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RELATION BETWEEN THE INCREASE OF SERUM CHOLESTEROL WITH AGE AND SHORT TERM SUSCEPTIBILITY OF SERUM CHOLESTEROL TO DIETARY FAT AND CHOLESTEROL IN MAN

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ABSTRACT

We have studied the relation between the increase of serum cholesterol with age and serum cholesterol response to dietary saturated fat and/or cholesterol. To this end we investigated in two groups of subjects both their spontaneous change in serum cholesterol over a period of 4 to 10 years and their responsiveness to dietary cholesterol and/or saturated fat in dietary experiments.

In 19 men and women first investigated in 1974 -1979, the mean increase of serum cholesterol with age over a period of 8 ± 2 years was 0.36 ± 0.52 mmol/l for the men and -0.20 ± 0.17 mmol/l for the women. In a six-week trial in 1985 they were given a low saturated fatty acid, low cholesterol diet for three weeks followed by a high saturated, high cholesterol diet for another three weeks. The average difference in serum cholesterol between periods was 0.96 ± 0.34 mmol/l, and it was unrelated to the increase with age ($r=-0.05$, $p=0.84$).

In another 23 subjects the mean serum cholesterol increased with age over a period of 4 years by 0.33 mmol/l. The mean response of their serum cholesterol to diet in four or more dietary experiments was 0.53 ± 0.32 mmol/l. Again we found no relation between the serum cholesterol increase with age and the cholesterol response to dietary saturated fat and/or cholesterol ($r=-0.37$, $p=0.08$). Our findings suggest that different mechanisms are responsible for differences between persons in the increase of serum cholesterol with age and in the responsiveness of serum cholesterol to dietary saturated fat and/or cholesterol as measured in dietary trials.

INTRODUCTION

Hypercholesterolemia is rare in children and young adults [1], but in affluent populations serum cholesterol levels rise with age [1-9]. As a consequence of this, hypercholesterolemia becomes widespread from age 40 onward. In non-Western populations with a very low fat and cholesterol intake, no or only a very small increase of serum cholesterol with age is found [10-13]. This suggests that the rise of serum cholesterol with age in affluent populations is due to some cumulative environmental effect, and is therefore preventable.

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The cause of the increase of serum cholesterol with age is unknown. A few studies found that changes in body mass index can explain some of the change in serum cholesterol [14,15]. Changes in food habits do not appear to explain the changes in serum cholesterol with age [16].

In a study of Beynen and Katan [17] an association was found between the increase of serum cholesterol with age and the individual sensitivity of serum cholesterol to dietary cholesterol in controlled trials. This suggested that there may be a relation between the rise of serum cholesterol with age caused by some cumulative effect of dietary lipid, and the response of serum cholesterol to diet in short-term experiments.

We have tested this hypothesis in two longitudinal studies which both combined a follow-up period of several years with one or more controlled dietary experiments lasting a number of weeks.

METHODS

Design and subjects

This paper presents data for two cohorts, coded HL and HY respectively, which were studied using different designs.

The HL-cohort. The design of the HL-study is given in Figure 1. Between 1974 and 1979, about 400 student volunteers underwent a medical examination, including the estimation of fasting serum cholesterol and triglycerides, body fatness and urinary excretion of glucose and protein, when they were screened for participation in dietary trials [18-22]. In the end 339 participated in the particular trial for which they had been screened; those that did not participate were lost to follow-up. The values obtained during the screening examination constituted the baseline values for the present study. In 1984 we managed to trace 238 out of the 339 original participants, and out of these 169 proved willing to participate in a follow-up measurement. To distinguish whether the increase of serum cholesterol with age is determined by changes in dietary fat and cholesterol intake and body fatness or whether other factors play a role, those 127 participants in the follow-up measurement whose body mass index had shown a change of less than 2 kg/m² over the follow-up period were invited to take part in a three-week trial, during which their dietary habits at baseline were recreated, the "back to the seventies" trial. Thirty-four subjects were willing to participate, and the results have been described [16]. Nineteen subjects proved willing to participate in a further six-week controlled trial. During this trial, the "dietary-response" trial, the participants had to consume first a control diet and then a diet high in saturated fat and cholesterol. Data from both trials, the "back to the seventies" trial and the "dietary response" trial were used to determine whether there is a relation between the increase of serum cholesterol with age and the individual responsiveness of serum cholesterol. Details of the "dietary response" trial are described in the present paper.

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The HY-cohort. The design of the HY-study is given in Figure 2. Its main aim was to study variability in serum cholesterol response to diet. In 1982, 94 subjects entered the study and participated in a first dietary trial of four weeks. Out of these, 41 were selected for further dietary trials. The selection was based on the serum cholesterol response of the subjects during the first trial. Only subjects with a serum cholesterol response in the first trial in the upper quartile or lower quintile of the response distribution were

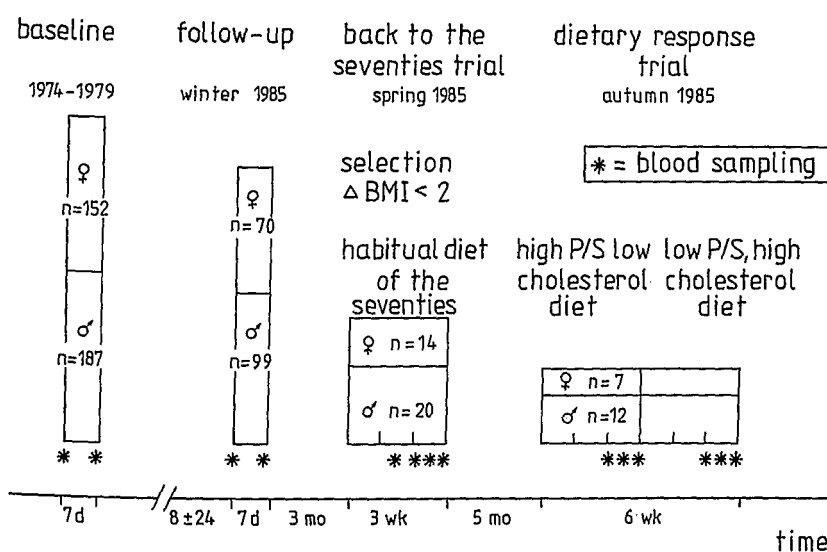


Fig. 1. Design of the study and the number of participants of the HL-cohort. At baseline and at follow-up measurement, serum cholesterol, weight and height were measured twice at a one week interval, and diets were recorded for two or three days. In the "back to the seventies" trial, serum was sampled four times and weight was measured each week. During the "dietary response" trial, serum was sampled six times and weight was measured weekly.

allowed to continue. In four further experiments over the period 1983-1986 various aspects of responsiveness were studied. Before each experiment, free-living values were measured of fasting serum cholesterol, body weight and in 1982 also height. These determinations provided the normal, habitual cholesterol level of each subject and its changes with the years.

Diets

The HL-cohort. The six-week "dietary response" trial performed at the conclusion of the HL-study (Fig 1) consisted of a 3-week period of a diet low in cholesterol and saturated fatty acids and high in polyunsaturated fatty acids, followed by a 3-week period of a diet

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high in cholesterol and saturated fatty acids and low in polyunsaturated fatty acids. Precise prescriptions for the daily menu were given to the participants in each period, and most products that contained fat and/or cholesterol were provided by us. These products provided 75% per cent of the daily fat intake and 61% per cent of the daily cholesterol intake. All participants had volunteered for and participated in previous dietary trials. They were highly motivated, and well aware of the requirements of the trial. The energy intake

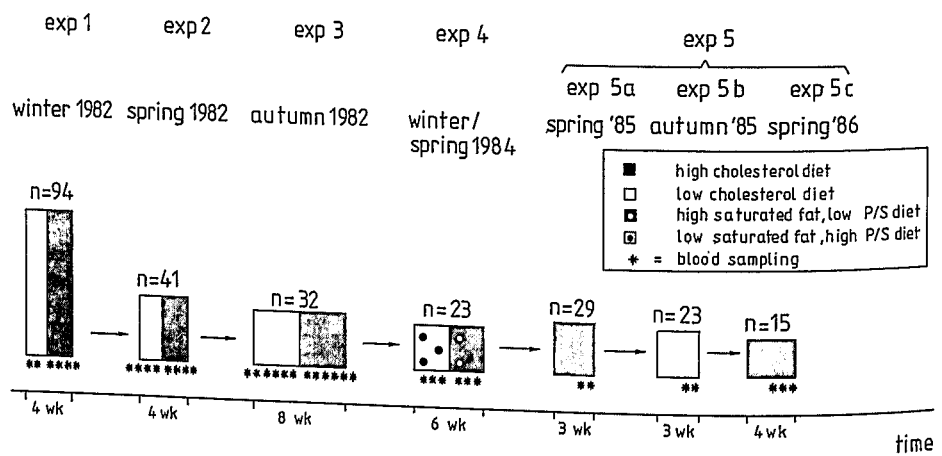


Fig. 2. Design of the study and number of participants of the HY-cohort. The duration of the experiments varied between 4 and 8 weeks. Blood was sampled throughout each experiment so as to measure responsiveness of serum cholesterol to diet. In addition, blood was sampled prior to and in between the experiments to measure the habitual serum cholesterol level.

was adjusted to individual needs. During the low cholesterol period we provided polyunsaturated margarine, sunflower oil, polyunsaturated salad dressing, peanut butter and nuts. In the high cholesterol period, butter, eggs, and salad dressing were distributed. In both periods cheese, cookies, jam or honey and sugar were also provided. Once a week the products were packaged with frozen cooling elements, and sent by express mail to the participants, who received them the same day. Each subject was allowed to consume each day one Megajoule worth of self-selected foodstuffs low in fat and/or cholesterol; these foodstuffs were specified in a list. Table 1 describes the foods eaten per day for each period. The subjects checked their body weight twice a week on their own weighing scales, and energy intake was adjusted when necessary. Although individual intake was adjusted at the first instance of weight loss, there was an average weight change of -0.8 kg (range: -2.5 to 0.3) over the 6 weeks of the experiment.

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Food intake was monitored through weekly 24 hour recalls. Mostly these recalls were done by telephone by specially trained dieticians. This also provided the opportunity to discuss problems and to emphasize the importance of good adherence. On the first day of each dietary period the subjects recorded their food intake for one day using portable electronic scales. This recording was done to give the subjects a more precise idea of the quantities of the foods that they had to eat during the next three weeks. The recalls and records were coded and nutrients calculated using the 1983 release of the Dutch nutrient data base 'UCV' [23].

The composition of the experimental diets is given in table 2. The diets were defined in terms of % of energy or of mg per Megajoule. Thus a higher energy intake involved a higher intake of all

TABLE 1
Typical daily menus for the low cholesterol low saturated fatty acid period and the high cholesterol high saturated fatty acid period of the "dietary response" trial of the HL-cohort

low saturated fat and cholesterol period	high saturated fat and cholesterol period
6 30-g slices of bread	6 30-g slices of bread
45 g polyunsaturated margarine	45 g butter
1 15-g slice of cheese	2 15-g slices of cheese
2 15-g packages of peanut butter	2 15-g slices of medium fat meat
3 15-g packages of jam or honey	2 15-g packages of jam or honey
400 g skimmed milk or low fat yoghurt	400 g milk (1.5% fat) or yoghurt
250 g potatoes	250 g potatoes
cooked vegetables	cooked vegetables
10 g polyunsaturated dressing	10 g cream-based salad dressing
10 g sunflower oil	100 g low fat meat
250 g of skimmed milk based dessert	250 g of milk based dessert (1.5% fat)
2 pieces of fruit	2 pieces of fruit
2 10-g cookies	2 10-g cookies
25 g sugar	30 g sugar
30 g nuts	

nutrients. Dietary adherence was also monitored by measuring the fatty acid composition of plasma cholesteryl esters [24]. The mean linoleic acid to oleic acid ratio in the plasma cholesteryl esters changed from 6.0 to 3.2 on going from the low cholesterol, low saturated fatty acid diet to the high cholesterol, high saturated fatty acid diet, which shows that the fatty acid composition of the diet was indeed markedly different between the two diet periods. After successful completion of the experiment the subjects were given an expense allowance of Hfl 200,= (about \$ 100,=).

The HY-cohort. Follow-up for the HY-cohort covered a period of 4 years, during which they frequently participated in dietary experiments. Four out of the five dietary experiments (Fig 2) of the HY-study consisted of a low cholesterol period followed by a high

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cholesterol period. In experiment 4, a low saturated, high polyunsaturated fatty acid period was followed by a high saturated, low polyunsaturated fatty acid period. In experiments 1, 2, and 4 natural mixed diets were provided daily as described [25-27]. Total diets were provided except for one Megajoule worth of self-selected foodstuffs low in fat and/or cholesterol; these were specified in a list. In experiments 3 and 5b the subjects prepared their hot meals at home. Individual packages were supplied two or three times a week in experiment 3 and once a week in experiment 5b. In experiment 5a the subjects had to consume extra eggs to increase the dietary cholesterol intake. The eggs were supplied by us. In experiment 5a dietary intake was monitored through weekly 24 hour recalls, and body weight was checked weekly.

The composition of the diets used during the experimental trials has been described elsewhere [25].

Blood collection and analysis

The HL-cohort. In the HL-study, fasting blood was sampled twice during the baseline measurements. Non-fasting blood was sampled twice during the first follow-up measurement and four times during the last two weeks of the "back to the seventies" trial. During the "dietary response" trial described in the present paper, non-fasting blood was sampled 3 times during each dietary period (Figure 1). As most of the participants did not live in Wageningen anymore, local hospitals were involved in blood collection. Serum samples were stored at -20 °C at the hospitals, transported to Wageningen in the frozen state, and then kept at -80 °C until analysis. At the end of the experiment serum total and HDL-cholesterol were determined in the same rigidly standardized laboratory that had also performed the baseline measurements in the seventies [26,28,29]. Reanalysis in 1983 of serum pools prepared and first analysed in 1975 showed that the analytical drift in absolute level of serum cholesterol in these pools had been less than 1.7%.

Table 3

Change of serum cholesterol with age over 6 to 10 years, corrected for changes in diet over that period and the serum cholesterol response in the dietary response trial: the HL cohort (mean \pm sd)

	men (n=12)	women (n=7)	all (n=19)
Age change (minus diet effects [#])	0.35 \pm 0.52	-0.20 \pm 0.46	0.15 \pm 0.56
Response in a dietary trial	0.94 \pm 0.36	0.98 \pm 0.32	0.96 \pm 0.34

[#] Change of serum total cholesterol between the "Back to the seventies" trial in the spring of 1985 and baseline measurement in the seventies

[|] Change of serum total cholesterol between the high cholesterol high saturated fatty acid period and the low cholesterol low saturated fatty acid period of the dietary response trial in the autumn of 1985

Table 2
 Characteristics and diet of the 19 subjects of the HL-study at the first follow-up measurement in the winter of 1985 and during the dietary response trial in the autumn of 1985 (Mean ± sd)

Characteristic	Follow-up measurements				Dietary response trial			
	men (n=12)	women (n=7)	low saturated fat and cholesterol period men (n=12)	high saturated fat and cholesterol period women (n=7)	low saturated fat and cholesterol period men (n=12)	high saturated fat and cholesterol period women (n=7)	low saturated fat and cholesterol period men (n=12)	high saturated fat and cholesterol period women (n=7)
Age (years)	30.0 ± 5.2	28.9 ± 3.4	71.2 ± 8.4	60.4 ± 2.8	70.3 ± 7.4	59.4 ± 3.2	70.3 ± 7.4	59.4 ± 3.2
Height (cm)	184.7 ± 8.0	172.1 ± 5.9	20.7 ± 1.8	20.2 ± 1.1	20.6 ± 1.7	20.1 ± 1.1	20.6 ± 1.7	20.1 ± 1.1
Weight (kg)	73.2 ± 8.3	61.6 ± 3.0	4.07 ± 0.72	3.99 ± 0.57	5.03 ± 0.58	4.97 ± 0.63	5.03 ± 0.58	4.97 ± 0.63
Body mass index (kg/m ²)	21.5 ± 1.9	20.8 ± 1.2	1.34 ± 0.31	1.57 ± 0.37	1.37 ± 0.29	1.60 ± 0.40	1.37 ± 0.29	1.60 ± 0.40
Serum total cholesterol (mmol/l)	5.00 ± 0.81	4.61 ± 0.69						
Serum HDL-cholesterol (mmol/l)	1.40 ± 0.27	1.64 ± 0.29						
Diet								
Energy (Megajoule)	11.6 ± 2.8	8.0 ± 2.1	11.2 ± 2.5	10.4 ± 2.4	11.6 ± 2.2	8.5 ± 1.3	11.6 ± 2.2	8.5 ± 1.3
Protein (%energy)	13.4 ± 1.1	14.2 ± 1.8	10.9 ± 1.4	12.6 ± 1.5	13.9 ± 1.0	14.1 ± 1.7	13.9 ± 1.0	14.1 ± 1.7
Fat (%energy)	37.4 ± 4.0	36.7 ± 2.5	37.5 ± 2.8	37.1 ± 1.6	36.9 ± 2.1	35.6 ± 2.6	36.9 ± 2.1	35.6 ± 2.6
Saturated fat (%energy)	16.9 ± 2.7	16.6 ± 2.0	10.1 ± 0.6	9.1 ± 0.7	19.4 ± 1.2	19.1 ± 1.3	19.4 ± 1.2	19.1 ± 1.3
Polysaturated fat (%energy)	5.4 ± 2.1	6.2 ± 1.8	15.5 ± 1.7	16.3 ± 0.9	3.2 ± 0.5	3.5 ± 0.4	3.2 ± 0.5	3.5 ± 0.4
Carbohydrates (%energy)	46.8 ± 4.1	45.3 ± 4.7	48.0 ± 2.2	47.3 ± 2.8	46.1 ± 3.2	47.8 ± 2.5	46.1 ± 3.2	47.8 ± 2.5
Sugars (%energy)	22.2 ± 4.4	19.6 ± 2.2	23.7 ± 3.7	23.4 ± 3.2	22.1 ± 4.2	22.2 ± 1.8	22.1 ± 4.2	22.2 ± 1.8
Alcohol (%energy)	2.4 ± 2.1	3.8 ± 3.7	3.6 ± 3.2	4.6 ± 0.9	2.9 ± 2.4	4.3 ± 0.6	2.9 ± 2.4	4.3 ± 0.6
Fiber (g/MJ)	3.2 ± 0.8	3.5 ± 0.7	3.9 ± 0.5	2.9 ± 2.4	3.7 ± 0.5	2.5 ± 2.2	3.7 ± 0.5	2.5 ± 2.2
Cholesterol (mg/MJ)	28.8 ± 10.3	31.3 ± 10.3	7.6 ± 1.0	7.6 ± 1.2	47.7 ± 3.8	44.6 ± 2.8	47.7 ± 3.8	44.6 ± 2.8

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The increase of serum cholesterol with age was calculated as the serum cholesterol at the end of the "back to the seventies" trial minus the baseline values measured six to ten years earlier. The short-term serum cholesterol response to diet was calculated as the mean of the three samples collected at the end of the high cholesterol, high saturated fatty acid period minus the mean of the three blood samples at the end of the low cholesterol, low saturated fatty acid period of the "dietary response" trial.

The HY-cohort. In the HY-study, blood was sampled after an overnight fast, serum was stored at -80°C and total and HDL-cholesterol were determined in a rigidly standardized laboratory as described earlier [26,28,29].

The increase of serum cholesterol with age of each person was calculated as the slope of the linear regression equation of eight to ten measurements of his or her serum cholesterol against time over the period 1982-1986. The response to diet within each experiment was calculated as the mean of the last two cholesterol values on the high cholesterol or high saturated fatty acid period minus the mean of the last two cholesterol values on the low cholesterol or low saturated fatty acid diet. The overall short-term serum cholesterol response to diet was calculated as the mean of four or five experiments per subject.

RESULTS

Changes in cholesterol

The HL-cohort. Characteristics of the 19 subjects of the HL-cohort who completed all phases of the study are given in table 2. Their mean increase of serum cholesterol with age was 0.35 ± 0.52 mmol/l for the men and -0.20 ± 0.46 mmol/l for the women (table 3). From the diaries no serious disease or other confounding events were in evidence during the response trial. In comparison with pre-experimental values, the low-cholesterol high-polyunsaturate diet given during the first 3 weeks of the response trial caused an average decrease of serum cholesterol of 0.81 ± 0.50 mmol/l. In the high cholesterol, high saturated fatty acid period serum total cholesterol rose by 0.96 ± 0.34 mmol/l or 23%. HDL-cholesterol rose by 2.3%. The mean response of the participants was somewhat lower than could be expected [30] from the composition of the diets in the two periods.

The HY-cohort. Characteristics of the HY-subjects are given in table 4. Regression analysis of the habitual serum cholesterol values on time over 4 years indicated that there had been a mean increase of serum cholesterol of 0.08 mmol/l per year. The mean response of serum cholesterol of these subjects during the dietary trials was 0.53 ± 0.32 mmol/l. No significant differences were found between men and women in their dietary response in either cohort.

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TABLE 4

Age, weight, body mass index, serum cholesterol increase between 1982 and 1986 and serum cholesterol response averaged over four or five trials: the HY-cohort (mean \pm sd)

Characteristic	men (n=14)	women (n=9)	all (n=23)
Age 1982	32.9 \pm 12.9	28.2 \pm 12.6	31.0 \pm 12.7
Weight 1982 (kg)	74.5 \pm 7.1	67.3 \pm 9.4	71.7 \pm 8.7
Height 1982 (cm)	182.9 \pm 6.1	169.6 \pm 4.4	177.7 \pm 8.6
Body mass index 1982 (kg/m ²)	22.2 \pm 1.6	23.4 \pm 3.2	22.7 \pm 2.3
Weight 1986 (kg)	75.8 \pm 6.9	69.5 \pm 9.3	73.3 \pm 8.3
Body mass index 1986 (kg/m ²)	22.6 \pm 1.2	24.2 \pm 3.1	23.2 \pm 2.3
Serum cholesterol increase in four years (mmol/l)	0.43 \pm 0.41	0.17 \pm 0.78	0.33 \pm 0.58
Serum cholesterol response [#]	0.61 \pm 0.28	0.42 \pm 0.36	0.53 \pm 0.32

[#] Averaged over 4 or 5 trials between 1982 and 1986

Relation between short-term serum cholesterol response to diet and serum cholesterol rise with age.

Figures 3 (HL) and 4 (HY) show the relation between the individual susceptibility of serum cholesterol to dietary manipulation, and the spontaneous increase of serum cholesterol with age. In both studies, negative, non-significant correlations were found between the increase of serum cholesterol with age and the short term serum cholesterol response to dietary cholesterol and/or saturated fat for both sexes.

DISCUSSION

In this study we did not find a significant relationship between serum cholesterol increase with age and the short-term susceptibility of serum cholesterol to dietary cholesterol and/or saturated fat. Thus we cannot confirm the relation reported by Beynen and Katan [17], who found that in 16 men studied six years apart there was correlation of 0.42 between the serum cholesterol increase with age and the response of serum cholesterol to short-term dietary manipulation. The results of the two cohorts were quite consistent. Combined analysis yielded a correlation r of -0.31 between the long-term rise of serum cholesterol with aging and the short-term response in dietary experiments ($n=42$; 95% confidence limits, -0.01 and -0.56). Thus the relation, if anything, was inverse rather than direct. Analyzing the data separately for men and women yielded similar results (men: $r=-0.29$, $n=26$, women: $r=-0.47$, $n=16$). It appears that no positive relation exist between the increase of serum cholesterol with age and the response in dietary trials.

The question remains what does cause the serum cholesterol concentration to increase with age. Rossouw et al. [31] suggested that this increase with age results from a cumulative effect of dietary saturated fat or cholesterol. However, observations from controlled

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trials lasting several years speak against this suggestion. It has been shown that after an increase in the intake of saturated fat or cholesterol, the rise of the serum cholesterol concentration is completed within a few weeks [32], and it is relatively stable thereafter. A reverse phenomenon, a rapid decrease of the serum cholesterol concentration, is seen on increasing the polyunsaturated and decreasing the saturated fat intake [33-36]. In elderly hypercholesterolemic men participating in a long term heart disease prevention trial, this diet-induced decrease in serum cholesterol was already completed at the first time of blood sampling after the start of the diet, and no further change appeared in the succeeding eight or eleven years [33,36]. This shows that diet-induced hypercholesterolemia is rapidly reversible, with no sign of a long term component in the effect.

In our study of the HL-cohort, we have shown that the serum cholesterol concentration increased with age, even though the saturated fat and cholesterol content of the diet did not [16]. Recreating the diet eaten at a younger age did not reproduce the low cholesterol levels of that time. On the other hand, in primitive populations on presumably constant diets serum cholesterol does not rise with age [10-13]. One possible explanation for this difference between primitive and affluent populations is changes in body mass

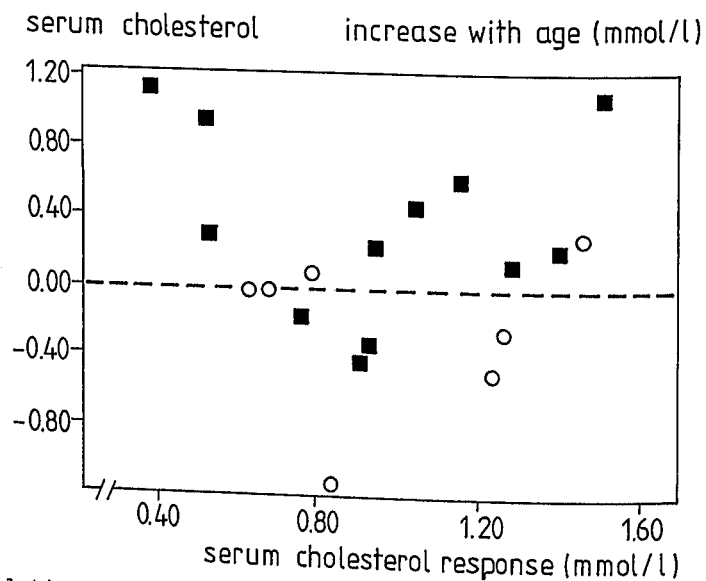


Fig. 3. Relation between the serum cholesterol response of HL cohort subjects during the "dietary response" trial (x-axis) and the individual serum cholesterol increase with age from the baseline measurements in 1974-1979 to the end of the "back to the seventies" trial in 1985 (y-axis) (■: men, n=12, ○: women, n=9; $r=-0.05$).

index; in affluent populations body fatness rises markedly with age.

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In many studies it has been found that higher body mass index or higher body fatness is associated with a higher serum cholesterol concentration [37-43], and there are also studies -including our own- which show that a change in body mass index over time is positively associated with a change in serum cholesterol [14-16, and Berns et al, unpublished]. The absence of similar changes in body mass index in non-affluent populations could explain, at least partly, the lack of an increase in serum cholesterol with time. The role of body fatness is emphasized by a different type of study [44] in which omnivorous and macrobiotic boys and men were compared. The omnivorous and macrobiotic boys had about the same body mass index. The adult macrobiotic men (aged between 30 and 39 years) were remarkably lean, with a body mass index of 20.9 kg/m². Their serum cholesterol level (3.8 mmol/l) was only 0.4 mmol/l higher than that of boys aged 6-11 years from the same dietary group. In contrast with this, the omnivorous men were fatter, with a body mass index of 24.4 kg/m², and they showed a serum cholesterol concentration that was 1.3 mmol/l higher than that of the omnivorous boys. Thus, various types of study suggest that an increase of body mass index is a prime suspect for the increase of serum cholesterol increase with age.

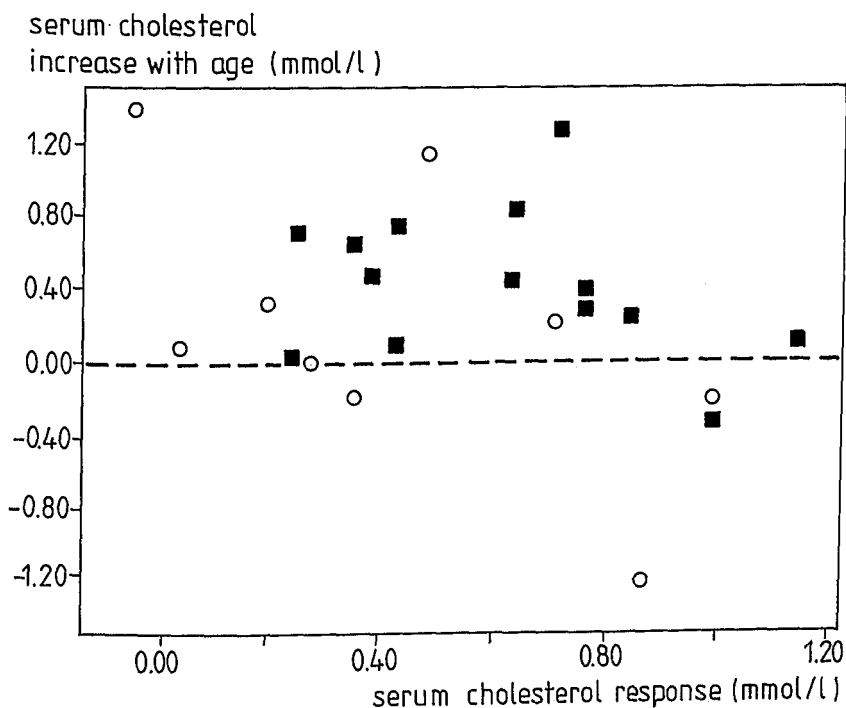


Fig. 4. The HY-cohort, relation between the mean serum cholesterol response of at least four dietary experiments (x-axis) and the serum cholesterol increase with age (calculated from linear regression analysis per subject, y-axis) (■: men, n=14, ○: women, n=9, $r=-0.37$).

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