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Congruence of individual responsiveness to dietary cholesterol and to saturated fat in humans

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Abstract Previous experiments have shown that differences between humans in the response of serum cholesterol to dietary cholesterol are at least partly reproducible and stable over a prolonged period. In this study it was investigated whether enhanced sensitivity to dietary cholesterol and saturated fat go together. The subjects had also participated in three or four experiments dealing with the reproducibility of the effect on blood cholesterol of either adding cholesterol to the diet in normal subjects (NORM-EGG group; n = 23) or of cessation of egg consumption in subjects with a high habitual egg intake (HAB-EGG group; n = 24). In the present experiment the NORM-EGG subjects were fed a mixed natural diet providing 21% of energy as polyunsaturated and 11% as saturated fat (P/S[2] ratio, 1.9) for 3 weeks, and one providing 5% of energy as polyunsaturated fat and 23% as saturated fat (P/S ratio, 0.2) for the next 3 weeks. The HAB-EGG group was fed the same diets in reverse order. The serum cholesterol concentrations were higher on the low P/S diet than on the high P/S diet (on average 23% in normal subjects and 16% in habitual egg eaters). The correlation coefficient between each subject's serum cholesterol response to fatty acids and his or her average response to dietary cholesterol in the dietary cholesterol experiments was 0.62 for the normal subjects ($P < 0.01$) and 0.15 for the HAB-EGG group. We conclude that modest differences in responsiveness of serum cholesterol to dietary saturated fat do exist in humans, and that, in people of normal cholesterol intake, responsiveness to dietary cholesterol and to saturated fat tend to go together. —**Katan, M. B., M. A. M. Berns, J. F. C. Glatz, J. T. Knuiman, A. Nobels, and J. H. M. de Vries.** Congruence of individual responsiveness to dietary cholesterol and to saturated fat in humans. *J. Lipid Res.* 1988. **29:** 883–892.

Supplementary key words saturated fatty acids • unsaturated fatty acids • HDL • essential fatty acids • controlled clinical trial

In several animal species, differences between individuals or inbred strains in the responsiveness of serum cholesterol to hypercholesterolemic diets have been well established (1–4), and they appear to have a genetic basis. Variability in the responsiveness of serum cholesterol to dietary cholesterol and to dietary fatty acid composition has also been found in humans (5–8). The differences in response to dietary cholesterol between humans are at least partly reproducible and stable over a prolonged period (9), and the same appears to hold for fatty acids (8).

What is unknown, however, is whether responders to dietary cholesterol and to dietary saturated fat are the same persons. The existence of a congruent response to dietary cholesterol and saturated fat would mean that there is a category in the population, probably large, with an increased sensitivity to diet in general, because cholesterol and saturated fat are the two major dietary influences on serum cholesterol. This category would then especially benefit from a diet low in saturated fat and cholesterol. The identification of subjects who are hyper- or hyporesponsive to both saturated fat and cholesterol might also help to elucidate the pathways through which saturated fatty acids elevate serum cholesterol. These pathways are still largely unknown, although progress in terms of LDL receptor regulation is being made (10). If, on the other hand, responses to these two dietary components are independent of each other, then the hyper/hyporesponder concept loses much of its relevance for the public health approach to hypercholesterolemia.

We now report on the results of a controlled trial that was set up to answer the question whether hyperresponders to dietary cholesterol are also hyperresponders to saturated fat in the diet.

Abbreviations: HDL, high density lipoproteins; VLDL, very low density lipoproteins; LDL, low density lipoproteins; P/S ratio, ratio of polyunsaturated fatty acids over saturated fatty acids.

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[2]P/S values are given here as a convenient descriptor only. We are well aware that there is no direct relation between the P/S value of a diet and its effect on lipoproteins.

METHODS

Experimental design

The present trial dealt with the effect of saturated fat in the diet on blood cholesterol in hyper- and hyporesponders to dietary cholesterol. For most subjects this was the fourth experiment in which they participated. We had earlier organized two parallel series of trials dealing with either the reproducibility of the effect of dietary cholesterol on blood cholesterol in volunteers of normal dietary habits (NORM-EGG group) or with the reproducibility of the effect of cessation of egg consumption on blood cholesterol in volunteers with a high habitual egg intake (HAB-EGG group). In the present experiment these two population samples were studied together for the first time.

Fig. 1 describes the sequence of experiments in NORM-EGG and HAB-EGG subjects, and the number of participants in each. In the first three experiments in the NORM-EGG group, a low-cholesterol baseline period was followed by a high-cholesterol period. Thus a subject's serum cholesterol was always measured on both diets, and the response was defined as the serum cholesterol at the end of the high-cholesterol period minus the level at the end of the baseline low-cholesterol period. In such a design, changes in subjects over the years, such as a slow rise of serum cholesterol with aging, are not reflected in the response. The diets were well controlled; details have been described (9, 11). After the present study (experiment 4), a fifth experiment was set up to study the relationship between response to dietary cholesterol and low density lipoprotein (LDL) kinetics. It consisted of three periods, high, low, and again high in dietary cholesterol, separated from each other by several months. Mean cholesterol intake was 1000 mg/day in the first and 140 mg/day in the second period. Two serum cholesterol values from the first period and two from the second period of this fifth trial were used to calculate one more estimate of responsiveness to dietary cholesterol; this estimate was then averaged with the responses from experiments 1–3 (see Table 4).

Experiments 1 and 2 in the HAB-EGG group entailed dietary prescriptions to omit cholesterol-rich foods, and contact with the subjects was limited. The response was defined as the change from pre-experimental values. Complete diets were provided to six subjects in HAB-EGG experiment 3. Details of these experiments have been described elsewhere (12–14).

Subjects

The subjects were healthy normolipemic volunteers. Their recruitment and screening have been described previously (9, 12). Baseline characteristics are given in

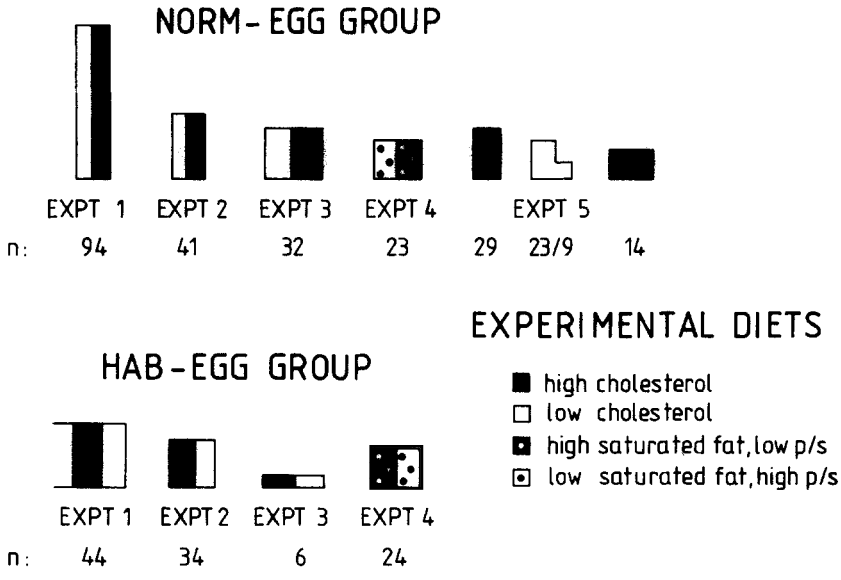

Fig. 1. Sequence of trials and number of participants in the two series of experiments. HAB-EGG subjects were recruited in 1976 (14) by calling for volunteers with a high habitual consumption of eggs. The NORM-EGG group consisted of volunteers of normal dietary habits first recruited in 1981 (9). Subjects were volunteers from the population of Wageningen and surroundings. The reduction in number of subjects from HAB-EGG experiment 2 to HAB-EGG experiment 3 and from NORM-EGG experiment 1 to NORM-EGG experiment 2 was deliberate (see Methods). Other causes for drop-out were: moving abroad, illness, pregnancy (one NORM-EGG subject), death (one HAB-EGG subject), and reluctance to continue. In addition, each experiment yielded some subjects whose data were not analyzed because of weight loss, minor illness, or poor compliance; they are not included in the number of participants.

Table 1. Most NORM-EGG subjects were students or University staff members. HAB-EGG subjects were older on average, lived further away from Wageningen, and were not connected with the University.

The large decrease in the number of subjects in the NORM-EGG group after the first experiment was intentional: 53 subjects with an intermediate response were dismissed. The attrition in numbers from NORM-EGG experiment 2 onwards was due to subjects moving, becoming ill or pregnant, or to refusal. For experiment 3 in the HAB-EGG group, which was a controlled dietary trial, only the 8 subjects that had shown the most extreme responses in the previous two experiments were invited to participate. One of them refused and another one withdrew during the experiment. Thus, for most subjects in the HAB-EGG group, our estimate of the responsiveness to cholesterol was based on two trials.

In order to enable as many subjects as possible to participate in the present fatty acid experiment, NORM-EGG subjects were offered a choice of three different runs. In run A, which ran from 24 October to 2 December 1983, 8 NORM-EGG persons participated; in run B, from 9 January to 17 February 1984, 12; and in run C, from 5 March to 13 April 1984, 7. Data of 4 NORM-EGG subjects were rejected prior to analysis because of illness, weight loss, less-than-perfect dietary adherence as judged by fatty acid composition of blood cholesteryl esters and erythrocytes, or combinations of these factors. In the HAB-EGG group, 15 subjects participated during run B, and another 12 during run C. Data of 3 subjects were eliminated because of illness, weight loss, or poor dietary adherence, as judged by the dieticians and confirmed by analysis of the fatty acid pattern of blood lipids.

The design of the study was thoroughly explained to the subjects, and informed consent was obtained. Prior approval was obtained from the Ethical Committee of the Department.

Diets

The response was defined as the change from pre-experimental values. The NORM-EGG subjects first received a mixed natural diet high in polyunsaturated and low in saturated fat (high P/S diet) for 3 weeks and then a diet low in polyunsaturated and high in saturated fat (low P/S) diet for another 3 weeks. HAB-EGG subjects received the same diets in reverse order. 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 (15, 16). In addition, low and high P/S bread rolls with exactly the same

TABLE 1. Baseline characteristics (mean ± SD) for all subjects who completed the study on hypo- and hyperresponsiveness to dietary saturated fat

Parameter[a]	NORM-EGG Group			HAB-EGG Group		
	Men (n = 14)	Women (n = 9)	All (n = 23)	Men (n = 10)	Women (n = 14)	All (n = 24)
Age at entry (yr)	38 ± 14	28 ± 9	34 ± 13	53 ± 14	54 ± 13	54 ± 13
Height (cm)	183 ± 6	169 ± 4	178 ± 9	177 ± 6	168 ± 6	172 ± 7
Weight (kg)	76 ± 7	64 ± 7	71 ± 9	84 ± 13	67 ± 13	74 ± 15
Body mass index (kg/m^2)	22.7 ± 1.9	22.3 ± 2.8	22.5 ± 2.2	25.9 ± 2.9	22.7 ± 2.9	24.4 ± 3.8
Serum cholesterol (mmol/l)[b]						
Total	4.97 ± 0.84	5.06 ± 0.63	5.01 ± 0.75	6.53 ± 1.13	6.13 ± 1.33	6.29 ± 1.24
HDL	1.29 ± 0.25	1.46 ± 0.24	1.36 ± 0.25	1.34 ± 0.26	1.59 ± 0.40	1.48 ± 0.37
Triglyceride (mmol/l)[c]	1.23 ± 0.65	0.95 ± 0.31	1.12 ± 0.55	1.77 ± 0.92	1.33 ± 0.80	1.51 ± 0.86
Habitual diet						
Energy (MJ/day)			9.7 ± 2.8			10.1 ± 2.5
Energy (kcal/day)			2331 ± 669			2414 ± 609
Protein (%)			15.1 ± 2.8			14.2 ± 2.6
Fat (%)			35.5 ± 6.3			44.6 ± 6.5
Polyunsaturated fatty acids (%)			6.3 ± 2.6			6.3 ± 2.1
Saturated fatty acids (%)			16.2 ± 3.3			18.9 ± 2.4
P/S ratio			0.41 ± 0.23			0.34 ± 0.13
Carbohydrates (%)			46.0 ± 7.1			38.5 ± 7.0
Cholesterol (mg/MJ)			28 ± 14			61 ± 22
Alcohol (%)			3.0 ± 4.2			2.5 ± 3.2

Subjects were volunteers from the general population of Wageningen and surroundings, The Netherlands, who had earlier participated in studies on dietary cholesterol (9, 12). NORM-EGG subjects were volunteers with normal dietary habits; HAB-EGG subjects had a high habitual consumption of eggs. Baseline data were obtained in October or December 1983.

[a] 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. Habitual dietary intake was estimated using the dietary history method, and nutrient intakes were calculated using the 1984 edition of the computerized extended Dutch food composition table (19). Macronutrients are presented as percent of energy.

[b] One mmol/liter = 39 mg/dl.

[c] One mmol/liter = 89 mg/liter.

nutrient composition as the corresponding diets were provided ad libitum during run C. Subjects were encouraged to take these whenever they felt hungry; this served to counteract weight loss. On week days, subjects from the NORM-EGG group and some from the HAB-EGG group came in at noon for their hot meal, which was served in a special dining room at the Department. Evening bread, breakfast, and other foods were distributed to them 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. For the other subjects from the HAB-EGG group, food packages were delivered by dieticians to the participants' homes two or three times a week. Only the P/S ratio varied between the two periods of the experiment; protein, carbohydrate, total fat, monounsaturated fatty acids, alcohol, and cholesterol were constant (**Table 2**).

For the high P/S diet, a day's menu providing 10 MJ (2,390 kcal) consisted of six slices of whole meal bread baked with sunflower oil (8 g of oil per 100 g of bread), 30 g of margarine rich in linoleic acid, 15 g of moderate-fat Gouda cheese, 30 g of sliced meat, 15 g of low-fat cheese spread, 350 g of low-fat milk, one piece of fruit, 20 g of selected cookies, 30 g of nuts, 8 g of egg, 8 g of egg yolk, 4 g of sunflower oil, 6 g of sugar, 200 g of potatoes, 200 g of vegetables, 60 g of a gravy rich in linoleic acid, 17 g of salad dressing, and 200 g of a dessert prepared with low-fat milk. Each subject was allowed to consume each day 1 MJ (240 kcal) worth of self-selected foodstuffs free from fat and cholesterol as specified in a list. Typical selections were alcoholic and soft drinks, fruit, and candy. Subjects recorded these free-choice items daily in a diary. In the low P/S diet, oils and fats rich in linoleic acid were replaced by butter, low-fat milk by full-cream milk, and 75 to 100 g of meat per day was added. Slight adjustments were made in other items. Egg and egg yolk consumption were reduced to equalize the cholesterol content of the two diets. Subjects were repeatedly urged not to change their free-choice selections and smoking and exercise habits over the course of the experiment; inspection of the diaries suggested that they had complied with these demands.

The nutrient composition of the diets was estimated by analysis of duplicate portions collected for one imaginary NORM-EGG and one HAB-EGG person with average intake on each diet (17, 18). As the fatty acid experiment ran in two different runs for group HAB-EGG and in three runs for group NORM-EGG, actually five duplicates of both diets were separately collected and analyzed. Differences between runs were slight and random. The contribution of free-choice items as recorded in the diary was calculated with the extended Dutch food composition table (19) and the results were combined with the analytical data.

Table 2 gives the mean nutrient intakes in the low and high P/S periods of the experiment. The diets were designed in terms of percent of energy or of mg of cholesterol per MJ, and were then specified in g per day for a range of energy intakes. Thus, a higher energy intake involved a higher intake of all nutrients, including cholesterol. The amount and composition of nutrients other than saturated and polyunsaturated fat were almost the same in the high and low P/S diets. Body weights were checked to the nearest 100 g twice a week and energy intake was adjusted when necessary to counter weight changes.

TABLE 2. Mean nutrient intakes in the low and high P/S periods of the fatty acid experiment

	Mean Nutrient Intakes			
	NORM-EGG Group		HAB-EGG Group	
Nutrient	High P/S Diet	Low P/S Diet	High P/S Diet	Low P/S Diet
Energy (± SD)				
MJ/day	12.5 ± 3.0	12.4 ± 2.7	10.8 ± 2.3	10.6 ± 2.1
kcal/day	2941 ± 717	2917 ± 646	2577 ± 550	2532 ± 502
Protein (%)	13	14	13	14
Fat (%)	45	44	45	45
Saturated fatty acids (%)	11	23	10	23
Monounsaturated fatty acids (%)	12	14	12	15
Polyunsaturated fatty acids (%)	21	5.3	21	5.1
P/S ratio	1.9	0.2	2.0	0.2
Carbohydrates (%)	39	39	40	39
Cholesterol (mg/MJ)	40	40	44	40
Alcohol (%)	2.2	2.0	1.9	2.0

Energy intakes were calculated from the amount of food provided plus one MJ (240 kcal) as free-choice items. Macronutrients are presented as percent of energy. Nutrient composition was determined by chemical analysis of duplicates of the diets provided; the contribution of free-choice items (mainly carbohydrates and alcohol) was calculated using a food composition table (19), and was added to the analytical data. The experiment took place in Wageningen, The Netherlands, in 1983 and 1984, in volunteers from the population in the Wageningen region.

Dietary adherence was monitored by estimating the fatty acid composition of plasma cholesteryl esters and erythrocyte membrane phospholipids (20, 21). The mean linoleic acid to oleic acid ratio in the plasma cholesteryl esters changed from 6.5 to 3.5 in the NORM-EGG group on going from the high P/S diet to the low P/S diet, and from 2.9 to 5.5 in the HAB-EGG group, which went from the low to the high P/S diet. In the erythrocyte membrane esters, the linoleic/oleic ratio changed from 1.02 to 0.83 in the NORM-EGG and from 0.69 to 0.92 in the HAB-EGG group.

Blood sampling and laboratory 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. Cholesterol in serum was measured enzymatically using the kit (Monotest) supplied by Boehringer-Mannheim GmbH (FRG). Serum calibrators were used as described (22). HDL cholesterol was measured by the manganese–heparin method (23) and triglycerides were determined as described by Soloni (24). The concentration of LDL cholesterol was estimated using the Friedewald equation (25). Sera of one subject were always analyzed within one batch. Within-batch reproducibility for internal control sera was less than 1.1% for total cholesterol, within 2.6% for HDL cholesterol, and within 3.6% for triglycerides. Bias for control sera provided by the Centers for Disease Control, Atlanta, GA, was within 1.1% for total cholesterol and within 3.3% for HDL cholesterol. Since posture influences serum levels (26), subjects stood and waited until their turn had come to sit down for venipuncture. Blood was sampled four times at the end of both dietary periods, with 3 or 4 days in between two successive measurements.

Statistical methods

Response to cholesterol was defined in earlier experiments (9, 12) as the mean of serum measurements made during the high-cholesterol period minus the mean of measurements during the low-cholesterol period. Similarly, response to saturated fat was now defined as the mean of measurements on the low P/S diet minus the mean of measurements on the high P/S diet. For this and all other data analyses, only the last three of the four measurements obtained in each dietary period were used (see Fig. 2).

Our null hypothesis was that a subject's (average) position in the distribution of responsiveness to dietary cholesterol, as previously determined, is not related to his position in the distribution of responses to saturated fatty acids. Under the null hypothesis, the Pearson correlation coefficient r between the response in the cholesterol feeding (NORM-EGG) or cholesterol removal (HAB-EGG) experiments and the response in the fatty acids experiment equals zero, and a person's response in the cholesterol feeding or removal experiments bears no relationship to his response in the fatty acid experiment.

The significance of correlation coefficients (see Table 4), was calculated using one-tailed tests, because responses in the cholesterol feeding or removal experiments and the fatty acids experiment should either be unrelated or positively related. A negative relationship is biologically implausible.

RESULTS

Energy intake and body weight

The actual energy intake during the trial (Table 2) was higher than estimated by the history method prior to the experiment (Table 1). In previous experiments of ours, survey methods also tended to underestimate actual energy requirements (9).

There were no significant differences in body weight between the high and low P/S periods. In the NORM-EGG group the average change was −0.28 ± 0.54 kg (mean ± SD). In the HAB-EGG group it was 0.43 ± 0.84 kg.

Effect of changing fatty acid composition on serum cholesterol

The effect of dietary fatty acid composition on lipids and lipoproteins is given in **Table 3** and **Fig. 2.** The low P/S diet caused an increase in total cholesterol of 23% (1.04 mmol/liter, 40 mg/dl) in the NORM-EGG group and of 16% (0.84 mmol/liter, 32 mg/dl) in the HAB-EGG group, both relative to levels on the high P/S diet. The higher total cholesterol concentration on the low P/S diet could largely be attributed to LDL cholesterol, which rose by 32% in the NORM-EGG and by 20% in the HAB-EGG group. On the low P/S high saturated-fat diet, HDL cholesterol rose on average by 8% (0.12 mmol/liter, 4.6 mg/dl) in the NORM-EGG group and by 6% (0.09 mmol/liter, 3.5 mg/dl) in the HAB-EGG group. Serum triglycerides increased on average by 21% in the NORM-EGG and by 10% in the HAB-EGG group when polyunsaturated fatty acids were replaced by saturated fatty acids.

The total cholesterol response showed a unimodal distribution with no evidence for subgroups of different responsiveness, even though the middle responders of group NORM-EGG had been removed after the first experiment (Fig. 1). The spread in responsiveness is somewhat overestimated by the SDs given in Table 3, because these SDs also contain a within-subject component. After subtraction of the within-subject component of variance from the total variance (9), a pure between-subject SD of

TABLE 3. Average levels (± SD) of serum lipids from subjects on a diet high in polyunsaturated and low in saturated fat (high P/S ratio) and on a diet low in polyunsaturated and high in saturated fat (low P/S ratio)

Serum Lipid	NORM-EGG Group (n = 23)			HAB-EGG Group (n = 24)		
	High P/S Diet	Low P/S Diet	Change	High P/S Diet	Low P/S Diet	Change
			mmol/l [a]			
Total cholesterol	4.45 ± 0.71	5.48 ± 0.90	1.04 ± 0.43*	5.32 ± 0.94	6.16 ± 1.21	0.84 ± 0.60*
HDL cholesterol	1.42 ± 0.26	1.54 ± 0.30	0.12 ± 0.10*	1.43 ± 0.40	1.52 ± 0.39	0.09 ± 0.10*
LDL cholesterol[b]	2.67 ± 0.70	3.53 ± 0.82	0.85 ± 0.37*	3.41 ± 0.90	4.10 ± 1.15	0.70 ± 0.48*
Triglycerides	0.77 ± 0.28	0.93 ± 0.39	0.15 ± 0.19*	1.09 ± 0.58	1.20 ± 0.51	0.11 ± 0.32

The participants were volunteers with normal dietary habits (NORM-EGG group) or with a high habitual cholesterol intake (HAB-EGG group). The study took place in Wageningen in 1983/1984. Group NORM-EGG consumed the high P/S diet first and was then switched to the low P/S diet. In group HAB-EGG, the order of the diets was reversed.

[a] Cholesterol: 1 mmol/liter = 39 mg/dl; triglycerides: 1 mmol/liter = 89 mg/liter.

[b] Calculated according to the Friedewald formula (25).

[c] *$P < 0.01$.

response was found of 0.37 mmol/l for the NORM-EGG and of 0.55 mmol/l for the HAB-EGG group. These SDs quantitate the true extent of heterogeneity in response to fat type between subjects.

Relation between response to dietary cholesterol and to fatty acids

The relationship between average response to dietary cholesterol and to fatty acids is depicted in **Fig. 3** (NORM-EGG group) and **Fig. 4** (HAB-EGG group). **Table 4** gives the correlation coefficients of the responses of serum cholesterol in the fatty acid experiment with the responses in each of the dietary cholesterol experiments separately, and with the average of these responses. There was a tendency to congruent responses to dietary cholesterol and saturated fat in the NORM-EGG group but not in the HAB-EGG group. The correlation coefficient of the response to fatty acids of the NORM-EGG subjects with the response to cholesterol as averaged over three or four

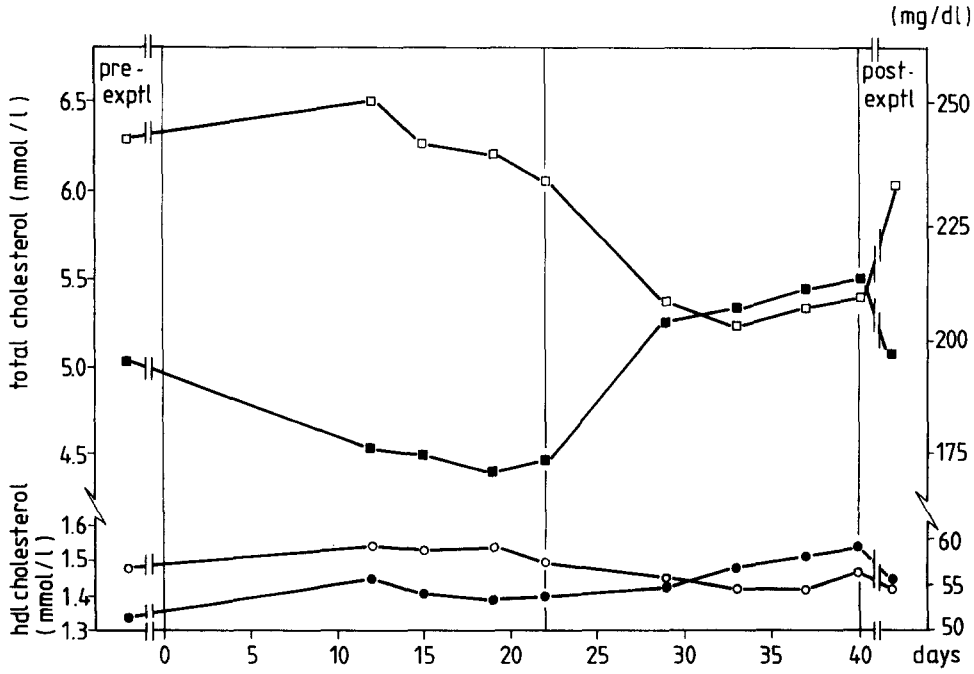

Fig. 2. Effect of polyunsaturated versus saturated fatty acids on serum total cholesterol (upper curves) and HDL cholesterol (lower curves) in volunteers with a high habitual intake (open symbols) or a normal habitual intake (closed symbols) of egg cholesterol. The volunteers with a high habitual egg intake (□, ○) first received a controlled diet high in saturated and low in polyunsaturated fatty acids, and then a diet low in saturates and high in polyunsaturates. The group with a normal egg consumption (■, ●) received the diets in reverse order. Pre-experimental values were obtained 4–12 weeks before the start, and post-experimental values 4–5 weeks after the completion of the study, while subjects consumed their habitual self-selected diets.

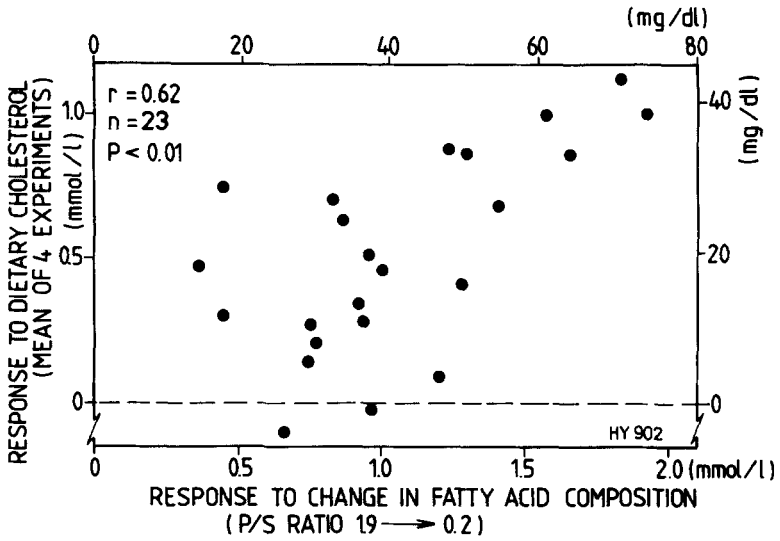

Fig. 3. Relationship between average response to a high cholesterol diet and to a diet rich in saturated fat in a group of volunteers with normal habitual egg intake (NORM-EGG group). The average response to dietary cholesterol was estimated on the basis of four different experiments for 17 participants and on the basis of three experiments for the other 6 participants. Response to saturated fat was estimated in the present experiment.

dietary cholesterol experiments was higher than that with the responses in each separate experiment. By taking the average of the responses in all dietary cholesterol experiments, the degrading influence of within-person variation on the correlation coefficient is minimized (9).

DISCUSSION

Impact of fatty acids relative to dietary cholesterol

Animal studies of the hyperresponder phenomenon have focused largely on dietary cholesterol (1–4). In man, however, serum total and LDL cholesterol concentrations are more sensitive to the number of double bonds in the dietary fatty acids than to the amount of cholesterol eaten, when dietary lipids are varied over the range allowed by natural mixed solid diets. The mean change in serum cholesterol in the present experiment was about twice that observed in preceding experiments in the same subjects when dietary cholesterol intake was manipulated over a wide range of intake. This confirms a large body of experimental evidence in man (27) where saturated fat intake was found to be the major influence on serum cholesterol.

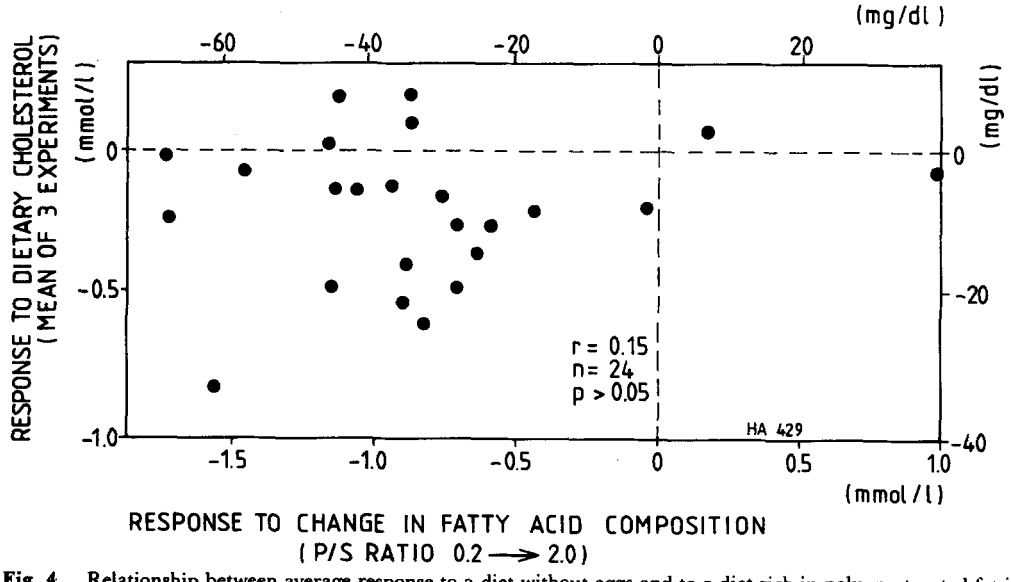

Fig. 4. Relationship between average response to a diet without eggs and to a diet rich in polyunsaturated fat in a group of habitual egg-eaters (HAB-EGG group). The average response to dietary cholesterol was based on three studies for 6 of the subjects, and on two experiments for the other 18 subjects. Response to polyunsaturated fat was estimated in the present experiment.

TABLE 4. Correlation coefficients of the response to a change in dietary P/S ratio, as estimated in the present experiment 4, with the response to a change in cholesterol intake as estimated in the same subjects in other experiments

Dietary Cholesterol Experiment	Correlation between Response to Cholesterol and to Saturated Fat			
	NORM-EGG Group		HAB-EGG Group	
	n	r	n	r
Exp. 1	23	0.33	24	0.18
Exp. 2	23	0.43*	24	0.18
Exp. 3	21	0.46*	6	−0.61
Exp. 5	17	0.39		
Average of 1, 2, and 3[a]	23	0.50**	24	0.15
Average of 1, 2, 3, and 5[a]	17	0.62**		

The number of subjects who participated in both the fatty experiment and in the particular dietary cholesterol experiment is indicated by n. Participants were volunteers from the general population of Wageningen and surroundings. Experiments 1–3 have been described previously (NORM-EGG group, ref. 9 and HAB-EGG group, ref. 12). Details on experiment 5 are given under Experimental Design in the Methods section.

[a]For the 2 subjects who had not participated in experiment 3, the average was taken over the available response values; based on three dietary cholesterol experiments for 6 subjects and on two experiments for the remaining 18 participants.

*, $P < 0.05$; **, $P < 0.01$.

The change in total cholesterol was largely due to LDL. Although HDL was also increased by saturated fat and lowered by polyunsaturated fat, these changes were much more modest. As a result, the low P/S diet caused a marked fall in the HDL/LDL cholesterol ratio. This agrees with earlier trials using mixed solid natural diets. On liquid formula diets with very high linoleic acid content, the fall in HDL tends to be more marked (28). The effect of dietary fat saturation on triglyceride and VLDL concentration has been noted before (29); it has been suggested that this is, in fact, the primary cause of the ensuing changes in LDL concentrations (30).

Hypo- and hyperresponders to fatty acids

Although almost every subject showed higher serum total and LDL cholesterol values on the low P/S diet than on the high P/S diet, a wide variation in responses was seen. Part of this apparent variation was probably due to biological fluctuations in serum cholesterol, which add a random term to the real dietary effect. The extent of the spontaneous within-subject variance was known in these subjects from previous experiments (9, 31). Correction for this term leaves a true between-subject SD of about 0.5 mmol/l. If we assume that the response to the dietary change in this study has a mean of about 1 mmol/l (Table 3) and a more or less normal distribution, then in subjects such as ours 66% will show a response between the mean minus and the mean plus one SD, i.e., between 0.5 and 1.5 mmol/l. The ± 2 SD interval of the response would range from 0 to 2 mmol/l and would include 95% of subjects; 2.5% of subjects would thus show no response at all, and another 2.5%, the true hyperresponders, would show an increment in serum cholesterol of 2 mmol/l or more when the dietary P/S ratio is reduced from 2.0 to 0.2.

The correlation in the NORM-EGG group between the response to fatty acids in the present experiment and to dietary cholesterol in preceding or following experiments suggests that in certain types of subjects there is such a thing as an innate reactivity of LDL levels to dietary intervention.

The mechanism involved is obscure. However, there is evidence that the effects of dietary cholesterol and of saturated fatty acids reinforce each other (32, 33), albeit that other, equally well-designed studies have failed to support this notion (34, 35). If such an interaction does exist, it would help to explain the concordance in our NORM-EGG subjects between the response to saturated fat in the present and to cholesterol in the previous studies. The impact on serum lipoproteins of the cholesterol consumed in the low P/S saturated fat diet period would be higher than that of the same amount of cholesterol (Table 2) consumed as part of the high P/S diet, and hyperresponders to dietary cholesterol might be more sensitive to this difference in impact than hyporesponders.

The low correlation in the HAB-EGG group contradicts the finding in the NORM-EGG group. We are unable to explain this discrepancy. One could speculate that it is due to a less reliable estimate of their responsiveness to dietary cholesterol, because most subjects in this group had only participated in two egg experiments of less-than-perfect design and dietary control. One could also surmise that the HAB-EGG and NORM-EGG groups might be metabolically different. HAB-EGG stands for habitual egg consumer, and it could be that the chronic high cholesterol intake in this group (12, 14) had changed their

metabolism. We have earlier found that a high habitual cholesterol intake tends to blunt the reactivity of serum cholesterol to changes in cholesterol intake in short-term controlled trials (11).

The fatty acid composition of the habitual diet was assessed earlier both by recall and by fat tissue biopsy analysis and was found to be unrelated to responsiveness (11). There was a weak and nonsignificant relation between linoleic acid content of the biopsies and response to dietary fatty acids in the NORM-EGG participants in the present experiment ($r = 0.36$, $n = 22$, $P = 0.10$). Thus the fatty acid composition of the HAB-EGG diet appears to have less influence on responsiveness in our subjects than the habitual cholesterol intake.

Public health implications

Not one of our subjects failed to show a rise in serum cholesterol when the number of double bonds in the dietary fat was reduced. This agrees with the conclusion of Jacobs, Anderson, and Hannan (8) that few if any persons are insensitive to a hypercholesterolemic diet, and it supports present day recommendations for a general reduction in saturated fat intake. Still, our results do imply that there are subjects in the normal population who not only hyperrespond to dietary cholesterol but also to saturated fat. The discrepancy between the two groups studied here shows that the extent of this concordance remains to be defined. Still, our findings emphasize the importance of hyperresponsiveness as a phenomenon, and may have implications for counseling subjects who attempt to lower their serum cholesterol by diet.

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REFERENCES

1. Clarkson, T. B., and M. R. McMahan. 1980. Individual differences in the response of serum cholesterol to change in diet: animal studies. *In* Childhood Prevention of Atherosclerosis and Hypertension. R. M. Lauer and R. B. Shekelle, editors. Raven Press, New York. 127–135.
2. Imai, Y., and H. Matsumara. 1973. Genetic studies on induced and spontaneous hypercholesterolemia in rats. *Atherosclerosis.* **18:** 59–64.
3. West, C. E., and D. C. K. Roberts. 1974. Cholesterol metabolism in two strains of rabbits differing in their cholesterolaemic response to dietary cholesterol. *Biochem. Soc. Trans.* **2:** 1275–1277.
4. Eggen, D. A. 1976. Cholesterol metabolism in groups of rhesus monkeys with high or low response of serum cholesterol to an atherogenic diet. *J. Lipid Res.* **17:** 663–673.
5. Quintão, E., S. M. Grundy, and E. H. Ahrens, Jr. 1971. Effects of dietary cholesterol on the regulation of total body cholesterol in man. *J. Lipid Res.* **12:** 233–247.
6. Nestel, R. J., and A. Poyser. 1976. Changes in cholesterol synthesis and excretion when cholesterol intake is increased. *Metabolism.* **25:** 1591–1599.
7. Mistry, P., N. E. Miller, M. Laker, W. R. Hazzard, and B. Lewis. 1981. Individual variation in the effects of dietary cholesterol on plasma lipoproteins and cellular cholesterol homeostasis in man. *J. Clin. Invest.* **67:** 493–502.
8. Jacobs, D. R., J. T. Anderson, P. Hannan, A. Keys, and H. Blackburn. 1983. Variability in individual serum cholesterol response to change in diet. *Arteriosclerosis.* **3:** 349–356.
9. Katan, M. B., A. C. Beynen, J. H. M. De Vries, and A. Nobels. 1986. Existence of consistent hypo- and hyperresponders to dietary cholesterol in man. *Am. J. Epidemiol.* **123:** 221–234.
10. Spady, D. K., and J. M. Dietschy. 1985. Dietary saturated triacylglycerols suppress hepatic low density lipoprotein receptor activity in the hamster. *Proc. Natl. Acad. Sci. USA.* **82:** 4526–4530.
11. Katan, M. B., and A. C. Beynen. 1987. Characteristics of human hypo- and hyperresponders to dietary cholesterol. *Am. J. Epidemiol.* **125:** 387–399.
12. Beynen, A. C., and M. B. Katan. 1985. Reproducibility of the variations between humans in the response of serum cholesterol to cessation of egg consumption. *Atherosclerosis.* **57:** 19–31.
13. Glatz, J. F. C., M. A. M. Berns, and M. B. Katan. 1985. Whole-body cholesterol synthesis and fecal neutral steroid and bile acid excretion in man on diets high or low in linoleic acid. *In* Cholesterol Metabolism in Health and Disease: Studies in the Netherlands. A. C. Beynen, M. J. H. Geelen, M. B. Katan, and J. A. Schouten, editors. Ponsen & Looijen, Wageningen, The Netherlands. 120–125.
14. Bronsgeest-Schoute, D. C., R. J. J. Hermus, G. M. Dallinga-Thie, and J. G. Hautvast. 1979. Dependence of the effects of dietary cholesterol and experimental conditions on serum lipids in man. III. The effect on serum cholesterol of removal of eggs from the diet of free-living, habitually egg-eating people. *Am. J. Clin. Nutr.* **32:** 2193–2197.
15. Van Raaij, J. M. A., M. B. Katan, C. E. West, and J. G. Hautvast. 1982. Influences of diets containing casein, soy isolate, and soy concentrate on serum cholesterol and lipoproteins in middle-aged volunteers. *Am. J. Clin. Nutr.* **35:** 925–934.
16. Brussaard, J. H., M. B. Katan, P. H. E. Groot, L. M. Havekes, and J. G. Hautvast. 1982. Serum lipoproteins of healthy persons fed a low-fat diet or a polyunsaturated fat diet for three months. A comparison of two cholesterol-lowering diets. *Atherosclerosis.* **42:** 205–219.
17. Stasse-Wolthuis, M., J. G. Hautvast, R. J. Hermus, M. B. Katan, J. E. Bausch, J. H. Rietberg-Brussaard, J. P. Velema, J. H. Zondervan, M. A. Eastwood, and W. G. Brydon. 1979. The effect of a natural high-fiber diet on serum lipids, fecal lipids, and colonic function. *Am. J. Clin. Nutr.* **32:** 1881–1888.
18. Knuiman, J. T., L. Räsänen, M. Ahola, C. E. West, and L. van der Snoek. 1987. The relative validity of reports of food intake of Dutch and Finnish boys aged 8 and 9 years and the effect of the duplicate portion technique on usual food intake. *J. Am. Diet Assoc.* **87:** 303–307.
19. Hautvast, J. G. 1975. Ontwikkeling van een systeem om gegevens van voedingsenquêtes met behulp van de computer te verwerken. *Voeding.* **36:** 356–361.
20. Angelico, F., M. Arca, A. Calvieri, A. Cantafori, P. Guc-

cione, P. Monini, A. Montali, and G. Ricci. 1983. Plasma and erythrocyte fatty acids: a methodology for evaluation of hypercholesterolemic dietary interventions. *Prev. Med.* **12:** 124–127.
21. Koot-Gronsveld, E. A. M., F. Schouten, J. T. Knuiman, C. E. West, and M. B. Katan. 1981. Errors in determination of fatty acid composition of cholesteryl esters after extraction from serum with n-octane. *Clin. Chem.* **27:** 1148–1149 (letter).
22. Katan, M. B., F. Van der Haar, D. Kromhout, and F. J. Schouten. 1982. Standardization of serum cholesterol assays by use of serum calibrators and direct addition of Liebermann-Burchard reagent. *Clin. Chem.* **28:** 683–686.
23. Van der Haar, F., C. M. Van Gent, F. J. Schouten, and H. A. van der Voort. 1978. Methods for the estimation of high density cholesterol, comparison between two laboratories. *Clin. Chim. Acta.* **88:** 469–481.
24. Soloni, F. G. 1971. Simplified manual micromethod for the determination of serum triglycerides. *Clin. Chem.* **17:** 529–534.
25. Friedewald, W., R. I. Levy, and D. W. Fredrickson. 1972. Estimation of the concentration of low density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin. Chem.* **18:** 499–502.
26. Dixon, M., and C. R. Paterson. 1978. Posture and the composition of plasma. *Clin. Chem.* **24:** 824–826.
27. Keys, A., J. T. Anderson, and F. Grande. 1965. Serum cholesterol response to changes in the diet. *Metabolism.* **14:** 747–765.
28. Katan, M. B. 1984. Diet and HDL. *In* Metabolic Aspects of Cardiovascular Diseases. Vol. 3. Clinical and Metabolic Aspects of High Density Lipoproteins. N. E. Miller and G. J. Miller, editors. Elsevier, Amsterdam, New York, and Oxford. 103–132.
29. Antonis, A., and I. Bersohn. 1961. The influence of diet on serum-triglyceride in South African white and Bantu prisoners. *Lancet.* **1:** 3–9.
30. Beynen, A. C., and M. B. Katan. 1985. Why do polyunsaturated fatty acids lower serum cholesterol? *Am. J. Clin. Nutr.* **42:** 560–563.
31. Rotterdam, E. P., M. B. Katan, and J. T. Knuiman. 1987. Importance of time interval between repeated measurements of total or high-density lipoprotein cholesterol when estimating an individual's baseline concentration. *Clin. Chem.* **33:** 1913–1915.
32. National Diet-Heart Study Research Group. 1986. The National Diet-Heart Study Final Report. *Circulation.* **37 (Suppl.1):** 260–273.
33. Schonfeld, G., W. Patsch, L. L. Rudel, C. Nelson, M. Epstein, and E. Olson. 1982. Effects of dietary cholesterol and fatty acids on lipoproteins. *J. Clin. Invest.* **69:** 1072–1080.
34. Hegsted, D. M., R. M. McGandy, M. L. Meyers, and F. J. Stare. 1965. Quantitative effects of dietary fat on serum cholesterol in man. *Am. J. Clin. Nutr.* **17:** 281–295.
35. Anderson, J. T., F. Grande, and A. Keys. 1976. Independence of the effects of cholesterol and degree of saturation of the fat in the diet on serum cholesterol in man. *Am. J. Clin. Nutr.* **29:** 1184–1189.