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Reproducibility of the Variations Between Humans in the Response of Serum Cholesterol to Cessation of Egg Consumption

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Summary

To find out whether the variable response of serum cholesterol levels to changes in cholesterol intake in man is due to constitutional differences in responsiveness, we have reinvestigated in 1982 34 healthy men and women, who habitually consumed at least 1 egg/day and had participated in a trial in 1976. Serum cholesterol was measured on the habitual diet (about 800 mg cholesterol/day), and after 3 weeks during which no eggs or egg-containing products were consumed (about 300 mg cholesterol/day). Serum cholesterol decreased by 0.16 ± 0.42 mmol/l (6 ± 16 mg/dl) in 1976 and by 0.31 ± 0.35 mmol/l (12 ± 14 mg/dl) in 1982 (mean $\pm$ SD). Individual responses varied from -1.0 to $+0.5$ mmol/l (-39 to $+19$ mg/dl). The correlation between the responses in 1976 and 1982 was $r = 0.32$ ($P < 0.05$). The decrease in serum cholesterol was most pronounced for subjects with a low body mass index and a high level of HDL-cholesterol. In men, the increase in serum cholesterol with age was correlated with the mean decrease in the trials ($r = 0.42$, $n = 16$, $P = 0.11$). In a controlled trial, 4 hypo- and 2 hyperresponders were given 11 mg cholesterol/MJ (11 mg/240 kcal; 116 mg/day) for 4 weeks followed by 72 mg/MJ for another 4 weeks; all other nutrients were kept constant. Almost all food

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was supplied and intakes were rigidly controlled. The 2 hyperresponders and 3 of the 4 hyporesponders were also hyper- and hyporesponsive under the controlled conditions. We conclude that part of the cholesterolemic response to dietary cholesterol in man is individually determined and stable for at least 6 years.

Key words: *Aging – Dietary cholesterol – Eggs – HDL-cholesterol – Hyperresponders – Hyporesponders – Obesity – Serum cholesterol*

Introduction

In animals the cholesterolemic response to dietary cholesterol can vary greatly among individuals of the same species. Certain individuals display only small changes in the level of serum cholesterol (nonresponders or hyporesponders), whereas others show pronounced increases (hyperresponders). This phenomenon has been well-established in monkeys [1,2], rabbits [3,4], rats [5,6] and pigeons [7,8], and it appears to be genetically determined [1,3,5].

Laboratory experiments suggest that human hyper- and hyporesponders to dietary cholesterol also exist [9–12], but the individual variation in the response of serum cholesterol is much less pronounced than that in experimental animals. The phenomenon of hyper- and nonresponsiveness to dietary cholesterol could be of major significance since genetic disorders such as familial hypercholesterolemia and combined hyperlipidemia account for only a small percentage of the prevalence of hypercholesterolemia in affluent countries.

It is not known whether the serum cholesterol response to dietary cholesterol in man is stable over a prolonged period. From the practical point of view it is also important to know whether free-living subjects show consistent individually-determined differences in cholesterolemic response to a low-cholesterol diet. In an attempt to answer these questions, we have reinvestigated 34 subjects who habitually consumed at least one egg/day and had participated in a trial in 1976 [13]. In the trial in 1976 a large variation between subjects was found in the cholesterolemic response to cessation of egg consumption. We have now found that 6–7 years later these differences were still partly reproducible. Part of the present work has appeared in abstract form [12,14].

Subjects and Methods

Experiments in 1976 and 1982

Subjects

In 1976 volunteers who habitually ate at least 1 egg/day were recruted for a study on 'The nutritive value of eggs'. Twenty-five men and 19 women participated in the experiment. By 1982, 3 of them had moved outside the Wageningen region, one had

died, and 4 subjects could not be traced. The remaining 36 were willing to participate in another experiment of the same design, but 2 of them withdrew during the experiment. Age, height and weight of the remaining 34 subjects (17 men and 17 women) in 1982 were (means ± SD, range): men, 49 ± 17, 23–78 years; women 54 ± 14, 27–77 years; men (n = 16), 177 ± 6, 165–186 cm; women, 167 ± 6, 157–177 cm; men (n = 16), 78.6 ± 12.8, 62.0–106.6 kg; women, 69.7 ± 12.1, 54.6–103.3 kg. From 1976 to 1982 body weight on the habitual diet had increased by 3.6 ± 5.1 kg in the men and by 2.4 ± 3.6 kg in the women. The experimental protocol was fully explained to the participants and informed consent was obtained. The study was approved by the Ethical Committee of the Department of Human Nutrition.

Design and diets

The experimental design is illustrated in Fig. 1. Originally all subjects habitually consumed at least one egg/day. Three subjects had stopped eating eggs after learning their response to cessation of egg consumption in the trial of 1976; the others still had an egg-rich diet. On our request, all subjects ate at least one egg/day during the 3 weeks before the egg-free period; they recorded the number of eggs consumed each day on a check list. In 1982 usual food intakes were estimated by the dietary history method. In 1976 the 24-h recall method was used as described [13]. Food intake data were converted into nutrients using the computerized Dutch food table [15].

In 1976 the subjects were asked to abstain from eggs, omelets, and other egg-containing products for 3 weeks. In 1982 the subjects were asked also not to eat other cholesterol-rich products such as shell-fish, liver and kidney. They were instructed in this by our dietitian, who also telephoned the subjects half-way through the low-cholesterol period to discuss possible problems. The subjects kept a diary to note illness, smoking habits, drug use and possible deviations from the diet. Nothing extraordinary was noted.

Non-fasting blood samples were taken twice within 3 days just prior to and again at the end of the low-cholesterol period.

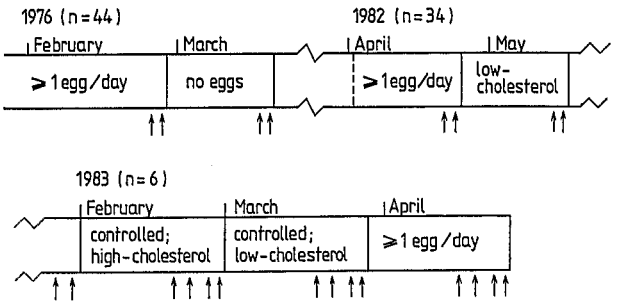

Fig. 1. Design of the repeated studies. Arrows indicate the days on which blood samples were taken. Ten of the 44 subjects who participated in 1976 were no longer available in 1982. Of the 34 subjects 3 had stopped eating eggs after the 1976 trial, but on our request they consumed at least 1 egg/day again for the 3 weeks preceding the 1982 trial.

TABLE I

COMPOSITION OF THE DIETS [a]

Values are mean ± SD. In the experiments in 1976 and 1982 the low-cholesterol diet was achieved by subjects omitting eggs and other cholesterol-rich products from their habitual free-living diets. In the experiment in 1983 all food was provided by the laboratory.

Dietary component	Experiment 1976 (n = 34)		Experiment 1982 (n = 34)		Experiment 1983 (n = 6)	
	Cholesterol intake		Cholesterol intake		Cholesterol intake	
	High	Low	High	Low	High	Low
Energy (kcal/day)	2398 ±650	2548 ±675	2512 ±579	2235 ±503	2664 ±508	2703 ±567
(MJ/day)	10.0 ± 2.7	10.7 ± 2.8	10.5 ± 2.4	9.4 ± 2.1	11.1 ± 2.1	11.3 ± 2.4
Total fat (% of energy)	43 ± 8	43 ± 7	43 ± 7	41 ± 1	42 ± 1	42 ± 1
Saturated fatty acids	16 ± 4	17 ± 4	17 ± 4	17 ± 4	18 ± 1	18 ± 1
Monosaturated fatty acids	16 ± 3	16 ± 3	16 ± 3	15 ± 3	11 ± 1	13 ± 1
Polyunsaturated fatty acids	7 ± 3	7 ± 3	6 ± 2	6 ± 2	8 ± 0	8 ± 0
P:S ratio	0.43± 0.25	0.44± 0.28	0.39± 0.18	0.40± 0.21	0.47± 0.02	0.45± 0.02
Total carbohydrate (% of energy)	42 ± 10	42 ± 9	39 ± 8	41 ± 8	45 ± 3	45 ± 3
Sugars	21 ± 8	20 ± 7	19 ± 7	20 ± 6	20 ± 1	19 ± 2
Starch	20 ± 7	21 ± 6	20 ± 4	21 ± 4	26 ± 3	27 ± 3
Total protein (% of energy)	14 ± 3	13 ± 3	16 ± 4	15 ± 3	11 ± 1	10 ± 1
Animal	10 ± 3	9 ± 3	12 ± 4	11 ± 4	6 ± 1	5 ± 0
Vegetable	4 ± 1	5 ± 1	4 ± 1	4 ± 1	5 ± 1	5 ± 1
Alcohol [b] (% of energy)	3	3	4	5	3	3
Dietary fiber (g/day)	26 ± 12	27 ± 10	26 ± 8	26 ± 10	31 ± 7	33 ± 9
Cholesterol (mg/day)	840 ±437	303 ±147	812 ±269	246 ± 95	640 ±114	114 ± 20

[a] The composition of the diets was estimated by 24-h recall in 1976 and by dietary history in 1982. Three subjects who had stopped eating eggs after the trial in 1976 again included eggs in their habitual diets for 3 weeks in 1982 on our request. Apart from the cholesterol content the composition of the habitual and experimental diets in 1976 and 1982 did not differ significantly. The composition of the diets in the controlled trial in 1983 was calculated from the composition of the foods supplied, plus some cholesterol-free items selected by the subjects and noted in their diaries. The latter amounted to 1 MJ (240 kcal) per day.

[b] SD not given because the distribution was skewed.

Study with controlled diets in 1983

Subjects

In 1983 4 subjects with consistently high and 4 with consistently low serum cholesterol responses to cessation of egg consumption in the previous trials were asked to participate in a controlled dietary trial. The characteristics of these subjects are shown in Table 3. One subject (No. 6) refused and another subject (No. 5) withdrew during the experiment.

Design and diets

The 6 subjects received controlled high- and low-cholesterol diets for periods of 4 weeks each. After that they returned to their habitual diet, which on our request included at least 1 egg/day for another 4 weeks. Non-fasting blood samples were taken 4 times during each period (Fig. 1).

The controlled diets (Table 1) consisted of regular foodstuffs and were identical except for the amount of cholesterol. Dietary cholesterol was provided by whole eggs and egg-yolk. Protein and fat intakes were balanced by adding egg-white, extra milk, meat and margarine and some olive oil to the low-cholesterol diet. Total diets were provided except for 1 MJ (240 kcal)/day, in which the subjects were free to choose from a list of foodstuffs not containing cholesterol. All foodstuffs were weighed out for each person in quantities appropriate to his or her energy needs. A typical package consisted of potatoes, other vegetables, salads, meat, sauce, pudding, brown bread high in saturated fat, margarine (P/S ratio = 0.19), meats, cheese, yoghurt and/or milk, marmalade, sugar and/or honey, apple juice or grapefruit juice, fruit and biscuits. Egg yolk was provided as whole eggs, and added to salads and puddings. Foodstuffs were delivered by AC Beynen at the subjects' home 3 times a week. The subjects noted any illness, drug use and departures from the diets in diaries. Nothing out of the ordinary was noted.

During this study duplicate portions of the high- and low-cholesterol diets were collected. In addition, feces were collected for the determination of steroids during the last 5 days of the high- and low-cholesterol periods. Diets and feces are being analysed and the results will be published later.

Cholesterol analyses

Total serum cholesterol was measured with the Liebermann-Burchard reagent under rigidly standardized conditions [16]. In 1982 HDL cholesterol was measured by the Mn–heparin precipitation method [17]. Serum samples collected during the experiment from an individual subject were analysed within 1 batch.

Statistics

Pearson correlation coefficients and their significance according to a 2-tailed test were calculated [18], and Student's 2-tailed *t*-test was used.

Results

Experiments in 1976 and 1982

Table 1 shows the composition of the high- and low-cholesterol diets in the 3 experiments. The mean daily egg consumption in the high-cholesterol period was 2.0 in 1976 and 1.7 in 1982. Removal of eggs and other cholesterol-rich products from the diet lowered cholesterol intake by 537 mg/day in 1976 and by 566 mg/day in 1982. Changes in other nutrients were slight and not significant.

The decrease in cholesterol intake during the experimental period was associated with a small, but statistically significant decrease in the level of serum cholesterol in 1976 (Table 2). From 1976 or 1982 the mean concentration of total cholesterol in the serum on the usual diet had increased slightly in both men and women. In 1982 the decrease in serum total cholesterol upon removal of cholesterol-rich products from the diet was almost twice that in 1976 (Table 2). Cessation of egg consumption also caused a lowering of the HDL cholesterol level (Table 2). This effect however, reached statistical significance only in the female subjects.

The mean body weight of the 34 subjects decreased by 0.3 ± 0.9 kg ($\pm$ SD) during the experiment in 1976 and by 0.5 ± 0.9 kg in 1982. No one lost more than 2 kg

TABLE 2

SERUM TOTAL CHOLESTEROL AND HDL CHOLESTEROL CONCENTRATIONS THROUGHOUT THE EXPERIMENTS IN 1976 AND 1982

Results are expressed as means $\pm$ SD. The values of each individual were means of determinations in blood drawn on 2 occasions within 3 days on each diet. Conversion factor: 1 mmol cholesterol/l = 38.7 mg/dl.

	Habitual, egg-rich diet	Egg-free, low-cholesterol diet	Change
Serum total cholesterol (mmol/l)			
1976			
All subjects (n = 34)	5.88 ± 1.07	5.72 ± 1.05	−0.16 ± 0.42 *
Men (n = 17)	5.82 ± 1.08	5.63 ± 1.10	−0.19 ± 0.40
Women (n = 17)	5.94 ± 1.09	5.81 ± 1.03	−0.14 ± 0.44
1982			
All subjects (n = 34)	6.17 ± 1.25	5.86 ± 1.13	−0.31 ± 0.35 *
Men (n = 17)	6.15 ± 1.37	5.81 ± 1.18	−0.35 ± 0.32 *
Women (n = 17)	6.20 ± 1.16	5.92 ± 1.11	−0.28 ± 0.39 *
HDL cholesterol (mmol/l)			
1982			
All subjects (n = 34)	1.38 ± 0.32	1.34 ± 0.30 [a]	−0.05 ± 0.12 *,[a]
Men (n = 17)	1.31 ± 0.30	1.29 ± 0.28	−0.02 ± 0.14
Women (n = 17)	1.46 ± 0.33	1.39 ± 0.33 [b]	−0.09 ± 0.08 *,[b]

* Significantly different from 0 at $P < 0.05$.

[a] n = 33, [b] n = 16.

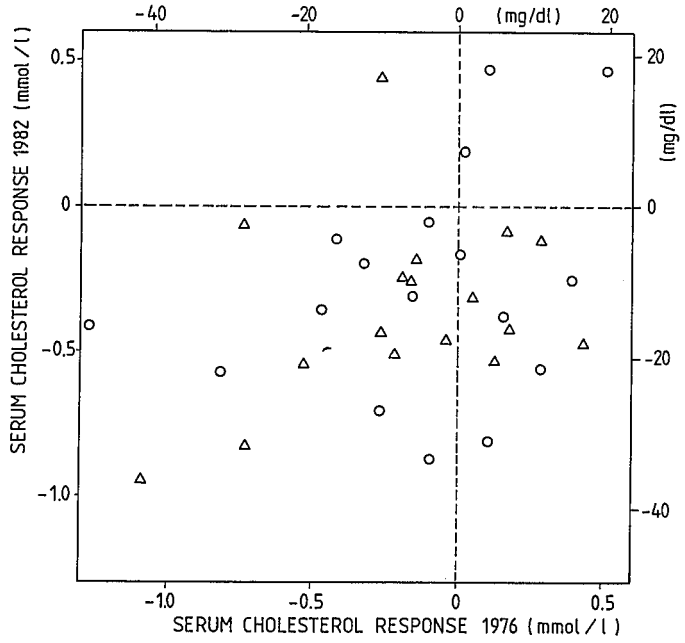

Fig. 2. Relationship between the cholesterolemic responses to cessation of egg consumption in the trials in 1976 and 1982. ○, women; △, men. The correlation was $r = 0.32$ ($P < 0.05$).

except for one women in 1976 who lost 3.7 kg, and one man in 1982 who lost 2.2 kg. Exclusion of these subjects from the analyses did not influence the conclusions.

We have calculated Pearson correlation coefficients between the decrease in serum cholesterol upon cessation of egg consumption and various parameters. Age was not related to the response. The body mass index (weight/height^2) was negatively associated with the decrease in serum cholesterol in 1976 ($r = -0.43$, $n = 34$; $P < 0.05$), but this was not seen in 1982 ($r = +0.06$, $n = 33$). The initial HDL cholesterol level was positively associated with the decrease in serum total cholesterol in 1982 ($r = +0.43$, $n = 33$; $P < 0.05$), the strongest correlation being found in the males ($r = +0.65$, $n = 17$; $P < 0.05$). Thus the fall in serum cholesterol when egg eating was stopped was higher for those subjects who were leaner and had higher HDL cholesterol levels.

The cholesterolemic response to cessation of egg consumption varied considerably between individuals. In 1976 it ranged from -1.27 to $+0.51$ mmol/l, and in 1982 from -0.94 to $+0.48$ mmol/l. However, the more extreme responses were probably due to chance, and were caused by the large spontaneous fluctuations of the serum cholesterol concentration within one person.

Figure 2 illustrates the correlation between the individual responses in 1976 ad 1982. It is clear that the response per subject was only partly reproducible from one study to another. Nevertheless, there was a significant, positive correlation between the responses in 1976 and 1982 ($r = 0.32$, $P < 0.05$). A one-tailed test for significance was used, as a negative correlation is inherently improbable.

TABLE 3

CHARACTERISTICS OF SUBJECTS WITH RESPONSES IN THE EXTREME QUARTILES IN BOTH 1976 AND 1982, AND THEIR CHOLESTEROLEMIC RESPONSES TO CONTROLLED DIETS IN 1983.

Serum cholesterol values are means of 2 or 4 samples taken on different occasions. Positive cholesterolemic responses indicate a rise in serum cholesterol when cholesterol intake is reduced. Conversion factor: 1 mmol cholesterol/l = 38.7 mg/dl.

Characteristics	Hyporesponders					Hyperresponders				
	1	2	3	4	Mean	5	6	7 [a]	8	Mean
Cholesterolemic response, 1976 (mmol/l)	+0.51	+0.11	+0.29	+0.17	+0.27	−1.09	−0.73	−0.81	−0.54	−0.79
Cholesterolemic response, 1982 (mmol/l)	+0.48	+0.47	−0.12	−0.08	+0.19	−0.94	−0.83	−0.59	−0.53	−0.72
Age, 1982 (years)	67	55	51	50	56	23	69	57	35	46
Sex	F	F	M	M		M	M	F	M	
Weight/height^2, mean of 1976 and 1982 (kg/m^2)	27.0	27.7	29.3	24.7	27.2	21.8	23.2	23.6	20.3	22.2
Initial serum cholesterol, 1976 (mmol/l)	6.66	5.42	5.90	5.16	5.79	5.51	8.82	6.12	5.47	6.48
Initial serum cholesterol, 1982 (mmol/l)	6.16	6.28	6.18	5.21	5.96	7.16	9.41	6.15	6.09	7.20
Initial serum cholesterol, 1983 (mmol/l)	5.67	6.46	6.20	5.50	5.96	6.87		5.21	5.79	5.96
Final serum cholesterol, 1983 (mmol/l)	5.52	6.22	5.89	5.91	5.89			5.91	5.78	5.85
Initial HDL cholesterol, 1982 (mmol/l)	1.08	1.11	1.04	1.42	1.16	1.62	1.45	2.15	1.61	1.71
Initial HDL cholesterol, 1983 (mmol/l)	1.29	1.08	1.16	1.36	1.22	1.58		1.90	1.49	1.66
Serum total cholesterol (mmol/l) on controlled diets in 1983										
High-cholesterol diet	6.44	6.34	5.40	5.39	5.89			5.85	6.03	5.94
Low-cholesterol diet	5.68	6.31	5.82	5.31	5.78			5.39	5.47	5.43
Response	−0.76	−0.03	+0.42	−0.08	−0.11			−0.46	−0.56	−0.51

[a] This subject had stopped eating eggs after the trial in 1976.

Follow-up study with controlled diets

Two subjects who were consistently hyperresponsive and 4 subjects who were consistently hyporesponsive to cessation of egg consumption (Table 3) participated in the trial with controlled diets in 1983.

During the high-cholesterol period the mean body weight of the 6 subjects changed by 0.0 ± 1.1 kg; during the low-cholesterol period there was an increase of 0.3 ± 0.8 kg. Body weight change during the entire controlled experiment ranged from -2.2 (subject No. 4) to 1.9 kg (subject No. 2).

In the 4 hyporesponders the decrease in cholesterol intake caused a mean reduction in serum cholesterol of 0.1 mmol/l (Table 3). The hyperresponders (Nos. 7 and 8) exhibited a mean decrease of about 0.5 mmol/l.

Discussion

There is a controversy about the effect of dietary cholesterol on the concentration of serum cholesterol in man [19,20]. The conflicting results are partly due to the poor design of various studies [21], but individual differences in the sensitivity of serum cholesterol to dietary cholesterol have also been invoked. The aim of the present work was to find out whether there are stable differences between individuals over a period of 6–7 years in the response of serum cholesterol to a reduction in cholesterol intake.

On a group mean level, the decrease in the intake of cholesterol from 800 to 300 mg should theoretically result in a decrease in serum cholesterol of 0.27 mmol/l [22]. This value agrees with the decrease of 0.31 mmol/l observed in 1982, but less so with the decrease of 0.16 mmol/l in 1976 (Table 2). We should note that in 1982 the dietary restrictions were somewhat more stringent, and that our contacts with the subjects were more intensive, which may have resulted in better adherence. Our findings add to the body of evidence that reduction of cholesterol intake to 250–300 mg/day causes a real but small decrease in the mean concentration of serum cholesterol of a population.

Although the mean effect was small, Fig. 2 and Table 3 show that there are individuals whose serum cholesterol was quite sensitive to changes in cholesterol intake. The response per subject was only partly reproducible (Fig. 2). This may have been due to the diets of the subjects not having been fully controlled. Either the habitual cholesterol intake or the compliance with the low-cholesterol diet or both may have varied within individuals between the experiments in 1976 and in 1982. Furthermore, only 2 blood samples per period were taken. In spite of these obscuring factors, Fig. 2 does suggest that the cholesterolemic response to a reduction in cholesterol intake in free-living subjects is individually determined. This corroborates our experiments with controlled diets [12]. The long-term stability of the trait suggests that at least part of the inter-individual variation in the cholesterolemic response to dietary cholesterol is explained by fundamental differences in cholesterol metabolism.

It could be argued that we observed a consistent response in serum cholesterol,

because some of our subjects consistently replaced their eggs by low-fat, low-cholesterol foods, while others replaced them by cheese or fat-rich meat. Dietary interviews cannot rigorously exclude this possibility [23,24]. However, the results of the experiment with controlled diets speak against this. The 2 subjects who were hyperresponsive in both 1976 and 1982 showed a marked decrease in serum cholesterol in 1983 (Table 3), when cholesterol was the only dietary variable. With exactly the same diets there was no decrease in serum cholesterol in 3 of the 4 hyporesponders. One putative hyporesponsive subject (No. 1) did not show the predicted lack of serum cholesterol response. We assume that this is due to misclassification of this subject in the trials of 1976 and 1982.

In the experiment of 1976 we found a negative association ($r = -0.43$, $n = 34$) between body mass index and the decrease in serum cholesterol. Thus hyperresponders were leaner than hyporesponders. This is illustrated by the lower body mass index of the hyperresponders in Table 3. Evidence has been presented that the cholesterolemic response to dietary cholesterol is related to the rate of whole-body cholesterol synthesis; individuals capable of depressing cholesterol synthesis most markedly, showed the smallest increase in serum cholesterol on a cholesterol-rich diet [10]. As cholesterol turnover is increased in obese subjects [25,26], it could be that in such subjects there is a wider range over which cholesterol synthesis can be down-regulated in response to an increased cholesterol intake, and that thus changes in cholesterol intake are compensated by changes in cholesterol synthesis.

The HDL cholesterol concentration was positively associated with the decrease in serum cholesterol after cessation of egg consumption ($r = +0.43$, $n = 33$). Thus HDL cholesterol levels tend to be higher in hyperresponders (see also Table 3). In our controlled studies [27] we have also found this relationship. It is possible that this relation is secondary to the relationship between body mass index and serum cholesterol response. High body mass indexes are associated with low HDL cholesterol concentrations [28].

One would expect that hyperresponders would show higher serum total cholesterol levels on their habitual diets than hyporesponders. We did find a correlation of $r = +0.25$ in 1976 and of $+0.48$ in 1982 ($n = 34$) between the serum cholesterol level on the habitual diet and the decrease in serum cholesterol when egg eating was stopped. However, these figures are probably inflated because these same levels were used in calculating the responses. As a consequence, any transient fluctuation that increased the baseline level increased the apparent response.

There was a mean increase of serum cholesterol on the habitual diet with age of 0.29 mmol/l for all subjects over the period 1976–1982 (Table 2). There was a relation between the rise in serum cholesterol with age and the sensitivity to dietary cholesterol in men (Fig. 3). However, the correlation failed to reach statistical significance ($r = 0.42$, $n = 16$, $P = 0.11$). In the females the parameters were not correlated ($r = -0.14$, $n = 15$). For the subjects studied in 1983 the mean increase with age was 0.17 mmol/l for 4 extreme hyporesponders and 0.95 mmol/l for 3 hyperresponders (Table 3). (In these calculations the data for subject no. 7 were not used because she had stopped eating eggs after 1976). Further research on a possible

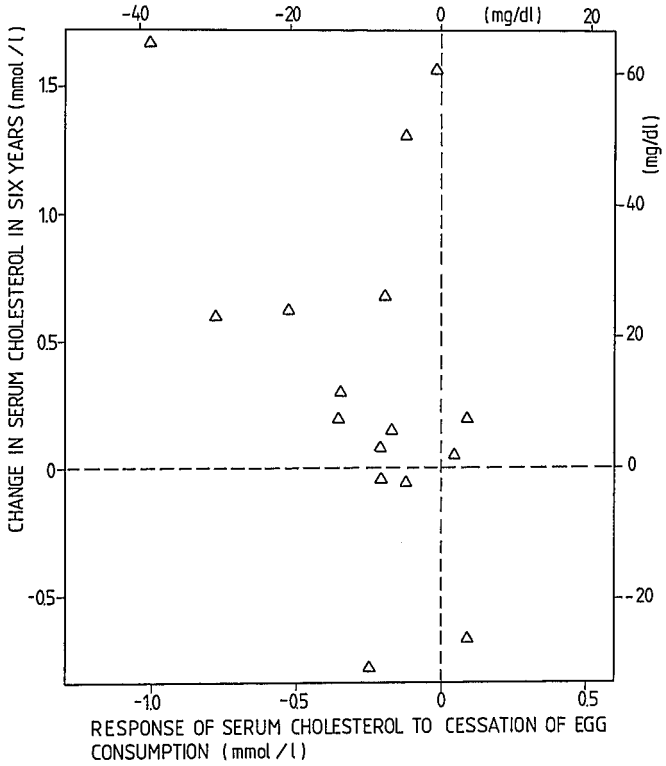

Fig. 3. Relationship between the short-term cholesterolemic response when egg eating is stopped and the change in serum cholesterol with age from 1976 to 1982 in men. One subject had stopped eating eggs after the trial of 1976; his data are not included. The response after cessation of egg consumption is the mean of that in the trials in 1976 and 1982. The correlation was $r = -0.42$ ($P = 0.11$, NS), or $r = 0.42$ if the sign of the response is first reversed so as to give hyperresponders the highest instead of the lowest response values.

relation between the increase in serum cholesterol with age and the sensitivity to dietary cholesterol seems mandated.

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