2, because knowledge of this might have influenced their behavior.

Experiment 2 started 26 days after the end of experiment 1, and all 41 subjects completed it successfully. The low-cholesterol diet was now given for 11 days and the high-cholesterol diet for 13 days. The diets were identical to those used in experiment 1 (table 2).

Body weight. Slight weight losses were observed in some subjects during the early part of experiment 2. This pointed to an increased energy requirement, since their intakes were the same as in experiment 1. There was a mean change in body weight of -0.8 kg from start to end (range -2.7 to $+0.7$ kg) for the group as a whole. Again there was no correlation of body weight changes with serum total or HDL cholesterol changes.

Serum cholesterol. At the start of experiment 2 the average concentration of cholesterol in serum had recovered from the effect of experiment 1, and so had the HDL-cholesterol concentration; both were less than 2 per cent above pre-experimental values. The 53 normoresponding subjects who had been dismissed after experiment 1 were checked six weeks after the return to their usual diets; at that time their HDL and total cholesterol levels had also returned to pre-experimental values.

The 41 participants in experiment 2 showed a mean increase of 1.5 per cent in total cholesterol after 10 days of the low-

TABLE 3

Average levels of serum total and HDL cholesterol on the low-cholesterol diet, and changes on the high-cholesterol diet (mean \pm standard deviation) in a study on hypo- and hyperresponders to cholesterol. The participants were volunteers from the general population. Subjects were divided into hypo-, normo-, and hyperresponders according to their response in experiment 1, and only hypo- and hyperresponders were reinvestigated in experiments 2 and 3. All experiments took place in Wageningen, the Netherlands, in 1982

| | Average levels of serum total and HDL cholesterol (mmol/liter) [†] | | | | |
|-------------------------------------|---|---------------------------------|----------------------------------|---------------------------------|----------------------------------|
| | Experiment 1 | Experiment 2 | | Experiment 3 | |
| | All subjects (n = 94) | Hypo- responders (n = 18) | Hyper- responders (n = 23) | Hypo- responders (n = 15) | Hyper- responders (n = 17) |
| Total cholesterol | | | | | |
| Low-cholesterol diet | 5.25 \pm 1.19 | 5.10 \pm 0.98 | 5.34 \pm 0.94 | 5.02 \pm 0.96 | 5.25 \pm 0.86 |
| Change on high- cholesterol diet | | | | | |
| After 6 days | +0.21 \pm 0.28** | 0.14 \pm 0.26* | 0.11 \pm 0.34 | | |
| After 13 days | +0.50 \pm 0.39** | 0.11 \pm 0.40 | 0.15 \pm 0.51 | 0.49 \pm 0.31** | 0.71 \pm 0.47** |
| After 24 days | | | | 0.47 \pm 0.36** [‡] | 0.82 \pm 0.39** |
| HDL cholesterol | | | | | |
| Low-cholesterol diet | 1.50 \pm 0.34 | 1.38 \pm 0.33 | 1.54 \pm 0.34 | 1.37 \pm 0.34 | 1.46 \pm 0.25 |
| Change on high- cholesterol diet | | | | | |
| After 6 days | +0.01 \pm 0.09 | 0.03 \pm 0.10 | 0.02 \pm 0.10 | | |
| After 13 days | +0.06 \pm 0.11** | 0.03 \pm 0.10 | 0.05 \pm 0.10 | 0.08 \pm 0.10** | 0.10 \pm 0.08** |
| After 24 days | | | | 0.11 \pm 0.12** [‡] | 0.18 \pm 0.09** |

* $p < 0.05$. ** $p < 0.01$, different from zero by two-tailed t test.

[†] Serum cholesterol, 1 mmol/liter = 39 mg/dl.

[‡] $p < 0.05$, different from hyperresponders by one-tailed t test.

cholesterol, high-saturated-fat baseline diet. The high-cholesterol diet caused a further mean increase of 2.8 per cent after six days and 3.0 per cent after 13 days (table 3). There was little if any difference after 13 days between the responses of the 18 putative hyporesponders (mean \pm SD, +2.7 \pm 7.8 per cent) and the 23 putative hyperresponders (+3.2 \pm 9.1 per cent). Thus the mean response of the 41 participants was much lower than was to be expected from the composition of the diets (23, 24) and from the mean response in experiment 1. Since this low overall response might have obscured differences between hypo- and hyperresponders, we opted for a third trial.

Experiment 3

Thirty-two of the participants in experiment 2 proved willing and able to take part in a third experiment, and all of them completed it successfully. Their response in

experiment 2 had been on average (\pm SD) 0.06 \pm 0.35 mmol/liter for the 15 hypo- and 0.28 \pm 0.38 mmol/liter for the 17 hyperresponders. Thus the correspondence between the responses in experiment 1 and experiment 2 was better for these 32 subjects than for the group of 41 as a whole, and the responses differed significantly between hypo- and hyperresponders ($p < 0.05$). Of the nine subjects who did not go on to experiment 3, eight were women and one was a man. Three of them had moved outside the Wageningen region, one woman had become pregnant, and five were unwilling to participate because of the burden on their personal lives or for unspecified reasons.

Experiment 3 lasted eight weeks instead of four; this enabled us to take more blood samples and thus reduce the effect of within-subject variation. A smaller proportion of the food was provided by us, and

subjects had to do more of the cooking themselves. Because the subjects were highly motivated and experienced we were confident that this would not threaten adherence, while it greatly reduced costs.

In view of the low response in experiment 2, cholesterol intake during the high-cholesterol period was now increased to a mean of 989 mg/day. The intake of other nutrients as determined in weekly interviews was essentially the same as in experiments 1 and 2 (table 2).

The experiment proceeded uneventfully and there were no dropouts. No serious disease or other confounding events were noted in the diaries.

Body weight. Weight changes from the first to the eighth week averaged 0.0 ± 1.2 kg (range: -2.3 to $+3.8$ kg). There was again no correlation with the cholesterol response ($r = 0.10$, nonsignificant).

Serum cholesterol. The serum cholesterol concentration on average did not change on the low-cholesterol, high-saturated-fat baseline diet compared with values obtained four to eight weeks before the experiment, and neither did HDL cholesterol. After addition of cholesterol to the diet, serum total cholesterol concentrations rose by a mean of 12.0 per cent after 13 days, very close to the final increase of 12.7 per cent after 24 days. HDL cholesterol concentrations for all subjects combined showed an increase of 6.4 per cent after 13 days and 10.1 per cent after 24 days. The subgroups of putative hyper- and hyporesponders both showed a significant increase of serum total and HDL cholesterol after cholesterol loading. The mean response in the hyperresponders was, however, significantly and markedly higher than in the hyporesponders (table 3). Using the four measurements made in the last two weeks of each period, we obtained a total cholesterol response of 0.82 ± 0.35 mmol/liter (32 ± 14 mg/dl) for the hyperresponders and a significantly ($p < 0.01$) lower value of 0.47 ± 0.26 mmol/liter (18 ± 10 mg/dl) for the hyporesponders.

Reproducibility of the response per person

Table 4 gives the standardized regression coefficients of the responses of serum cholesterol in experiments 2 and 3 on the responses in the preceding experiments for the 32 subjects who participated in all three experiments. Under the null hypothesis any deviation of an individual's response from the group mean in a given experiment would be due to random within-subject fluctuations and would be extinguished in subsequent experiments. Table 4 shows that this hypothesis is untenable because the regression coefficients relating responses in different experiments were significantly different from zero.

Figure 2 shows the mean responses in the three experiments of the 17 subjects who had been labeled "hyperresponders" and the 15 subjects marked initially as "hyporesponders" based on their response in experiment 1. It is clear that part of the initial difference is lost in subsequent experiments, but on average the groups remained distinct in their response to dietary cholesterol.

Quantitation of variance between subjects

In the simplest model the variance in the response to dietary cholesterol between subjects observed in one experiment is made up of the true variance between subjects that would be observed if the number of measurements per subject were infinite,

TABLE 4

Standardized regression coefficients of the response of serum cholesterol to dietary cholesterol in follow-up experiments as a function of the response in previous experiments, in 32 subjects in a study on hypo- and hyperresponders to cholesterol. The participants were volunteers from the general population. All experiments took place in Wageningen, the Netherlands, in 1982

| | β | p^* |
|-----------------------------|---------|-------|
| Experiment 2 - experiment 1 | 0.34 | <0.05 |
| Experiment 3 - experiment 1 | 0.53 | <0.01 |
| Experiment 3 - experiment 2 | 0.37 | <0.05 |

* Statistical significance, one-tailed.

and an error term largely caused by the random fluctuations of serum cholesterol within each person: $SD^2\text{-observed} = SD^2\text{-between} + SD^2\text{-within}$. In experiment 3, six measurements of serum cholesterol were made for each person on each diet under equilibrium conditions; as stated above, average serum cholesterol concentrations on the low-cholesterol diet were constant all through the first diet period, and had stabilized at the higher level after 13 days on

the high-cholesterol diet. This made it possible to estimate within one experiment both the standard deviation within subjects and the total standard deviation.

The interdependence of serial cholesterol values per person was first estimated. Application of von Neumann's Q (25) yielded autocorrelation coefficients of 0.09 for the low- and 0.07 for the high-cholesterol period. These low correlation coefficients suggested that the values within one period were independent. The spontaneous variability of cholesterol on constant diets was then calculated for each person as the standard deviation of the mean of his or her six values per period. The average of this standard deviation of serum cholesterol levels within subjects (calculated as the root of the mean variance) was 0.24 mmol/liter for the hyporesponders, 0.30 for the hyperresponders, and 0.27 (11 mg/dl) for the group as a whole. Values for the two diet periods were very similar. The resulting standard deviation within persons of the change in cholesterol in response to the egg yolk diet was calculated and the observed total standard deviation of the response was corrected for this within-person component (table 5). This yielded a true standard deviation between subjects of the responsiveness to dietary cholesterol of 0.29 mmol/liter (11 mg/dl).

The distribution of responses within the hypo- and hyperresponder groups shows a great deal of overlap (figure 2, experiment

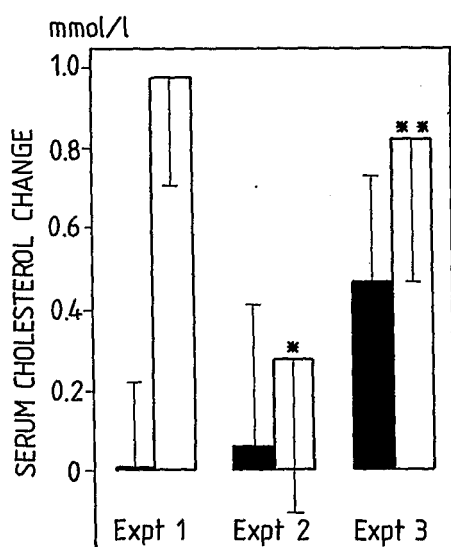


FIGURE 2. Mean of the responses of 15 putative hypo- (dark bars) and 17 hyperresponders (open bars) to cholesterol in the three experiments. The groups were taken from the tails of the response distribution in experiment 1 and retested in experiments 2 and 3. Thin bars denote one standard deviation. Group means were significantly different by one-tailed t test (* $p < 0.05$; ** $p < 0.01$).

TABLE 5

The observed variance of the response of serum cholesterol in experiment 3, and its partitioning into within- and between-person components in a study on hypo- and hyperresponders to cholesterol. Variance is given as square of standard deviation (SD), in mmol/liter; 1 mmol/liter = 38.7 mg/dl. The participants were volunteers from the general population. All experiments took place in Wageningen, the Netherlands, in 1982

| | All | Hyporesponders | Hyperresponders |
|-------------------------|-------------------|-------------------|-------------------|
| n | 32 | 15 | 17 |
| SD^2 observed* | 0.33 ² | 0.23 ² | 0.36 ² |
| SD^2 within persons† | 0.16 ² | 0.14 ² | 0.17 ² |
| SD^2 between persons‡ | 0.29 ² | 0.18 ² | 0.31 ² |

* Values differ somewhat from those in table 3 and under experiment 3 because all six cholesterol measurements per period were used in deriving them.

† Calculated as $SD^2(\text{low})/6 + SD^2(\text{high})/6$, where SD^2 is the average within-subject variance of the absolute cholesterol level and "low" and "high" refer to the diet periods.

‡ Calculated as SD^2 observed minus SD^2 within persons.

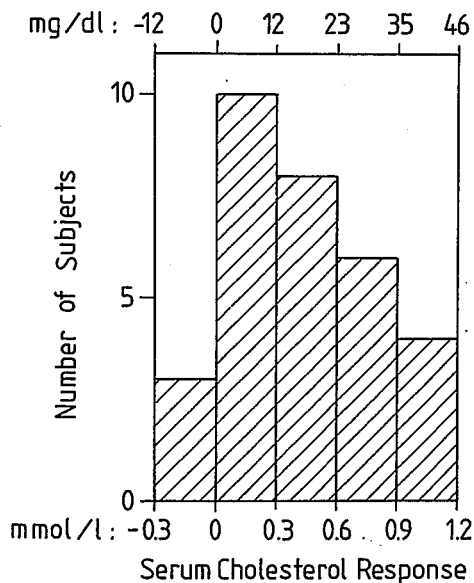


FIGURE 3. Distribution of the response of serum cholesterol to dietary cholesterol in the 32 subjects who participated in all three experiments. The response was defined for each subject as the mean of his responses in the three experiments.

3), and when combined they form a unimodal distribution (figure 3). The same held for the responses in each separate experiment. Still, it could be argued that combination of both groups in one analysis might lead to overestimation of the dispersion of the response. However, separate analyses (table 5) yielded values of 0.18 mmol/liter (7 mg/dl) for the dispersion (1 SD) of the responsiveness within the hypo- and 0.31 mmol/liter (12 mg/dl) within the hyperresponder group. Thus the value of 0.29 mmol/liter (11 mg/dl) obtained above for the combined groups cannot have been much of an overestimation of the spread in responsiveness in the population from which our subjects were drawn.

DISCUSSION

The concept of hyper- and hyporesponders derives from animal experiments, where this phenomenon has been firmly established (3-7). Indications for the existence of hyper- and hyporesponsiveness to dietary cholesterol in man have also been found (26-28), but the dietary challenge was limited to one experiment and the re-

producibility of the response is not known. Our experiments are the first to show that the differences in response to dietary cholesterol between humans are at least partly reproducible and stable over a prolonged period, and to quantitate the extent of these differences in a normal population.

Effect of egg yolk cholesterol on serum cholesterol

Our data provide some explanation for the conflicting results on the effect of dietary cholesterol on serum cholesterol in man (8, 29). Although it has been pointed out (30, 31) that some investigators may have failed to find a response of serum cholesterol to dietary cholesterol simply because of poor experimental design, our results suggest that people do exist whose serum cholesterol is resistant to dietary cholesterol. However, people who are consistently insensitive to dietary cholesterol are probably rare. Our studies, employing large numbers of subjects and rigid dietary control, show once again that, on average, dietary cholesterol from egg yolk does raise serum cholesterol, although the effect is more modest than the effect of changes in saturated fat intake (22, 23). The mean effects observed (table 3) were intermediate between those predicted by the formula of Keys et al. (23), which predicts an increase of about 13 mg/dl (0.34 mmol/liter) for experiments 1 and 2 and 18 mg/dl (0.46 mmol/liter) for experiment 3, and those predicted by the formula of Hegsted et al. (24) which predicts a rise of about 27 mg/dl (0.69 mmol/liter) for experiments 1 and 2 and of 43 mg/dl (1.11 mmol/liter) for experiment 3. Our data thus reaffirm the qualitative validity of these formulas.

The concentration of manganese-heparin-soluble HDL cholesterol rose significantly in experiments 1 and 3 after cholesterol loading (table 3). However, both the relative and the absolute increase in serum cholesterol concentration were larger in other, presumably atherogenic lipoproteins. As a result the HDL to total cholesterol ratio, which was 0.29 on the low-

cholesterol diet, changed significantly ($p < 0.001$); on the high-cholesterol diet it decreased by 0.02 in experiment 1 and by 0.01 in experiment 3. Although these differences seem small, they are of the same order of magnitude as those between men with coronary heart disease and healthy controls (32). Thus egg yolk cholesterol caused an unfavorable decrease in the mean ratio of HDL to total serum cholesterol. Higher increases in HDL cholesterol after dietary cholesterol loading have been found in other trials, but part of the increase in HDL may have been due to increases in fat intake which accompanied the increased cholesterol consumption in those studies (for a review see reference 33).

Reproducibility of individual differences in response

Finding human hyper- and hyporesponders proved to be harder than we had anticipated. Our second experiment was inconclusive because this time the response was very low in almost all subjects. The cause of this low response is unclear. It cannot be explained as regression to the mean, because the average serum cholesterol responses in experiment 1 amounted to 0 per cent for the hypo- and +20 per cent for the hyperresponders. Complete regression to the mean should therefore have resulted in a mean response of +10 per cent in both groups, which is substantially higher than the observed values of +2.7 per cent for the hypo- and 3.2 per cent for the hyperresponders.

The duration of the high-cholesterol period of 13 days should have been sufficient to allow a rise in serum cholesterol to become manifest; Keys et al. (22) estimated that at eight days about 70 per cent of the ultimate effect of dietary cholesterol is achieved, and Connor et al. (34) found that after cholesterol loading a new stable level is reached in about two weeks. Our own experience in experiment 3 (table 3) shows that levels did not increase any more after 13 days.

Poor dietary adherence is not the expla-

nation either, because the excretion of cholesterol plus its neutral and acidic metabolites with the feces was on average 390 mg/day higher during the high-cholesterol than during the low-cholesterol period (Katan et al., unpublished data). Intake was planned to increase by 570 mg/day; the difference with the excretion value can be explained satisfactorily by suppression of endogenous synthesis on the high-cholesterol diet. Thus there is objective evidence for a lack of response of serum cholesterol in the face of a substantial rise in intake. The nine subjects who did not go on to experiment 3 showed the same rise in fecal excretion of steroids as the 32 who did continue, showing that dietary compliance had been satisfactory in both groups.

One might speculate that the high-cholesterol diet consumed in experiment 1 had made subjects refractory to the effect of dietary cholesterol in experiment 2, which followed it closely. There is some evidence for such "memory-effects" in rats (35), but no such effects were found in a long-term study in baboons (36). Connor and coworkers (37) also found no evidence that people habituated to a low-cholesterol diet show an exaggerated response to dietary cholesterol loading. The matter thus remains open.

In the third experiment, performed half a year later with 32 of the 41 original subjects, marked responses to dietary cholesterol were found once more, and these were significantly correlated with the individual changes in the first and to a lesser extent in the second study (table 4 and figure 2).

Although the regression coefficients for the relations between responses in repeated experiments were significant, they were still low, and quite a number of subjects who appeared hyperresponsive in one experiment proved to be hyporesponsive in another experiment. This is caused by the diet-independent within-person variability of serum cholesterol. We estimated this variability in experiment 3 by analysis of variance, and obtained a within-person standard deviation of serum cholesterol of

0.27 mmol/liter (11 mg/dl). This standard deviation is caused by random diet-independent fluctuations, and it is of the order of magnitude of the response to dietary cholesterol itself. The responses observed for separate persons thus contain large error terms (table 5), which degrade the correlation between responses measured in independent experiments. Jacobs et al. (38) found an average within-person standard deviation for the absolute level of serum cholesterol in the controlled studies of Keys and coworkers of 17.8 mg/dl or 0.46 mmol/liter, which is appreciably higher than our value. Part of the difference may be due to reduction of laboratory error since the time when those experiments were performed. Also our calculations span a shorter period than those of Jacobs et al. (38).

Distribution of responsiveness within the population

Subtraction of the within-subject variance from the total observed variance in response when the diet was changed gave an estimated between-subject standard deviation of the response of about 0.29 mmol/liter (11 mg/dl). This is thus a measure of the true differences in responsiveness between subjects.

One can now calculate the distribution of the true long-term responses to a change in cholesterol intake in a group of subjects comparable to ours, if one assumes that the responses follow a normal (Gaussian) distribution. For instance, at a mean response of 0.58 mmol/liter (22 mg/dl)—a realistic figure, as evident from table 3—and a standard deviation of 0.29 mmol/liter, 16 per cent of subjects would have a true long-term response of less than half the mean response, i.e., 0.29 mmol/liter (11 mg/dl) or less. Another 16 per cent would have a responsiveness of more than 150 per cent of the mean, i.e., 0.87 mmol/liter (34 mg/dl) or over. About 2 per cent would show no increase at all. Our figures agree reasonably well with those of Jacobs et al. (38) who, using the same criteria, labeled 9 per cent of their subjects as hypo- and another

9 per cent as hyperresponding. The data of Jacobs et al. referred to middle-aged mentally retarded men and to diets that differed in several components. Our data extend these findings to the specific effect of dietary cholesterol alone in men and women spanning a large range of ages. Our results suggest that differences in responsiveness to dietary cholesterol in man are real but that they are smaller than has hitherto been assumed on the basis of animal studies and single experiments in humans (28).

Our best estimate of each person's sensitivity is the average of his responses in the three experiments combined. As shown in figure 3 the distribution of individual average responses has a single maximum with no evidence for discrete subgroups, even though these subjects had originally been drawn from the opposite tails of the response distribution in experiment 1. The intraindividual fluctuations discussed above would probably obscure any discrete peaks present in the true responsiveness distribution.

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