INCREASE IN BODY FATNESS AS A MAJOR DETERMINANT OF CHANGES IN SERUM TOTAL CHOLESTEROL AND HIGH DENSITY LIPOPROTEIN CHOLESTEROL IN YOUNG MEN OVER A 10-YEAR PERIOD

MONIQUE A. M. BERNS, JEANNE H. M. DE VRIES, AND MARTIJN B. KATAN

Berns, M. A. M., J. H. M. de Vries, and M. B. Katan (Dept. of Human Nutrition, Agricultural U., Bomenweg 2, P.O. Box 8129, 6700 EV Wageningen, The Netherlands). Increase in body fatness as a major determinant of changes in serum total cholesterol and high density lipoprotein cholesterol in young men over a 10-year period. Am J Epidemiol 1989;130:1109–22.

Serum cholesterol rises with age in most Western (and Westernized) populations. To identify causes of this rise, the authors studied 315 young Dutch men in 1976 in the district of Utrecht, The Netherlands, when they were aged 18 or 19 years, and again in various towns in the same region 10 years later, in 1986. These men formed the lower and upper quartiles of the distribution of changes in body mass index (weight (kg)/height (m)^2) from 1976 to 1986 in a larger cohort of men representative of all Dutch men aged 18 or 19 years in 1976. In 10 years, mean serum total cholesterol ($\pm$ standard deviation) had increased by 1.20 $\pm$ 0.88 mmol/liter (46 $\pm$ 34 mg/100 ml), and high density lipoprotein (HDL) cholesterol had decreased by 0.12 $\pm$ 0.21 mmol/liter (4.6 $\pm$ 8.1 mg/100 ml). The mean increase in body mass index was 2.7 $\pm$ 2.5 kg/m^2, and the mean increase in body fat percentage (assessed from skinfolds) was 3.3 $\pm$ 4.6 g/100 g. The mean subscapular:tricipital skinfold thickness ratio—an indicator of body fat distribution—had not changed. In multiple regression analysis, the change in body mass index was the only significant ($p < 0.001$) determinant of changes in serum total cholesterol; an increase of 1 kg/m^2 in body mass index was associated with an increase of 0.20 mmol/liter (standard error, 0.02) in serum total cholesterol. Changes in body mass index and in smoking habits both contributed significantly toward explanation of changes in HDL cholesterol and in the HDL cholesterol:total cholesterol ratio. If smoking habits were adjusted for, HDL cholesterol decreased by 0.02 mmol/liter and the HDL cholesterol:total cholesterol ratio decreased by 0.012 (standard error, 0.001) for every 1 kg/m^2 increase in body mass index. Changes in body fat distribution, as assessed by skinfold ratio, were not associated with changes in lipids. By interpolation, the authors estimated that for the full cohort of men, including the second and third quartile of body mass index changes, the mean rise in cholesterol had been 1.15 mmol/liter (44 mg/100 ml), of which 0.47 mmol/liter could be explained by the estimated rise in body mass index of 2.4 kg/m^2. An increase in body fatness between ages 19 and 29 years is a powerful determinant of the rise in total cholesterol and the fall in HDL cholesterol occurring over that period of time.

aging; cholesterol; diet; lipoproteins, HDL cholesterol; men; obesity; smoking

Both longitudinal and cross-sectional studies have shown that serum cholesterol levels increase with age in Western (1–5) and Westernized (6, 7) populations. This

Abbreviations: HDL, high density lipoprotein; LDL, low density lipoprotein.

increase is the main source of the wide-spread mild hypercholesterolemia seen in such populations from age 40 years onward. The causes of this change with age have not been clarified. Changes in the nutrient composition of the diet have not been shown to play a major role (4). However, the absence of a rise in serum cholesterol with age in primitive populations does suggest that the rise of cholesterol levels is not an inexorable consequence of growing older, and that life-style factors could be important. One of these factors could be a combination of high energy intake and low energy expenditure leading to fatness. In some studies, changes in body mass index (weight (kg)/height (m)^2) were indeed found to explain a small proportion of the changes in serum cholesterol in young adults (2, 4, 5).

Recently, it has become clear that the lipoprotein profile and the risk of coronary heart disease and other diseases is influenced not just by the amount of fat in the body but also by its distribution. Thus, a change in body fat topography could be another cause of the rise of cholesterol with age. Indeed, both Seidell et al. (8) and Enzi et al. (9) found that, as people age, not only do they become fatter but the fat tends to concentrate more in the abdominal region at the expense of peripheral subcutaneous fat.

Cross-sectional data (10) suggest that, as people age, not only do total cholesterol and low density lipoprotein (LDL) cholesterol go up, but in males high density lipoprotein (HDL) cholesterol tends to

decrease as well. On the other hand, longitudinal data of Hubert et al. (2) could not show a significant decrease of HDL cholesterol over a period of eight years.

The changes of serum total cholesterol and HDL cholesterol with age and their determinants clearly require further study. Therefore, we examined the impact of changes in various measures of obesity and of changes in smoking habits on serum total cholesterol and HDL cholesterol over a period of 10 years in a cohort of 315 men initially aged 18 or 19 years.

MATERIALS AND METHODS

Selection of the subjects

In the Netherlands, all males aged 18 years are submitted to a one-day medical examination to determine their fitness for military service. In 1976, Bos (11) used the occasion of the medical examination to carry out an ancillary study concerning coronary risk factors in men examined in one of the nation's seven army examination districts, Utrecht (11). Of the 3,066 men approached by Bos, 3,058 were willing to participate; between August 30 and December 3, 1976, data were collected on 3,017 of them (table 1). The district of Utrecht is located in the central Netherlands. It contains two cities with more than 100,000 inhabitants and another two cities with 50,000 to 100,000 inhabitants; about half of the subjects were living in one of these four cities. The other half came from smaller villages in the same region. In 1986, we were able to trace 1,970 of the 3,017 original subjects, and we approached them by letter. We asked them if they would be willing to participate in a new study, and we inquired about their present weight. Of the 1,159 subjects (59 percent) who were willing to participate, 242 were dropped by us because they lived too far away, leaving 917 subjects.

Because our resources did not allow us to study all of these subjects in depth, we decided to select subjects with extreme values of the variables of interest, because in

TABLE 1

Numbers of subjects at various stages of a study on body fatness and serum cholesterol, and selection procedure for subjects reexamined at follow-up: men aged 18 or 19 years in 1976 living in the district of Utrecht, the Netherlands

Stage of study	No. excluded	No. included
Examined for army service in the district of Utrecht, 1976		3,066
Examined in ancillary examination for coronary risk factors, 1976, and data still available in 1986		3,017
Traced and approached in 1986		1,970
Replies		1,463
Refusals	185	
Reply incomplete or received too late for processing	+119	
	304	
Willing to be reexamined, and present weight reported		1,159
Living close enough to examination centers		917
Change in body mass index (weight (kg)/height (m)^2) inside ranges of interest (−1.16 to 1.25 kg/m^2 and >3.50 kg/m^2)		476
No reply to final invitation	54	
Medications or diseases affecting lipoproteins	33	
Ill at time of follow-up examination	26	
Could not be fitted into examination schedule	+48	
Not reexamined at follow-up	161	
Reexamined at follow-up, 1986		315

a linear model this would allow the most accurate estimation of regression coefficients for the number of subjects investigated. Because we were interested in the impact of longitudinal changes in body fatness and body fat distribution on serum total cholesterol and HDL cholesterol, we made an estimate of the present body mass index and of the change in body mass index over the previous 10 years using the reported present weight and the height and weight measured 10 years earlier. Then we ranked subjects by their change in body mass index and selected the two extreme quartiles for further study. This produced two groups: One group consisted of 235 subjects whose change in body mass index over the 10 years of follow-up ranged from −1.16 to 1.25 kg/m^2 (mean = 0.45 kg/m^2). They represented the lowest 26 percent of the total distribution. In the other group of 241 subjects, the change ranged from 3.51 to 11.08 kg/m^2 (mean = 4.89 kg/m^2); they represented the highest 26 percent of the distribution of body mass index changes.

These 476 persons were invited to participate in the present study. The letter of invitation also contained a standard medical questionnaire used by us in screening subjects for participation in dietary trials (12). A total of 54 subjects did not answer this second invitation, 33 were dropped for medical reasons (i.e., use of medications, the presence of diseases which can affect serum lipoproteins, or reported changes in body weight of more than 5 kg in the past few weeks), another 26 because of illness at the time of the examination, and the other 48 because they could not be fitted into the examination schedules. The remaining 315 persons (166 from the "constant weight" group and 149 from the "weight increase" group) were investigated twice at a one-week interval. The full selection scheme is presented in table 1.

Anthropometric measurements

In 1976, the baseline measurements took place in the Knoop barracks in Utrecht. Body weight and height were measured in

a standardized manner, with the examinees dressed in their underwear. Body weight was measured to the nearest 0.1 kg with a beam balance, which was calibrated before the study. The bicipital (front of the upper arm), tricipital (back of the upper arm), subscapular (back, below the shoulder blade), and suprailiac (side, just above the hipbone) skinfolds were measured with a Harpenden skinfold caliper by one member of the team (11).

In 1986, the measurements took place in hospitals in the central Netherlands or at our department at Agricultural University. Four thoroughly trained nutrition students and two members of the department staff carried out the measurements. Weight and height were measured twice at a one-week interval, with the subjects wearing their underwear only. The electronic balances used were checked regularly with 60-kg calibration weights. The thickness of skinfolds was measured in triplicate with a Harpenden skinfold caliper; if subjects were right-handed, they were measured at the left hand side, and if they were left-handed, they were measured at the right hand side of the body. The circumferences of the waist and hip were measured with a measuring tape in duplicate at the first of the two examinations.

In both 1976 and 1986, body fat percentage was estimated from the sum of the thicknesses of the bicipital, tricipital, subscapular, and suprailiac skinfolds according to the method of Durnin and Womersley (13).

Smoking, physical activity, and socioeconomic status

In 1976, the subjects filled out a questionnaire concerning their smoking habits and socioeconomic status. Socioeconomic status was derived from the socioeconomic status of the parents (11). In 1986, the same questionnaire as in 1976 was used to determine smoking habits (11). Socioeconomic status was now derived from the level of education and the occupation of the participants themselves; they were graded into three categories—low, middle, and high (14). The participants' level of physical activity was also now determined using a published questionnaire developed in our department (15). Three aspects were measured: physical activity at work, sports activities, and other physical activity during leisure time. The level of activity was coded on a scale ranging from 1 (low) to 5 (high) for each of these three dimensions of physical activity (15).

Diet

Diet was assessed in 1986 but not in 1976. At the first of the two appointments in 1986, subjects were given a dietary history questionnaire which they filled out at home. At the second appointment, the questionnaire was discussed point by point with the subject, and a food frequency list was also obtained. The dietary history and the food frequency list were combined, and a mean daily diet was calculated using the 1985 release of the Dutch national nutrient data base UCV (16).

Blood sampling and analysis

In 1976, nonfasting blood was sampled from an antecubital vein into an evacuated tube and was allowed to clot. Serum was obtained by low-speed centrifugation for 10 minutes; the sera were stored at 4 C and were transported within one week to our laboratory in Wageningen, where they were stored for a maximum of two weeks at −20 C before analysis. Cholesterol was analyzed with a method equivalent to that of Abell et al. (17) as previously described (18). At that time, our laboratory was certified by the Centers for Disease Control, Atlanta, Georgia, as meeting the Centers for Disease Control/World Health Organization criteria for accuracy and precision in cholesterol analysis; bias for control sera provided by the Centers for Disease Control was within 1.1 percent of the target value for total cholesterol. HDL cholesterol was determined in a rigidly standardized manner by manganese-heparin precipitation as described earlier (19).

In 1986, nonfasting blood was sampled at both examinations from an antecubital vein using evacuated tubes. Serum was obtained by low-speed centrifugation. The serum samples were stored in the hospitals at −20 C, transported within one week in the frozen state to Wageningen, and kept at −80 C until analysis. Serum total cholesterol and HDL cholesterol were determined in the same rigidly standardized laboratory that had performed the baseline analyses in 1976. Sera were analyzed for total cholesterol and HDL cholesterol and for triglycerides using enzymatic methods and strict quality control (18, 20–22). The maximum interval between collection of sera and analysis was three months. After introduction of the enzymatic cholesterol method into our laboratory in 1983, reanalysis of serum pools prepared and first analyzed in 1975 showed that the analytic drift of the absolute level of serum total cholesterol in those pools had been less than 1.7 percent over the eight-year period of storage from 1976 to 1983 (4). At the time of the follow-up analyses in 1986, the combined within- and between-run coefficient of variation for control sera was 0.8 percent for total cholesterol, 1.7 percent for HDL cholesterol, and 0.3 percent for triglycerides. As in 1976, accuracy was checked by analysis of serum pools provided by the Centers for Disease Control. Mean bias with regard to target values was −0.7 percent for total cholesterol, −4.4 percent for HDL cholesterol, and 0.9 percent for triglycerides.

Cholesterol values are expressed here in mmol/liter; 1 mmol/liter equals 38.7 mg/ 100 ml.

RESULTS

Characteristics of the subjects

The 1,159 subjects who said in 1986 that they were willing to be reexamined reported present weights ranging from 48.5 to 120 kg (mean ± standard deviation = 76.5 ± 10.6 kg). These reported weights allowed us to calculate hypothetical changes in body mass index over the previous 10 years and

to select subjects at the upper and lower ends of the distribution of body mass index for further investigation.

In table 2, baseline characteristics of these 315 participants are compared with those of nonparticipants, that is, those 2,702 subjects examined in 1976 but not in 1986 (cf. table 1). No differences were found between participants and nonparticipants in initial HDL cholesterol or in socioeconomic status. The initial body mass index and serum total cholesterol and the percentage of subjects smoking at baseline were lower in participants than in nonparticipants, but the extent of the differences was very slight.

In table 3, baseline characteristics are presented for subgroups of participants categorized according to the increase in their body mass index over the 10-year follow-up period. The middle group of 58 subjects arose because the self-reported weights of these subjects did not equal their weights as actually measured, so that their true change in body mass index fell between 1.25 and 3.5 kg/m². No differences were found in baseline total cholesterol or HDL cholesterol, body mass index, or socioeconomic status between subjects who had subsequently gained an appreciable amount of weight and those whose weight had remained stable. Only the percentage of subjects who smoked at baseline was larger in the group that subsequently was to gain a large amount of weight.

Table 3 also presents the changes in serum lipids and body fatness from baseline to follow-up for all subjects combined and for the various categories of weight gain separately. The mean total cholesterol level for all 315 participants combined had increased by 1.20 mmol/liter (46 mg/100 ml) or 28 percent, while the mean HDL cholesterol level had decreased by 0.12 mmol/liter (4.6 mg/100 ml) or 10 percent. The HDL cholesterol:total cholesterol ratio had decreased by 0.09 or 29 percent. On average, all body fat indices had risen except for the subscapular:tricipital skinfold thickness ratio, which equalled 1.15 mm/mm in 1976

TABLE 2

Baseline (1976) characteristics (mean ± standard deviation) of subjects in a study on body fatness and serum cholesterol, by whether or not they were reexamined at follow-up (1986): men aged 18 or 19 years at baseline living in the district of Utrecht, the Netherlands

	Reexamined in 1986	Not reexamined in 1986	
		All subjects	Eligible subjects*
No.	315	2,701	161
Age on October 16, 1976 (years)	18.5 ± 0.5	18.5 ± 0.5	18.4 ± 0.5
Height (cm)	180.6 ± 6.7	180.2 ± 6.9	179.6 ± 6.3
Weight (kg)	68.1 ± 8.2	68.6 ± 9.8	68.9 ± 9.0
Body mass index (weight (kg)/height (m)²)	20.8 ± 2.0	21.2 ± 2.7†	21.4 ± 2.6
Body fat % (g/100 g)	14.1 ± 3.9	14.2 ± 4.6	14.8 ± 4.7
Subscapular:tricipital skinfold thickness ratio	1.14 ± 0.28	1.18 ± 0.28†	1.17 ± 0.26
Serum cholesterol (mmol/liter)			
Total cholesterol	4.23 ± 0.74	4.33 ± 0.79†	4.44 ± 0.79
High density lipoprotein (HDL) cholesterol	1.30 ± 0.27	1.29 ± 0.28	1.29 ± 0.27
HDL cholesterol:total cholesterol ratio	0.31 ± 0.08	0.30 ± 0.08†	0.30 ± 0.07
Cigarette smokers (%)	47	53‡	47
No. of cigarettes smoked per day§	12 ± 6.3	14 ± 8.3‖	14.0 ± 7.5
Socioeconomic status	2.7 ± 0.7	2.8 ± 0.7	2.9 ± 0.7

* Subjects were eligible for reexamination if their body mass index had changed by less than 1.25 kg/m² or more than 3.5 kg/m² in 10 years.

† Significantly different between the two categories ($p < 0.05$, two-tailed t test).

‡ Significantly different between the two categories (chi-square test).

§ Smokers only.

‖ Significantly different between the two categories ($p < 0.01$, two-tailed t test).

and 1.14 mm/mm in 1986. The mean weight reported by these 315 subjects in their answer to our first letter was, on average, 0.8 kg (range, −6.6–7.1 kg) lower then the actual weight measured by us. The difference between actual and reported weight was 0.8 ± 2.0 kg for the subgroup with little weight change and 0.9 ± 2.6 kg for those 241 subjects who had become appreciably fatter over the previous 10 years.

Large differences between the subgroups were found for changes in serum total cholesterol, HDL cholesterol, and the percentage of subjects who started or stopped smoking. Of course, the subgroups also showed different increments in body mass index and body fat percentage, because that was the criterion by which the subgroups had been formed. Total cholesterol had increased by 0.74 mmol/liter (29 mg/100 ml) in subjects with a small change in body mass index and by 1.69 mmol/liter (65 mg/ 100 ml) in subjects with an increase in body mass index of more than 3.5 kg/m². The mean changes in HDL cholesterol and in the ratio of HDL cholesterol to total cholesterol were −0.06 mmol/liter (2.3 mg/100 ml) and −0.06, respectively, for subjects with a small change in body mass index, and −0.18 mmol/liter (7.0 mg/100 ml) and −0.12, respectively, for subjects with an increase in body mass index of more than 3.5 kg/m². The distribution of fat over the body, as assessed by the thickness of the subscapular skinfold (on the back) divided by the thickness of the tricipital skinfold (at the back of the upper arm) had not changed in either group.

In table 4, the composition of the diet and the level of physical activity in 1986 are given. Baseline dietary and activity data were, unfortunately, not available. Subjects who had accumulated more body fat in the previous 10 years turned out to have a higher present intake of cholesterol ($p <$

TABLE 3

Baseline (1976) characteristics (mean ± standard deviation) and changes over 10 years in 315 participants in a study on body fatness and serum cholesterol, by category of change in body mass index over 10 years: men aged 18 or 19 years at baseline living in the district of Utrecht, the Netherlands

	Baseline level in 1976, by subsequent change in body mass index				Change in level (1976-1986), by change in body mass index			
	<1.25 kg/m²	1.25-3.5 kg/m²*	>3.5 kg/m²	All	<1.25 kg/m²	1.25-3.5 kg/m²*	>3.5 kg/m²	All
No.	123	58	134	315	123	58	134	315
Height (cm)	180.6 ± 7.0	181.5 ± 5.7	180.2 ± 6.8	180.6 ± 6.7	0.4 ± 1.4	0.2 ± 1.2	0.6 ± 1.4	0.5 ± 1.4
Weight (kg)	68.4 ± 8.26	69.2 ± 8.3	67.2 ± 8.2	68.1 ± 8.2	1.1 ± 2.7	6.9 ± 2.6	17.5 ± 5.2	9.1 ± 8.4
Body mass index (weight (kg)/height (m)²)	20.9 ± 2.0	21.0 ± 2.2	20.7 ± 2.0	20.8 ± 2.0	0.3 ± 0.8	2.0 ± 0.6	5.1 ± 1.5	2.7 ± 2.5
Body fat % (g/100 g)	14.1 ± 3.9	14.3 ± 3.6	14.1 ± 4.0	14.1 ± 3.9	-0.5 ± 2.9	2.3 ± 2.2	7.3 ± 3.2	3.3 ± 4.6
Serum cholesterol (mmol/liter)								
Total cholesterol	4.28 ± 0.77	4.21 ± 0.75	4.20 ± 0.70	4.23 ± 0.74	0.74 ± 0.63†	1.04 ± 0.74†	1.69 ± 0.90†	1.20 ± 0.88
High density lipoprotein (HDL) cholesterol	1.32 ± 0.28	1.29 ± 0.23	1.28 ± 0.26	1.30 ± 0.27	-0.06 ± 0.21‡	-0.10 ± 0.18	-0.18 ± 0.21	-0.12 ± 0.21
HDL cholesterol:total cholesterol ratio	0.32 ± 0.08	0.31 ± 0.07	0.31 ± 0.08	0.31 ± 0.08	-0.06 ± 0.05†	-0.08 ± 0.04†	-0.12 ± 0.06†	-0.09 ± 0.06
Cigarette smokers (%)§	40	50	52	47				
Started smoking (%)					11	10	7	9
Stopped smoking (%)					3	10	17	11
No. of cigarettes smoked per day‖	12.1 ± 6.5	13.8 ± 5.6	11.9 ± 6.5	12.3 ± 6.4	3.3 ± 7.3	2.4 ± 8.6	3.6 ± 8.9	3.5 ± 8.5
Socioeconomic status§	2.7 ± 0.7	2.7 ± 0.7	2.8 ± 0.7	2.7 ± 0.7				

* Subjects in this category were originally assigned to the upper (>3.5 kg/m²) or lower (<1.25 kg/m²) quartile of body mass index change based on their self-reported weight in 1986, but had to be recategorized after they had been weighed by us.

† All categories were significantly different by one-way analysis of variance.

‡ The category of subjects with a change in body mass index of less than 1.25 kg/m² was significantly different from the other categories by one-way analysis of variance.

§ Differences between the categories were analyzed by chi-square test.

‖ Smokers only.

TABLE 4

Diet and physical activity (mean ± standard deviation) of 315 participants in a study on body fatness and serum cholesterol in 1986, by category of change in body mass index (weight (kg)/height (m)²) over the preceding 10 years: men aged 28 or 29 years living in the district of Utrecht, the Netherlands

	Change in body mass index in 10 years (1976–1986)		
	<1.25 kg/m²	1.25–3.5 kg/m²	>3.5 kg/m²
No.	123	58	134
Diet			
Energy (kcal/day)	3,222 ± 877	3,230 ± 795	3,361 ± 842
(MJ/day)*	13.5 ± 3.7	13.5 ± 3.3	14.1 ± 3.5
Protein (% of energy)	13.4 ± 2.1	13.4 ± 1.9	13.6 ± 2.2
Fat (% of energy)	39.1 ± 5.9	39.1 ± 4.4	39.4 ± 5.6
Saturated fatty acids	15.6 ± 2.5	15.5 ± 2.2	15.5 ± 2.4·
Polyunsaturated fatty acids	6.7 ± 2.1	6.9 ± 1.8	6.6 ± 1.8
Carbohydrates (% of energy)	43.9 ± 6.3	44.1 ± 5.2	42.3 ± 5.3
Mono- and disaccharides	20.9 ± 5.9	21.6 ± 5.0	21.0 ± 5.5
Polysaccharides	22.5 ± 5.2†	22.0 ± 4.0	20.7 ± 3.9
Alcohol (% of energy)	4.6 ± 4.7	4.4 ± 3.7	5.6 ± 4.5
Cholesterol (mg/MJ)*	29.0 ± 6.6	30.2 ± 7.0	32.6 ± 7.0‡
Dietary fiber (g/MJ)*	2.5 ± 0.9†	2.3 ± 0.5	2.2 ± 0.6
Keys score (mmol/liters)§	5.62 ± 0.22	5.62 ± 0.19	5.64 ± 0.18
Physical activity‖			
At work	2.6 ± 0.8	2.7 ± 0.8	2.8 ± 0.7
Sports	2.6 ± 0.8	2.7 ± 0.9	2.7 ± 0.7
Leisure time	2.7 ± 0.7†	2.6 ± 0.6	2.4 ± 0.6
Total	8.0 ± 1.3	7.9 ± 1.3	7.9 ± 1.2

* One megajoule (MJ) equals 239 kcal.

† Subjects with a change in body mass index of less than 1.25 kg/m² differed significantly from the other two subgroups by one-way analysis of variance.

‡ Subjects with a change in body mass index of more than 3.5 kg/m² differed significantly from the other two subgroups by one-way analysis of variance.

§ The Keys score quantitates the effect of diet on serum cholesterol. It was calculated as $168/38.7 \pm 1.35$ $(2S - P)/38.7 + 1.5 \times Z^{0.5}/38.7$, where S is the percentage of daily energy intake provided by saturated fatty acids, P is the percentage provided by polyunsaturated fatty acids, and Z is the cholesterol intake in mg/1,000 kcal.

‖ Scored in arbitrary units on a scale from 1 (low) to 5 (high).

0.001) and a lower intake of polysaccharides and dietary fiber. However, there were no differences between subgroups in the Keys score, a composite score of saturated fat, polyunsaturated fat, and cholesterol intake that summarizes dietary influences on serum cholesterol (23). Differences in physical activity were minimal, except for a higher activity level during leisure time in the group that had gained the least amount of weight.

Relations of changes in smoking and body fat indices to changes in serum lipids

Univariate linear regression coefficients were calculated to examine the association of changes in lipids (HDL cholesterol and total cholesterol and their ratio) with the changes in body fat indices (body mass index, body fat percentage as calculated from the sum of skinfolds, and the ratio of subscapular to tricipital skinfold thickness) and with changes in smoking habits. As is shown in table 5 and figure 1, the change in total cholesterol was strongly associated with changes in either of two indicators of body fatness—body mass index or body fat percentage. The association with the change in the subscapular:tricipital skinfold thickness ratio was significant, but was in the direction opposite of what we had expected. In subjects in whom the skinfold

TABLE 5

Univariate regression of changes in total cholesterol, high density lipoprotein (HDL) cholesterol, and the HDL cholesterol:total cholesterol ratio on changes in body mass index (weight (kg)/height (m)^2), body fat percentage, subscapular:tricipital skinfold thickness ratio, and smoking habits over a 10-year period: 315 men examined initially at age 18 or 19 years in 1976 in the district of Utrecht, The Netherlands, and reexamined in 1986

	Dependent variable								
Independent variable	Change in total cholesterol (mmol/liter)			Change in HDL cholesterol (mmol/liter)			Change in HDL cholesterol:total cholesterol ratio		
	b^*	SE*	p	b	SE	p	b	SE	p
Change in body mass index (kg/m^2)	0.191	0.017	<0.001	−0.024	0.005	<0.001	−0.012	0.001	<0.001
Change in body fat % (g/100 g)†	0.092	0.010	<0.001	−0.007	0.003	0.005	−0.005	0.001	<0.001
Change in subscapular:tricipital skinfold ratio	−0.525	0.166	0.002	0.036	0.040	0.38	0.032	0.011	0.004
Change in smoking habits‡	−0.122	0.122	0.32	−0.057	0.029	0.05	−0.004	0.008	0.66

* b, regression coefficient; SE, standard error.
† As calculated from the sum of four skinfold thicknesses.
‡ Changes in smoking habits were scored as follows: 0, no change; 1, started smoking; −1, stopped smoking.
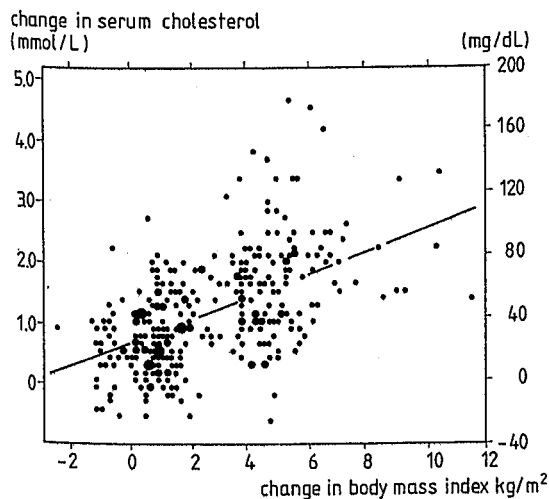

FIGURE 1. Relation between changes in body mass index (weight (kg)/height (m)^2) and serum total cholesterol over a 10-year period (1976–1986): 315 men initially examined at age 18 or 19 years in the district of Utrecht, the Netherlands.

FIGURE 2. Relation between changes in body mass index (weight (kg)/height (m)^2) and serum high density lipoprotein (HDL) cholesterol over a 10-year period (1976–1986): 315 men initially examined at age 18 or 19 years in the district of Utrecht, the Netherlands.

thickness on the back had decreased relative to that on the upper arm, total cholesterol had risen more than in subjects in whom fat accumulation had centered more on the back and less on the arms.

HDL cholesterol had decreased as either indicator of body fatness had increased; the relation to the change in body mass index is given in figure 2. HDL chol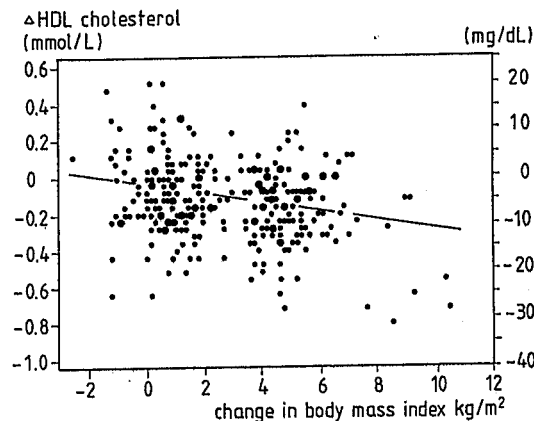esterol had also changed inversely with the number of cigarettes smoked. There was no association between changes in HDL cholesterol and changes in the subscapular:tricipital skinfold thickness ratio. Changes in the HDL cholesterol:total cholesterol ratio were significantly associated with changes in all three body fatness indicators. In univariate analysis, the HDL cholesterol:total cholesterol ratio did not change significantly with changes in smoking behavior.

To correct for the interrelations between changes in smoking behavior, body fatness,

TABLE 6

Multiple regression of changes in total cholesterol, high density lipoprotein (HDL) cholesterol, and the HDL cholesterol:total cholesterol ratio on changes in body mass index (weight (kg)/height (m)²) and smoking habits over a 10-year period: 315 men examined initially at age 18 or 19 years in 1976 in the district of Utrecht, The Netherlands, and reexamined in 1986*

	Dependent variable								
Independent variable	Change in total cholesterol (mmol/liter)			Change in HDL cholesterol (mmol/liter)			Change in HDL cholesterol:total cholesterol ratio		
	b†	SE†	p	b	SE	p	b	SE	p
Constant	0.681	0.062	<0.001	−0.055	0.017	0.001	−0.065	0.004	<0.001
Change in body mass index (kg/m²)	0.195	0.017	<0.001	−0.024	0.005	<0.001	−0.012	0.001	<0.001
Change in smoking habits‡				−0.072	0.029	0.001	−0.015	0.007	0.03

* Body fat percentage and skinfold ratio were included in the multiple regression analysis, but regression coefficients did not differ significantly from zero.

† b, regression coefficient; SE, standard error.

‡ Changes in smoking habits were scored as follows: 0, no change; 1, started smoking; −1, stopped smoking.

and lipoproteins, we used stepwise multiple regression analysis (table 6). The only independent variable that then made a significant contribution to the explanation of changes in total cholesterol was the change in body mass index. An increase of 1 kg/m² in body mass index was associated with an increase of 0.20 mmol/liter (8 mg/100 ml) in total cholesterol. Going by the present weights reported by the 1,159 subjects willing to be reexamined, their estimated average change in body mass index over the follow-up period was 2.4 kg/m². This hypothetical figure is probably close to the truth, because for the 315 subjects actually reexamined, the mean estimated change, based on reported weight, was 2.6 kg/m², and the actual mean change, based on measured weights, was 2.7 kg/m². If we enter the value of 2.4 kg/m² into the multiple regression equation derived for the 315 subjects actually reexamined, we arrive at a hypothetical rise in total cholesterol of 1.15 mmol/liter (44 mg/100 ml) for the full group of 1,159 subjects. Of this rise of 1.15, 0.47 mmol/liter or 41 percent can be explained by the rise in body mass index, and 0.68 mmol/liter remains unexplained (table 6).

Changes in body mass index and in smoking habits both contributed significantly toward explanation of the changes in HDL cholesterol and in the ratio of HDL

cholesterol to total cholesterol. When smoking was held constant, an increase of 1 kg/m² in body mass index was associated with a decrease of 0.02 mmol/liter in HDL cholesterol and a decrease of 0.012 in the HDL cholesterol:total cholesterol ratio. An estimated decrease in HDL cholesterol for all 1,159 subjects who had reported their present weights to us by letter can be calculated in the same manner as was done above for total cholesterol. For all 1,159 subjects willing to participate, HDL cholesterol was estimated to have decreased by 0.11 mmol/liter over the period of 10 years. Smoking was held constant in this calculation because we had no information on the changes in the smoking habits of the 844 subjects not reexamined in 1986. Of the change of 0.11 mmol/liter, 0.05 mmol/liter or 50 percent could be explained by the estimated change in body mass index of 2.4 kg/m². In multiple regression analysis, changes in fat distribution as assessed by the subscapular:tricipital skinfold thickness ratio were not significantly associated with the change in either total cholesterol or HDL cholesterol.

DISCUSSION

Representativeness of the sample

At baseline in 1976, the mean serum cholesterol level of 4.23 mmol/liter (164 mg/

dl) in our 315 participants was quite similar to that in the 2,702 subjects not rescreened and to the levels found in other studies in young men in the Netherlands at that time (24). The body mass index at baseline was slightly lower in this sample than that found by Rookus et al. (25) in a study in 1980 in Ede. They found a mean body mass index for men aged 20–22 years of 22.6 kg/m², whereas we found a body mass index of 20.8 kg/m². Some of the difference can be ascribed to differences in age; in 1976, our study population was almost three years younger than the population studied by Rookus et al. (25). Values for height, weight, and cigarette use in the original examinees were also fairly similar to those in young men in the country as a whole (26, 27). This was to be expected, because the army examination covers almost the entire population of 18- and 19-year-old men, and in a population as small and homogeneous as that of the Netherlands, regional differences in the variables of interest are small (19). As table 2 shows, the 315 participants in the follow-up study in 1986 had approximately the same baseline characteristics as the nonparticipants except for small differences in body mass index and serum total cholesterol. The mean body weight of 76.3 kg reported by our 315 participants in 1986 was also quite similar to the mean weight of 76.5 kg reported by the 844 subjects who volunteered for rescreening but were not seen in 1986. Thus, for the major variables of interest, the sample studied by us at follow-up was fairly representative of all Dutch males originally aged 18 or 19 years in 1976.

Changes over 10 years in weight and body mass index

Over the follow-up period of 10 years, the mean weight had increased by 9 kg and the body mass index by 2.7 kg/m². These changes are much higher than those that can be derived from the study of Rookus et al. (25), who found an increase in weight of 0.4 kg per year or in body mass index of 0.16 kg/m² in a four-year mixed longitudinal study of subjects aged 19–31 years. In

other longitudinal studies (28–33), increases in weight of 0.6–0.8 kg per year were found in subjects of the same initial age as our participants. In the cross-sectional Lipid Research Clinics Prevalence Study (10), the mean body mass index was 22.1 for white males aged 15–19 years and 25.6 for those aged 25–29 years, which yields a difference similar to that found by us longitudinally. Thus, the increase in body fatness in our subjects was fairly typical of that experienced during early adulthood by men in affluent societies.

Total cholesterol. The increase in serum total cholesterol of 0.12 mmol/liter per year over the 10-year period is the same as that found by Clark et al. (33). In an earlier study, we found that alumni of our department had experienced an increase in total cholesterol of 0.08 mmol/liter per year over the 6- to 10-year period that had elapsed since their student days (4). In a longitudinal study by Gillum et al. (34) spanning a follow-up period of 20 years and in a study by Hubert et al. (2) with a follow-up period of eight years, an increase in total cholesterol of 0.04 mmol/liter per year was found. In both studies, the initial cholesterol levels were higher than those in our subjects. In the Lipid Research Clinics Prevalence Study, mean plasma total cholesterol was 149.9 mg/dl (3.87 mmol/liter) for white males aged 15–19 years and 182.2 mg/dl (4.71 mmol/liter) for those aged 25–29 years, a difference of 0.84 mmol/liter (10). Thus, the rise of total cholesterol with age in our population was similar to that found in other populations.

HDL cholesterol. In addition to a substantial rise in total cholesterol, our subjects had experienced a fall in HDL cholesterol of, on average, 0.12 mmol/liter (4.6 mg/dl). Baseline and follow-up determinations were both performed in the same rigidly standardized laboratory, under supervision of the same chief technician, and using serum calibrators always prepared in the same way. The duration and temperature of storage of sera were also such that they probably did not have a material effect on HDL levels (35). Therefore, the fall in

HDL was probably real, and it predicts a substantial increase in coronary heart disease risk (36, 37). Long term longitudinal data on the development of HDL cholesterol with aging are scarce. In the Lipid Research Clinics Prevalence Study, HDL in white males showed little difference between subjects aged 15–19 years (46.1 mg/dl) and subjects aged 25–29 years (44.7 mg/dl) (10). However, these are cross-sectional data.

Determinants of changes in total cholesterol and HDL cholesterol

Total cholesterol. The change in body mass index was the major explanatory variable for changes in total cholesterol. In multiple regression analysis, its explanatory power was superior to that of changes in body fatness as assessed by skinfold thickness measurements. Possibly this is due to the lesser precision of the measurement of skinfolds as opposed to measurements of height and weight.

An association between a rise in body mass index and increases in serum cholesterol was also found by Hubert et al. in a longitudinal study with 379 men and 497 women who were approximately the same age as our participants (2). In that study, a change in body mass index of 1 kg/m² was accompanied by a change of 0.11 mmol/liter in total cholesterol over a period of eight years. Our study demonstrated an even greater impact of changes in body mass index on total cholesterol, which increased by 0.20 mmol/liter for every 1 kg/m² gained. At the follow-up examination, the intakes of those nutrients that influence total cholesterol did not differ between the subgroups, as is shown by the equal Keys scores in table 4. Although baseline diets were also not measured, there is no reason to assume that those who were later to grow fatter were eating less saturated fat and cholesterol and more unsaturated fat at baseline than those whose weights remained stable. Therefore, the association between the rise in body mass index and the rise in total cholesterol is probably not explained by a change in the composition of the diet, except that in those subjects who grew fatter, caloric intake exceeded requirements. Subjects whose body mass index had increased by more than 3.5 kg/m² had a lower level of physical activity in their leisure time than subjects who had not grown fatter, the indices being 2.4 ± 0.6 and 2.7 ± 0.7, respectively ($p < 0.01$). In the absence of baseline data, it is impossible to tell which occurred first, the difference in activity or the difference in body fatness.

Our data (reference 4 and the present study) thus suggest that the rise in body fatness itself rather than a change in dietary composition was a major cause of the rise of serum cholesterol with age. For the other cohorts mentioned above, no dietary data were presented.

The rise of cholesterol with body fatness may be due to overproduction of very low density lipoproteins in the obese (38). Very low density lipoproteins contain cholesterol, and in addition, they are the precursors of low density lipoproteins, the main carriers of cholesterol in plasma.

HDL cholesterol. In our study, changes in body mass index and in smoking habits were the only significant determinants of the change in HDL cholesterol. The relation of body fatness and smoking to HDL cholesterol is known from cross-sectional studies. The mechanism responsible for it is essentially unknown. Shennan et al. (39) also reported that changes in smoking habits were related to changes in HDL cholesterol. Hubert et al. (2) found that, besides the variables already mentioned, changes in alcohol consumption explained a significant proportion of the variance in change in HDL cholesterol. We could not measure changes in alcohol consumption because no data were available about the alcohol consumption in 1976.

Although the change in the subscapular:tricipital skinfold thickness ratio was associated with the change in serum cholesterol in univariate analysis (table 5), it did not remain significant in the multiple

regression analysis. Thus, changes in body fat topology did not appear to be major determinants of changes in lipids and lipoproteins. Of course, the skinfold ratio that we used is a less-than-perfect measure of body fat distribution. In addition, it could be that errors in the measurements of the skinfold thicknesses obscured the real changes in these variables. Longitudinal assessment of changes in body fat distribution by more accurate methods is therefore warranted. For now, our study shows that the increase in body weight is the most important determinant of the age-related increase in serum total cholesterol, and that changes in body weight and smoking habits are important determinants of unfavorable changes in HDL cholesterol with aging.

REFERENCES

1. Baumgartner RN, Roche AF, Chumlea WC, et al. Fatness and fat patterns: associations with plasma lipids and blood pressures in adults, 18 to 57 years of age. Am J Epidemiol 1987;126:614–28.
2. Hubert HB, Eaker ED, Garrison RJ, et al. Lifestyle correlates of risk factor change in young adults: an eight-year study of coronary heart disease risk factors in the Framingham offspring. Am J Epidemiol 1987;125:812–31.
3. Hubert H, Castelli W, Garrison R. Longitudinal study of coronary heart disease risk factors in young adults: The Framington Offspring Study. (Abstract). Am J Epidemiol 1983;118:443.
4. Berns MAM, de Vries JHM, Katan MB. Determinants of the increase of serum cholesterol with age: a longitudinal study. Int J Epidemiol 1988; 17:789–96.
5. Hershcopf RJ, Elahi D, Andres R, et al. Longitudinal changes in serum cholesterol in man: an epidemiologic search for an etiology. J Chronic Dis 1982;35:101–14.
6. Moulopoulos SD, Adamopoulos PN, Diamantopoulos EI, et al. Coronary heart disease risk factors in a random sample of Athenian adults: The Athens Study. Am J Epidemiol 1987;126:882–92.
7. Orchard TJ, Rodgers M, Hedley AJ, et al. Changes in blood lipids and blood pressure during adolescence. Br Med J 1980;280:1563–7.
8. Seidell JC, Oosterlee A, Thijssen MAO, et al. Assessment of intra-abdominal and subcutaneous abdominal fat relation between anthropometry and computed tomography. Am J Clin Nutr 1987;45:7–13.
9. Enzi G, Gasparo M, Biondetti PR, et al. Subcutaneous and visceral fat distribution according to sex, age, and overweight, evaluated by computed tomography. Am J Clin Nutr 1986;44:739–46.
10. The Lipid Research Clinics population studies data book; vol 1. The prevalence study. Washing-

ton, DC: US Department of Health and Human Services, 1980. (NIH publication no. 80-1527).
11. Bos JS. Electrocardiografie en coronair risicoprofiel van jonge Nederlandse mannen (keurlingen voor de militaire dienstplicht). (In Dutch). PhD thesis. Utrecht: State University of Utrecht, 1985.
12. Van Raaij JMA, Katan MB, West CE, et al. Influence of diets containing casein, soy isolate, and soy concentrate on serum cholesterol and lipoproteins in middle-aged volunteers. Am J Clin Nutr 1982;35:925–34.
13. Durnin JVGA, Womersley J. Body fat assessed from total body density and its estimation from skinfold thickness: measurements on 481 men and women aged from 16 to 72 years. Br J Nutr 1974;32:77–97.
14. Westerlaak JM, Kropman JA, Collaris JWM. Beroepenklapper. (In Dutch). Nijmegen: Instituut Toegepaste Sociologie, 1975.
15. Baecke JAH, Burema J, Frijters JER. A short questionnaire for the measurement of habitual physical activity in epidemiological studies. Am J Clin Nutr 1982;36:936–42.
16. Kommissie UCV. UCV table. (In Dutch). The Hague: Voorlichtingsbureau voor de Voeding (Nutrition Education Bureau), 1985.
17. Abell LL, Levy BB, Brody BB, et al. A simplified method for the estimation of total cholesterol in serum and demonstration of its specificity. J Biol Chem 1952;195:357–66.
18. Katan MB, Van Der Haar F, Kromhout D, et al. Standardization of serum cholesterol assays by use of serum calibrators and direct addition of Liebermann-Burchard reagent. Clin Chem 1982; 28:683–6.
19. Van Der Haar F, Kromhout D. Food intake, nutritional anthropometry, and blood chemical parameters in 3 selected Dutch schoolchildren populations. Wageningen: H. Veenman & Zonen B.V., 1978.
20. Rösschlau P, Bernt E, Gruber W. Enzymatische bestimmung des gesamt-cholesterins in serum. (In German). Z Klin Chem Klin Biochem 1974;12: 403–9.
21. Warnick GR, Benderson J, Albers JJ. Dextran sulfate-Mg^{2+} precipitation procedure for quantitation of high-density-lipoprotein cholesterol. Clin Chem 1982;28:1379–88.
22. Sullivan DR, Kruijswijk Z, West CE, et al. Determination of serum triglycerides by an accurate enzymatic method not affected by free glycerol. Clin Chem 1985;31:1227–8.
23. Keys A, Anderson JT, Grande F. Serum cholesterol response to changes in the diet. III. Differences among individuals. Metabolism 1965;14: 766–75.
24. Knuiman JT, Katan MB. Cholesterolniveaus in serum in Nederland in vergelijking met die in de Verenigde State. (In Dutch). Ned Tijdschr Geneeskd 1985;129:2500–5.
25. Rookus MA, Burema J, Van't Hof MA, et al. The development of the body mass index in young adults. I. Age-reference curves based on a four-year mixed longitudinal study. In: Rookus MA, ed. Body mass index in young Dutch adults: its

development and the etiology of its development. Wageningen: PUDOC (Centre for Agricultural Publishing and Documentation), 1986:31–49.

26. Centraal bureau voor de statistiek. Statistisch zakboek 1978. (In Dutch). The Hague: Staatsuit-geverij/CBS publikaties, 1978.

27. Valkenburg HA, Hofman A, Klein F, et al. Een epidemiologisch onderzoek naar risico-indicato-ren voor hart-en vaatziekten (EPOZ). I Bloed-druk, serumcholesterolgehalte, Quetelet-Index on rookgewoonten in een open bevolking van vijf jaar en ouder. (In Dutch). Ned Tijdschr Geneeskd 1980;124:183–9.

28. Friedlaender JS, Costa PT, Bosse R, et al. Lon-gitudinal physique changes among healthy white veterans at Boston. Hum Biol 1977;49:541–58.

29. Hsu P, Mathewson FAL, Rabkin SW. Blood pres-sure and body mass index patterns: a longitudinal study. J Chronic Dis 1977;30:93–113.

30. Cronk CE, Roche AF, Chumlea WC, et al. Lon-gitudinal trends of weight/stature^2 in childhood in relationship to adulthood body fat measures. Hum Biol 1982;54:751–64.

31. Gillum RF, Taylor HL, Brozek J, et al. Indices of obesity and blood pressure in young men followed 32 years. J Chronic Dis 1982;35:211–19.

32. Khoury P, Morrison JA, Mellies MJ, et al. Weight change since age 18 years in 30- to 55-year-old whites and blacks. JAMA 1983;250:3179–87.

33. Clark DA, Allen MF, Wilson FH. Longitudinal study of serum lipids: 12-year report. Am J Clin Nutr 1967;20:743–52.

34. Gillum RF, Taylor HL, Brozek J, et al. Blood lipids in young men followed 32 years. J Chronic Dis 1982;35:635–41.

35. Demacker PN, Jansen AP. Stability of high-density-lipoprotein cholesterol in frozen sera. Clin Chem 1983;567–8.

36. Miller NE, Førde OH, Thelle DS, et al. The Tromsø Heart study. High density lipoprotein and coronary heart disease: a prospective case-control study. Lancet 1977;1:965–7.

37. Gordon T, Castelli WP, Hjortland MC, et al. High density lipoprotein as a protective factor against coronary heart disease: The Framingham Study. Am J Med 1977;62:707–14.

38. Kesäniemi YA, Beltz WF, Grundy SM. Compari-sons of metabolism of apolipoprotein B in normal subjects, obese patients, and patients with coro-nary heart disease. J Clin Invest 1985;76:586–95.

39. Shennan NM, Seed M, Wynn V. Variation in serum lipid and lipoprotein levels associated with changes in smoking behaviour in non-obese Cau-casian males. Atherosclerosis 1985;58:17–25.