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Determinants of the Increase of Serum Cholesterol with Age: A Longitudinal Study

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In Western populations, the level of serum cholesterol increases with age, whereas in certain developing countries this increase is absent. In order to determine which factors are related to this increase, the authors investigated 99 men and 70 women whose serum cholesterol, habitual food intake, and body mass index were measured between 1974 and 1979 when they were students (baseline values) and again in 1985 (follow-up measurement).

Serum cholesterol had increased by 0.59 mmol/l (14%) in men, and by 0.34 mmol/l (7%) in women. The effect of changes in habitual food intake on serum cholesterol was quantified as the Keys score. The mean increase of the Keys score corresponded to a change in cholesterol of 0.12 mmol/l for both men and women. Body mass index had increased by 0.9 kg/m² in men and by 0.5 kg/m² in women. Regression analysis showed that in men change in body mass index partly explained the change in serum cholesterol ($r = 0.20$). In women none of the independent variables could explain changes in serum cholesterol.

In order to determine what the effect of ageing was independent of changes in body mass index and dietary changes, 34 volunteers who had participated in the follow-up measurements and whose change in body mass index had been less than 2 kg/m², were prescribed a diet for three weeks. This diet had the same composition as their habitual diet at baseline measurement. It lowered serum cholesterol levels by 0.1 mmol/l over the three weeks of the trial, and thus could not abolish the rise of serum cholesterol with age. The authors conclude that the increase of serum cholesterol between ages 20 and 30 is not caused by changes in food intake, and that in these subjects only a small proportion of the increase is related to changes in body mass index.

Epidemiological and experimental studies have shown that the risk of coronary heart disease increases with increasing plasma concentrations of total or LDL-cholesterol.[1-3] Populations with high intakes of saturated fat and cholesterol tend to have higher total and LDL-cholesterol levels. Although marked differences in cholesterol levels between populations can already be found in children and young adults,[4] frank hypercholesterolaemia at this stage of life is infrequent. However, serum cholesterol levels rise with age, and as a consequence of this, hypercholesterolaemia becomes widespread in affluent populations from age 40 onwards. In cross-sectional studies the strongest increase, of 0.2 to 0.8 mmol/l, is found between 20 and 30 years of age,[5-10] followed by a gradual increase until 60 years of age.[6,10] In longitudinal studies an increase of serum cholesterol with age has also been found.[11-14] Thus, the prevalence of mild hypercholesterolaemia rises with age.

Among populations with a very low fat and cholesterol intake, no or only a small increase of serum cholesterol with age is found.[15-17] Total serum cholesterol is also very low in these populations—3.5 mmol/l.[18] It is unlikely that genetic differences explain differences in the increase of serum cholesterol with age between populations. Migrants from areas where cholesterol levels are low (Japan) to areas where cholesterol levels are high (USA) experience an increase in cholesterol levels. The children of migrants have cholesterol levels similar to those of native born individuals.[19] In a cross-sectional study in China, a rise with age was seen in urban but not in rural Chinese.[20] This suggests that the rise of cholesterol with age in affluent populations is preventable. However, reliable data on the longitudinal increase of cholesterol with age and its correlates within subjects are rare, and few have addressed the age range from 20 to 30 years when the rise is most rapid.

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The purpose of our study was to investigate the increase of serum cholesterol with age over a period of 6–10 years as a function of changes in body mass index and habitual food consumption. In order to separate the effect of dietary from that of other determinants, the observational study was followed by a trial in a subsample of subjects who were prescribed a diet similar to their baseline habitual diet eight years earlier.

METHODS

Design and Subjects

In Figure 1 the design of the study is given. In 1974, serum cholesterol was measured in 75 nutrition students who participated in a trial on the effect of vitamin C tablets versus placebo on serum cholesterol. No effect of vitamin C on serum cholesterol was found at that time, so these measurements were considered baseline values. Between 1975 and 1979 baseline values were obtained during the screening of about 360 students who applied for participation in dietary trials.[21–25] During this screening phase the subjects had to record their food intake, and they underwent a medical examination of fasting serum cholesterol and triglycerides, body fatness, and urinary excretion of glucose and protein. About 40 subjects with either serum cholesterol levels above 5.7 mmol/l, serum triglyceride levels above 1.6 mmol/l, diastolic blood pressure above 95 mm Hg, or a body fat percentage, as calculated from the sum of tricipitalis, bicipitalis, subscapular and suprailiaca skinfolds, above 20% for men or above 30% for women were dismissed and lost to follow-up. Another 51, for a variety of reasons, did not enter the trial for which they had volunteered; as the list linking their names with their code numbers was destroyed and they were lost to follow-up. Thus of 339 subjects who had participated in a trial between 1974 and 1979, 238 could be traced and were approached in 1984. Out of these 169 (69%) proved willing to participate in a follow-up measurement. Sixty-two subjects were not willing and seven were pregnant and therefore not eligible.

There were no significant differences in baseline serum cholesterol ($p = 0.6$), Keys score ($p = 0.07$) and body weight ($p = 0.6$) between the 169 subjects who were available for follow-up and the 170 non-responders who were not.

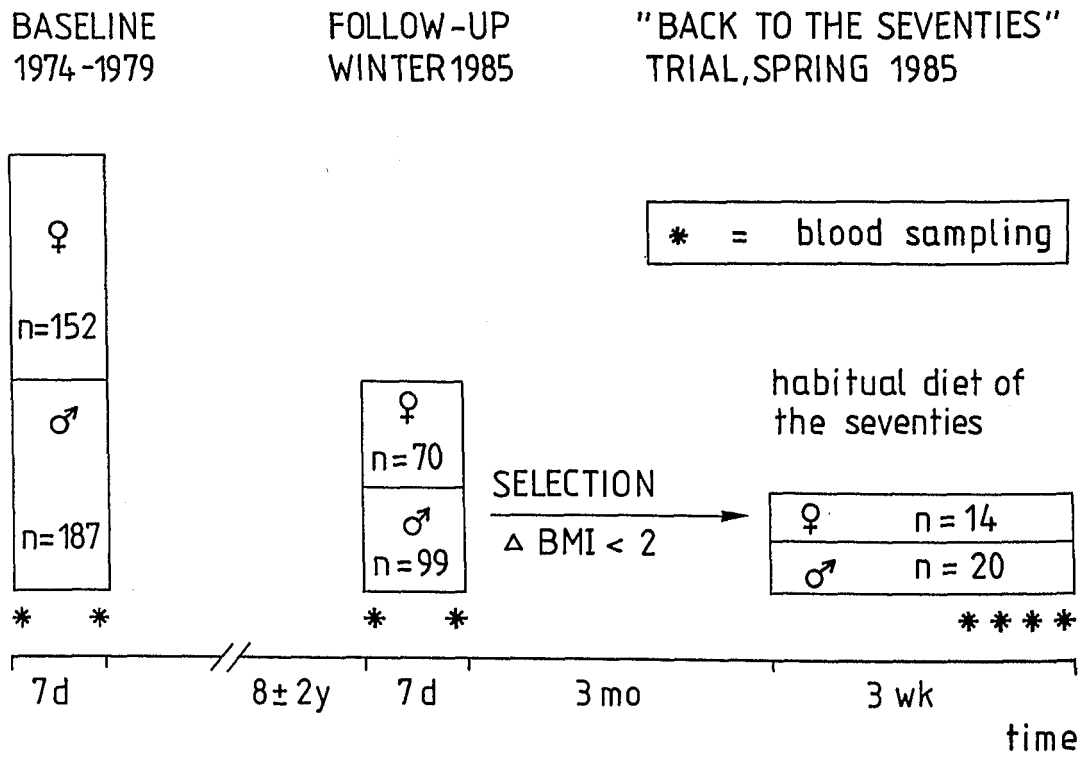

FIGURE 1 *Design of the study and the number of participants at baseline measurement (1974–1979), at follow-up measurement (December 1984–February 1985), and during the 'back to the seventies' trial (April–May 1985). At baseline and at follow-up measurements serum cholesterol, weight and height were 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.*

Data collection for the follow-up measurement took place from December 1984 until March 1985. The baseline and follow-up measurements were both made in the winter months, to minimize the influence of seasonal fluctuations.

In order to find out to what extent the increase of cholesterol with age is determined by changes in dietary fat and cholesterol intake, 127 subjects whose body mass index had shown a change from baseline to follow-up measurement of less than 2 kg/m^2 were invited to take part in a three-week 'back to the seventies' trial, during which they were to consume a diet similar to that at baseline. Thirty-four subjects were willing to participate in this trial.

Diets

Food intake at baseline was recorded for two days by the participants studied in 1974 and 1975, and quantities were estimated in household units. Participants studied between 1976 and 1979 recorded their baseline food intake for three days, weighing all items on a balance. At the time of the follow-up measurement the participants were again given instructions on how to record their food intake. For each individual the days of the week and the method of registration were replicates of those employed at the time of his or her baseline measurements.

The 'back to the seventies' dietary trial lasted three weeks. Participants were prescribed a diet providing 15 energy per cent as saturated fat, 5 energy per cent as polyunsaturated fat, 33 energy per cent as total fat and 27 mg cholesterol per megajoule (1MJ = 239 kcal). These values were the same as the average habitual diet of all subjects at baseline.[26] The energy intake was adjusted to the individual needs of the subject. The subjects checked their body weight twice a week and if necessary energy intake was adjusted. Once each week a 24-hour recall was taken by telephone.

Food intake data were coded and nutrients were calculated using the 1983 release of the national Dutch nutrient data base 'UCV'.[27] To quantify the effect of habitual diet on serum cholesterol, the Keys score was calculated. The Keys score, expressed in mmol/l, equals $168/38.7 + 1.35(2S - P)/38.7 + 1.5\sqrt{Z}/38.7$, with S = energy per cent saturated fatty acids, P = energy per cent polyunsaturated fatty acids and Z = cholesterol in mg per 1000 kcal.[28]

Blood Collection and Analysis

Baseline blood samples were obtained in Wageningen from fasting subjects. At the time of the follow-up measurement many participants did not live in Wageningen any more, so 12 hospitals all over the country were involved in blood and data collection. Non-fasting blood was sampled twice, at a one-week interval. Recent food intake has been shown not to affect serum total or HDL-cholesterol levels.[29] 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.

Weight and height were measured without shoes, while the subjects were wearing light indoor clothing. Balances used at collaborating hospitals were checked by us before the measurements.

In the 'back to the seventies' trial non-fasting blood was sampled four times during the last two weeks of the experiment.

In 1974 serum cholesterol was measured according to Abell,[30] with reference sera from our Department. From 1975 until 1979 baseline serum cholesterol was measured using the Liebermann-Burchard reagent and serum calibrators calibrated with the Abell method.[31] At that time our laboratory was certified by the Center for Disease Control (CDC), Atlanta GA as meeting the CDC/WHO criteria for accuracy and precision in cholesterol analysis. For the follow-up measurements, cholesterol was assayed with the Boehringer CHOD-PAP Monotest enzyme kit, using serum calibrators provided and certified by the Netherlands 'Foundation for Quality Control of Chemical Analyses for Epidemiological Research' (KCA).[32] To exclude any drift in analytical levels, a number of serum pools made and analysed by our department in 1973 and 1974 were retrieved from the freezer and re-analysed in 1983. Samples of these pools had also kindly been analysed in 1973–1975 by Dr Gerald Cooper, CDC, using the Abell method as reference. As shown in Table 1 the mean change in laboratory level over the time between baseline and follow-up measurement was only 0.06 mmol/l (2 mg/dl), and thus analytical levels were very stable.[33]

HDL-cholesterol was determined in a rigidly standardized laboratory as earlier described.[34,35]

Statistical Analysis

Differences in serum cholesterol, body mass index (weight/height^2) and Keys score between baseline and follow up measurement were calculated.

All possible subset regression[36–38] was used, with change in serum cholesterol as dependent variable, and changes in body mass index, Keys score, and for women also changes in oral contraceptive use, as independent variables.

TABLE 1 *Serum cholesterol values in human serum pools prepared and assayed at the time of baseline measurement and assayed again shortly before the follow-up measurements. Pools had been stored at −20°C, CDC, Centers for Disease Control. Further details are available.*[31,33]

Serum pool	1973–1975 This lab (mmol/l)	1973–1975 CDC (mmol/l)*	1983 This lab (mmol/l)‡	Absolute difference (mmol/l)
C	2.22*	2.22	2.31	0.09
W	4.68†	4.62	4.67	−0.01
A	5.51*	5.59	5.55	0.04
X	7.06†	7.06	7.20	0.14

* Analysed by the method of Abell *et al.*[30]

† Analysed by the routine method.[31] Measurements in 1975 of 141 random sera by both the routine[31] and the Abell[30] method yielded a mean difference between methods at that time of 0.06 mmol/l[33].

‡ Analysed with the Boehringer CHOD-PAP Monotest enzymatic method and serum calibrators calibrated by the Abell method. Mean of 26 or 27 determinations per pool; variation coefficients ranged from 1.1 to 1.5%.

TABLE 2 *Characteristics and diets of 169 student volunteers as measured at baseline in 1974–1979 in Wageningen, The Netherlands, and changes over the period from baseline until the follow-up measurement in 1985.*

Characteristics	Mean ± sd at baseline		Mean change ± sd between baseline and follow-up measurement	
	Women (n = 70)	Men (n = 99)	Women (n = 70)	Men (n = 99)
Age (years)	21.4 ± 2.8	22.2 ± 4.2	8.2 ± 2.2	7.6 ± 1.9
Height (cm)	169.4 ± 6.5	182.3 ± 6.9	0.4 ± 0.8	0.6 ± 1.1
Weight (kg)	60.6 ± 7.0	70.7 ± 6.4	1.6 ± 5.1*	3.6 ± 4.8**
Body mass index (kg/m²)	21.1 ± 1.8	21.3 ± 1.7	0.50 ± 1.77*	0.91 ± 1.43***
Serum total cholesterol (mmol/l)	4.71 ± 0.70	4.33 ± 0.73	0.34 ± 0.67***	0.59 ± 0.47***
Serum HDL-cholesterol (mmol/l)†	1.54 ± 0.48	1.41 ± 0.27	−0.00 ± 0.28	−0.17 ± 0.24***
Habitual diet				
Energy (MJ/day)	8.1 ± 1.9	12.2 ± 2.9	0.5 ± 1.9	−0.6 ± 2.7
Protein (%Energy)	15.1 ± 2.2	13.3 ± 2.2	−0.7 ± 2.7	0.3 ± 2.6
Fat (%Energy)	34.7 ± 5.5	34.1 ± 5.6	3.2 ± 7.3	2.5 ± 7.2
Saturated fatty acids (%Energy)	15.4 ± 2.5	14.7 ± 2.5	1.7 ± 3.6	1.6 ± 3.8
Polyunsaturated fatty acids (%Energy)	5.5 ± 1.8	6.2 ± 2.0	0.3 ± 2.3	−0.1 ± 2.2
P/S ratio	0.37 ± 0.14	0.44 ± 0.17	−0.02 ± 0.18	−0.05 ± 0.20
Carbohydrates (%Energy)	47.3 ± 6.0	48.3 ± 5.5	−3.2 ± 7.6	−3.6 ± 7.3
Sugars (%Energy)	23.9 ± 6.0	21.1 ± 5.2	−3.8 ± 6.9	−2.4 ± 6.3
Alcohol (%Energy)	2.9 ± 3.2	4.3 ± 4.6	0.7 ± 4.3	−1.2 ± 5.8
Fibre (g/MJ)	3.7 ± 1.1	3.5 ± 1.1	−0.4 ± 1.3	−0.3 ± 1.1
Cholesterol (mg/MJ)	30.9 ± 12.3	27.6 ± 10.3	1.2 ± 17.5	0.9 ± 14.8
Keys score (mmol/l)‡	5.65 ± 0.23	5.56 ± 0.22	0.12 ± 0.33**	0.12 ± 0.33***

† Serum HDL-cholesterol was measured in 13 women and 25 men only.

‡ The Keys score[28] was calculated as $168/38.7 + 1.35(2S–P)/38.7 + 1.5\sqrt{Z}/38.7$, where S = energy % saturated fatty acids, P = energy % polyunsaturated fatty acids, Z = cholesterol in mg per 1000 kcal.

*$p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

RESULTS

Baseline and First Follow-up Measurements

Details of age, height, body weight, body mass index, serum total cholesterol and habitual diet at baseline, and changes over the period from baseline until follow-up measurement are given in Table 2. The Keys score quantifies the effect of habitual diet on serum cholesterol; in controlled trials, an increase of 1 in the Keys score has on average produced an increase in the serum cholesterol concentration of 1 mmol/l.[28] Since their baseline measurements, the subjects had aged by about eight years and on average the Keys score of their diet had increased by 0.12 mmol/l. Their height had not changed perceptibly, but they had gained a significant though modest amount of weight; body mass index had increased by on average 2% in women and by 4% in men.

The mean increase of serum total cholesterol was 0.34 mmol/l or 7% in women and 0.59 mmol/l or 14% in men. The mean increase of serum cholesterol was

significantly larger in men than in women. A Spearman correlation coefficient of 0.69, $p<0.01$, was found between the level of serum cholesterol at baseline and at follow-up measurement. This shows that the rank order of the serum cholesterol values of the subjects at both measurements remained fairly stable.

Univariate product-moment correlation coefficients between the increase of serum cholesterol with age, and the body mass index, Keys score, the age at first measurement and the changes in these variables with time are given in Table 3. A significant correlation was found only between the change in serum total cholesterol and the change in body mass index in men.

All possible subset regression showed also that in men the change in body mass index partly explained the change in serum cholesterol. The adjusted r squared was 0.04. A change of 1 kg/m^2 in body mass index contributed a change of 0.07 mmol/l in serum cholesterol. In women neither the change in body mass index, nor the change in Keys score, nor the change in contraceptive use could explain the change in serum cholesterol.

We also analysed the data cross-sectionally, relating differences between subjects in serum cholesterol at baseline or follow-up with differences in age and other factors at the same point in time. All possible subset regression was used to examine this. Age appeared to be the most important factor determining the level of serum cholesterol both at baseline level and at follow-up measurement for men and women. In men the cross-sectional data showed a difference of 0.09 mmol/l for each year of age in the data set obtained at baseline, and of 0.07 mmol/l per year at follow-up measurement, highly similar to the longitudinal data which showed a mean increase of serum cholesterol with time of 0.08 mmol/l per year. In women, cross-sectional analysis produced a difference of 0.09 mmol/l for each difference of one year in age at baseline and of 0.06 mmol/l per year at follow-up, which is higher than the longitudinal increase of serum cholesterol with time of 0.04 mmol/l per year.

TABLE 3 *Pearson correlations of change of serum cholesterol in 99 men and 70 women with baseline body mass index, Keys score, and age, measured in 1974–1979 and with changes in body mass index and Keys score over the period from baseline until follow-up measurement.*

Variable	Correlation with change in serum cholesterol	
	Women	Men
Age at entry	−0.08	0.05
Initial body mass index	−0.03	0.02
Change in body mass index	0.07	0.22*
Initial Keys score	0.08	−0.04
Change in Keys score	−0.12	−0.02

* $p < 0.05$.

The 'Back to the Seventies' Trial

In the 'back to the seventies' trial only subjects with a change in body mass index of less than 2 kg/m^2 were allowed to participate. This selection was made because we wanted to find out what the increase of serum cholesterol with age was, independent of changes in body mass index and dietary changes. Out of the 127 eligible subjects 34 proved willing to participate. Baseline characteristics and diets of these 34 volunteers, and changes over the period from baseline until the end of the 'back to the seventies' trial are given in Table 4. The increase of serum cholesterol with age from baseline to follow-up measurement for the group of 34 participants was 0.13 mmol/l for women and 0.45 mmol/l for men, as opposed to 0.30 mmol/l and 0.53 mmol/l in the total group of 127 subjects with a change in body mass index of less than 2 kg/m^2, and 0.34 mmol/l and 0.59 mmol/l for the full group of 169 subjects. Over the three weeks of the trial serum cholesterol levels decreased by 0.1 mmol/l. This agreed with what could be expected from changes in nutrient consumption as evaluated by the Keys score of the 'back to the seventies' diet, compared with the habitual diet of those 34 participants as assessed at the follow-up measurement. The diet given, which was actually somewhat lower in saturated fat, polyunsaturated fat and cholesterol than the diet consumed in the seventies, still did not abolish the age-related rise in serum cholesterol.

DISCUSSION

At baseline, our subjects had mean cholesterol values (Table 2) in the range considered optimal for the prevention of coronary heart disease. Similar values are found for 20-year-olds in other affluent populations. Those in the USA[7] are even lower than in the Netherlands, as noted previously.[39] However, from the age of 20 onwards serum cholesterol started to creep up, by about 0.08 mmol/l per year in men and 0.04 mmol/l per year in women. These longitudinal changes are almost identical to what can be calculated from the cross-sectional data of the LRC prevalence study.[8] We are not aware of any other longitudinal studies over this age span. As a consequence of the dismissal of people with serum cholesterol levels above 5.7 mmol/l, it is possible that the increase of serum cholesterol with age was overestimated somewhat in our sample due to regression to the mean. However the number of people excluded for this reason was only about 6% of

TABLE 4 *Characteristics and diets of 34 student volunteers as measured at baseline in 1974–1979 in Wageningen, and changes over the period from baseline until the end of the 'back to the seventies' trial†.*

Characteristics	Mean ± sd at baseline		Mean change ± sd	
	Women (n = 14)	Men (n = 20)	Women (n = 14)	Men (n = 20)
Age (years)	21.9 ± 2.9	22.1 ± 3.0	8.5 ± 2.0	7.6 ± 1.9
Height (cm)	169.5 ± 1.0	183.5 ± 1.7	0.4 ± 1.0	0.6 ± 1.7
Weight (kg)	61.0 ± 2.7	71.7 ± 3.0	1.5 ± 2.7	1.8 ± 3.0*
Body mass index (kg/m²)	21.0 ± 1.0	21.3 ± 1.9	0.5 ± 0.9	0.4 ± 1.0
Serum total cholesterol (mmol/l)	4.86 ± 0.84	4.44 ± 0.68	0.03 ± 0.61	0.34 ± 0.43**
Serum HDL-cholesterol (mmol/1)‡	1.42 ± 0.18	1.55 ± 0.29	0.04 ± 0.35	−0.27 ± 0.11**
Habitual diet				
Energy (MJ/day)	8.4 ± 2.0	13.8 ± 4.5	−0.7 ± 1.8	−3.1 ± 2.6**
Protein (%Energy)	15.2 ± 1.8	13.1 ± 2.6	−0.5 ± 2.0	0.4 ± 2.9
Fat (%Energy)	36.4 ± 7.6	37.0 ± 5.5	−4.7 ± 8.7	−5.3 ± 6.0**
Saturated fatty acids (%Energy)	16.1 ± 3.1	15.2 ± 2.4	−1.6 ± 3.7	−0.9 ± 2.9
Polyunsaturated fatty acids (%Energy)	5.9 ± 1.1	7.1 ± 2.2	−0.4 ± 1.8	−1.8 ± 2.3**
P/S ratio	0.38 ± 0.08	0.48 ± 0.20	0.01 ± 0.13	−0.10 ± 0.22
Carbohydrates (%Energy)	46.5 ± 8.7	47.0 ± 6.1	2.4 ± 9.0	3.9 ± 5.7**
Sugars (%Energy)	24.7 ± 6.6	19.8 ± 5.3	−0.2 ± 6.4	2.7 ± 6.1
Alcohol (%Energy)	1.8 ± 2.1	2.9 ± 3.4	2.8 ± 3.4**	1.1 ± 4.3
Fibre (g/MJ)	3.6 ± 1.1	3.4 ± 0.8	0.3 ± 1.4	−0.4 ± 0.9
Cholesterol (mg/MJ)	30.7 ± 13.3	27.5 ± 9.0	−5.6 ± 14.1	0.2 ± 15.5
Keys score (mmol/l)§	5.69 ± 0.28	5.56 ± 0.24	−0.14 ± 0.03	−0.00 ± 0.31

† The trial was performed in the spring of 1985 with subjects living at their homes in various places in the Netherlands.
‡ Serum HDL-cholesterol was measured in 3 women and 5 men.
§ The Keys score[28] was calculated as $168/38.7 + 1.35(2S-P)/38.7 + 1.5\sqrt{Z}/38.7$, where S = energy % saturated fatty acids, P = energy % polyunsaturated fatty acids, Z = cholesterol in mg per 1000 kcal.
* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, two-tailed t-test.

the total number screened, so the influence of excluding them was small. The levels of serum cholesterol of the people excluded ranged between 5.7 mmol/l and 8.5 mmol/l, so that genetic abnormalities among them were probably rare.

Changes in food habits did not explain the serum cholesterol increase with age. These findings were confirmed by the 'back to the seventies' trial; serum cholesterol decreased by only 0.1 mmol/l when subjects went back to the diet that was habitual at baseline measurements, leaving a residual increase of 0.03 mmol/l in women and of 0.34 mmol/l in men. The duration of the 'back to the seventies' trial of three weeks should have been sufficient to show the influence of diet on the change in serum cholesterol, as reviewed by Katan *et al.*[40] Thus the residual change appears to have been a consequence of determinants other than diet. As far as we know, no other data are available on food consumption changes as determinants of the serum cholesterol increase with age in man.

In males, changes in body mass index were found to be related to changes in serum cholesterol; a change of 1 kg/m² was accompanied by a change of 0.07 mmol/l in serum cholesterol. This value is almost equal to that found in the Framingham Offspring study and in the Baltimore study.[13,14] In ours as in other studies, only a small part of the change in serum cholesterol can be explained by changes in body mass index.

There are some suggestions as to other determinants of the increase in serum cholesterol with age.

Rossouw *et al* suggested that there is a cumulative effect of chronic diet-induced lipid overload.[41] Long-term dietary intervention studies have, however, provided no evidence for slow effects of diet on serum lipids; in general, changes in serum cholesterol were completed within a few weeks after a change in diet.[42,43] Ageing is accompanied by a decrease in LDL-receptor activity,[44,45] and Mistry *et al* suggested that in people with a lower receptor activity cholesterol levels are more susceptible to the effect of dietary cholesterol.[46] These findings together suggest that as people grow older they become more sensitive to cholesterol-elevating factors in the diet. However, in our studies of hypo-and hyper-responders to dietary cholesterol we found no evidence whatever for enhanced susceptibility in older people of serum cholesterol to diet.[40]

One determinant that may be important is body fat distribution. As people age they not only become fatter, but in men the fat also tends to concentrate more in the abdominal region at the expense of peripheral subcutaneous fat.[47,48] Larsson concluded that an abdominal adipose tissue distribution ('apple-shape' as opposed to 'pear-shape') predicts ischaemic heart disease and death independently of other obesity indices, but not independently of serum cholesterol concentration.[49] It is possible that the change of serum cholesterol with age is related to accumulation of adipose tissue in the abdomen, but until now no longitudinal studies on this have been carried out.

We conclude that in this population the increase of serum cholesterol between ages 20 and 30 was not caused by changes in dietary habits, but that some of the increase can be explained by changes in body mass index.

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