SLAATS

BLOOD CHOLESTEROL
a public health perspective

Monique Verschuren

Stellingen

1. Ondanks een lange traditie van voedingsvoorlichting is het totaal cholesterolgehalte in de algemene Nederlandse bevolking in de afgelopen decennia niet substantieel gedaald.
Dit proefschrift

2. Voor de vertaling van epidemiologisch onderzoek naar volksgezondheidsbeleid is het van groot belang te kijken naar absolute risico's en niet alleen naar relatieve risico's.
Dit proefschrift

3. Eventuele gezondheidsrisico's van een laag totaal cholesterolgehalte vormen in Nederland geen belangrijk volksgezondheidsprobleem.
Dit proefschrift

4. Dat een gezonde leefstijl met betrekking tot coronaire hartziekte ontdaan is van elk 'joie de vivre' wordt gelogenstraft door de observatie in de Zeven Landen Studie, dat de sterfte aan coronaire hartziekte in de mediterrane Zuideuropese cohorten een factor 4 lager was dan die in de Noordeuropese cohorten.
Dit proefschrift

5. Het ontbreken van betrouwbare (trend-) gegevens over de incidentie van hart- en vaatziekten maakt het onmogelijk na te gaan welk deel van de daling in sterfte aan hart- en vaatziekten toe te schrijven is aan primaire preventie.

6. Bij het rekenen met persoonsjaren follow-up in epidemiologisch onderzoek, moet rekening worden gehouden met het feit dat 5 jaar follow-up van 50.000 mensen andere informatie levert dan 25 jaar follow-up van 10.000 mensen.

7. De snelheid waarmee nieuwe produkten op de markt verschijnen is vele malen groter dan de snelheid waarmee de NEVO tabel kan worden uitgebreid met nieuwe produkten.

8. Door de grote aandacht voor genetica en de bijna dagelijkse ontdekking van een nieuwe mutatie of polymorfisme, kan bij de leek gemakkelijk fatalisme ontstaan over de mogelijkheid van het individu met betrekking tot primaire preventie.

9. De publikatiedruk op wetenschappers, 'publish or perish', kan vruchtbare, onbaatzuchtige samenwerking flink in de weg staan.

10. De tijdwinst die scan-kassa's de klant aanvankelijk opleverden bij Albert Heijn is inmiddels verdwenen door tijdrovende handelingen als het verstrekken van air-miles, kristalzegels en gewone zegels.

Stellingen behorend bij het proefschrift "Blood cholesterol — a public health perspective" van Monique Verschuren

Wageningen, 4 oktober 1995

BLOOD CHOLESTEROL

a public health perspective

Monique Verschuren

Promotor: Dr ir D. Kromhout
             Hoogleraar Volksgezondheidsonderzoek

BLOOD CHOLESTEROL
a public health perspective

Wilhelmina Maria Monica Verschuren

Proefschrift
ter verkrijging van de graad van doctor
in de landbouw- en milieuwetenschappen
op gezag van de rector magnificus,
dr C.M. Karssen,
in het openbaar te verdedigen
op woensdag 4 oktober 1995
des namiddags om vier uur in de Aula
van de Landbouwuniversiteit te Wageningen

Voor Bennie

Cholesterol is poisonous,
so never never eat it;
sugar too may murder you,
there is no way to beat it.
Fatty food may do you in,
dessert, do avoid it.
Some food was rich in vitamins,
but processing destroyed it.
So let your life be ordered
by each documented fact,
and die of malnutrition
but with arteries intact.

.

ABSTRACT

BLOOD CHOLESTEROL — A PUBLIC HEALTH PERSPECTIVE

PhD Thesis. Agricultural University Wageningen, the Netherlands and the National Institute of Public Health and the Environment, Bilthoven, the Netherlands.

W.M. Monique Verschuren

Changes in total cholesterol levels (TC) were studied using data from three epidemiological studies: about 30,000 men and women aged 37-43 were examined between 1974 and 1980 (CB Project), about 80,000 men aged 33-37 between 1981 and 1986 (RIFOH Project) and 42,000 men and women aged 20-59 from 1987 to 1992 (Monitoring Project on CVD Risk Factors). In men a decline in TC of 6.5% was observed between 1974 and 1992. However, the largest decrease took place between 1981 and 1986 in men in a limited age range (33-37 years), and there were indications that this decrease was not generalizable to other age groups. From 1987 to 1992, a decrease of 7% in HDL cholesterol levels (HDL-C) was observed in men, leading to an increase in the non-HDL-C/HDL-C ratio. In women, no changes in TC and HDL-C were observed.

Analyses of data from 36,000 men and women aged 20-59 years showed that between ages 30 and 50 about 19-38% of the gender difference in TC was explained by differences in body mass index (BMI) and cigarette smoking between men and women. After age 50, the higher TC in women compared to men was largely due to the effect of the menopause. The TC increase associated with menopause was 0.45 mmol/l in non-smokers and 0.28 mmol/l in smokers. The difference between a low-risk and a high-risk lifestyle was 0.58 mmol/l for TC and 0.38 mmol/l for HDL-C in men, and 0.40 mmol/l for TC and 0.45 mmol/l for HDL-C in women.

Twelve year follow-up of 50,000 men and women aged 30-54 (CB Project) showed that the adjusted relative risk for coronary heart disease (CHD) mortality for the highest compared to the lowest cholesterol quintile was 3.0 (95% CI 1.8-5.1) in men and 3.8 (95% CI 1.1-13.1) in women. It was estimated that a TC reduction of 0.6 mmol/l was associated with a 20% lower CHD mortality. Low TC was not associated with non-cardiovascular mortality. All-cause mortality was positively related to total cholesterol, with a 60% and 46% higher risk in the highest compared to the lowest TC quintile for men and women respectively.

Twenty-five year follow-up of the Seven Countries Study, in which over 12,000 men aged 40-59 at baseline participated, showed that relative risks for CHD mortality were similar in different cultures, but the absolute risks were strikingly different. At a cholesterol level of about 5.4 mmol/l age-standardized CHD mortality rates varied from 4% to 5% in Japan and Mediterranean Southern Europe to 15% in Northern Europe after adjustment for age, smoking and blood pressure. It was concluded that other factors, such as diet, typical for low-risk countries, modify the effect of TC on CHD mortality. In the Seven Countries Study, in non-smokers no association of TC with cancer mortality was observed, while non-cardiovascular/non-cancer mortality was elevated only at TC below 4.15 mmol/l. In smokers, cancer mortality and non-cardiovascular/non-cancer mortality were inversely associated with TC. All-cause mortality showed a J-shaped association with TC in non-smokers (lowest all-cause mortality for TC between 4.15 and 5.15 mmol/l), while all-cause mortality was unrelated to TC in smokers. Absolute mortality rates were higher in smokers than in non-smokers for all endpoints.

Lowering the average TC level in the population is concluded to contribute to a reduction in the burden of CHD. Low cholesterol levels are not considered an important public health concern in the Netherlands. Changes in the lipid profile should preferably be achieved by lifestyle interventions such as a diet low in saturated fat and rich in fruits and vegetables, no cigarette smoking, a desirable body mass index (less than 25 kg/m²) and a physically active lifestyle. Such a lifestyle will not only have a favorable impact on coronary heart disease, but is also compatible with recommendations on the prevention of other chronic diseases such as diabetes and cancer.

Contents

1 General Introduction

The problem of coronary heart disease

Cardiovascular diseases form an important public health problem in the Netherlands, causing about 50,000 deaths every year.[1] Coronary heart disease constitutes about half of the cardiovascular mortality. The majority of coronary heart disease mortality is due to acute myocardial infarction. In 1992, more than 9,000 men and 7,000 women died of acute myocardial infarction. Between 1972 and 1990, age-standardized mortality rates for coronary heart disease decreased in the Netherlands by about 29% in men and 38% in women.[2] It is not quite clear to what extent changes in cardiovascular risk factors have contributed to the decline in coronary heart disease mortality and to what extent improvements in medical care have played a role. However, although age-standardized mortality rates decreased substantially, the increasing population size and proportion of elderly persons has led to the absolute number of deaths decreasing only slightly in men and remaining the same in women. No accurate nationwide data are available on the incidence of coronary heart disease. It is important to know whether the declining mortality rates are due to primary prevention of the disease or to better survival after a first event. If the latter is the case, the burden of the disease to the population will increase, which has large implications for the health-care system. An indication that higher survival rates play a role is the fact that standardized hospital admission rates have been increasing steadily over the past 20 years.[1]

Serum total cholesterol and coronary heart disease

Not only in the Netherlands, but also in a number of other industrialized countries, it was noticed that since the Second World War, increasing affluence has been accompanied by an epidemic level of coronary heart disease. As early as the 1950s it was suspected that serum total cholesterol was an important risk factor for coronary heart disease and that

diet was an important determinant of the serum total cholesterol concentration.[3] A great number of studies to investigate the role of serum total cholesterol in the etiology of coronary heart disease started around that time. It became clear that serum total cholesterol was one of three major risk factors for coronary heart disease, the other two being blood pressure and cigarette smoking.[4-7]

Cholesterol metabolism

For an understanding of the role of cholesterol in the etiology of coronary heart disease, insight in the atherosclerotic process is needed. Cholesterol is transported through the body in lipoproteins.[8] The liver is the central organ in cholesterol metabolism. Low density lipoproteins (LDL) transport cholesterol from the liver to the tissues throughout the body. About 70% of the cholesterol is contained in LDL particles. The removal of LDL from the plasma is mediated by the LDL receptors that are present at the cell surface[9], and most of the removal occurs by the liver. However, LDL is also taken up by the arterial wall. High LDL concentrations in the plasma will increase LDL concentrations in the intima, where oxidation of LDL can take place. It has been shown that especially the oxidized form of LDL plays an important role in causing the atherosclerotic lesions.[10] Oxidized LDL is taken up rapidly by macrophages causing the formation of foam cells. Furthermore, oxidized LDL is cytotoxic, causing damage to the endothelial wall. Oxidation of LDL and the formation of foam cells give rise to the formation of fatty streaks, the early stages of the atherosclerotic lesion. From there on progression takes place to more serious lesions.[11] Although the LDL fraction is the atherogenic component of the total cholesterol level in plasma, in large-scale epidemiological studies total cholesterol is measured as an indicator of LDL levels because of the strong correlation between total cholesterol and LDL cholesterol levels.

High density lipoproteins (HDL) transport cholesterol from the tissues back to the liver (reverse cholesterol transport) where it can be degraded and excreted in the gut in the form of bile acids, or incorporated again, primarily in LDL particles. About 20% of cholesterol is transported in HDL particles. The mechanisms underlying this reverse cholesterol transport by HDL particles is much less understood than that of cholesterol transport by LDL particles. Epidemiological studies have shown that HDL cholesterol is inversely associated with the occurrence of coronary heart disease.[12]

Cholesterol lowering and mortality

Although many observational studies showed the importance of serum total cholesterol levels in the etiology of coronary heart disease, the most convincing evidence that a factor is causally related to a disease is provided by intervention studies. After the observation that persons with a high cholesterol level are at an increased risk for coronary heart disease, intervention trials were initiated to show that lowering cholesterol levels would lower coronary heart disease risk. A large number of trials have been carried out to assess the effect of cholesterol lowering on coronary heart disease morbidity and mortality. A meta-analysis of 28 randomized controlled trials showed that lowering total cholesterol levels led to a decrease in coronary heart disease mortality.[13] In a number of trials, however, total mortality did not decrease as a consequence of cholesterol lowering. This raised concern about unfavorable side-effects of cholesterol lowering[14], especially since in many countries guidelines had been adopted for lowering of elevated cholesterol levels.[15-16] Several meta-analyses of randomized trials reached different conclusions about the effect of cholesterol lowering on non-cardiovascular mortality.[17-20] The most recent meta-analysis by Law et al. shed some light on these inconsistencies and showed that dietary trials did not show increased mortality in the intervention group while drug trials did, and that secondary prevention trials did not show increased mortality while primary prevention trials did.[21] This would imply that dietary intervention has no adverse effects, and that drug intervention should be limited to individuals with existing coronary heart disease. A recent secondary prevention trial with the newest type of cholesterol-lowering drugs (HMG-CoA reductase inhibitors) showed both a reduction in coronary heart disease and total mortality.[22]

Low cholesterol levels

Apart from concern about possible side-effects of cholesterol lowering in individuals with a high cholesterol level, concern was also recently raised about an increased risk for non-cardiovascular mortality in individuals with a low cholesterol level.[23,24] If a low cholesterol level were related to non-cardiovascular mortality, this would mean that cholesterol lowering would not be beneficial to persons with a relatively low cholesterol level. However, although excess non-cardiovascular mortality at low cholesterol levels has been observed in many studies, the question to what extent the observed associations are causal

13

is still in debate. The fact that this association was present in cohorts based on a sample of the general population and not observed in cohorts consisting of young or working persons ("healthy" cohorts), suggests that the association arises from reversed causality or bias.[25,26]

Outline of the thesis

Given the importance of cholesterol in the etiology of coronary heart disease it is important to have information on cholesterol levels in the general population. This information is necessary to determine the number of persons at increased risk and to be able to anticipate future developments and demands of health care. Since 1974 a number of projects have been carried out in the Netherlands that have measured cholesterol levels in the general population. On the basis of these projects, changes in total cholesterol levels over the period 1974-1986 are described in Chapter 2. In Chapter 3, total and HDL cholesterol levels in the general population aged 20-59 are reported as well as changes over the period 1987-1992. To be able to prevent hypercholesterolemia, it is important to have insight into biological and lifestyle factors that determine total cholesterol levels. In Chapters 4 and 5 the effect of age and gender on total and HDL cholesterol levels is presented, as well as the contribution of body mass index, smoking, alcohol consumption, physical activity and (in women) the menopause. The public health impact of cholesterol levels depends on the strength of the relation between total cholesterol and subsequent mortality from different causes. The relation between total cholesterol and mortality in a large cohort of Dutch men and women is described in Chapter 6. The relation between total cholesterol and coronary heart disease mortality in different cultures is reported in Chapter 7, and in Chapter 8 the association between total cholesterol and mortality from causes other than coronary heart disease is described. In the general discussion (Chapter 9) the public health implications of the results are discussed.

References

1. Nederlandse Hartstichting. Cardiovascular diseases in the Netherlands. Morbidity and mortality statistics. The Hague: Nederlandse Hartstichting, 1994 (in Dutch).

2. Jansen J, Kromhout D. Coronary heart disease. In: Ruwaard D, Kramers PGN, (eds). Public health status and forecasts. The health status of the Dutch population over the period 1950-2010. The Hague: Sdu Uitgeverij Plantijnstraat; p 379-86 (in Dutch).

3. Keys A. The cholesterol problem. Voeding 1952;13:539-555.

4. The Pooling Project Research Group. Relationship of blood pressure, serum cholesterol, smoking habit, relative weight and ECG abnormalities to incidence of major coronary events: final report of the Pooling Project. J Chron Dis 1978;31:201-306.

5. Stamler J, Wentworth D, Neaton J. Is the relationship between serum cholesterol and risk of premature death from coronary heart disease continuous and graded ? Findings in 356,222 primary screenees of the multiple risk factor intervention trial (MRFIT). JAMA 1986;256:2823-28.

6. Anderson KM, Castelli WP, Levy D. Cholesterol and mortality: 30 years of follow-up from the Framingham Study. JAMA 1987;257:2176-80.

7. Neaton JD, Blackburn H, Jacobs DR, et al. Serum cholesterol level and mortality findings for men screened in the multiple risk factor intervention trial. Arch Intern Med 1992;152:1490-1500.

8. Assmann G. Lipid metabolism and atherosclerosis. Stuttgart/New York:Schattauer, 1982.

9. Brown MS, Goldstein JL. A receptor-mediated pathway for cholesterol homeostasis. Science 1986;232:34-47.

10. Steinberg D, Parthasarathy S, Carew TE, et al. Beyond cholesterol: modifications of low-density lipoprotein that increase its atherogenicity. N Engl J Med 1989;320:915-24.

11. Fuster V. Mechanisms leading to myocardial infarction: insights from studies of vascular biology. Circulation 1994;90:2126-46.

12. Gordon DJ, Probstfield JL, Garrison RJ, et al. High-density lipoprotein cholesterol and cardiovascular disease: four prospective American studies. Circulation 1989;79:8-15.

13. Law MR, Wald NJ, Thompson SG. By how much and how quickly does reduction in serum cholesterol concentration lower risk of ischemic heart disease ? BMJ 1994;308:367-73.

14. Davey Smith G, Pekkanen J. Should there be a moratorium on the use of cholesterol lowering drugs? BMJ 1992;304:431-4.

15. Consensus Conference: Lowering blood cholesterol to prevent heart disease. JAMA 1985;253:2080-6.

16. Study Group European Atherosclerosis Society. Strategies for prevention of coronary artery disease: a policy statement of the European Atherosclerosis Society. Eur Heart J 1987;8:77-88.

17. Yusuf S, Wittes J, Friedman L. Overview of results of randomized clinical trials in heart disease. II. Unstable angina, heart failure, primary prevention with aspirin, and risk factor modification. JAMA 1988;260:2259-63.

18. Oliver MF. Reducing cholesterol does not reduce mortality. J Am Coll Cardiol 1988;12:814-7.

19. Muldoon MF, Manuck SB, Matthews KA. Lowering cholesterol concentrations and mortality: a quantitative review of primary prevention trials. BMJ 1990;301:309-14.

20. Holme I. An analysis of randomized trials evaluating the effect of cholesterol reduction on total mortality and coronary heart disease incidence. Circulation 1990;82:1916-24.

21. Law MR, Thompson SG, Wald NJ. Assessing possible hazards of reducing serum cholesterol. BMJ 1994;308:373-9.

22. Scandinavian Simvastatin Survival Study Group. Randomized trial of cholesterol lowering in 4444 patients with coronary heart disease: the Scandinavian Simvastatin Survival Study. Lancet 1994;344:1383-9.

23. Jacobs DR, Blackburn H, Higgins M, et al. Report of the conference on low blood cholesterol:mortality associations. Circulation 1992;86:1596-9.

24. Neaton JD, Wentworth D. Serum cholesterol, blood pressure, cigarette smoking and death from coronary heart disease: overall findings and differences by age for 316,099 white men. Arch Intern Med 1992;152:56-64.

25. Klag MJ, Ford DE, Mead LA, et al. Serum cholesterol in young men and subsequent cardiovascular disease. N Engl J Med 1993;328:313-8.

26. Persson B, Johansson BW. The Kockum Study: twenty-two years of follow-up. Acta Med Scand 1984;216:485-93.

2 Trend in serum total cholesterol level in the Netherlands from 1974 to 1986

Abstract

Background. Age-standardized mortality rates from coronary heart disease have declined in the Netherlands since 1972. The extent to which changes in cardiovascular risk factors might have contributed to this decline is, however, not known.

Methods. Data from two screening projects on cardiovascular risk factors were used to analyze the trend in serum total cholesterol level in the Netherlands between 1974 and 1986. Cholesterol levels were measured in a single reference laboratory of the World Health Organization throughout the entire study period. Between 1974 and 1980, about 30,000 men and women aged 37 to 43 years (mean age, approximately 40 years) were screened.

Results. A decrease in mean serum total cholesterol level was observed until the end of 1977, when it was followed by an increase. This resulted in a net change over the entire study period of -0.07 mmol/liter (3 mg/dl) in men and -0.03 mmol/liter (1 mg/dl) in women. Between 1981 and 1986, about 80,000 men aged 33 to 37 years (mean age, 35 years) were screened. During this period, a decrease of 0.20 mmol/liter (8 mg/dl) in the mean total cholesterol level was observed.

Conclusion. In spite of the decline in the mean total cholesterol level, the prevalence of cholesterol values of ≥ 6.5 mmol/liter (≥ 251 mg/dl) in young adult men was still high in 1986 (16%). A further reduction is therefore desirable. The decline in the mean total cholesterol level in young adults might indicate that a further decline in mortality from coronary heart disease can be expected.

Introduction

In 1972, mortality from coronary heart disease reached its peak in the Netherlands. Since that time the age-standardized death rate for coronary heart disease fell from 2,537/10^6 in 1972 to 1,810/10^6 in 1988 for men and from 1,697/10^6 in 1972 to 1,146/10^6 in 1988 for women.[1] The extent to which this fall can be attributed to primary prevention or improved health care is not known. To estimate the effect of primary prevention, it is of interest to know what happened with regard to the established risk factors for coronary heart disease during the last decades. Serum total cholesterol is a major risk factor for coronary heart disease.[2] From 1974 to 1986, two screening projects on cardiovascular risk factors were carried out in the Netherlands. Throughout the entire study period, all measurements of serum total cholesterol were made in a single laboratory that serves as the lipid reference laboratory for the Netherlands and participates in the standardization program of the World Health Organization. The standards of the World Health Organization for cholesterol determination were met throughout the entire study. These continuously collected data provided a unique opportunity for analyzing the trend in serum total cholesterol in young adults in the Netherlands over a period of 13 years. The results of these analyses are reported here.

Materials and Methods

Study population

The screening projects were carried out according to a standardized protocol. From October 1974 to December 1980 a total of about 50,000 men and women from five different towns in the Netherlands were examined in the Consultation Bureau Heart Project. The project was begun in 1974 in two towns: Doetinchem, a small town in a rural area in the eastern part of the country with about 40,000 inhabitants, and Tilburg, a town in the southwest with about 150,000 inhabitants. Because the project was considered successful, three other cities were added to the study in 1976: Amsterdam, the capital, located in the western part of the country, with more than 700,000 inhabitants, and Leiden in the west and Maastricht in the south, both with about 100,000 inhabitants. In each town, a list of all inhabitants in a defined age range was obtained from the civil registry. These individuals were invited by mail to participate in the examination. They had to send

back a registration card in order to be scheduled for the examination. After 4 weeks, a reminder was mailed to all who had not yet responded. Everyone who registered was scheduled for the examination and received a confirmation notice 1 to 2 weeks before the appointment was scheduled.

The response rate was about 80% at the beginning of the study period, and dropped to about 70% at the end of the study.[3] The age range differed from city to city: in the small town of Doetinchem, the ages of the participants ranged from 30 to 54 years; in Amsterdam, from 40 to 42 years; in Maastricht, from 35 to 40 years; in Tilburg, from 38 to 42 years; and in Leiden, from 30 to 35 years. Because age is a very strong predictor of serum total cholesterol, it was necessary to exclude the possibility that the observed trend would be confounded by age. Therefore, a subsample with a narrower age range was selected for trend analyses. All respondents aged 37 to 43 years were included in the trend analyses. This resulted in a stable mean age across the entire study period, and small fluctuations in age could be corrected with regression analysis because the relation between age and serum total cholesterol is linear within this age range. Because of the age restriction, Leiden was excluded, and the number of respondents eligible for trend analysis was reduced to about 30,000.

The examination included measurement of blood pressure, weight, and height. The subject was not required to fast before blood was drawn for determination of serum total cholesterol. All subjects completed a questionnaire requesting information about smoking habits, cardiovascular complaints, family history of premature cardiovascular mortality, physical activity, history of hypertension, and diabetes.

In 1981 the study protocol changed. The decision made to examine men only, because they were at higher risk for cardiovascular diseases than women. From July 1981 to October 1986, about 80,000 men with a mean age of 35 years (range, 33-37) were screened in the study Risicofactoren Onderzoek Hart- en Vaatziekten (Risk Factor Project on Coronary Heart Disease, hereafter referred to as the RIFOH Project). This project was carried out in the same five towns in which the Consultation Bureau Heart Project took place. Potential respondents were again identified from the civil registry and invited to participate according to the same procedure followed in the Consultation Bureau Heart Project. For each 2-year period, certain birth cohorts were invited to participate: from 1981 to 1982, all men born in 1946 and 1947; from 1983 to 1984, all men born in 1948 and

1949; and from 1985 to 1986, all men born in 1950 and 1951. This ensured a narrow age range across the entire study period, and no respondents had to be excluded in the trend analyses. The average response rate was 65%; in Amsterdam, it was approximately 55%, and in the other towns approximately 70%. The main purpose of the RIFOH Project was to measure the three major risk factors for coronary heart disease: serum total cholesterol, blood pressure, and cigarette smoking. Weight and height were measured, and the questionnaire was restricted to questions about smoking habits, cardiovascular complaints, and history of hypertension and diabetes.

Cholesterol determination

Throughout the entire study measurements of serum total cholesterol were made at the Central Clinical Chemistry Laboratory of the University Hospital Dijkzigt in Rotterdam. This laboratory participates in the standardization program of the World Health Organization through the World Health Organization Regional Lipid Center for Europe in Prague, Czechoslovakia. It is the lipid reference laboratory for standardized determination of cholesterol in the Netherlands and also participates in the network of reference laboratories of the national Heart, Lung, and Blood Institute and the Centers for Disease Control in the United States.

During the entire study period, the quality standards for certified laboratories of the World Health Organization as adopted by the Centers for Disease Control, were met, ensuring that the measurement of cholesterol concentration differed no more than 5% from the true level. However, this is not sufficient for a valid trend analysis. Changes in cholesterol over time, if any, were expected to be small. Therefore, a small laboratory drift, unimportant for clinical practice, could disturb the trend analyses. Therefore, the internal quality control data were also analyzed to detect possible small changes over time.

The laboratory routinely checked the precision and accuracy of the cholesterol measurements. Three control sera (sometimes two) were included in all series of measurements, or "runs". In each run, two out of three control sera had to yield values within a range of two standard deviations from the previously determined mean value. Otherwise, the run was rejected. The stability of lyophilized serum is such that a shelf-life of 2 years is typical. Therefore, several batches of control sera were used during the study period. Consequently, the entire study period was divided into periods during which a certain combination of control sera was used. During such a period, the regression coefficient for the change in the level of a control serum over time (months) was

calculated with regression analysis. If one or more of the sera had a statistically significant regression coefficient, the null hypothesis that there was no laboratory drift was rejected. Correction was made throughout that period with the average regression coefficient of the two or three control sera. If only one out of three regression coefficients was statistically significant, correction was still based on the mean of the three regression coefficients. For example, if the mean correction factor was -0.01 mmol/liter/month over a certain period, 0.01 mmol/liter was subtracted from all cholesterol values of the respondents in month 1, 0.02 mmol/liter was subtracted in month 2, etc. During the Consultation Bureau Heart Project the magnitude of the correction factor was -0.022 mmol/liter/month between October '74 and April '75; -0.017 mmol/liter/month between June '75 and January '77; -0.0051 mmol/liter/month between February '77 and February '78; and -0.0054 mmol/liter/month between March '78 and May '79. No correction was necessary between June '79 and December '80. The total study period of the RIFOH Project was also divided into five periods, because of different combinations of control sera. The correction factor was -0.0195 mmol/liter/month between July 1981 and July 1982; 0.0049 mmol/liter/month between August 1982 and June 1983; and 0.009 mmol/liter/month between July 1983 and March 1984. Between April 1984 and May 1985, no correction was necessary, and between June 1985 and December 1986, the correction factor amounted to -0.0016 mmol/liter/month.

During the Consultation Bureau Heart Project, cholesterol was determined according to a direct Liebermann-Burchard method as described by Huang et al.[4] At the beginning of the RIFOH Project in 1981 the laboratory switched from this method to an enzymatic method described by Katterman et al..[5] After a test period of 2 months, cholesterol was measured for 4 months in more than 5,000 serum samples using both the Huang and the enzymatic methods. The equation for conversion from Huang to enzymatic values was estimated with regression analysis, as follows:

total cholesterol (enzymatic) = 1.004 * total cholesterol (Huang) - 0.299 mmol/liter.
All cholesterol values of the Consultation Bureau Heart Project reported in this paper were converted to enzymatic values with this equation. This was done because the prevalence of hypercholesterolemia was analyzed according to the criteria of the Netherlands Cholesterol Consensus.[6] These criteria are based on enzymatically determined cholesterol values. According to this consensus, total cholesterol values below 5.0 mmol/liter (194 mg/dl) are considered "ideal"; values between 6.50 and 7.99 mmol/liter (251-309 mg/dl)

are considered "moderately elevated"; and values of 8.0 mmol/liter or more ($\geq$ 310 mg/dl) are considered "highly elevated".

Statistical Methods

Analyses were carried out on a personal computer using the Statistical Package for the Social Sciences for personal computers, SPSS/PC+, version 2.0.[7] Regression analyses were carried out with time (months) as the independent variable and total cholesterol as dependent variable. Because regression analysis with 80,000 respondents is very time consuming on a personal computer, weighted regression analyses were performed using monthly means. Both methods give exactly the same results. Adjustments for age and laboratory drift were made on an individual basis, adjustment for town was done by analysis of covariance. Trends in the mean age of the study population across the study period or changes in the cholesterol determination level of the laboratory could cause an artificial time trend. To exclude age effects, the relation between age and total cholesterol was quantified in a regression analysis. This regression coefficient was used to extrapolate every individual's cholesterol value to what the value would be if that person were 40 years of age (Consultation Bureau Heart Project) or 35 years of age (RIFOH Project). To exclude laboratory drift effects, the data of the measurements of the control sera were used, and each individual's cholesterol value was adjusted for laboratory drift as described above under "Cholesterol determination". There were differences in the mean cholesterol level between towns. During the study period, the number of respondents per town differed. An increasing number of respondents from a town with the lowest mean cholesterol level could cause an artificially decreasing time trend. Therefore, dummy variables for town were included in all regression models, Amsterdam being the reference.

Because the trend could differ between towns, a test was performed to determine whether a calculation of an overall regression coefficient for the towns together was justified. This was done by comparing the total variance explained by calculating separate regression equations for each town with the variance explained by calculating an overall regression equation with town included as a dummy variable.[8] Fitting separate regression equations per town did not significantly increase the explained variance, both for the Consultation Bureau Heart Project and the RIFOH Project. Therefore, overall regression equations with town included in the model as a dummy variable were used. Because the data of the Consultation Bureau Heart Project indicated a decrease followed by an increase, a test was performed to determine whether a V-shaped regression line should be fitted. This was

done by comparing the variance explained by a V-shaped regression line with the variance explained by a straight regression line. The V-shaped line significantly increased the explained variance. The "breakpoint" in the line was determined by optimizing the explained variance. The analysis was performed with two time variables: one for the total period (β_1) and one for the period after the breakpoint (β_2). The change in cholesterol across the first period (up to the breakpoint) is given by β_1, the change across the second period (after the breakpoint) is given by $\beta_1+\beta_2$. For the RIFOH Project, no increase in explained variance was achieved by allowing an extra time variable; therefore, a straight regression line was fitted.

All tables presented here contain crude data, data adjusted for age and town and data adjusted for age, town, and laboratory drift. In the text of this paper, however, only data adjusted for age, town, and laboratory drift are discussed.

Results

Consultation Bureau Heart Project (1974-1980)
Table 1 gives mean total cholesterol levels per year for men and women in the four towns. After adjustment for age, town and laboratory drift, a statistically significant decrease in mean total cholesterol of 0.10 mmol/liter (4 mg/dl) per year was observed in men from October 1974 until December 1977; between January 1978 and December 1980, a statistically significant increase of 0.09 mmol/liter (4 mg/dl) per year was observed (table 2, figure 1). In women a statistically significant decrease in mean total cholesterol of 0.08 mmol/liter (3 mg/dl) per year was observed until August 1977. This decline was followed by an increase of 0.06 mmol/liter (2 mg/dl) per year from September 1977 to December 1980 (table 2, figure 1). Across the entire study period, a net decrease in mean cholesterol levels of 0.07 mmol/liter (3 mg/dl) in men and 0.03 mmol/liter (1 mg/dl) in women was observed.

The decrease in mean total cholesterol followed by an increase was also reflected in the prevalence of moderately and highly elevated cholesterol values, according to the criteria of the Netherlands Cholesterol Consensus. Regression coefficients for changes in the prevalence of hypercholesterolemia are given in table 2. The percentage of distribution per year for the four serum cholesterol categories according to the Netherlands Cholesterol

Consensus is given in table 3 for men and women. For comparison, the distribution according to the US National Cholesterol Education Program[9] is given in table 3 as well. Table 2 shows a statistically significant decline of 2.1 percentage points per year in the prevalence of moderately elevated cholesterol values (6.50-7.99 mmol/liter, 251-309 mg/dl) in men until December 1977. In women, this decline was 1.8 percentage points per year until August 1977. This decrease was followed by an increase of 1.4 percentage points per year in men and of 1.0 percentage points per year in women until December 1980. The prevalence of highly elevated cholesterol values (≥ 8.0 mmol/liter, or ≥310 mg/dl) showed a statistically significant decrease in men of 0.7 percentage points per year, followed by an increase of 0.5 percentage point per year until December 1980.

Table 1. *Mean total cholesterol (mmol/l)[a] adjusted for age and laboratory drift in men and women aged 37-43 years: Findings of the Consultation Bureau Heart Project*, the Netherlands, 1974-1980

	1974[b]	1975[c]	1976	1977	1978	1979	1980	N
Men								
Amsterdam			5.56	5.63	5.49	5.59	5.64	3,478
Doetinchem	5.67	5.50	5.37	5.35	5.34	5.42		1,818
Maastricht			5.55	5.51	5.44	5.53	5.77	3,672
Tilburg	5.88	5.71	5.57	5.48	5.55	5.55	5.71	5,163
Total mean	5.81	5.66	5.53	5.51	5.47	5.55	5.70	14,131
S.D.	1.14	1.11	1.13	1.05	1.03	1.06	1.10	
Women								
Amsterdam			5.16	5.20	5.17	5.22	5.27	4,180
Doetinchem	5.36	4.99	4.91	4.95	4.98	4.89		1,999
Maastricht			5.06	5.13	5.02	5.17	5.33	3,796
Tilburg	5.35	5.21	5.15	5.12	5.12	5.22	5.28	5,372
Total mean	5.36	5.16	5.10	5.13	5.08	5.19	5.29	15,347
S.D.	0.99	1.01	1.00	0.94	0.95	0.96	1.00	

a: 1 mmol/l = 38.67 mg/dl
b : In 1974 and 1975 the Consultation Bureau Heart Project was only
   carried out in Doetinchem and Tilburg
c : In 1980 no respondents aged 37-43 were examined in Doetinchem

Table 2. *Regression coefficients (β)[a] for the relation between total cholesterol and time, and the net change across the entire study period for men and women aged 37-43 years: Findings from the Consultation Bureau Heart Project, the Netherlands, 1974-1980*

	Crude	Adjusted[b]	Adjusted[c]
Men (N=14,128)			
Mean			
Intercept[d]	5.91	5.95	5.77
ß (Oct '74-Dec '77)	-0.13***	-0.13***	-0.10***
ß (Jan '78-Dec '80)	+0.08***	+0.05**	+0.09***
Net change Oct '74-Dec '80[e]	-0.19	-0.28	-0.07
% Moderately elevated (6.50 - 7.99 mmol/l)			
Intercept	24.07	24.00	20.24
ß (Oct '74-Dec '77)	-2.89***	-2.65***	-2.08***
ß (Jan '78-Dec '80)	+1.38*	+0.70	+1.37**
Net change Oct '74-Dec '80	-5.27	-6.49	-2.67
% Highly elevated ($\geq$ 8.0 mmol/l)			
Intercept	4.18	4.59	3.64
ß (Oct '74-Dec '77)	-0.76***	-0.87***	-0.69***
ß (Jan '78-Dec '80)	+0.33	+0.34	+0.53**
Net change Oct '74-Dec '80	-1.49	-1.80	-0.66
Women (N=15,381)			
Mean			
Intercept	5.41	5.45	5.29
ß (Oct '74-Aug '77)	-0.10***	-0.10***	-0.08***
ß (Sep '77-Dec '80)	+0.05**	+0.02	+0.06***
Net change Oct '74-Dec '80	-0.12	-0.22	-0.03
% Moderately elevated (6.50 - 7.99 mmol/l)			
Intercept	12.72	13.48	11.10
ß (Oct '74-Aug '77)	-1.99***	-2.22***	-1.83***
ß (Sep '77-Dec '80)	+0.92*	+0.48	+1.03***
Net change Oct '74-Dec '80	-2.76	-4.87	-1.90
% Highly elevated ($\geq$ 8.0 mmol/l)			
Intercept	1.63	1.60	1.28
ß (Oct '74-Aug '77)	-0.31*	-0.25*	-0.19
ß (Sep '77-Dec '80)	+0.20*	+0.05	+0.11
Net change Oct '74-Dec '80	-0.23	-0.56	-0.19

a: ß in mmol/l per year for mean cholesterol and in percent per year for changes in prevalence of moderately/highly elevated values; 1 mmol/l = 38.67 mg/dl

b: Adjusted for town and age

c: Adjusted for town, age and laboratory effects

d: Intercept is the weighted average of each town's intercept, weighted by the sample size from the town

e: calculated as the predicted value in the first month minus the predicted value in the last month, based on the regression equation

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

Table 3. *Percentage distribution of cholesterol categories according to the Cholesterol Consensus of the Netherlands and of the United States for men and women aged 37-43 years: Findings of the Consultation Bureau Heart Project, the Netherlands, 1974-1980*[a]

Total cholesterol (mmol/l)[b]	1974	1975	1976	1977	1978	1979	1980
Men							
N	449	1070	3370	2968	2801	1736	1737
Netherlands Consensus							
< 5.0	24.5	29.4	33.9	33.2	33.5	31.6	26.1
5.0 - 6.49	49.2	48.8	47.8	50.4	51.2	50.9	52.2
6.5 - 7.99	22.7	19.3	16.0	14.6	13.8	15.6	18.7
>= 8.0	3.6	2.5	2.3	1.9	1.5	2.0	3.0
American Consensus							
< 5.17	29.2	34.3	39.8	39.8	40.7	37.5	32.1
5.17 - 6.19	36.7	36.4	35.2	37.0	37.1	37.9	37.9
>= 6.20	34.1	29.3	25.0	23.2	22.2	24.6	30.0
Women							
N	511	1171	3457	3362	2982	1946	1918
Netherlands Consensus							
< 5.0	39.5	48.0	49.4	48.3	50.5	44.8	41.0
5.0 - 6.49	48.3	42.8	42.5	44.1	42.8	46.7	48.2
6.5 - 7.99	11.4	7.9	7.1	7.0	6.1	7.5	9.5
>= 8.0	0.8	1.3	1.0	0.7	0.6	1.0	1.2
American Consensus							
< 5.17	45.2	55.5	56.9	56.1	57.2	53.4	48.0
5.17 - 6.19	37.4	30.0	30.6	31.3	31.7	33.3	35.7
>= 6.20	17.4	14.5	12.5	12.6	11.1	13.3	16.3

a: based on age and laboratory drift adjusted cholesterol values
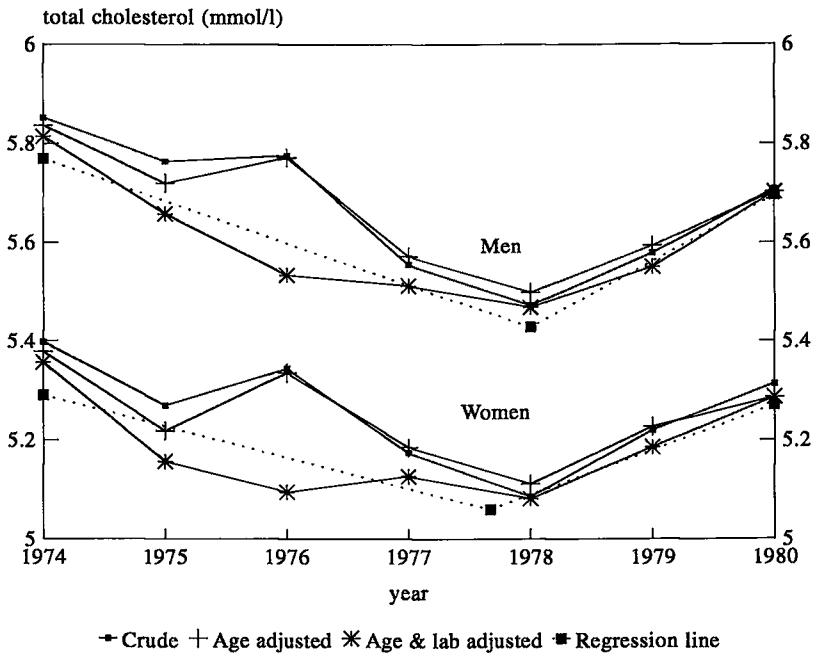b: 1 mmol/l = 38.67 mg/dl

Figure 1. *Crude and adjusted mean serum total cholesterol in men and women aged 37-43 over the period 1974-1980. The Consultation Bureau Heart Project, The Netherlands.*

In women, a decrease of 0.2 percentage points per year was observed until August 1977, followed by an increase of 0.1 percentage points per year until December 1980; this was not statistically significant. Across the entire study period, the net decrease in the prevalence of moderately elevated cholesterol levels was 2.7 percentage points in men and 1.9 percentage points in women. The net decrease in the prevalence of highly elevated cholesterol values was 0.7 percentage points in men and 0.2 percentage points in women.

RIFOH Project (1981-1986)

Mean total cholesterol levels per year for the five towns are given in table 4. Statistically significant changes were seen in all cholesterol parameters (table 5, figure 2). After adjusting for age, town, and laboratory drift, the changes became smaller, but remained statistically significant, mean total cholesterol decreasing by 0.04 mmol/liter (1.5 mg/dl) per year. The total decline in mean cholesterol level across the study period amounted to

27

0.20 mmol/liter (8 mg/dl). The prevalence of cholesterol values between 6.50 and 7.99 mmol/liter (251-309 mg/dl) decreased significantly by 0.8 percentage points per year. The prevalence of cholesterol values of 8.0 mmol/liter or more (≥310 mg/dl) decreased by 0.2 percentage points per year. The total decline in the prevalence of moderately and highly elevated cholesterol levels over the entire study period was 4.4 and 1.0 percentage points respectively.

Table 4. *Mean total cholesterol (mmol/l)[a] adjusted for age and laboratory drift in men aged 33-37: Findings of the RIFOH Project*, the Netherlands, 1981-1986

	1981	1982	1983	1984	1985	1986	N
Amsterdam	5.68	5.66	5.63	5.52	5.46	5.51	21,622
Doetinchem	5.66	5.64	5.61	5.55	5.53	5.54	13,411
Leiden	5.59	5.54	5.59	5.51	5.49	5.40	14,151
Maastricht	5.66	5.67	5.56	5.54	5.54	5.56	16,345
Tilburg	5.63	5.65	5.63	5.56	5.51	5.55	15,138
Total mean	5.64	5.64	5.61	5.53	5.50	5.52	80,667
S.D.	1.10	1.13	1.12	1.08	1.10	1.08	

a: 1 mmol/l = 38.67mg/dl

Table 6 gives the percentage of distribution per year for the different serum cholesterol categories according to the Netherlands Cholesterol Consensus and, for comparison, according to the US National Cholesterol Education Program. The percentage of distribution and the mean total cholesterol levels per year suggest that cholesterol levels were stable over the period from 1981 to 1983. In fact, during the first 2 years of the RIFOH Project, month-to-month variation was greater than it was during the rest of the study period. When separate regression equations were fitted over the first 2 years (1981/1982) and over the next 4 years (1983-1986), a non-significant regression coefficient was found for the first period and a statistically significant negative regression coefficient was found for the second. However, the separate regression equations did not significantly increase the explained variance ($F = 1.46$, $p > 0.10$). Therefore, one regression coefficient encompassing the entire period was calculated.

Table 5. *Regression coefficients (ß)[a] for the relation between total cholesterol and time, and the total change across the study period, for men aged 33-37 years. Findings from the RIFOH Project, The Netherlands, 1981-1986*

	Crude	Adjusted[b]	Adjusted[c]
Mean total cholesterol			
intercept[d]	5.72	5.72	5.67
ß	-0.05***	-0.05***	-0.04***
total change '81-'86	-0.27	-0.27	-0.20
% Moderately elevated (6.50-7.99 mmol/l)			
intercept	19.99	19.18	18.13
ß	-1.12***	-1.12***	-0.84***
total change '81-'86	-5.89	-5.87	-4.42
% Highly elevated (≥ 8.0 mmol/l)			
intercept	3.45	3.25	3.04
ß	-0.27***	-0.25***	-0.20***
total change '81-'86	-1.43	-1.34	-1.04

a: ß in mmol/l per year for mean cholesterol and in percent per year for changes in prevalence of moderately/highly elevated values; 1 mmol/l = 38.67 mg/dl

b: Adjusted for town and age

c: Adjusted for town, age and laboratory effects

d: Intercept is the weighted average of each town's intercept, weighted by the sample size from the town

***: $p < 0.001$

Table 6. *Percentage of distribution of cholesterol categories according to the Cholesterol Consensus of the Netherlands and of the United States for men aged 33-37 years: Findings of the RIFOH Project, the Netherlands, 1981-1986*[a]

Total cholesterol (mmol/l)[b]	1981	1982	1983	1984	1985	1986
N	8,083	12,251	17,608	14,860	15,873	11,992
Netherlands Consensus						
< 5.0	29.6	30.3	30.7	33.5	34.3	33.6
5.0-6.49	50.9	49.1	50.3	49.3	48.8	50.2
6.5-7.99	16.7	17.7	16.2	15.0	14.6	14.1
>= 8.0	2.7	2.8	2.8	2.2	2.2	2.1
American Consensus						
< 5.17	36.1	35.9	37.2	39.8	40.8	39.8
5.17-6.19	36.7	36.0	36.2	36.0	35.6	36.9
>=6.20	27.2	28.2	26.6	24.3	23.6	23.3

a: based on age and laboratory drift adjusted cholesterol values
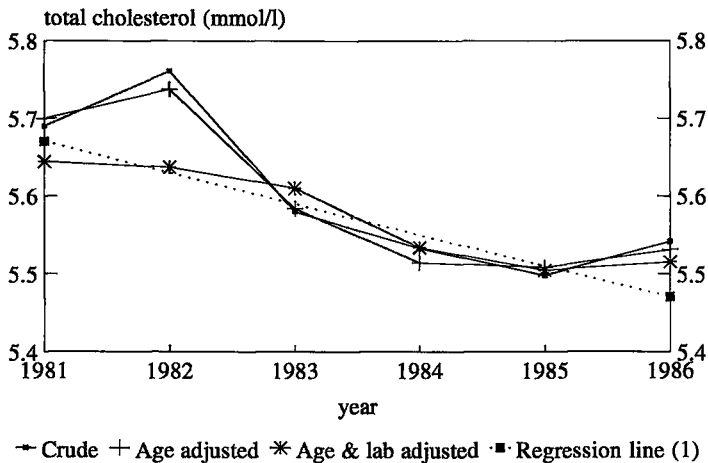b: 1 mmol/l = 38.67 mg/dl

Figure 2. *Crude and adjusted mean serum total cholesterol per year in men aged 33-37 over the period 1981-1986. The RIFOH Project, The Netherlands.*

Discussion

An advantage of the present study was the availability of continuous cholesterol measurements across the study period from large numbers of respondents. This enabled us to describe changes in cholesterol level in detail for a 13-year period. In analyzing a long-term trend in serum total cholesterol level, the accuracy of the cholesterol data is very important. In our study, cholesterol measurements were made in a single laboratory, which serves as the lipid reference laboratory for the Netherlands, throughout the study period. Although the criteria for the World Health Organization were met throughout the study period, adjustments were made for changes in the determination level of the laboratory. This was done because even when a laboratory stays within the range allowed by the World Health Organization, considerable differences in determination level can occur.[10] Because the changes in cholesterol level over time were small, even a small laboratory drift would cause a substantial error in the trend analysis. Our results show that, in certain years, (for example, 1976 and 1982), cholesterol levels with and without adjustment for laboratory drift differed considerably. This illustrates the necessity of adjusting for the possible effects of laboratory drift when comparing cholesterol levels over time.

From 1974 to 1980, a decrease in the mean total cholesterol level followed by an increase was observed in men and women (mean age, 40 years). This resulted in a net decrease across this period of 0.07 mmol/liter (3 mg/dl) in men and 0.03 mmol/l (1 mg/dl) in women. From 1981 to 1986, a decrease of 0.20 mmol/liter (8 mg/dl) in the mean total cholesterol level was observed in men with a mean age of 35 years. Unfortunately, during this period, no information was obtained about cholesterol levels in women. However, the data of the Consultation Bureau Heart Project suggest that trends in men and women were in the same direction, although the changes in women were somewhat smaller than those in men.

With respect to the implications for public health, it is important to know whether the trend observed in relatively young adults can be generalized to the total population. Data from the Zutphen Study[11], the Dutch contribution to the Seven Countries Study, were used to analyze changes in mean total cholesterol levels in men of other age groups. The Zutphen Study is a longitudinal study of risk factors for cardiovascular diseases begun in 1960 with 872 men aged 40 to 59 years. In 1960, the average serum total cholesterol level

of these men was 6.09 mmol/liter (236 mg/dl). In 1985, a new small random sample of men aged 40 to 59 years was examined in Zutphen. The mean total cholesterol level of this new sample was compared with that of the original cohort of the Zutphen Study. The mean total cholesterol level of the men aged 40 to 59 years in 1985 was 0.2 mmol/liter (8 mg/dl) higher than that of the men aged 40 to 59 years in 1960. Men aged 65 to 69 years were examined in the Zutphen Study in 1970, 1977-1978, and 1985. Mean total cholesterol levels were 6.05 mmol/liter in 1970, 5.91 mmol/liter in 1977-1978, and 6.34 mmol/liter in 1985 (234, 229, and 245 mg/dl, respectively). From these data, it can be concluded that the U-shaped relation between serum cholesterol and time observed in the present study in 40-year-old men between 1974 and 1980 was also present in men aged 65 to 69 years examined in Zutphen in 1970, 1977-78 and 1985. The results from the present study for men aged 35 years suggested a decline in serum total cholesterol between 1981 and 1986. The data for men aged 40 to 59 years and 65-69 examined in 1985 in Zutphen, however, did not indicate a decline, in serum total cholesterol in these age groups, but an increase, suggesting that the decline in serum total cholesterol in the 1980s is restricted to young adults.

At present, a number of other countries are experiencing favorable changes in mortality from coronary heart disease. Changes in serum total cholesterol levels in different countries during the last decades are, however, different in magnitude. At the beginning of the Seven Countries Study in the late 1950s and early 1960s, average serum total cholesterol levels in middle-aged men in the United States and the Netherlands were comparable (about 6.1 mmol/liter, or 235 mg/dl).[12] Today, the average serum total cholesterol level in Dutch adults is about 0.5 mmol/liter (19 mg/dl) higher than that of their counterparts in the United States.[13,14] This is the result of a decline in serum total cholesterol levels during the last decades in the United States. This decline was illustrated with data of three national surveys in men and women aged 20 to 74 years (the National Health Examination Survey and the first and second National Health and Nutrition Examination Surveys).[15] A decrease in mean serum total cholesterol of 0.16 mmol/liter (6 mg/dl) in men and 0.21 mmol/liter (8 mg/dl) in women was observed between 1960 and 1980. In men, the largest decrease was seen in the age group of 35 to 44 years; in women, the decrease was largest in the oldest age group, 65 to 74 years. The Minnesota Heart Survey compared the total cholesterol values of men and women in 1973-1974 with values in 1985-1987.[16] In all age strata, a decline in the mean total cholesterol level was observed, averaging 0.21 mmol/liter (8 mg/dl) for men and 0.28 mmol/liter (11 mg/dl) for

women. In Finland, where serum total cholesterol levels and mortality from coronary heart disease are high, mean serum total cholesterol levels in men and women aged 30 to 59 years declined by 0.8 mmol/liter (31 mg/dl) and 0.6 mmol/liter (23 mg/dl), respectively, between 1972 and 1982.[17] In the Nine Communities Study in Italy, no change in mean serum total cholesterol in men and women aged 20 to 59 years was found between 1978 and 1983.[18] The change in mean total cholesterol level observed in the present study between 1981 and 1986 amounted to 3% to 4%. A decrease in mean total cholesterol level of a few percent can be important with respect to mortality from coronary heart disease. It is known from intervention studies that lowering total cholesterol is associated with a reduction of the incidence of and mortality from coronary heart disease. It is estimated that a reduction of 1% in serum total cholesterol will result in a reduction of 2% to 3% in coronary heart disease.[19,20] However, in observational studies like the present one, quantification of the effect of the observed change in total cholesterol level on the observed change in mortality pattern remains difficult. First, the trend in total cholesterol may differ according to age. The Dutch data suggest that a decreasing trend in total cholesterol is limited to young adults. Therefore, relations between changes in serum total cholesterol and mortality from coronary heart disease should not be analyzed for the total population but for specific age strata. Second, there is a lag between the occurrence of a decrease in mean serum total cholesterol level and the resulting decline in mortality from coronary heart disease. A decrease in the total cholesterol level in the population has an effect on mortality years later.[19-21] Third, other risk factors might also change. In the present study substantial changes in cigarette smoking from 1974 to 1986 were observed, while changes in blood pressure and body mass index (weight/height^2) were small (unpublished results, Consultation Bureau Heart Project and RIFOH Project).[22]

A recent investigation in the Netherlands compared mortality from coronary heart disease and hospital admission rates for acute ischemic heart disease for the period from 1971 to 1973 with those from 1985 to 1987.[23] For all 10-year age strata of subjects $\geq$ 30 years of age, mortality rates for ischemic heart disease had declined substantially (15-50%). The patterns for men and women were quite similar. However, hospital admission rates for age strata of $\geq$ 50 years increased, while hospital admission rates for the age strata of 30 to 39 and 40 to 49 years decreased. This is an indication that reduction of mortality in the older age strata may be caused by improved medical care, while in the younger age strata, an effect of primary prevention also plays a role. This is in accordance with our finding of a decline in total cholesterol level in relatively young adults in the RIFOH Project, and

an indication of an increase in total cholesterol levels in middle-aged and elderly men in the Zutphen Study.

From the present study, it can be concluded that serum total cholesterol levels in young adults in the Netherlands are declining, especially in the 1980s. Data from other Dutch investigations (Kromhout D, unpublished results) indicate that this decline is limited to younger age groups.[23] Detailed information about trends in serum total cholesterol levels in different age groups is, however, lacking. Because mean total cholesterol levels and the prevalence of hypercholesterolemia in the Netherlands are still high, a further lowering of average total cholesterol levels in the Dutch population seems a useful tool for enhancing the decline in morbidity and mortality from coronary heart disease.[24]

References

1. Overledenen naar doodsoorzaak, leeftijd en geslacht (A1-serie). Centraal Bureau voor de Statistiek, Voorburg, jrg. 1969-1988 (in Dutch).

2. Stamler J, Wentworth D, Neaton JD. Is the relationship between serum cholesterol and risk of premature death from coronary heart disease continuous and graded? JAMA 1986;256:2823-28.

3. Meijer J, van Geuns HA, Sluijter DP. CB Heart Project in the Netherlands. Screening for risk factors of coronary heart disease in consultation bureaus for tuberculosis. Hart Bulletin 1976;7:42-6.

4. Huang TC, Chen CP, Wefler V, et al. A stable reagent for the Liebermann Burchard reaction. Anal Chem 1961;33:1405-7.

5. Katterman R, Jaworek D, Möller G. Multicentre study of a new enzymatic method of cholesterol determination. J Clin Chem Clin Biochem 1984;22:245-51.

6. Erkelens DW. Cholesterol consensus in the Netherlands. Eur J Clin Nutr 1989;43:89-96.

7. SPSSPC/PC+ V2.0 Base Manual. SPSS Inc. Chicago. ISBN 0-918469-55-4, 1983.

8. Kleinbaum DG, Kupper LL, Muller KE. Applied regression analysis and other multivariable methods. PWS-KENT Publishing Company, Boston, 1988:265-79.

9. Report of the National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. Arch Intern Med 1988;148:36-79.

10. Boerma GJM. Welke kwaliteitseisen moeten aan bepalingen van totaal-cholesterol, HDL-cholesterol en triglyceriden worden gesteld? Hart Bull suppl 1987;1:35-40.

11. Kromhout D, De Lezenne Coulander C, Obermann-de Boer GL, et al. Changes in food and nutrient intake in middle-aged men from 1960 to 1985 (the Zutphen Study). Am J Clin Nutr 1990;51:123-9.

12. Keys A. Coronary heart disease in seven countries. Circulation 1970;41:suppl.I:1-211.

13. Knuiman JT, Katan MB. Cholesterolniveaus in serum in Nederland in vergelijking met die in de Verenigde Staten. Ned Tijdschr Geneesk 1985;129:2500-5.

14. Sempos C, Fulwood R, Haines C, et al. The prevalence of high blood cholesterol levels among adults in the United States. JAMA 1989;262:45-52.

15. National Heart, Lung and Blood Institute Collaborative Lipid Group. Trends in serum cholesterol levels among US adults aged 20 to 74 years. JAMA 1987;257:937-42.

16. Burke GL, Sprafka JM, Folsom AR, et al. Trends in CHD mortality, morbidity and risk factor levels from 1960 to 1986: The Minnesota Heart Survey. Int J Epidemiol 1989;18(suppl. 1):S73-S81.

17. Tuomilehto J, Puska P, Korhonen H. Trends and determinants of ischaemic heart disease mortality in Finland: with special reference to a possible levelling off in the early 1980s. Int J Epidemiol 1989; 18 (suppl. 1):S109-17.

18. The Research Group ATS-RF2-OB43 of the Italian National Research Council. Time trends of some cardiovascular risk factors in Italy. Results from the Nine Communities Study. Am J Epidemiol 1987;126:95-103.

19. Lipid Research Clinics Program. The Lipid Research Clinics Coronary Primary Prevention Trial results. II. The relationship of reduction in incidence of coronary heart disease to cholesterol lowering. JAMA 1984;251:365-74.

20. Manninen V, Elo O, Frick H, et al. Lipid alterations and decline in the incidence of coronary heart disease in the Helsinki Heart Study. JAMA 1988;260:641-51.

21. Rose G. Incubation period of coronary heart disease. Br Med J 1982;284:1600-01.

22. Blokstra A, Kromhout D. Trends in obesity in young adults in the Netherlands from 1974 to 1986. Int J Obes 1991;15:513-21.

23. Hoogendoorn D. Some remarks on the epidemic of acute myocardial infarction. Ned Tijdschr Geneeskd 1990;134:592-5 (in Dutch).

24. Rose G. The strategy of prevention: Lessons from cardiovascular disease. Br Med J 1981;282:1847-51.

3 Trends in total and HDL cholesterol levels in the Netherlands from 1987 to 1992

Abstract

Background. To gain insight into the prevalence of and trends in plasma cholesterol levels in the general population of the Netherlands, a monitoring project was carried out from 1987 to 1992.

Methods. Each year a random sample of men and women aged 20-59 years in three towns in the Netherlands was invited to participate in the study. Overall response rate was 50% for men and 57% for women and a total of almost 42,000 men and women participated. Total (TC) and HDL cholesterol (HDL-C) was measured and the non-HDL-C/HDL-C ratio was computed. Data were age-standardized to the Dutch population distribution aged 20-59 years.

Results. The prevalence of hypercholesterolemia (TC $\geq$ 6.5 mmol/l) in men ranged from 5% in the youngest (20-29 years) to 29% in the oldest age group (50-59 years), and from 4% to 38% in women. Low HDL-C levels ($\leq$ 0.9 mmol/l) in men ranged from 15% in the youngest to 26% in the oldest age group, and in women from 4% in the youngest to 7% in the oldest age group. The lipid profile of those with a higher educational level was more favorable than that of the less educated. From 1987 to 1992, in men, TC decreased by 0.12 mmol/l, HDL-C decreased by 0.07 mmol/l and the non-HDL-C/HDL-C ratio increased by 0.22. In women no statistically significant changes were observed. Changes over time did not differ according to age and educational level.

Conclusion. Prevalence of hypercholesterolemia is still high in the Netherlands. During the period 1987-1992 lipid profile worsened in men and remained stable in women.

Introduction

In recent decades both longitudinal and intervention studies have shown that plasma total cholesterol (TC) is an important risk factor for coronary heart disease (CHD).[1-4] More recently, evidence has accumulated that a low high density lipoprotein level (HDL-C) is an independent risk factor for coronary heart disease.[5-6] Coronary heart disease mortality in the Netherlands has declined during the last two decades, but it is still the most important cause of death.[7] Detailed information on the distribution of plasma total and HDL cholesterol levels and other risk factors in (subgroups of) the Dutch population was not available. For primary prevention it is important to know whether changes in CHD risk factors are taking place and whether high-risk groups can be identified in the general population. Therefore, in 1987 a monitoring project was started to gain insight into the distribution of the major CHD risk factors and changes in these risk factors. From 1987 to 1992 almost 42,000 men and women aged 20-59 years participated in this project. In this paper the data on plasma total and HDL cholesterol will be reported.

Methods

Study population

The Monitoring Project on Cardiovascular Disease Risk Factors has been carried out in the Netherlands from 1987 to 1992.[8] The aim of this project was to monitor the major risk factors for cardiovascular diseases, e.g. plasma cholesterol, blood pressure and smoking habits. The project was carried out at the municipal health services in three towns in the Netherlands: Amsterdam, the capital city in the western part of the country with about 700,000 inhabitants; Doetinchem, a small town in a rural area in the eastern part of the country with about 40,000 inhabitants, and Maastricht, a town in the south with about 100,000 inhabitants. Each year a new random sample of men and women aged 20-59 years was selected from the municipal registry of each town and invited to participate in the study. The overall response rate was 50% for men and 57% for women. In the years 1987-1992 a total of almost 42,000 men and women were examined.

To examine to what extent selection bias had taken place, a non-response survey was conducted. For this survey all non-respondents in the period August-December 1991 were

selected.[9] If possible a telephone interview was conducted, if no telephone number could be obtained a questionnaire was mailed that was identical to the one used in the telephone interviews. Because information on biological risk factors such as plasma cholesterol could not be obtained from the non-respondents, educational level was considered an important indicator variable.[10-12]

Data collection

The examination included measurement of weight, height and blood pressure.[8] A total of 30 ml blood was drawn (non-fasting). A questionnaire was filled out providing information about demographic variables, education, presence and family history of cardiovascular diseases, presence of a number of other chronic diseases, use of medication, smoking habits, selected dietary habits (by means of a short semi-quantitative food frequency questionnaire), physical activity, psycho-social factors and (for women) reproductive history. Education was assessed at seven levels ranging from primary education or less to university education. For the present analyses education was collapsed into three categories: low (intermediate secondary education or less), medium (intermediate vocational or higher secondary education) or high (higher vocational or university education).

Cholesterol determination

Total and HDL cholesterol determinations were performed at the Clinical Chemistry Laboratory of the University Hospital 'Dijkzigt' in Rotterdam. This laboratory takes part in the standardization program of the World Health Organization (WHO) through the WHO Regional Lipid Center for Europe in Prague, Czechoslovakia and the Centers for Disease Control (CDC), Atlanta, USA. It is the Lipid Reference Laboratory for standardized cholesterol determinations in the Netherlands and also serves as an international member of the Cholesterol Reference Method Laboratories Network in the USA.[13]

Plasma total cholesterol was determined enzymatically using a Boehringer testkit.[14] HDL lipoproteins were isolated by precipitation of the apo-B-containing lipoproteins with magnesium phosphotungstate.[15] Subsequently the cholesterol content of the HDL lipoproteins was determined, in the same way as total cholesterol. During the entire study period the quality control standards of the WHO were met. Furthermore, the internal quality control data of the laboratory were analyzed to detect whether, within the range

allowed by the WHO criteria, there had been laboratory 'drifts' that would influence the observed trend in cholesterol. If this was the case, the cholesterol values had to be corrected, as was done in earlier projects.[16]

Small adjustments were necessary over three periods. In two periods there had been a problem with calibration of the enzymatic method to the Abell-Kendal method, which is considered to be the 'gold standard'. From May 1990 to March 1991 a calibration error resulted in an overestimation of the samples by 0.92%. Over this period all cholesterol values were divided by 1.0092. From April 1991 to December 1992 there was a drift in the calibration serum of 0.05% per month. Therefore, over this period the cholesterol values were multiplied by $(1.0005)^{month}$, where 'month' equals 1 for April 1991, 2 for May 1991, ... and 21 for December 1992.

Quality control sera at a low ($\pm$ 1 mmol/l), medium ($\pm$ 4.5 mmol/l) and high level ($\pm$ 7 mmol/l) were, as a rule, included in all series of measurements. A shift in the values of the control sera indicates a shift in the results of the enzymatic method. With regression analysis the change in the level of a control serum over the study period was quantified. In the period September 1990 to March 1991 (after adjustment for the calibration error) the method shifted towards a lower level by 0.3% per month. Therefore, cholesterol values in this period are multiplied by $(1.003)^{month}$, where month equals 1 for September 1990 and 7 for March 1991. The coefficients of variation for the quality control sera at the low, medium and high level were 2.6%, 1.6% and 2.3% respectively, based on about 3,000 measurements for each level of the control serum.

Cutoff point for hypercholesterolemia was 6.5 mmol/l (250 mg/dl), according to the Netherlands Cholesterol Consensus.[17] For low HDL cholesterol the internationally used cutoff point of 0.9 mmol/l (35 mg/dl) was used.[18] The non-HDL-C/HDL-C ratio was computed, but no internationally used cutoff points are available for this parameter.

Statistical analyses
Compared to the Dutch population aged 20-59, in our study sample the older age groups were overrepresented. Therefore cholesterol levels were age-standardized to the age distribution of the Dutch population aged 20-59 years in 1990, by weighing age-specific data based on 5-year age groups. Because the primary goal of our analyses was to assess

changes over time, an age-standardized mean was computed for each month. Subsequently, monthly means were used in the analyses.

Statistical analyses were carried out using the statistical package SAS (version 6.07) on a minicomputer.[19] All pregnant women were excluded from the analyses. Trends over time were analyzed using linear regression analysis with time (month) as independent variable and the age-standardized monthly mean of the cholesterol parameter as dependent variable. Differences between age-standardized yearly means (computed as the average of the age-standardized monthly means) were tested by analysis of variance, using the Scheffé-test for multiple comparisons. Differences in trends between the three study centers, between different age groups and between different educational strata were examined using interaction terms. By including interaction terms (study center*month, age group*month and educational level*month) in the regression models we investigated if the interaction terms significantly increased the explained variance (tested with an F-statistic).[20]

Results

Levels
In table 1 the total number of people examined is presented by age, gender and educational level. In table 2 the observed age and gender results are presented for the total study period. Total cholesterol levels and the prevalence of hypercholesterolemia increased strongly with age in both men and women. In the age group 50-59 years mean TC levels in women exceeded those in men. Overall HDL-C decreased slightly with age, resulting in an increase of low HDL-C with age. Mean HDL-C was about 0.25 mmol/l higher in women compared to men. The non-HDL-C/HDL-C ratio was lower in women at all ages.

For all cholesterol parameters, age-adjusted levels were more favorable with increasing educational level. The differences between the different educational classes were larger in women than in men (figures 1a-c).

Trends
In table 3 age-standardized annual means and prevalences are given which, due to the standardization, represent the levels in the general population aged 20-59 in the Netherlands. Statistically significant differences between the annual means are indicated.

41

Table 1. *Number of people examined in the Monitoring Project on Cardiovascular Disease Risk Factors 1987-1992, by 10-year age group, gender and educational status*

Age	Educational Level		
	Low	Medium	High
Men			
20-29	1493	1416	560
30-39	2354	1228	1435
40-49	3435	934	1154
50-59	3722	777	799
Total	11004	4355	3948
Women			
20-29	1594	1747	685
30-39	3244	1088	1240
40-49	4395	715	868
50-59	5004	562	465
Total	14237	4112	3258

Table 2. *Mean, standard deviation (SD) and prevalence of high-risk levels of total and HDL cholesterol and non-HDL-C/HDL-C ratio in men and women aged 20-59 by 10-year age strata. Monitoring Project on Cardiovascular Disease Risk Factors, The Netherlands, 1987-1992*

	Men				Women			
	20-29	30-39	40-49	50-59	20-29	30-39	40-49	50-59
N	3531	5110	5622	5405	4070	5687	6062	6135
Total cholesterol								
mean (mmol/l)[a]	4.75	5.35	5.82	5.98	4.87	5.03	5.50	6.23
SD (mmol/l)	0.94	1.06	1.10	1.06	0.88	0.91	0.99	1.13
% $\geq$ 6.5 mmol/l	4.5	13.6	24.1	29.2	4.2	6.2	14.5	37.7
HDL cholesterol								
mean (mmol/l)	1.14	1.12	1.11	1.09	1.38	1.36	1.39	1.36
SD (mmol/l)	0.25	0.26	0.28	0.28	0.30	0.31	0.33	0.34
% $\leq$ 0.9 mmol/l	15.1	20.3	22.2	25.6	3.8	4.8	5.1	7.1
non-HDL-C/HDL-C ratio								
mean	3.37	4.06	4.56	4.82	2.68	2.90	3.21	3.88
SD	1.37	1.77	2.02	1.85	1.03	1.21	1.40	1.60

[a]: 1 mmol/l = 38.7 mg/dl

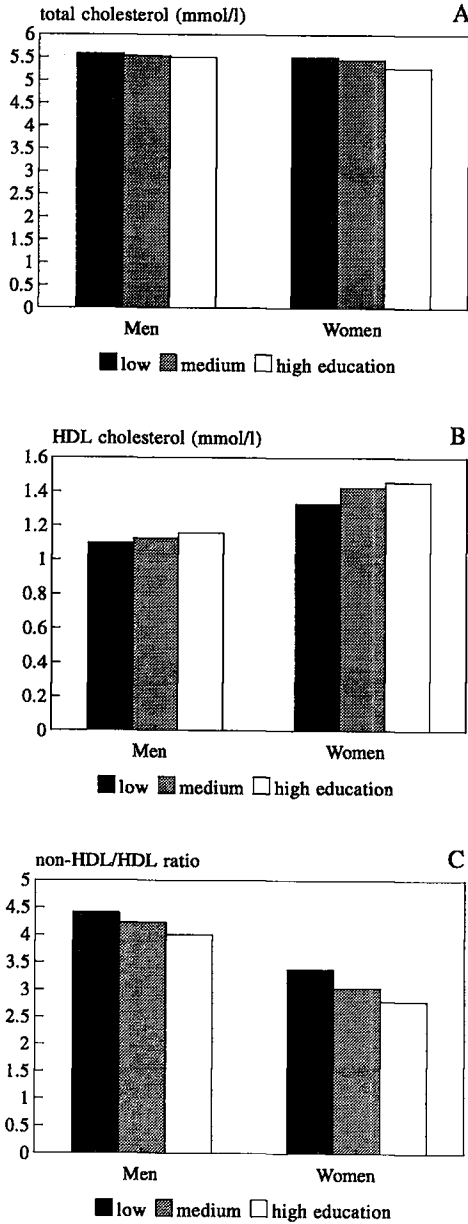**Figure 1.** *Mean total cholesterol (a), HDL cholesterol (b), and non-HDL-C/HDL-C ratio (c) by educational level in men and women, adjusted for age. The Monitoring Project on Cardiovascular Disease Risk Factors 1987-1992. All differences between educational levels are statistically significant (p< 0.01), except the difference in total cholesterol between the medium and high educational level in men.*

total cholesterol (mmol/l)   A

HDL cholesterol (mmol/l)   B
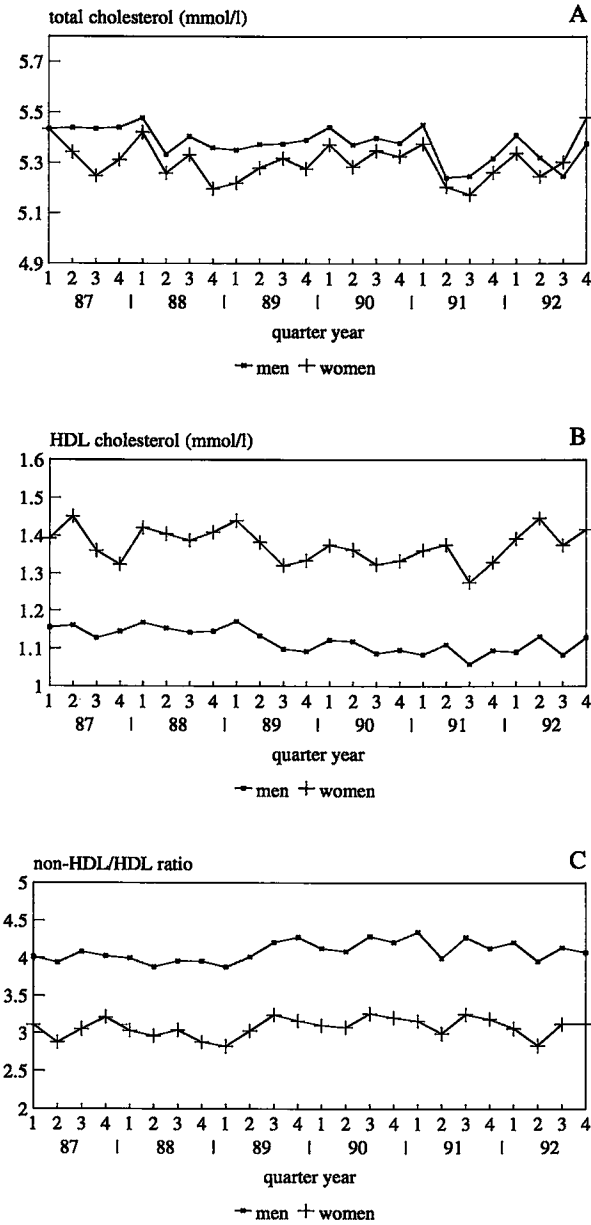

non-HDL/HDL ratio   C

Figure 2. *Mean total cholesterol level (a), HDL cholesterol level (b) and non-HDL-C/HDL-C ratio (c) per quarter year in men and women aged 20-59 in the period 1987-1992. Data are age-standardized to the age distribution of the Dutch population aged 20-59. The Monitoring Project on Cardiovascular Disease Risk Factors.*

44

Table 3. *Mean levels of total and HDL cholesterol and the non-HDL-C/HDL-C ratio and prevalence of high-risk levels of these per year for men and women aged 20-59 years. Data are age-standardized based on the age distribution of the Dutch population aged 20-59 years in the year 1990. Monitoring Project on Cardiovascular Disease Risk Factors, The Netherlands, 1987-1992*

Risk factor	Men						Women					
	1987	1988	1989	1990	1991	1992	1987	1988	1989	1990	1991	1992
N	2979	3835	3477	3248	3515	2614	3227	4264	3924	3603	3919	3007
Total cholesterol												
mean (mmol/l)[a]	5.44	5.39	5.37	5.40	5.31[b]	5.34	5.32	5.30	5.27	5.33	5.25	5.34
% ≥ 6.5 mmol/l	17.6	16.1	16.1	16.2	14.6	14.3	14.0	13.2	12.9	13.1	11.7	14.0
HDL cholesterol												
mean (mmol/l)	1.15	1.15	1.12	1.11[c]	1.09[bc]	1.11[c]	1.38	1.41	1.37	1.35	1.34[c]	1.41[d]
% ≤ 0.90 mmol/l	17.2	16.5	20.9	20.9	23.2[bc]	22.1[c]	4.8	3.6	5.0	5.7	5.1	5.5
non-HDL-C/HDL-C ratio												
mean	4.02	3.95	4.10	4.18[c]	4.19[c]	4.10[c]	3.06	2.98	3.07	3.16	3.15	3.03

a : 1 mmol/l = 38.7 mg/dl
b : significantly different from 1987
c: significantly different from 1988
d: significantly different from 1991

Table 4. *Regression coefficients for changes in cholesterol fractions over time, based on age-standardized data. Monitoring Project on Cardiovascular Disease Risk Factors, The Netherlands, 1987-1992*

		Men	Women
Total cholesterol			
Mean	intercept (mmol/l)[a]	5.44	5.31
	ß (mmol/l/year)	-0.021***	-0.001
	95% CL[b]	(-0.032;-0.010)	(-0.014;+0.012)
% ≥ 6.5 mmol/l	intercept (%/year)	17.8	13.5
	ß (%/year)	-0.66***	-0.14
	95% CL	(-1.02;-0.29)	(-0.46;+0.17)
HDL cholesterol			
Mean	intercept (mmol/l)	1.16	1.39
	ß (mmol/l/year)	-0.012***	-0.005
	95% CL	(-0.016;-0.008)	(-0.012;+0.002)
% ≤ 0.90 mmol/l	intercept (%/year)	16.1	4.0
	ß (%/year)	1.35***	0.30*
	95% CL	(+0.85;+1.84)	(+0.05;+0.56)
non-HDL-C/HDL-C ratio			
Mean	intercept (mmol/l)	3.98	3.02
	ß (mmol/l/year)	0.037**	0.019
	95% CL	(+0.014;+0.060)	(-0.002;+0.039)

a: 1 mmol/l = 38.7 mg/dl
b: 95% CL = 95% confidence limits
*: p<0.05, **: p<0.01, ***: p<0.001

In the figures 2a-c the changes over time in TC, HDL-C and the non-HDL-C/HDL-C ratio in men and women are shown by plotting age-standardized means per quarter (January-March, etc.). To establish a consistent upward or downward trend linear regression analysis was used (table 4). No differences in changes over time were found between the three educational levels, between study centers or between 10 year age groups, with the exception of women aged 50-59 (in whom the decrease in TC over time was stronger than for other age groups). Therefore only overall results are presented.

In men mean TC decreased statistically significantly by 0.02 mmol/l per year, resulting in an increase of hypercholesterolemia of 0.7 percentage points per year. In women no statistically significant changes in TC were observed. HDL-C decreased in men by 0.01 mmol/l per year and the prevalence of low HDL-C levels increased by 1.4 percentage points per year. In women the decrease in mean HDL-C was not statistically significant, while the increase in the prevalence of low HDL-C by 0.3 percentage points per year did reach statistical significance. The non-HDL-C/HDL-C ratio increased in both men and women, although only in men statistically significantly.

Discussion

An advantage of the present study was the continuous measurement of plasma cholesterol levels in a standardized laboratory in a large number of persons during a 6-year period. The figures show that the range of variation in mean cholesterol levels per quarter can be of the order of magnitude of 0.1-0.2 mmol/l, even though each mean value is based on about 1,000 persons. Therefore, comparing just two points in time can lead to spurious conclusions.

The overall response rate in our study was about 50% in men and about 57% in women. To assess possible selection bias that could have taken place, a non-response survey was carried out, in which education was considered an important variable for assessment of bias with regard to biological risk factors. In men the proportion of low, medium and high educational level was 56%, 23% and 22% respectively among respondents, and 52%, 27% and 21% respectively among non-respondents. For women the proportion of low, medium and high education was 63%, 20% and 17% respectively among respondents and 57%, 26% and 17% respectively among non-respondents.[9] Although we have no information on actual cholesterol levels of the non-respondents, the distribution with respect to education is an indication that the respondents of our study seem to be reasonably representative of the total group invited to participate in the study. It must also be noted that the non-response survey revealed that about 10% of the addresses obtained from the municipal registries were outdated, thus contributing substantially to the observed non-response in our study.

During the study the response rate dropped by about 5%. Because this percentage is small compared to the total non-response, and because the difference between respondents and

non-respondents in educational level was not striking, it seems unlikely that a decrease in response rate of this magnitude can explain the observed trends.

Levels

The results of the present study showed that, especially in middle age, hypercholesterolemia is a common risk factor in the Netherlands. When our data were standardized to the general population aged 20-59 years, about 1 out of 6 men and 1 out of 8 women had hypercholesterolemia. When our data are compared with data from other industrialized countries (obtained in the MONICA Project at the beginning of the 1980s), it shows that TC levels in Scandinavia (Denmark, Sweden, Finland and Iceland) and some eastern European countries (former Soviet Union, East Germany and Czechoslovakia) are on average about 0.5 mmol/l higher than in the Netherlands.[21] Levels in Spain, Italy, Canada and Australia are comparable to those in the Netherlands and TC levels in the United States are about 0.5 mmol/l lower.[21] This shows that in other industrialized countries (except for the United States) hypercholesterolemia is as prevalent or even more prevalent than in the Netherlands.

Mean HDL-C levels in men were about 0.25 mmol/l lower than in women. Consequently, about 1 in 5 men had low HDL-C levels compared to about 1 in 17 women. Compared with a recent study in 38 year old men in 6 European countries, in which HDL-C was measured in the same laboratory, HDL-C in Dutch men was similar to that in Polish men, but 0.10 to 0.15 mmol/l lower than that in men from Sweden, Belgium and Italy.[22] A similar study in 38 year old women showed that differences in HDL-C between women were less marked.[23]

Trends

In the period 1987-1992 mean TC levels declined in men by about 0.1 mmol/l while in women no significant change was observed. A conclusion about the long term trend in TC levels in the general Dutch population is difficult, because data are scarce and concern a limited age range only. Previously we have analyzed data of two screening projects carried out between 1974-1980 (in men and women aged 37-43) and 1981-1986 (in men aged 33-37).[16] Between 1974 and 1980 a decrease in TC of 0.07 mmol/l in men and 0.03 mmol/l in women was observed, followed by a decline of 0.2 mmol/l in men between 1981-1986. Because there were indications that this decline was not generalizable to other age groups, it is difficult to make inferences for the general population during this period.[24] So far, the

available data point into the direction of relatively stable TC levels over the past 17 years, with the exception of a decline in relatively young adult men in the early eighties, and a recent decline of 0.1 mmol/l in 1991/1992 in men.

This suggests that the marked decline in CHD mortality over the past decades, is not likely to be explained by changes in cholesterol levels. Of the major risk factors, the decline in the prevalence of cigarette smoking in the last decades probably has contributed most to the decline in CHD mortality. Furthermore, survival of acute myocardial infarction has increased, due to therapeutic improvements. When only survival rates increase without a decrease in the incidence of the disease the burden of illness in the population will increase. Unfortunately, data on the incidence of CHD are scarce. Data of a regional registration on the incidence of myocardial infarction did not indicate a decreasing incidence of CHD since the beginning of the 1970s.[25] A study that compared hospital admission rates for acute CHD in the period 1971-1973 with that for 1985-1987 showed a decrease in hospital admission rates for persons below the age of 50 years, but an increase for those over 50 years.[26] Therefore, there is no reason to diminish primary preventive efforts.

In many industrialized countries efforts have been made to decrease cholesterol levels in the general population. In the United States a number of studies have shown that TC levels have decreased substantially over the last decades.[27-30] Because of these changes, cholesterol levels in the US are now lower than in the Netherlands, while at the beginning of the 1960s cholesterol levels in middle-aged men in the US and the Netherlands were comparable.[21,31] Also in Finland and Denmark TC levels decreased substantially between 1972 and 1982, followed by a smaller or no decrease between 1982 and 1987.[32-34] These data show that it is feasible to achieve a substantial change in cholesterol level in the general population.

The decline in HDL-C in men is an unfavorable development. Data on trends in HDL-C in other countries and long term trend data for the Netherlands are to our knowledge not available. The observed changes in total and HDL cholesterol levels could not be explained by changes in BMI, smoking, alcohol consumption, physical activity and educational level over the study period. When these variables were included in the regression analyses the coefficients for the change in cholesterol over time hardly changed.

49

Over the study period the non-HDL-C/HDL-C ratio increased. If this ratio is interpreted as a measure of CHD risk that combines both the unfavorable effect of TC and the favorable effect of HDL, this would mean that over the total study period the lipid profile of Dutch men worsened, while it remained the same in Dutch women.

The changes that occurred over the period 1987-1992 were comparable in different age, gender and educational strata. It could be expected that public health education about healthy lifestyles reaches higher educated people more easily than the less educated. However, also other studies have found that changes were similar in different socio-economic strata.[28,35] An explanation for the observed changes could be that certain lifestyle factors changed throughout the population. In this context, it would be interesting to examine changes in nutritional patterns in the Netherlands, for example with respect to fatty acid composition of the diet, concurrent with changes in TC levels.

Recently, data have become available of the second National Food Consumption Survey, carried out in 1992. When these data were compared with the first survey carried out in 1987/1988, it showed that consumption of saturated fat had decreased by about 2 percentage points in both men and women.[36]

In the spring of 1991 a campaign was launched to reduce fat intake in the Dutch population. For simplicity, it focused on a reduction in total fat intake. Evaluation of the campaign showed that in women the change in attitude towards products with a low (saturated) fat content was more pronounced than in men.[37] These observations are however not compatible with the observation that the decline in TC was significant in men but not in women.

The present study showed that, especially in middle age, prevalence of high TC (in men and women) and of low HDL-C (in men) is high in the Netherlands. Less educated respondents showed a more unfavorable risk profile than better educated respondents. Despite all the efforts of public health education over recent decades, TC levels did not change substantially in the Netherlands. It is disappointing to see that the most striking change over the past 5 years has been a decrease in HDL-C level. Based on the combination measure non-HDL-C/HDL-C ratio, it can be concluded that the lipid profile worsened in men and remained the same in women.

Until now most primary prevention activities have been focused on TC levels. Because the evidence for the importance of HDL-C in the prevention of coronary heart disease is

increasing, lifestyle changes should be aimed at both lowering TC and increasing HDL-C. To achieve this, apart from dietary changes to lower TC levels, attention should be given to stimulating physical activity, prevention and treatment of obesity and discouraging of smoking.

References

1. Stamler J, Wentworth D, Neaton JD. Is the relationship between serum cholesterol and risk of premature death form coronary heart disease continuous and graded? Findings in 356,222 primary screenees of the multiple risk factor intervention trial (MRFIT). JAMA 1986;256:2823-28.

2. Anderson KM, Castelli WP, Levy D. Cholesterol and mortality. 30 years of follow-up from the Framingham Study. JAMA 1987;257:2176-80.

3. Lipid Research Clinics Program. The Lipid Research Clinics Coronary Primary Prevention Trial results. II. The relationship of reduction in incidence of coronary heart disease to cholesterol lowering. JAMA 1984;251:365-74.

4. Manninen V, Elo MO, Frick H, et al. Lipid alterations and decline in the incidence of coronary heart disease in the Helsinki Heart Study. JAMA 1988;260:1237-45.

5. Gordon DJ, Probstfield JL, Garrison RJ, et al. High-density lipoprotein cholesterol and cardiovascular disease. Four prospective American studies. Circulation 1989;79:8-15.

6. Jacobs DR, Mebane IL, Bangdiwala SI, Criqui MH, Tyroler HA. High density lipoprotein cholesterol as a predictor of cardiovascular disease mortality in men and women: the follow-up study of the Lipid
Research Clinics Prevalence Study. Am J Epidemiol 1990;131:32-47.

7. Overledenen naar doodsoorzaak, leeftijd en geslacht (A1-serie). Voorburg, Netherlands: Centraal Bureau voor de Statistiek, 1969-1988.

8. Kromhout D, Obermann-De Boer GL, van Kampen-Donker M, Verschuren WMM. The Monitoring Project on Cardiovascular Disease Risk Factors 1987. Report 5289001. National Institute of Public Health and Environmental Protection, Bilthoven, the Netherlands, 1989 (in Dutch).

9. Verschuren WMM, Leer E van, Blokstra A, et al. Cardiovascular risk factors in the Netherlands. Neth J Cardiol 1993;4:205-10.

10. Kraus JF, Borhani NO, Franti CE. Socioeconomic status, ethnicity, and risk of coronary heart disease. Am J Epidemiol 1980;111:407-14.

11. Jacobsen BK, Thelle DS. Risk factors for coronary heart disease and level of education: The Tromso Heart Study. Am J Epidemiol 1988;127:923-32.

12. Duijkers TJ, Kromhout D, Spruit IP, Doornbos G. Inter-mediating risk factors in the relation between socioeconomic status and 25-year mortality (the Zutphen Study). Int J Epidemiol 1989;18:658-62.

13. Myers GL, Cooper GR, Winn CL, Smith SJ. The Center for Disease Control - National Heart, Lung and Blood Institute Lipid Standardization Program. Clin Lab Med 1989;9:105-35.

14. Katterman R, Jaworek D, Möller G. Multicenter study of a new enzymatic method of cholesterol determination. J Clin Chem Clin Biochem 1984;22:245-51.

15. Lopes-Virella MF, Stone P, Ellis S, Colwell JA. Cholesterol determination in high-density lipoproteins separated by three different methods. Clin Chem 1977;23:882-4.

16. Verschuren WMM, Al M, Blokstra A, Boerma GJM, Kromhout D. Trend in serum total cholesterol level in 110,000 young adults in the Netherlands, 1974 to 1986. Am J Epidemiol 1991;134:1290-1302.

17. Anonymous. Netherlands Cholesterol Consensus Update. Hart Bulletin 1992;(suppl)23:9-21 (in Dutch).

18. European Atherosclerosis Society. Strategies for the prevention of coronary heart disease: A policy statement of the European Atherosclerosis Society. Eur Heart J 1987;8:77-88.

19. SAS Institute Inc., SAS/STAT User's Guide, Version 6, Fourth Edition, Volume 1 and 2, Cary, NC: SAS Institute Inc., 1989.

20. Kleinbaum DG, Kupper LL, Muller KE. Applied regression analysis and other multivariable methods. Boston MA: PWS-KENT Publishing Company, 1988:265-79.

21. WHO. The WHO MONICA Project. A worldwide monitoring system for cardiovascular diseases. World Health Stat Ann 1989:27-149.

22. Seidell JC, Cigolini M, Deslypere JP, Charzewska J, Ellsinger BM, Cruz A. Body fat distribution in relation to serum lipids and blood pressure in 38-year old European men: the European fat distribution study. Atherosclerosis 1991;86:251-60.

23. Seidell JC, Cigoline M, Charzewska J, et al. Indicators of fat distribution, serum lipids, and blood pressure in European women born in 1948 - The European Fat Distribution Study. Am J Epidemiol 1989;130:53-65.

24. Kromhout D, De Lezenne Coulander C, Obermann-de Boer GL, et al. Changes in food and nutrient intake in middle-aged men from 1960 to 1985 (the Zutphen Study). Am J Clin Nutr 1990;51:123-9.

25. Ruwaard D, Kramers PGN (ed.). Public health status and forecasts. The health status in the Netherlands over the period 1950-2010. National Institute of Public Health and Environmental Protection. The Hague, Sdu Uitgeverij Plantijnstraat, 1993, p 381 (in Dutch).

26. Hoogendoorn D. Some remarks on the epidemic of acute myocardial infarction. Ned Tijdschr Geneeskd 1990;134:592-5 (in Dutch).

27. Sytkowski PA, Kannel WB, D'Agostino RB. Changes in risk factors and the decline in mortality from cardiovascular disease. The Framingham Heart Study. N Engl J Med 1990;322:1635-41.

28. National Center for Health Statistics-National Heart, Lung, and Blood Institute Collaborative Lipid Group. Trends in serum cholesterol levels among US adults aged 20 to 74 years. Data from the National Health and Nutrition Examination Surveys, 1960 to 1980. JAMA 1987;257:937-42.

29. Burke GL, Sprafka JM, Folsom AR, Hahn LP, Luepker RV, Blackburn H. Trends in serum cholesterol levels from 1980 to 1987. The Minnesota Heart Survey. N Engl J Med 1991;324:941-6.

30. Johnson CL, Rifkind BM, Sempos CT, et al. Declining serum total cholesterol levels among US adults. The National Health and Nutrition Examination Surveys. JAMA 1993;269:3002-8.

31. Keys A. Coronary heart disease in seven countries. Circulation 1970;41(suppl I):1-211.

32. Vartianen E, Korhonen HJ, Pietinen P, et al. Fifteen-year trend in coronary risk factors in Finland, with special reference to North Karelia. Int J Epidemiol 1991;20:651-62.

33. Tuomilehto J, Puska P, Korhonen H, et al. Trends and determinants of ischaemic heart disease mortality in Finland: with special reference to a possible levelling off in the early 1980s. Int J Epidemiol 1989;18(Suppl.1):S109-S117.

34. Sjol A, Grunnet K, Schroll M. Secular trends in serum cholesterol, high density lipoproteins and triglycerides 1964-1987. Int J Epidemiol 1991:105-13.

35. Winkleby MA, Fortmann SP, Rockhill B. Trends in cardiovascular disease risk factors by educational level: The Stanford Five-City Project. Prev Med 1992;21:592-601.

36. Lower fat consumption in the second National Food Consumption Survey. Is the 'Fat Watch' campaign successful? Löwik MRH, Hulshof KFAM, Kistemaker C. Voeding 1993;54;27-9 (in Dutch).

37. Feen de Lille JCJF van der, Wedel M, Jonkers R, et al. Evaluation national campaign 'Watch your fat intake'. TNO reportnumber V 91.272 (in Dutch). Zeist, the Netherlands, 1991.

4 Total and HDL cholesterol levels in premenopausal and postmenopausal women

Abstract

Background. In the Netherlands total cholesterol levels in women increase considerably around middle age. This led us to investigating the role of the menopause in levels of total and HDL cholesterol.

Methods. The Netherlands Monitoring Project on Cardiovascular Disease Risk Factors was carried out between 1987 and 1991. To examine the effect of the menopause on cholesterol levels, women aged 45-54 who were either premenopausal (N=2,567) or who had had a naturally occurring menopause (N=1,657) were selected.

Results. After adjustment for age, smoking and body mass index, non-smoking postmenopausal women were found to have a 0.45 mmol/l higher total cholesterol level than non-smoking premenopausal women, while in smoking women this difference was 0.28 mmol/l. HDL cholesterol levels were 0.04 mmol/l higher in postmenopausal women compared to premenopausal women after adjustment for age, BMI, smoking, alcohol consumption and physical activity. No interaction with smoking was observed for HDL cholesterol levels.

Conclusion. Menopause plays an important role in the marked increase in total cholesterol levels in middle-aged women, more so in non-smokers than in smokers. Menopause was associated with a small favorable effect on HDL cholesterol levels.

Introduction

It has been shown that total cholesterol is a major risk factor for coronary heart disease in men as well as in women.[1-4] Total cholesterol increases markedly around middle-age. While in younger age groups total cholesterol is lower in women than in men, above age 50 total cholesterol levels in women usually exceed those in men.[5] In our study population, 45% of the women aged 55-59 had hypercholesterolemia, defined according to the classification of the Netherlands Cholesterol Consensus as a total cholesterol of 6.5 mmol/l or more.[6,7] In aging populations therefore, hypercholesterolemia in women is a growing public health concern.

Knowledge on factors influencing cholesterol levels in women are important with respect to prevention of hypercholesterolemia. Most women go through the menopause between age 45 and 55. It is known that the menopause influences total cholesterol levels although estimates of the magnitude of this effect differ.[8-19] In studies that provide information on HDL cholesterol levels, no effect of menopause on HDL cholesterol levels is reported.[8,10,19]

From 1987 to 1991 the Netherlands Monitoring Project on Cardiovascular Disease Risk Factors has been carried out.[7] In this project over 36,000 men and women have been examined for cardiovascular risk factors such as plasma cholesterol, blood pressure, smoking and body mass index. These data provided the opportunity to investigate the relation between menopause, body mass index and cholesterol levels cross-sectionally in over 4,000 women aged 45-54 years.

Materials and methods

Study population
The Monitoring Project on Cardiovascular Disease Risk Factors has been carried out in the Netherlands from 1987 to 1991. The aim of this project was to monitor major risk factors for cardiovascular diseases, e.g. plasma cholesterol, blood pressure and smoking habits. The project was carried out at the municipal health services in three towns in the Netherlands: Amsterdam, the capital city in the west, Doetinchem in the east and Maastricht in the south. Each year a new random sample of men and women aged 20 to

59 is selected in each town. Because age and menopausal status are highly correlated, it is necessary to compare premenopausal and postmenopausal women in a narrow age range, to reduce the possibility of ascribing an effect of aging to the occurrence of menopause. Therefore, the analyses comparing premenopausal and postmenopausal women were restricted to women aged 45 to 54 years. The overall response rate for women in this age groups was 64%.

Data collection

The examination included measurement of weight, height and blood pressure, blood sampling and a questionnaire.[7] The respondents were weighed without shoes, after emptying their pockets, with an accuracy of 0.1 kg. Height was measured with an accuracy of 0.5 cm, while the respondent stood upright against the wall with the feet at a 45° angle. Body mass index (BMI) was calculated as weight(kg)/height(m)². Total and HDL cholesterol were determined in a non-fasting blood sample, at the Clinical Chemistry Laboratory of the University Hospital 'Dijkzigt' in Rotterdam. This laboratory takes part in the standardization program of the WHO through the WHO Regional Lipid Center for Europe in Prague, Czechoslovakia and the Centers for Disease Control (CDC), Atlanta, Georgia, USA.[20] Plasma total cholesterol was determined enzymatically using a Boehringer testkit.[21] HDL lipoproteins were isolated by precipitation of the apo-B-containing lipoproteins with magnesium phosphotungstate.[22] Subsequently the cholesterol content of the HDL lipoproteins was determined, as described for total cholesterol.

The questionnaire provided information about demographic variables, presence and family history of cardiovascular and a number of other chronic diseases, medication, smoking habits, physical activity, psycho-social factors and reproductive history. Menopausal status was evaluated by means of a question about regularity of menstruation. Response categories to this question were: regular, irregular, not applicable because of pregnancy, not applicable because of the menopause and not applicable due to surgery. The first two categories were classified as premenopausal. Women reporting that they no longer menstruated because of the menopause were classified as postmenopausal. Women reporting they did not menstruate due to surgery were excluded from the analysis because the type of operation (hysterectomy and/or oophorectomy) was not known, and therefore hormonal status could not be determined.

In the analyses present cigarette use was dichotomized into non-smoker versus smoker ($\geq$ 1 cigarette per day). Alcohol consumption (glasses/day) was used as a continuous variable. One question about physical activity at work and one about leisure time was asked. Physical activity during leisure time was used in the analyses. Answering categories were: no activity, exercise for at least four hours per week, regular exercise, heavy exercise. The first category was classified as inactive, the others as active.

Statistical analyses

The analyses were carried out on a personal computer using the statistical package SAS.[23] Regression analyses were performed using plasma total and HDL cholesterol as dependent variables. The total cholesterol difference between premenopausal and postmenopausal women was estimated in a regression model in which menopausal status, age, BMI and smoking were included as independent variables. For HDL cholesterol also alcohol consumption and physical activity were included in the regression model. It was tested whether the difference in total cholesterol between premenopausal and postmenopausal women was modified by cigarette smoking, body mass index (kg/m²) and age (45 to 49 years versus 50 to 54 years). For HDL cholesterol also interaction with physical activity or alcohol consumption was examined. By including interaction terms in the model (menopausal status*smoking, menopausal status*body mass index, etc.) it was tested whether the interaction terms significantly increased the explained variance (tested with an F-statistic).

Results

In table 1 some basic characteristics of premenopausal and postmenopausal women are given. The postmenopausal women were on average 3 years older than the premenopausal women. Total cholesterol levels were higher in postmenopausal women than in premenopausal women, cigarette smoking was more prevalent in postmenopausal women while consumption of alcohol was more prevalent in premenopausal women. No difference between the two groups was observed in HDL cholesterol level, body mass index and physical activity.

In table 2 results of the multivariate regression model are presented. The coefficient for menopause from this model can be interpreted as the difference in total or HDL

cholesterol between premenopausal and postmenopausal women, adjusted for the other variables in the model. The effect of menopause on total cholesterol levels was different in non-smokers compared to smokers, while no significant interactions of menopause with body mass index and age were observed. The estimated increase in total cholesterol due to menopause was 0.45 mmol/l in non-smokers and 0.28 mmol/l in smokers (0.448 minus 0.166). The increase in total cholesterol with age was 0.047 mmol/l per year, while the increase in total cholesterol per unit increase in BMI amounted to 0.026 mmol/l. The difference in total cholesterol between smokers and non-smokers was 0.28 mmol/l. In figure 1 the difference in total cholesterol between premenopausal and postmenopausal women according to smoking status is shown, estimated from the regression equation.

Table 1. *Basic characteristics (mean (SD)) of premenopausal and postmenopausal women aged 45-54 years. The Netherlands Monitoring Project on Cardiovascular Disease Risk Factors, 1987-1991*

	Premenopausal N=2,567		Postmenopausal N=1,657	
	mean	SD	mean	SD
Age (years)	48.7	2.40	51.7[1]	2.23
Total cholesterol (mmol/l)	5.63	1.03	6.18[1]	1.08
HDL cholesterol (mmol/l)	1.37	0.32	1.37	0.33
Body Mass Index (kg/m²)	25.96	4.39	26.02	4.22
Smokers (%)	32.1	46.7	41.2[a]	49.2
Alcohol users (%)	54.5	49.8	47.5[a]	50.0
Physically active (%)	62.8	51.6	65.6	52.5

a: significantly different from premenopausal (p<0.001)

Table 2. *Menopause and lifestyle factors in relation to total and HDL cholesterol levels in 4,224 women aged 45-54 years. The Netherlands Monitoring Project on Cardiovascular Disease Risk Factors, 1987-1991*

	Total cholesterol (mmol/l)		HDL cholesterol (mmol/l)	
	β[a]	SE[b]	β	SE
Menopause (no/yes)	0.448	0.047	0.040	0.011
Menopause*smoking	-0.166	0.015	-	-
Age (years)	0.047	0.007	-0.003	0.002
Body Mass Index (kg/m²)	0.026	0.004	-0.020	0.001
Cigarette smoking (no/yes)	0.284	0.044	-0.140	0.010
Alcohol consumption (glass/day)	-	-	0.069	0.004
Physical activity (no/yes)	-	-	0.030	0.010

a: regression coefficient
b: standard error
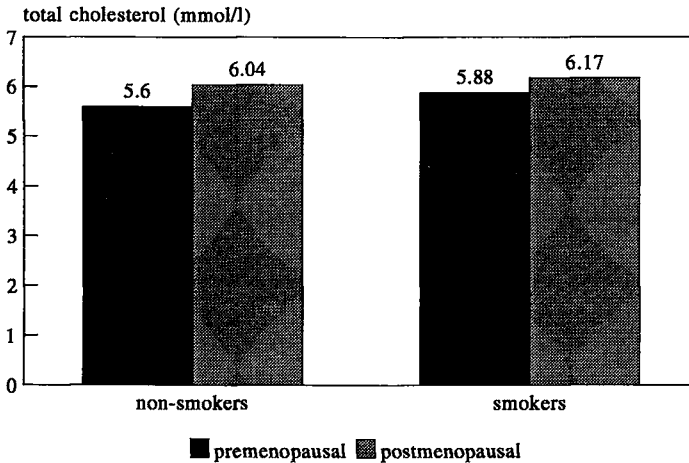
total cholesterol (mmol/l)

Figure 1. *Total cholesterol level in premenopausal and postmenopausal women according to smoking status. Estimated from the regression equation (table 2) for a woman aged 50 with a BMI of 26 kg/m².*

After multivariate analysis, HDL cholesterol was 0.04 mmol/l higher in postmenopausal compared to premenopausal women. HDL cholesterol was not associated with age. BMI was inversely related to HDL cholesterol: HDL cholesterol was 0.025 mmol/l lower for each unit increase in BMI. Smoking was associated with a 0.140 mmol/l lower HDL cholesterol level. Per glass of alcohol consumed an increase of 0.069 mmol/l in HDL cholesterol was observed. Physically inactive women had a 0.03 mmol/l lower HDL cholesterol level than physically active women. No interaction of menopause with age, smoking, body mass index, alcohol consumption and physical activity was observed for HDL cholesterol levels.

Discussion

Some limitations of the present study must be kept in mind when interpreting the results. Menopausal status was self-reported in our study, which might give rise to misclassification. Bonithon-Kopp et al. validated self-reported menopause against serum concentrations of follicle-stimulating hormone and estradiol.[12] The menopause was confirmed for 94% of the women who reported a natural menopause, but only for 70%

of the women who had had a hysterectomy. In our study, women with a surgical menopause (hysterectomy or oophorectomy) were excluded because of unknown hormonal status. Mean total cholesterol levels for these women were intermediate between those for premenopausal and naturally postmenopausal women, confirming the heterogeneous hormonal status of this group (results not shown).

No information on the use of hormones was obtained in the present study, but at the time of the study hormone replacement therapy was only used by a small proportion of the postmenopausal women in the Netherlands. A study of about 12,000 women (aged 40-73 years) attending a routine breast cancer screening in the Netherlands, showed that three percent of all postmenopausal women used estrogens. Estrogen use was highest among perimenopausal women: about 10%.[13] An American study showed that in 1989/1990 about 3.5% of all women who had had a natural menopause used estrogens as opposed to over 20% of the women with a surgical menopause.[24] These results show that it is not likely that the results of the present study, in women with a natural menopause, are strongly biased due to lack of information on estrogen use. Since estrogen use is associated with lower total cholesterol levels, any bias that would occur leads to an underestimation of the cholesterol increase due to menopause.

In the present cross-sectional study, the estimated increase in total cholesterol due to menopause amounted to 0.45 mmol/l in non-smokers and 0.28 mmol/l in smokers after adjustment for age, smoking and body mass index. We know of no other study that reported the effect of menopause stratified by cigarette smoking status. However, interaction between cigarette smoking and hormonal status has been suggested by the fact that smokers have an earlier menopause than non-smokers.[25-26] It has been proposed that smokers may metabolize estrogen at a different rate from non-smokers.[27] Our results are compatible with this hypothesis: due to the fact that the estrogen concentration is much lower after menopause, the difference in total cholesterol between non-smokers and smoker diminishes.

In a number of other cross-sectional studies higher total cholesterol levels for postmenopausal compared to premenopausal women were reported. However, the reported magnitude of the effect of menopause differs. Bonithon-Kopp and co-workers reported an increase in total cholesterol due to menopause of 0.36 mmol/l in women aged 45 to 54 years.[8] In the Evans County Study, an increase of 0.65 mmol/l was found for white

women.[9] In both studies adjustment for smoking, age and body mass index was made. In studies that did not adjust for differences in BMI or smoking, differences in total cholesterol between premenopausal and postmenopausal women ranged from 0.09 to 0.62 mmol/l.[10-14]

Smoking and BMI are associated with menopausal age: smoking is inversely and BMI is positively associated with age at menopause.[25,26] Therefore, differences in BMI and smoking prevalence influence the observed difference in total cholesterol between premenopausal and postmenopausal women in different age strata. The fact that smoking women have an earlier menopause is reflected in the present study by the higher percentage of smokers in the group of postmenopausal women compared to the group of premenopausal women.

It can be argued that comparing premenopausal and postmenopausal women in a cross-sectional study can not prove that menopause precedes the cholesterol increase. Data from the Framingham Study indicate that the effect of menopause on total cholesterol is a more or less 'immediate' effect, taking place within 1 to 2 years.[16] In the Framingham Study the longitudinal increase in total cholesterol in women who had become postmenopausal was about 0.30 mmol/l higher than the increase in controls who remained premenopausal.[16] Because of its longitudinal design, the Framingham Study is one of the few studies able to show convincingly that menopause precedes the rise in serum total cholesterol. Lindquist observed an increase of about 0.40 mmol/l over a 6-year period for women whose menopausal status changed during that period.[15] The results of these longitudinal studies are well in range with most cross-sectional studies. A Dutch study reported a total cholesterol increase of 1.1 mmol/l over an 8-year period in women who became postmenopausal during that period.[17] From the present study it can be estimated that the increase in total cholesterol over an 8-year period, based on an effect of menopause of 0.45 mmol/l in non-smokers and 0.28 mmol/l in smokers and an increase of 0.048 mmol/l per life-year, would be 0.83 mmol/l in non-smokers and 0.66 in smokers.

HDL cholesterol does not change much with age. Our results indicated a small positive effect of menopause on HDL cholesterol in multivariate but not in univariate analyses. This was due to the fact that smoking is inversely related to HDL cholesterol levels, and the percentage of smokers was higher in postmenopausal women while HDL cholesterol levels were similar. Therefore, after adjustment for smoking, HDL cholesterol levels were

higher in postmenopausal women. No interaction with age, body mass index, smoking, alcohol consumption or physical activity was observed. Also in the Minnesota Heart Study HDL cholesterol levels were higher in women with a natural menopause compared to premenopausal women after adjustment for age, smoking, body mass index, physical activity and alcohol consumption, but this difference (0.07 mmol/l) was not statistically significant.[19] Other studies that examined HDL cholesterol levels, however, did not find an effect of menopause on HDL cholesterol levels.[8,10]

It can be concluded that menopause plays an important role in the marked increase in total cholesterol levels in middle-aged women. In the present study, menopause was associated with a small increase in HDL cholesterol levels.

References

1. Castelli WP, Garrison RJ, Wilson PWF, Abbott RD, Kalousdian S, Kannel WB. Incidence of coronary heart disease and lipoprotein cholesterol levels. The Framingham Study. JAMA 1986;256:2835-8.

2. Lipid Research Clinics Program. The Lipid Research Clinics Coronary Primary Prevention Trial results. II. The relationship of reduction in incidence of coronary heart disease to cholesterol lowering. JAMA 1984;251:365-74.

3. Neaton JD, Blackburn H, Jacobs D, et al. Serum cholesterol level and mortality: findings for men screened in the Multiple Risk Factor Intervention Trial. Arch Intern Med 1992;152:1490-1500.

4. Manolio TA, Pearson TA, Wenger NK, Barrett-Connor E, Payne GH, Harlan WR. Cholesterol and heart disease in older persons and women. Review of an NHLBI Workshop. Ann Epidemiol 1992;161-76.

5. Expert Panel: Report of the National Cholesterol Education Program Panel on detection, evaluation, and treatment of high blood cholesterol in adults: NHLBI. Arch Intern Med 1988;148:36-9.

6. Erkelens DW. Cholesterol Consensus in the Netherlands. Eur J Clin Nutr 1989;43:89-96.

7. Verschuren WMM, Leer EM van, Blokstra A, et al. Cardiovascular disease risk factors in the Netherlands. Neth J Cardiol 1993;4:205-10.

8. Bonithon-Kopp C, Scarabin PY, Darne B, Malmejac A, Guize L. Menopause related changes in lipoproteins and some other cardiovascular risk factors. Int J Epidemiol 1990;19:42-8.

9. Baird DD, Tyroler HA, Heiss G, Chambless LE, Hames CG. Menopausal change in serum cholesterol. Black/white differences in Evans County, Georgia. Am J Epidemiol 1985;122:982-93.

10. Shibata H, Haga H, Suyama Y, Kumagai S, Seino T. Serum total and HDL cholesterol according to reproductive status in Japanese females. J Chron Dis 1987;40:209-13.

11. Shibata H, Matsuzaki T, Hatano S. Relationship of relevant factors of atherosclerosis to menopause in Japanese women. Am J Epidemiol 1979;109:420-4.

12. Lindquist O, Bengtsson C. Serum lipids, arterial blood pressure and body weight in relation to the menopause: results from a population study of women in Göteborg, Sweden. Scand J Clin Lab Invest 1980;40:629-36.

13. Weiss N. Relationship of menopause to serum cholesterol and arterial blood pressure: The United States' Health Examination Survey of adults. Am J Epidemiol 1972;96:234-41.

14. Kannel WB, Hjortland MC, McNamara PM, Gordon T. Menopause and risk of cardiovascular disease. The Framingham Study. Ann Intern Med 1976;85:447-52.

15. Lindquist O. Intraindividual changes of blood pressure, serum lipids and body weight in relation to menstrual status: results from a prospective population study of women in Göteborg, Sweden. Prev Med 1982;11:162-72.

16. Hjortland MC, McNamara PM, Kannel WB. Some atherogenic concomitants of menopause: The Framingham Study. Am J Epidemiol 1976;103:304-11.

17. Beresteijn EC van, Korevaar JC, Huijbregts PC, Schouten EG, Burema J, Kok F. Perimenopausal increase in serum cholesterol: a 10-year longitudinal study. Am J Epidemiol 1993;137:383-92.

18. Stevenson JC, Crook FD, Godsland IF. Influence of age and menopause on serum lipids and lipoproteins in healthy women. Atherosclerosis 1993;98:83-90.

19. Demirovic J, Sprafka JM, Folsom AR, Laitinen D, Blackburn H. Menopause and serum cholesterol: differences between blacks and whites. The Minnesota Heart Survey. Am J Epidemiol 1992;136:155-64.

20. Myers GL, Cooper GR, Winn CL, Smith SJ. The Centers for Disease Control - National Heart, Lung and Blood Institute Lipid Standardization Program. Clinic Lab Med 1989;9:105-35.

21. Katterman R, Jaworek D, Möller G. Multicenter study of a new enzymatic method of cholesterol determination. J Clin Chem Clin Biochem 1984;22:245-51.

22. Lopes-Virella MF, Stone P, Ellis S, Colwell JA. Cholesterol determination in high-density lipoproteins separated by three different methods. Clin Chem 1977;23:882-4.

23. SAS Institute Inc. SAS/STAT User's Guide, Version 6, Fourth Edition, Volume 1 and 2, Cary, NC: SAS Institute Inc., 1989.

24. Derby CA, Hume AL, Barbour MM, McPhillips JB, Lasater TM, Carleton RA. Correlates of postmenopausal estrogen use and trends through the 1980s in two southeastern New England communities. Am J Epidemiol 1993;137:1125-35.

25. McKinlay SM, Bifano NL, McKinlay JB. Smoking and age at menopause. Ann Intern Med 1985;103:350-6.

26. Willett W, Stampfer MJ, Bain C, et al. Cigarette smoking, relative weight, and menopause. Am J Epidemiol 1983;117:651-8.

27. Baron J. Smoking and estrogen-related disease. Am J Epidemiol 1984;119:9-22.

5

Gender differences in total and HDL cholesterol: the role of lifestyle factors, body mass index, menopause and aging

Abstract

Background. We wanted to examine to what extent the gender differences in total (TC) and HDL cholesterol (HDL-C) are explained by differences in body mass index (BMI), cigarette smoking, drinking and physical activity (PA) and to what extent the TC increase with age can be limited through a healthy lifestyle.

Methods. Between 1987 and 1991, about 36,000 men and women aged 20-59 were examined for cardiovascular disease risk factors. The association of BMI, smoking, alcohol consumption, PA, age and menopause with TC and HDL-C was estimated in a regression model. From this model, it was estimated what the gender difference in total and HDL cholesterol would be if men and women had equal levels of BMI, smoking, alcohol consumption and physical activity. Furthermore, the total cholesterol increase with age was compared to the predicted increase based on a low-risk lifestyle (BMI=22.5 kg/m², no smoking, moderate alcohol consumption, physically active).

Results. Between age 30 and 50, 19-38% of the gender difference in TC was explained by differences in BMI and smoking. After age 50, the higher TC in women was to a large extent due to the effect of the menopause. The difference in HDL-C between men and women would be 0.02-0.04 mmol/l larger if levels of BMI, smoking, alcohol consumption and PA had been similar. A low-risk lifestyle would, in men, lead to a 0.3 mmol/l less increase in total cholesterol between age 20 and 59 and in women to a 0.15 mmol/l less increase.

Conclusion. Between age 30 and 50 38%, at most, of the gender difference in TC is explained by differences in risk factor levels. After age 50, the gender difference in TC is mainly due to the occurrence of menopause in women. Differences in risk factor levels do not account for the gender difference in HDL cholesterol. A low-risk lifestyle can to some extent limit the cholesterol increase with age.

Introduction

In western countries, coronary heart disease (CHD) is a major cause of death, and the incidence increases with age. A high total cholesterol and a low HDL cholesterol level are important risk factors for coronary heart disease.[1-5] Plasma total cholesterol levels increase with age, but the pattern of increase is different in men and women. In men there is a strong increase until about age 45, while in women the increase is especially strong after that age.[6] This different relation with age can be due to a different effect of aging in men and women, but could also be the result of different levels of the determinants of total cholesterol in men and women at various ages. Determinants that are positively associated with total cholesterol levels are body mass index (BMI) (kg/m²), cigarette smoking and, for women, the menopause.[7-10] HDL cholesterol shows almost no relation with age, but the difference in HDL cholesterol between men and women is striking: women have consistently higher HDL cholesterol levels than men.[11] A number of lifestyle factors influence HDL cholesterol levels: alcohol consumption and physical activity are positively associated with HDL cholesterol[12-15] and smoking and BMI negatively.[8,16]

With data from the Dutch Monitoring Project on Cardiovascular Disease Risk Factors[17] we tried to gain insight in the extent to which cigarette smoking, alcohol consumption, physical activity, BMI and menopause can explain the gender differences in total and HDL cholesterol at various ages. Furthermore, the extent to which the increase in total cholesterol with age in men and women can be prevented by keeping modifiable determinants of total cholesterol at a desirable level was evaluated.

Methods

Study population
The Monitoring Project on Cardiovascular Disease Risk Factors has been carried out in the Netherlands from 1987 to 1991.[17] The aim of the project was to monitor the major risk factors for cardiovascular diseases: plasma cholesterol, blood pressure and smoking habits. The project was carried out at the municipal health services in three towns in the Netherlands: Amsterdam, the capital city in the west, Doetinchem in the east and Maastricht in the south. Each year a new random sample of men and women aged 20 to

59 years was selected in each town. In the years 1987-1991 over 36,000 men and women have been examined. The overall response rate was 51% in men and 58% in women.[18]

Data collection

The examination included measurement of weight, height and blood pressure, blood sampling and a questionnaire. The respondents were weighed without shoes, after emptying their pockets, with an accuracy of 0.1 kg. Height was measured with an accuracy of 0.5 cm, while the respondent stood upright against the wall with the feet at a 45° angle. Body mass index (BMI) was calculated as weight(kg)/height(m)^2. Total and HDL cholesterol were determined in a non-fasting blood sample at the Clinical Chemistry Laboratory of the University Hospital 'Dijkzigt' in Rotterdam. This laboratory takes part in the standardization program of the WHO through the WHO Regional Lipid Center for Europe in Prague, Czechoslovakia and the Centers for Disease Control (CDC), Atlanta, USA.[19] Plasma total cholesterol was determined enzymatically using a Boehringer testkit.[20] HDL lipoproteins were isolated by precipitation of the apo-B-containing lipoproteins with magnesium phosphotungstate.[21] Subsequently the cholesterol content of the HDL lipoproteins was determined, as described for total cholesterol.

The questionnaire provided information about demographic variables, presence and family history of cardiovascular and a number of other chronic diseases, medication, smoking habits, alcohol consumption, physical activity, psycho-social factors and, for women, reproductive history.

In the analyses cigarette smoking was dichotomized into non-smoking and smoking (≥ 1 cigarette/day). Alcohol consumption was used as a continuous variable (glasses/day). Two questions about physical activity were asked: one about work and one about leisure time. Since most people had sedentary jobs, physical activity during leisure time was used in the analyses. There were four answering categories: no activity, exercise for at least four hours per week, regular exercise, heavy exercise. The first category was classified as inactive, the others as active.

Menopausal status was based on a question about regularity of menstruation. The response categories were: regular, irregular, not applicable because of pregnancy, not applicable because of menopause and not applicable due to surgery. The first two categories were classified as premenopausal. All pregnant women (N=306) were excluded from the analyses. All women who reported that they no longer menstruated because of the

menopause were classified as postmenopausal. All women who did not menstruate due to surgery were excluded from the analysis because the type of operation (hysterectomy and/or oophorectomy) was not known, and therefore hormonal status could not be determined.

Statistical methods

The analyses were carried out using the statistical package SAS 6.07.[22] All analyses were performed with plasma total and HDL cholesterol as dependent variables. Regression models were built using the following strategy. Gender, menopausal status, age and the known determinants (BMI and smoking for total cholesterol and BMI, smoking, alcohol consumption and physical activity for HDL cholesterol) were entered into the regression model. Only variables that are known to be causally related to total and HDL cholesterol levels were entered into the model, to prevent the entry in the model of factors that are merely markers for other (causative) factors. Because the increase with age was not linear, the term age^2 was introduced. Differences in the coefficients between men and women were assessed by including interaction terms in the regression model of gender with the other variables. It was tested whether the interaction terms increased the explained variance with an F-statistic. The most parsimonious model was used. Separate regression coefficients for men and women were calculated from the interaction terms.

The regression equations were used to estimate the extent to which differences in levels of these factors explain the difference in total and HDL cholesterol between men and women. It was calculated what the mean total and HDL cholesterol levels of the male respondents would have been if they would have had the same average risk factor profile as females of the same age. This was done by calculating the mean levels of BMI and percentage of smokers, drinkers and physically active persons for women for each one year age group. Subsequently, the male regression coefficients were applied to this female risk factor profile to obtain the simulated values for men. These values were then compared with the predicted values from the regression equation for men and women. Cholesterol levels associated with a low-risk and high-risk lifestyle were also calculated based on the regression equations.

Results

Basic characteristics of men and women are given in table 1. Mean age, total cholesterol, BMI, percentage of smokers and the number of cigarettes smoked were somewhat higher in men compared to women. HDL cholesterol was 0.25 mmol/l higher in women than in men. Alcohol consumption was more frequent in men and the amount of alcohol consumed per day was about twice as high in men. About two thirds of the men and women were physically active.

Table 1. *Basic characteristics[a] (mean (SD)) of men and women aged 20-59. The Netherlands Monitoring Project on Cardiovascular Disease Risk Factors, 1987-1991*

	Men N=17,143[b]		Women N=17,168[b]	
Age (years)	41.5	(10.9)	40.3	(11.0)
Total cholesterol (mmol/l)	5.55	(1.15)	5.49	(1.11)
HDL cholesterol (mmol/l)	1.12	(0.27)	1.37	(0.32)
BMI (kg/m^2)	25.3	(3.4)	24.6	(4.2)
Smokers (%)	40.6	(49.5)	39.6	(49.4)
Cigarettes/day[c]	16.4	(9.1)	14.6	(8.3)
Drinkers (%)	76.7	(42.3)	50.2	(50.0)
Drinks/day[d]	2.2	(1.9)	1.2	(1.2)
Physically active (%)[e]	66.2	(47.3)	63.0	(48.3)

a : Men and women differ statistically significantly at p < 0.001 for all variables, for % smokers
   the difference is statistically significant at p < 0.05.
b : minimum N
c : mean is based on smokers only
d : mean is based on drinkers only
e : physically active during leisure time

Gender differences in total and HDL cholesterol
In figure 1 and 2 total and HDL cholesterol levels by age are shown. Under the age of 25 years women had a higher mean total cholesterol than men, between the ages 25 to 50 total cholesterol was higher in men, and after about age 50 women had again higher values. HDL cholesterol was fairly constant at all ages and was about 0.25 mmol/l higher in women than in men.

Table 2. *Regression equations[a] for total and HDL cholesterol for men and women*

Total cholesterol (mmol/l)

Men   =   0.73 + 0.060*BMI[b] + 0.132*Smoking[c] + 0.128*Age - 0.0011*Age^2

Women   =   4.21 + 0.029*BMI + 0.179*Smoking - 0.022*Age + 0.0007*Age^2 + 0.356*Menopausal status[d]

HDL cholesterol (mmol/l)

Men   =   1.646 - 0.023*BMI - 0.097*Smoking + 0.036*Alcohol[e] + 0.038*Activity[f]

Women   =   1.786 - 0.018*BMI - 0.131*Smoking + 0.073*Alcohol + 0.038*Activity + 0.024*Menopausal status

a: If coefficients differ between men and women this difference is statistically significant (p<0.01)
b: BMI: Body Mass Index (kg/m^2)
c: Smoking : no/yes
d: Menopausal status: 0=premenopausal, 1=postmenopausal
e: Alcohol consumption (glasses/day)
f: Physical activity during leisure time: 0=inactive, 1=active

Table 3. *Difference in total and HDL cholesterol (mmol/l) between men and women as predicted from the regression equations shown in table 2, and as predicted from the same regression equations when simulated that men had the same levels of BMI and lifestyle factors as women*

Age group	Total cholesterol		HDL cholesterol	
	from regression model	if BMI and smoking were similar	from regression model	if BMI, smoking alcohol and physical activity were similar
20-24	-0.30	-0.37	-0.23	-0.25
25-29	0.02	-0.06	-0.23	-0.25
30-34	0.26	0.16	-0.25	-0.27
35-39	0.37	0.29	-0.25	-0.27
40-44	0.42	0.33	-0.26	-0.28
45-49	0.31	0.25	-0.27	-0.29
50-54	-0.03	-0.06	-0.26	-0.29
55-59	-0.42	-0.40	-0.26	-0.30
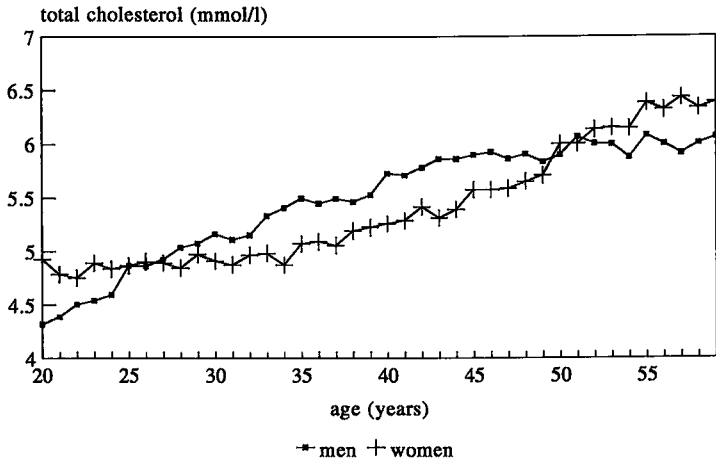
Figure 1. *Mean plasma total cholesterol according to age (yrs) in 36,000 men and women. The Netherlands Monitoring Project on Cardiovascular Disease Risk Factors 1987-1991.*
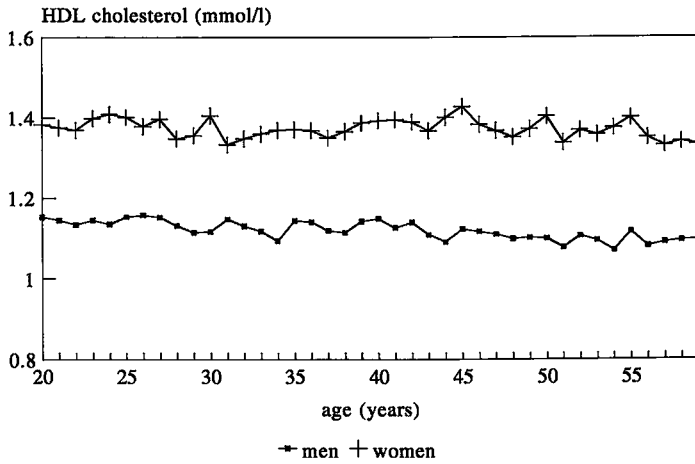
Figure 2. *Mean HDL cholesterol level according to age (yrs) in 36,000 men and women. The Netherlands Monitoring Project on Cardiovascular Disease Risk Factors 1987-1991.*

73

total cholesterol (mmol/l)
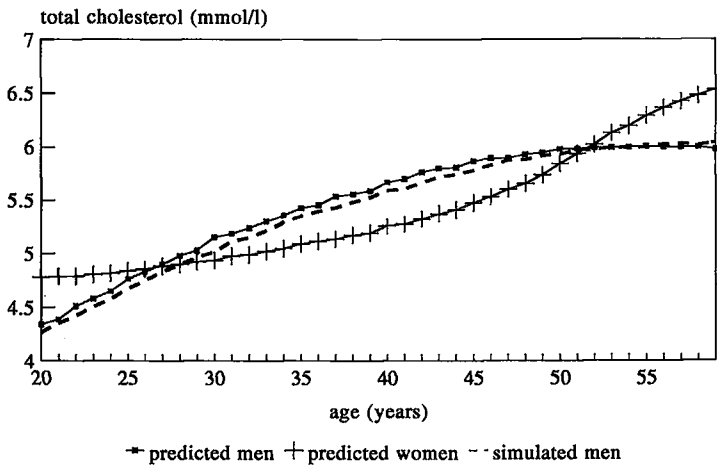
Figure 3. *Predicted total cholesterol values for men and women, and simulated for men with the same risk factor profile (BMI and smoking) as women of the same age.*

HDL cholesterol (mmol/l)
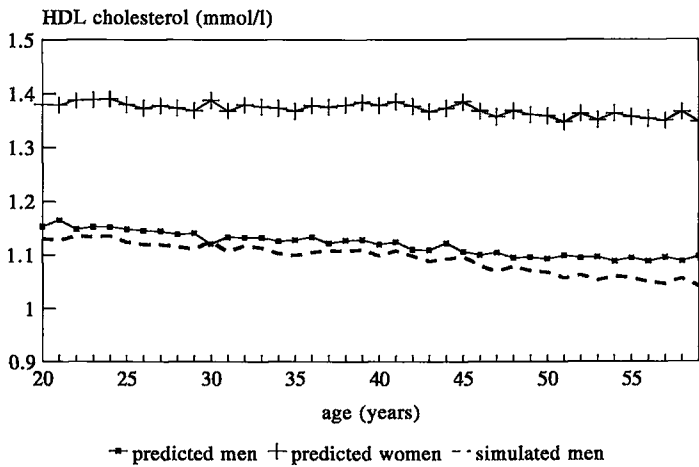
Figure 4. *Predicted HDL cholesterol values for men and women, and simulated for men with the same risk factor profile (BMI and lifstyle factors) as women of the same age.*

In table 2 the associations of BMI, smoking, alcohol consumption, physical activity and menopause with total and HDL cholesterol levels in men, premenopausal and postmenopausal women are shown. From these equations the predicted total and HDL cholesterol levels for men and women (based on their own level of BMI and lifestyle factors) and the simulated values for men (assuming the same average level of BMI and lifestyle factors as females of the same age) were calculated (figure 3 and 4). In table 3 the gender difference in total and HDL cholesterol is summarized per 5-year age category based on the predicted level for men and the simulated level for men. Until about age 55 the total cholesterol level in men would be lower if they would have the same BMI and smoking level as their female contemporaries. Between age 30 and 50 about 19-38% of the total cholesterol difference was explained by differences in BMI and smoking. Above age 50, mean total cholesterol levels in women exceed those in men, and BMI and lifestyle factors hardly explain the difference between men and women. The sharp increase in women around age 50 is to a great extent the result of the occurrence of the menopause. The difference in HDL cholesterol between men and women was not explained by differences in BMI and lifestyle factors. On the contrary, if men would have the same levels of BMI, smoking, drinking and PA as women, the difference in HDL cholesterol between men and women would be about 0.02-0.04 mmol/l larger.

Cholesterol difference between a low-risk and high-risk lifestyle
To examine the extent to which the increase with age can be prevented through lifestyle factors, two situations were simulated: a 'low-risk' scenario (BMI=22.5 kg/m^2 (mean level at age 20) and no cigarette smoking) and a 'high-risk' scenario (BMI=30 kg/m^2 (cutoff point for grade II obesity) and cigarette smoking ($\geq$ 1 cigarette/day)). Mean observed, predicted, low-risk and high-risk total cholesterol values by age are presented in figures 5 and 6 for men and women respectively. The low-risk line gives an indication to what level the rise with age can be restrained on average, by keeping BMI constant at a low level and abstaining from smoking. From figure 5 and 6 it can be seen that the total cholesterol level for the low-risk scenario at age 55-59 is about 0.3 mmol/l lower than the observed average level in men, and in women about 0.15 mmol/l lower. The difference between the low and high-risk lines gives an indication of the impact of a 'low-risk' versus a 'high-risk' lifestyle. This difference was greater in men (0.58 mmol/l) than in women (0.40 mmol/l).

total cholesterol (mmol/l)

age (years)

 ⁺– observed + predicted – · low-risk · ·· high-risk

Figure 5. *Mean observed[a], predicted[b], minimum predicted[c], and maximum predicted[d] total cholesterol according to age (years) in men. The Netherlands Monitoring Project on Cardiovascular Disease Risk Factors 1987-1991.*
[a] : *as measured*
[b] : *as predicted from a regression model with age, BMI and cigarette smoking*
[c] : *predicted value, based on the assumptions: BMI=22.5 kg/m²; no smoking*
[d] : *predicted values based on the assumptions: BMI=30 kg/m²; cigarette smoking*

total cholesterol (mmol/l)

age (year)

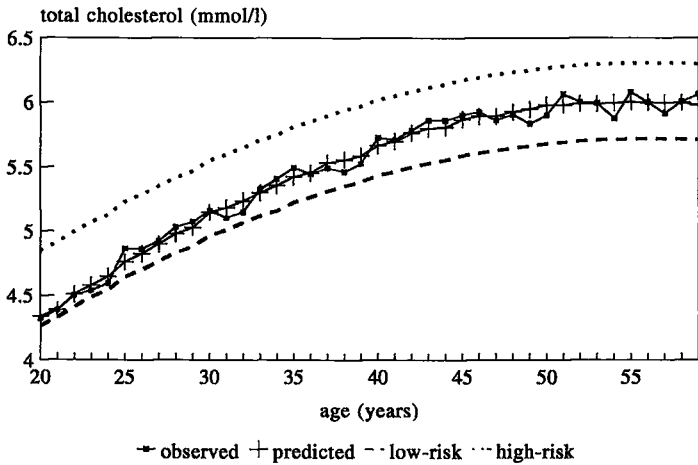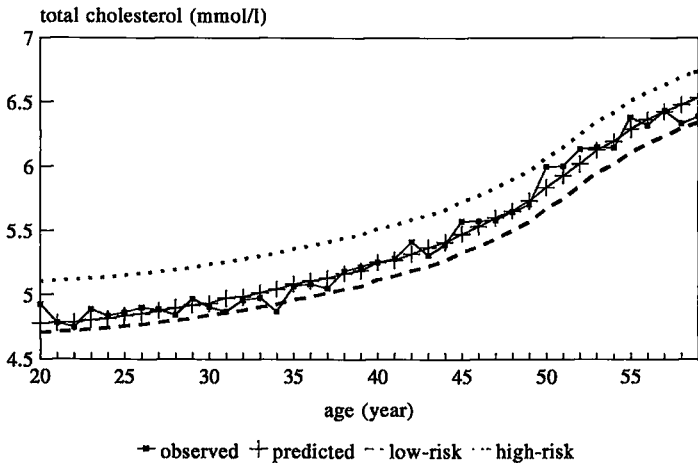 ⁺– observed + predicted – · low-risk · ·· high-risk

Figure 6. *Mean observed[1], predicted[2], minimum predicted[3], and maximum predicted[4] total cholesterol according to age (years) in women. The Netherlands Monitoring Project on Cardiovascular Disease Risk Factors 1987-1991.*
[a,b,c,d] : *footnotes, see figure 5*

For HDL cholesterol the low-risk scenario was based on a BMI of 22.5 kg/m^2, no cigarette smoking, 2 alcoholic consumptions per day and a physically active lifestyle; the high-risk scenario was based on a BMI of 30 kg/m^2, cigarette smoking ($\geq$ 1 cigarette/day), no alcohol consumption and a physically inactive lifestyle. For men, the HDL cholesterol level for the low and high-risk scenarios were 1.24 and 0.86 mmol/l respectively (not shown). The HDL-C from the low-risk scenario was about 0.1 mmol/l higher than the average observed HDL-C. In women, menopause was associated with a small increase in HDL cholesterol. Due to the increasing proportion of postmenopausal women with age, the HDL scenario lines increased slightly with age in women. The level of the scenarios was about 1.57 mmol/l in the low-risk scenario and about 1.12 mmol/l in the high-risk scenario (not shown). For women the low-risk scenario was associated with a 0.2 mmol/l higher HDL-C than the observed HDL-C. The difference in HDL-C between the low-risk and the high-risk scenario was 0.38 mmol/l in men and 0.45 mmol/l in women.

Discussion

One of the advantages of the present study was, that a large number of men and women (both premenopausal and postmenopausal) in a broad age range were examined in a standardized way. Therefore, this study could give reliable estimates of the relations between lifestyle habits and cholesterol levels in men and women. The figures in which both the observed and predicted values are plotted suggest that the models fitted the data well.

With respect to the explanation of the cholesterol rise with age, it is important to note that our data are cross-sectional. In a number of both cross-sectional and longitudinal studies the observed pattern of the total cholesterol rise with age was similar, suggesting that cross-sectional studies give a fairly accurate picture of the total cholesterol changes with age.[6,23-26]

Another issue in relation to generalizability of the results is possible selection bias due to non-response. A non-response survey was carried out among all non-respondents in the period August-December 1991 (N=1600). They were approached by telephone or, if no telephone number could be obtained, by mailed questionnaire. Educational level was considered an important indicator variable because it is known to be associated with risk

factor levels.[27-29] For men the percentage of low, medium and high educational level was 56%, 23% and 22% respectively among respondents, and among non-respondents 52%, 27% and 21% respectively. For women these percentages were 63%, 20% and 17% respectively among respondents and 57%, 26% and 17% respectively among non-respondents. Therefore we concluded that the participants in our study seemed to be representative of the total group invited to participate in the study.[18]

The associations between BMI and lifestyle factors and total and HDL cholesterol levels differed between men and women. The stronger unfavorable effect of smoking in women, and the stronger unfavorable effect of BMI in men is in accordance with other studies.[7,24-26,30,31] The stronger effect of BMI on total cholesterol in men compared to women can be explained by the fact that men have more abdominal fat than women, and this is known to be more atherogenic.[32-33] Differences in fat distribution between men and women were however not measured in the present study. Alcohol consumption is known to increase HDL cholesterol levels.[12,13] A stronger effect in women than in men, as was observed in the present study, has also been observed in the Framingham Study and the LRC Prevalence Study.[26,13]

The difference in total cholesterol between men and women at different ages is an intriguing phenomenon. With our data we tried to gain insight into factors that can explain this difference. Until about age 50, on average BMI was lower in women than in men, and therefore until this age men would have lower total cholesterol levels if they would have the same BMI as women. Because total cholesterol before age 25-30 is higher in women than in men, this lower TC would lead to an increased gender difference. From age 50 onwards BMI is similar in men and women, and therefore around this age the gender difference is mainly explained by the occurrence of the menopause in women. Other factors, that were not measured in the present study, might also play a role. We had no detailed information to analyze differences in for example saturated fat intake, but a recent survey in the Netherlands did not show substantial differences in saturated fat intake between men and women, nor between younger and older adults.[34]

The difference in HDL cholesterol between men and women was not explained by differences in BMI and the lifestyle factors studied (figure 4). If men would have the same risk factor levels as women, the difference in HDL cholesterol between men and women would be even 0.02-0.04 mmol/l larger, due to the lower level of alcohol consumption in

women compared to men. Gender was the most important determinant of the difference in HDL cholesterol. During puberty, HDL cholesterol levels drop in boys due to the effect of male sex hormones, while in girls the levels remain constant.[35] From puberty onwards men have lower HDL cholesterol levels than women. Recent publications suggest that differences in fat distribution (waist-to-hip ratio) between men and women, play an important role in explaining the gender difference in HDL cholesterol.[33,36,37]

The different scenarios (figure 5 and 6) gave an indication of the predicted mean total cholesterol levels at different ages, resulting from a low-risk lifestyle (BMI=22.5 kg/m², no smoking) and a high-risk lifestyle (BMI=30 kg/m², smoking) in comparison to average levels that were observed in the general population. The increase between age 20 and 60 in average cholesterol levels in the general population amounts to about 1.7 mmol/l in men and 1.6 mmol/l in women. It was estimated that the average cholesterol level at age 55-59 resulting from the low-risk scenario compared to the observed level would be about 0.3 mmol/l lower in men and about 0.15 mmol/l lower in women. Therefore, a substantial part of the increase of total cholesterol with age remained unexplained. Part of this increase may be explained by factors not included in the analyses, or it may be an 'unpreventable' aging effect, due to changes in cholesterol metabolism. Grundy and coworkers showed that with aging the clearance rate of LDL diminishes and the production rate increases, resulting in an increase of LDL levels with age.[38] Miller however only found a decrease in clearance rate with aging, and no change in production rate.[39]

For total cholesterol the difference between a 'low-risk' and a 'high-risk' lifestyle was larger in men (0.58 mmol/l) than in women (0.40 mmol/l), due to the fact that the unfavorable effect of BMI was twice as strong in men compared to women. For HDL cholesterol the difference between the two scenarios is 0.38 mmol/l for men and 0.45 mmol/l for women. The larger difference between the two scenarios in women compared to men is due to the stronger HDL cholesterol raising effect of alcohol in women.

It can be concluded that the effects of lifestyle factors on total and HDL cholesterol are different in men and women. Between age 30 and 50, about 19-38% of the difference in total cholesterol is explained by differences in levels of body mass index and smoking. After age 50, the difference is largely due to the effect of menopause. The difference in HDL between men and women would be even slightly larger if men had the same levels of body mass index, smoking, alcohol consumption and physical activity as women. A

low-risk lifestyle can diminish the cholesterol increase with age on average with about 0.3 mmol/l in men and with about 0.15 mmol/l in women. Women have the disadvantage that menopause, an unpreventable life event, has a strong cholesterol raising effect.

References

1. Castelli WP, Garrison RJ, Wilson PWF, Abbott RD, Kalousdian S, Kannel WB. Incidence of coronary heart disease and lipoprotein cholesterol levels. The Framingham Study. JAMA 1986;256:2835-8.

2. Stamler J, Wentworth D, Neaton JD. Is the relationship between serum cholesterol and risk of premature death from coronary heart disease continuous and graded? Findings in 356,222 primary screenees of the multiple risk factor intervention trial (MRFIT). JAMA 1986;256:2823-8.

3. Lipid Research Clinics Program. The Lipid Research Clinics Coronary Primary Prevention Trial Results. II. The relationship of reduction in incidence of coronary heart disease to cholesterol lowering. JAMA 1984;251:365-74.

4. Gordon DJ, Probstfield JL, Garrison RJ et al. High-density lipoprotein cholesterol and cardiovascular disease. Four prospective American studies. Circulation 1989;79:8-15.

5. Jacobs DR, Mebane IL, Bangdiwala SI, Criqui MH, Tyroler HA. High density lipoprotein cholesterol as a predictor of cardiovascular disease mortality in men and women: The Follow-up Study of the Lipid Research Clinics Prevalence Study. Am J Epidemiol 1990;131:32-47.

6. Kreisberg RA, Kasim S. Cholesterol metabolism and aging. Am J Med 1987;82(suppl 1B):54-60.

7. Ashley FW Jr, Kannel WB. Relation of weight change to changes in atherogenic traits: The Framingham Study. J Chron Dis 1974;27:103-14.

8. Craig WY, Palomaki GE, Haddow JE. Cigarette smoking and serum lipid and lipoprotein concentrations: an analysis of published data. Br Med J 1989;298:784-8.

9. Bonithon-Kopp C, Scarabin PY, Darne B, Malmejac A, Guize L. Menopause-related changes in lipoproteins and some other cardiovascular risk factors. Int J Epidemiol 1990;19:42-8.

10. Baird DD, Tyroler HA, Heiss G, Chambless LE, Hames CG. Menopausal change in serum cholesterol. Black/white differences in Evans County, Georgia. Am J Epidemiol 1985;122:982-93.

11. Rifkind BM, Tamir I, Heiss G, Wallace RB, Tyroler HA. Distribution of high density and other lipoproteins in selected LRC Prevalence Study populations. Lipids 1979;14:105-112.

12. Castelli WP, Doyle JT, Gordon T, et al. Alcohol and blood lipids. The Cooperative Lipoprotein Phenotyping Study. Lancet 1977;I:153-5.

13. Ernst N, Fisher M, Smith W, et al. The association of plasma high-density lipoprotein cholesterol with dietary intake and alcohol consumption. The Lipid Research Clinics Program Prevalence Study. Circulation 1980;62(Suppl IV):41-52.

14. Folsom AR, Caspersen CJ, Taylor HJ, et al. Leisure time physical activity and its relationship to coronary risk factors in a population-based sample. The Minnesota Heart Survey. Am J Epidemiol 1985;121:570-9.

15. Dannenberg AL, Keller JB, Wilson PWF, Castelli WP. Leisure time physical activity in the Framingham Offspring Study. Description, seasonal variation, and risk factor correlates. Am J Epidemiol 1989;129:76-88.

16. Heiss G, Johnson NJ, Reiland S, et al. The epidemiology of plasma high-density lipoprotein cholesterol levels. The Lipid Research Clinics program prevalence study. Circulation 1980;62(suppl IV):116-36.

17. Kromhout D, Obermann-De Boer GL, Verschuren WMM, Blokstra A. The Monitoring Project on Cardiovascular Disease Risk Factors 1988. Report 5289002. National Institute of Public Health and Environmental Protection, Bilthoven, the Netherlands, 1989 (in Dutch).

18. Verschuren WMM, Leer E van, Blokstra A, et al. Cardiovascular disease risk factors in the Netherlands. Neth J Cardiol 1993;4:205-10.

19. Myers GL, Cooper GR, Winn CL, Smith SJ. The Centers for Disease Control - National Heart, Lung and Blood Institute Lipid Standardization Program. Clinic Lab Med 1989;9:105-35.

20. Katterman R, Jaworek D, Möller G. Multicenter study of a new enzymatic method of cholesterol determination. J Clin Chem Clin Biochem 1984;22:245-51.

21. Lopes-Virella MF, Stone P, Ellis S, Colwell JA. Cholesterol determination in high-density lipoproteins separated by three different methods. Clin Chem 1977;23:882-4.

22. SAS Institute Inc., SAS/STAT User's Guide, Version 6, Fourth Edition, Volume 1 and 2, Cary, NC: SAS Institute Inc., 1989.

23. Nanas S, Pan WH, Stamler J, et al. The role of relative weight in the positive association between age and serum cholesterol in men and women. J Chron Dis 1987;40:887-92.

24. 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.

25. Davis CE, Frantz ID, Heiss G, et al. Lipoprotein cholesterol distributions in selected North American populations: The Lipid Research Clinics Program Prevalence Study. Circulation 1980;62:302-15.

26. Wilson PWF, Garrison RJ, Abbott RD, Castelli WO. Factors associated with lipoprotein cholesterol levels. The Framingham Study. Arteriosclerosis 1983;3:273-81.

27. Kraus JF, Borhani NO, Franti CE. Socioeconomic status, ethnicity, and risk of coronary heart disease. Am J Epidemiol 1980;111:407-14.

28. Jacobsen BK, Thelle DS. Risk factors for coronary heart disease and level of education: The Tromso Heart Study. Am J Epidemiol 1988;127:923-32.

29. Duijkers TJ, Kromhout D, Spruit IP, Doornbos G. Inter-mediating risk factors in the relation between socioeconomic status and 25-year mortality (the Zutphen Study). Int J Epidemiol 1989;18:658-62.

30. Criqui MH, Wallace RB, Heiss G, Mishkel M, Schonfeld G, Jones GTL. Cigarette smoking and plasma high-density lipoprotein cholesterol. The Lipid Research Clinics Program Prevalence Study. Circulation 1980;62(Suppl IV):IV70-IV76.

31. Halfon ST, Green MS, Heiss G. Smoking status and lipid levels in adults of different ethnic origins: The Jerusalem Lipid Research Clinic Program. Int J Epidemiol 1984;13:177-183.

32. Larsson B, Svärdsudd K, Welin L, Wilhelmsen L, Björntorp P, Tibblin G. Abdominal adipose tissue distribution, obesity, and risk of cardiovascular disease and death: 13 year follow up of participants in the study of men born in 1913. Br Med J 1984;288:1401-4.

33. Freedman DS, Jacobsen SJ, Barboriak JJ, et al. Body fat distribution and male/female differences in lipids and lipoproteins. Circulation 1990;81:1498-1506.

34. Ministry of Welfare, Public Health and Culture. Wat is eaten in the Netherlands? (in Dutch). ISBN 90 346 1710 6, 1988. Rijswijk, Ministry of Welfare, Public Health and Culture.

35. Orchard TJ, Rodgers M, Hedley AJ et al. Changes in blood lipids and blood pressure during adolescense. Br Med J 1980;280:1563-67.

36. Seidell J, Cigolini M, Charzewska J et al. Fat distribution and gender differences in serum lipids in men and women from four European communities. Atherosclerosis 1991;87:203-210.

37. Ostlund RE, Staten M, Kohrt WM, Schultz J, Malley M. The ratio of waist-to-hip circumference, plasma insulin level, and glucose intolerance as independent predictors of the HDL2 cholesterol level in older adults. NEJM 1990;322:229-42.

38. Grundy SM, Vega GL, Bilheimer DW. Kinetic mechanisms determining variability in low density lipoprotein levels and rise with age. Arteriosclerosis 1985;5:623-30.

39. Miller NE. Why does plasma low-density lipoprotein concentration in adults increase with age? Lancet 1984;i;263-6.

6 Total cholesterol and mortality at a relatively young age: do men and women differ?

Abstract

Background. The relation between total cholesterol (TC) and mortality from coronary heart disease (CHD), cardiovascular disease (CVD), cancer, non-cardiovascular/non-cancer and all causes in men and women was investigated.

Methods. Between 1974 and 1980 about 23,000 men and 26,000 women aged 30-54 were examined and followed for mortality for on average 12 years. Mortality rates for the above mentioned endpoints for cholesterol quintiles were calculated and relative risks were estimated using Cox' proportional hazard (survival) analysis. Adjustment was made for age, smoking, systolic blood pressure and body mass index.

Results. Mortality rates for all endpoints were higher in men than in women. A strong positive association between total cholesterol and CHD and CVD mortality was observed in both men and women. The relative risk for the highest compared to the lowest quintile for CHD mortality was 3.0 (95% confidence interval 1.8 to 5.1) in men and 3.8 (95% confidence interval 1.1 to 13.1) in women, and for CVD mortality 2.8 (95% confidence interval 1.8 to 4.2) in men and 2.9 (95% confidence interval 1.4 to 6.0) in women. No increase in non-cardiovascular mortality at low cholesterol levels was observed. All-cause mortality was significantly elevated in the highest cholesterol quintile compared to the lowest, relative risk 1.6 (95% confidence interval 1.3 to 2.0) in men and 1.5 (95% confidence interval 1.1 to 1.9) in women.

Conclusion. Total cholesterol was a strong predictor of CHD, CVD and all-cause mortality in women as well as in men. It was estimated that a cholesterol reduction of 0.6 mmol/l was associated with a reduction of about 20% in CHD mortality, 15% in CVD mortality and 6% in all-cause mortality.

Introduction

There is growing awareness that cardiovascular diseases are not only an important public health problem in men but also in women.[1,2] In the Netherlands, about 40% of total mortality is caused by cardiovascular diseases, both in men and women.[3]

Because research on cardiovascular diseases has been carried out mostly in middle-aged men, longitudinal data on women are still relatively scarce. In the Netherlands, a screening project on cardiovascular disease risk factors has been carried out between 1974 and 1980 in which 50,000 men and women aged 30-54 were examined.[4] Mortality follow-up of this cohort has recently been completed, and to our knowledge, this is one of the largest cohorts that provides information on total cholesterol in relation to mortality in women.

Although it has been shown in men that the relationship between total cholesterol and coronary heart disease mortality is strong and graded,[5,6] the risk of low cholesterol levels is debated.[7] There are indications that the benefit with respect to coronary heart disease mortality at low cholesterol levels would be offset by an increase in mortality from cancer and other diseases.[7,8] Therefore, we also investigated the associations between total cholesterol and non-cardiovascular mortality.

Methods

Study population
The Consultation Bureau Heart Project was carried out from 1974 to 1980 in five towns in the Netherlands: Amsterdam, Doetinchem, Maastricht, Leiden and Tilburg.[4] The response rate varied from 70% to 80%. The project was aimed primarily at the age group of around 40 years, but in some towns a wider age range was taken. The age range of the population examined was 30 to 54, with about 75% of the respondents aged 35 to 45.

Examination
Questionnaire information about smoking habits, cardiovascular complaints and the respondents history of myocardial infarction, stroke, hypertension and diabetes mellitus was obtained. Blood pressure, weight and height were measured. A non-fasting blood

sample was taken in which total cholesterol was measured at the Central Clinical Chemistry Laboratory of the University Hospital Dijkzigt in Rotterdam. This laboratory participated in the standardization program of the World Health Organization (WHO). Total cholesterol was determined according to a direct Liebermann-Burchard method.[9] In more recent projects, cholesterol has been determined enzymatically, and cutoff points for hypercholesterolemia according to the Netherlands Cholesterol Consensus are based on enzymatic values.[10] Therefore, cholesterol values were converted based on a comparison study carried out in 1981 in which cholesterol was measured with both methods in over 5,000 serum samples. The following equation for conversion from Huang to enzymatic values was obtained[11]:

$$\text{total cholesterol}_{enzymatic} = 1.004* \text{ total cholesterol}_{Huang} - 0.299 \text{ mmol/l.}$$

Mortality follow-up

Mortality follow-up was started in 1986 and completed in 1993. The administration cards of the project were retrieved from the archives and computerized. The vital status was checked through the municipal registries. Censor date was the date at which the information was obtained from the municipal registry (for the living) or date of death. If a person had moved with unknown destination, the date at which the person was dropped from the municipal registry was used as censor date. For 49,202 of the 50,887 persons examined, the administration card was still present in the archive. For 49,018 persons (96.3%) mortality follow-up was successfully completed, for 48,949 persons a total cholesterol value was obtained. A total of 1319 persons had died. From 1289 subjects the cause of death was obtained from the Central Bureau of Statistics, while for 30 persons who died outside the Netherlands, such information could not be obtained. Causes of death were coded according to the 9th revision of the International Classification of Diseases (ICD-9).[12] For deaths that had occurred before January 1st 1979 (n=89), the 8th revision (ICD-8) had been used. For the endpoints used in the present analyses, all codes from ICD-8 remained in the same category when defined according to ICD-9. Cause-specific endpoints used in the analyses were coronary heart disease (ICD 410-414), cardiovascular disease (ICD 390-459), cancer (ICD 140-239) and non-cardiovascular/non-cancer diseases (ICD 0-139, 240-389, 460-999).

Statistical methods

SAS statistical programs were used for the analyses.[13] Cholesterol was divided into gender specific quintiles and into categories of 0.5 mmol/l width. Cox proportional hazards (survival) analysis was used to estimate relative risks (RR). Adjustment for smoking was made based on a dichotomous variable 'current smoking yes/no', while age, systolic blood pressure and body mass index (weight(kg)/height(m)^2) were entered into the model as continuous variables. For cause-specific analyses subjects with an unknown cause of death were excluded. Two deceased subjects did not have a cholesterol measurement. Excluding prevalent cases of angina, stroke and myocardial infarction (N=1349) did not substantially alter the results and therefore they were included in the analyses.

Results

Mean age was 39 years, mean total cholesterol level was 5.55 mmol/l in men and 5.19 mmol/l in women. For all cardiovascular risk factors levels were higher in men than in women (table 1). In men, cardiovascular diseases (CVD), cancer and non-cancer/non-cardiovascular diseases each contributed for about 1/3 to total mortality. In women cancer was the predominant cause of death (table 2).

Table 1. *Characteristics of 49,018 men and women aged 30-54 years at baseline. The Netherlands Consultation Bureau Project on Cardiovascular Diseases, 1974-1980*

	Men N=23,389[a]		Women N=25,629[a]	
	mean	SD[b]	mean	SD[b]
Age (years)	39.2	4.3	39.4	4.4
Total cholesterol (mmol/l)	5.55	1.10	5.19	1.00
Systolic blood pressure (mmHg)	132.8	16.2	126.7	17.6
Diastolic blood pressure (mmHg)	81.1	11.2	77.8	11.0
Body Mass Index (kg/m²)	24.9	3.0	24.3	3.7
Smoking (%)	65.6	47.5	47.0	49.9

a: maximum number of valid observations
b: SD = standard deviation

Mean total cholesterol level per quintile ranged from 4.18 mmol/l in the lowest to 7.14 mmol/l in the highest quintile in men, and from 3.94 mmol/l to 6.69 mmol/l in women (table 3). For men the total number of person-years was over 275,000 (mean 11.8 years) and for women over 300,000 (mean 12.0 years).

For coronary heart disease, the highest compared to the lowest quintile showed a three-fold higher risk in men, and an almost four-fold higher risk in women after adjustment for age, smoking, blood pressure and body mass index (table 4). For cardiovascular diseases, the highest compared to the lowest quintile showed an almost three-fold higher risk in both men and women. For both coronary heart disease and cardiovascular diseases, the RR's for men were statistically significantly elevated in the fourth and fifth quintile, for women only in the fifth quintile.

Table 2. *Main causes of death in 49,018 men and women aged 30-54 years at baseline, after (on average) 12 years of follow-up. The Netherlands Consultation Bureau Project on Cardiovascular Diseases, 1974-1980*

Cause of death	ICD-9 codes	Men		Women	
		N	%	N	%
Cardiovascular diseases	**390-459**	**290**	**35.5**	**92**	**18.4**
Coronary heart disease	410-414	194	23.7	38	7.6
Cerebrovascular disease	430-439	38	4.6	35	7.0
Other cardiovascular diseases	390-409, 415-429, 440-459	58	7.1	19	3.8
Cancer	**140-239**	**276**	**33.7**	**292**	**58.3**
Digestive	150-159	66	8.1	66	13.2
Respiratory[a]	160-169	116	14.2	27	5.4
Breast	175	-	-	95	19.0
Other cancers	140-149, 170-173, 175-239	73	8.9	31	6.2
Non-Cardiovascular/non-cancer	**0-139, 240-389,459-999**	**232**	**28.4**	**107**	**21.4**
Respiratory diseases	460-519	15	1.8	10	2.0
Digestive diseases	520-570	32	3.9	7	1.4
Accidents[b]	800-999	112	13.7	59	11.8
Other non-CVD/non-cancer	0-139, 240-389, 580-799	73	8.9	31	6.2
Unknown		**20**	**2.4**	**10**	**2.0**
Total	**0-999**	**818**	**100.0**	**501**	**100.0**

a: predominantly lung cancer: n=108 in men and n=26 in women
b: including suicide: n=54 in men and n=30 in women

Table 3. *Total number examined, mean age (years) and total cholesterol level (mmol/l) by cholesterol quintile and gender*

Quintile	Men				Women			
	N	Total cholesterol			N	Total cholesterol		
		range	mean	SD[1]		range	mean	SD
1	4,904	≤ 4.62	4.18	0.40	5,185	≤ 4.32	3.94	0.35
2	4,114	4.63 - 5.12	4.90	0.14	5,165	4.33 - 4.82	4.63	0.14
3	5,405	5.13 - 5.73	5.45	0.17	5,403	4.83 - 5.32	5.12	0.14
4	4,060	5.74 - 6.33	6.00	0.17	4,826	5.33 - 5.93	5.65	0.17
5	4,868	≥ 6.34	7.41	0.76	4,988	≥ 5.94	6.69	0.73
Total	23,351	2.1 - 17.8	5.55	1.10	25,567	1.91 - 16.3	5.19	1.00

1: SD = standard deviation

In both men and women, no associations between cholesterol quintiles and mortality from cancer or non-cardiovascular/non-cancer causes were observed (table 4). Also no associations with site-specific cancers nor with subgroups of non-cardiovascular/non-cancer causes mentioned in table 2 were observed (results not shown).

All-cause mortality was significantly elevated in the highest cholesterol quintile, by 60% in men and by 46% in women (table 4). To explore the shape of the relation between total cholesterol level and all-cause mortality in more detail, cholesterol was divided into 9 categories, with 0.5 mmol/l steps (figure 1). The middle category (5.5-5.99 mmol/l) was used as the reference category. A total cholesterol level of 7.5 mmol/l or more, gave in men a relative risk of 2.3 (95% confidence interval 1.8 to 3.0) and in women a relative risk of 1.8 (95% confidence interval 1.2 to 2.8) (table 5). Risk increased continuously with increasing cholesterol level in women. In men the lowest all-cause mortality was observed in the category 4.5-4.99 mmol/l. The relative risk for total cholesterol below 4.0 mmol/l compared to a level of 5.5-5.99 mmol/l in men was 1.18 (95% confidence interval 0.81 to 1.71).

Table 4. *Relative risks (RR) for mortality from coronary heart disease (CHD), total cardiovascular diseases (CVD), cancer, non-CVD/non-cancer and all causes according to serum total cholesterol quintiles in men and women*

	Men					Women				
	Cholesterol quintiles					Cholesterol quintiles				
	Q1 low	Q2	Q3	Q4	Q5 high	Q1 low	Q2	Q3	Q4	Q5 high
Coronary heart disease										
rate[a]	2.77	3.30	3.77	9.84	15.64	0.48	0.64	0.62	1.20	3.32
RR[b]	1.0	1.14	1.29	3.37	5.32	1.0	1.34	1.28	2.50	6.86
RR[c]	1.0	0.93	0.94	2.29	3.03	1.0	1.16	1.00	1.65	3.80
CI[d]	-	0.47-1.84	0.50-1.75	1.31-4.01	1.79-5.14	-	0.26-5.21	0.22-4.48	0.42-6.45	1.10-13.1
Cardiovascular diseases										
rate	4.85	5.57	6.91	12.35	22.77	1.45	2.09	2.15	2.75	6.65
RR	1.0	1.12	1.39	2.48	4.53	1.0	1.45	1.50	1.91	4.60
RR	1.0	0.93	1.05	1.76	2.77	1.0	1.30	1.23	1.38	2.85
CI	-	0.55-1.58	0.65-1.68	1.13-2.76	1.84-4.18	-	0.56-3.05	0.53-2.85	0.61-3.16	1.36-5.99
Cancer										
rate	8.83	10.52	7.86	10.89	12.51	8.05	8.68	7.38	11.86	11.63
RR	1.0	1.20	0.89	1.24	1.41	1.0	1.06	0.91	1.45	1.42
RR	1.0	1.14	0.82	1.11	1.24	1.0	1.00	0.82	1.24	1.11
CI	-	1.78-1.69	0.52-1.22	0.75-1.65	0.86-1.80	-	0.68-1.47	0.55-1.21	0.86-1.79	0.77-1.62
Non-cardiovascular/non-cancer										
rate	7.62	6.39	8.01	9.42	10.43	2.74	2.73	3.54	2.92	5.48
RR	1.0	0.84	1.08	1.25	1.38	1.0	1.01	1.30	1.08	2.02
RR	1.0	0.80	0.98	1.09	1.15	1.0	0.96	1.21	0.95	1.67
CI	-	0.50-1.26	0.65-1.47	0.72-1.67	0.72-1.72	-	0.49-1.88	0.64-2.27	0.48-1.88	0.91-3.06
All-cause										
rate	21.30	22.48	22.94	32.66	45.71	12.24	13.50	13.07	17.53	23.77
RR	1.0	1.06	1.08	1.53	2.13	1.0	1.10	1.06	1.42	1.92
RR	1.0	0.95	0.90	1.24	1.60	1.0	1.01	0.93	1.17	1.46
CI	-	0.74-1.23	0.71-1.15	0.98-1.56	1.29-1.99	-	0.74-1.37	0.69-1.27	0.87-1.57	1.10-1.94

a: crude mortality rate per 10,000 person-years
b: RR unadjusted
c: RR adjusted for age, smoking, systolic blood pressure and body mass index
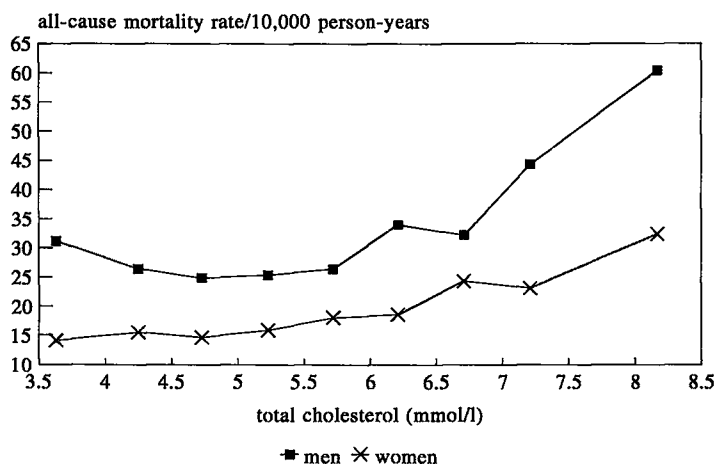d: 95% confidence interval

all-cause mortality rate/10,000 person-years

Figure 1. *All-cause mortality rates (/10,000 person-years) according to serum total cholesterol category in men and women aged 30-54, after (on average) 12 years of follow-up, adjusted for age, smoking, blood pressure and body mass index.*

Table 5. *All-cause mortality according to cholesterol categories of 0.5 mmol/l width in men and women: number of persons, adjusted[a] mortality rates, adjusted[a] relative risks and 95% confidence interval per category. Fifth quintile is used as the reference category*

Range (mmol/l)	Men				Women			
	N	Rate	RR	95% CI	N	Rate	RR	95% CI
< 4.0	1,269	31.14	1.18	0.81-1.71	2,311	14.09	0.78	0.52-1.17
4.0-4.49	2,288	26.39	1.00	0.73-1.6	3,812	15.54	0.86	0.62-1.20
4.5-4.9	3,812	24.81	0.94	0.73-1.23	5,388	14.64	0.81	0.60-1.10
5.0-5.49	4,380	25.33	0.96	0.75-1.23	5,174	15.90	0.88	0.65-1.18
5.5-5.99	4,216	26.39	1.00	-	3,899	18.07	1.00	-
6.0-6.49	3,061	34.00	1.29	1.01-1.65	2,473	18.61	1.03	0.73-1.44
6.5-6.99	2,054	32.20	1.22	0.93-1.61	1,327	24.39	1.35	0.94-1.95
7.0-7.49	1,133	44.34	1.68	1.25-2.25	632	23.13	1.28	0.78-2.08
≥ 7.5	1,159	60.43	2.29	1.76-2.97	561	32.35	1.79	1.16-2.77
Total	23,372	29.69			25,577	16.25		

a: mortality rate per 10,000 person-years adjusted for age, smoking, blood pressure and body mass index.

Discussion

During the follow-up period only 38 women died of coronary heart disease compared to 194 men, showing that at middle-age, coronary heart disease is already a prominent cause of death in men, but not yet in women. The association between total cholesterol and coronary heart disease mortality was comparable in men and women: risk was higher in the fourth and fifth quintile, although in women only the estimate for the fifth quintile was statistically significant. The relative risk for the highest compared to the lowest quintile was 3.0 in men and 3.8 in women. A slightly higher relative risk for women compared to men has been observed in other studies as well.[1,14] In all studies, however, absolute risk was lower in women than in men.

In the present study a single measurement of total cholesterol was obtained, leading to underestimation of the relative risk, a phenomenon often referred to as regression dilution bias.[16] Law et al. estimated the magnitude of the dilution factor to be 1.4.[17] When we apply this dilution factor to the relative risk for the highest quintile in the present study, the observed ('diluted') relative risk of 3.03 for men would correspond to an undiluted relative risk of 4.7 ($3.03^{1.4}$), and the relative risk for the highest quintile in women would increase from 3.8 to 6.5.

The relative risk's for coronary heart disease in women in the present study were high compared to other studies, even after adjustment for age, smoking, blood pressure and body mass index.[1,14] This might be due to the fact that the respondents were relatively young at the time of examination: three-quarters of the respondents were aged 35-45 years. A number of studies have shown that the relative risk is higher, the younger the age at examination.[18-20] Law et al. estimated that after 13 years of follow-up, a 0.6 mmol/l decrease in total cholesterol level was associated with a 31 % decrease in coronary heart disease mortality in men aged 35-44 at baseline, while in men aged 55-64 this decrease was only 12 %.[17]

The Dutch Cholesterol Consensus applies the same cutoff point for hypercholesterolemia (total cholesterol ≥ 6.5 mmol/l) to men and women.[10] Because HDL cholesterol levels are on average 0.25 mmol/l higher in women than in men[15], this cutoff point is in fact more strict for women. In the present study coronary heart disease risk in women with cholesterol levels above 5.9 mmol/l was almost four times that of women with levels

below 4.3 mmol/l. Therefore, the Consensus criteria seem justified. However, since absolute risk is much lower in women compared to men, the decision to treat hypercholesterolemia in women has to take the absolute risk (and therefore also other coronary heart disease risk factors besides total cholesterol) into account.

Recently, concern has been raised about an increased mortality risk at the lower end of the cholesterol distribution.[7] A number of studies reported an increased risk of non-cardiovascular mortality at a low cholesterol level, or even inverse associations over the entire cholesterol range.[8,21-26] In many studies these associations remained after exclusion of the first few years of follow-up, contradicting the hypothesis that the associations were due to pre-existing diseases at baseline.[8,21,24-26] In most studies in which these observations were found, study subjects were middle-aged men. It has been suggested that in 'healthy' cohorts, consisting of working persons[27] or relatively young persons[28], these associations are not present. In the present study of relatively young persons, no association was observed between total cholesterol levels and mortality from cancer and non-cardiovascular/non-cancer causes. Also for site specific cancers (digestive, respiratory and breast) and for subgroups of non-cardiovascular/non-cancer mortality (respiratory diseases, digestive diseases and accidents) no association with total cholesterol level was observed. Our data thus support the healthy cohort hypothesis.

In both men and women, all-cause mortality was increased by 60% and 46% respectively in the highest compared to the lowest cholesterol quintile. To examine the possibility of an increase in all-cause mortality at the lower end of the cholesterol distribution in more detail, smaller categories than quintiles were used. In women all-cause mortality increased continuously with increasing cholesterol level. In men all-cause mortality was lowest at a cholesterol level of 4.5-4.9 mmol/l, but the difference with mortality rates at cholesterol levels below 4.5 mmol/l was not statistically significant. In the MRFIT Study all-cause mortality at cholesterol levels below 4.1 mmol/l was increased in men.[8] In a recent meta-analysis all-cause mortality was increased at low cholesterol levels in men but not in women.[7] In the NHANES I Epidemiological Follow-up Study (NHEFS) all-cause mortality at low cholesterol levels was increased in both men and women, but this increase was only statistically significant in persons aged over 60 at baseline.[28] Our results support the hypothesis that the relation between total cholesterol and mortality is dependent on age at baseline.

With respect to public health an important question is how much of the mortality could be avoided by lowering cholesterol levels. Based on the cholesterol distribution and the RR's, it was calculated[29] that a decrease in total cholesterol of 0.6 mmol/l in this population would reduce coronary heart disease mortality by 19% in men and 20% in women and all-cause mortality by 7% in men and 5% in women. This stresses the importance of cholesterol lowering in the primary prevention of cardiovascular and all-cause mortality. Due to the lower mortality rates in women, these percentages correspond with lower absolute numbers of lives saved in women compared to men.

The present study showed that total cholesterol is a strong risk factor for coronary heart disease and all-cause mortality in men as well as in women. No significant increase in all-cause mortality at low cholesterol levels was observed. Our results add to the evidence that increased non-cardiovascular mortality at low cholesterol levels is limited to subjects who are middle-aged or older at baseline.

References

1. Manolio TA, Pearson TA, Wenger NK, Barrett-Connor E, Payne GH, Harlan WR. Cholesterol and heart disease in older persons and women. Review of an NHLBI Workshop. Ann Epidemiol 1992;2:161-176.

2. Anonymous. Women and cardiovascular diseases (in Dutch). Dutch Heart Foundation, The Hague, 1994. ISBN 90-75131-02-X.

3. Ruwaard D, Kramers PGN (editors). Public Health Status and Forecasts. The health status of the Dutch population over the period 1950-2010. National Institute of Public Health and Environmental Protection. The Hague, Sdu Uitgeverij Plantijnstraat, 1993, p377-392 (in Dutch).

4. Meijer J, Geuns HA van, Sluyter DP. Screening for risk factors of coronary heart disease in consultation bureaus for tuberculosis. CB Heart Project in The Netherlands. Hart Bulletin 1976;7:42-46.

5. Stamler J, Wentworth D, Neaton JD. Is the relationship between serum cholesterol and risk of premature death from coronary heart disease continuous and graded ? Findings in 356,222 primary screenees of the multiple risk factor intervention trial (MRFIT). JAMA 1986;256:2823-28.

6. Chen Z, Peto R, Collins R, MacMahon S, Lu J, Li W. Serum cholesterol concentration and coronary heart disease in a population with low cholesterol concentrations. BMJ 1991;303:276-82.

7. Jacobs DR, Blackburn H, Higgins M, Reed D, Iso H, McMillan G, et al. Report of the conference on low blood cholesterol: mortality associations. Circulation 1992;86:1046-1060.

8. Neaton JD, Blackburn H, Jacobs DR, Kuller L, Lee D-J, Sherwin R, et al. Serum cholesterol level and mortality findings for men screened in the Multiple Risk Factor Intervention Trial. Arch Intern Med 1992;152:1490-1500.

9. Huang TC, Cheng CP, Wefler V, Raftery A. A stable reagent for the Liebermann-Burchard reaction: application to rapid serum cholesterol determination. Anal Chem 1961;33:1405-7.

10. Anonymous. Netherlands Cholesterol Consensus Update. Hart Bulletin 1990;23(suppl.):9-21 (in Dutch).

11. Verschuren WMM, Al M, Blokstra A, Boerma GJM, Kromhout D. Trend in serum total cholesterol level in 110,000 young adults in the Netherlands, 1974-1986. Am J Epidemiol 1991;134:1290-1302.

12. WHO. International Classification of Diseases. 9th Revision, vol. 1 and 2. World Health Organization, Geneva, 1977

13. SAS Institute Inc. SAS/STAT User's Guide, Version 6, Fourth Edition, Volume 1 and 2, Cary, NC: SAS Institute Inc., 1989

14. Isles CG, Hole DJ, Hawthorne CM, Lever AF. Relation between coronary risk and coronary mortality in women of the Renfrew and Paisley survey: comparison with men. Lancet 1992;339:702-06.

15. Verschuren WMM, Leer EM van, Blokstra A, Seidell JC, Smit HA, Bueno de Mesquita HB, et al. Cardiovascular disease risk factors in The Netherlands. Neth J Cardiol 1993;4:205-10.

16. MacMahon S, Peto R, Cutler J, Collins R, Sorlie P, Neaton J, et al. Blood pressure, stroke, and coronary heart disease: part 1. Prolonged differences in blood pressure: prospective observational studies corrected for the regression dilution bias. Lancet 1990;335:765-774.

17. Law MR, Wald NJ, Wu T, Hackshaw A, Bailey A. Systematic underestimation of associations between serum cholesterol concentration and ischemic heart disease in observational studies; data from the BUPA study. BMJ 1994;308:363-6.

18. Klag MJ, Ford DE, Mead LA, He J, Whelton PK, Liang K-Y, et al. Serum cholesterol in young men and subsequent cardiovascular disease. N Engl J Med 1993;328:313-8.

19. Law MR, Wald NJ, Thompson SG. By how much and how quickly does reduction in serum cholesterol concentration lower risk of ischaemic heart disease? BMJ 1994;308:367-73.

20. Anderson KM, Castelli WP, Levy D. Cholesterol and mortality. 30 Years of follow-up from the Framingham Study. JAMA 1987;257:2176-2180.

21. Kark JD, Smith AH, Hames CG. The relationship of serum cholesterol to the incidence of cancer in Evans County, Georgia. J Chronic Dis 1980;33:311-22.

22. International Collaborative Group. Circulating cholesterol level and risk of death from cancer in men aged 40 to 69 years. JAMA 1982;248:2853-9.

23. Hiatt RA, Fireman BH. Serum cholesterol and the incidence of cancer in a large cohort. J Chron Dis 1986;39:861-70.

24. Schatzkin A, Hoover RN, Taylor PR, Ziegler RG, Carter CL, Albanes D, et al. Site-specific analysis of total serum cholesterol and incident cancer in the National Health and Nutrition Examination Survey I Epidemiologic Follow-up Study. Cancer Research 1988;48:452-8.

25. Isles CG, Hole DJ, Gillis CR, Hawthorne VM, Lever AF. Plasma cholesterol, coronary heart disease and cancer in the Renfrew and Paisley survey. BMJ 1989;298:920-4.

26. Davey Smith G, Shipley MJ, Marmot MG, Rose G. Plasma cholesterol concentration and mortality. The Whitehall Study. JAMA 1992;267:70-76.

27. Persson B, Johansson BW. The Kockum Study: twenty-two years of follow-up. Acta Med Scand 1984;216:485-93.

28. Harris T, Feldman JJ, Kleinman JC, Ettinger WH Jr, Makuc DM, Schatzkin AG. The low cholesterol-mortality association in a national cohort. J Clin Epidemiol 1992;45:595-601.

29. Wahrendorf J. An estimate of the proportion of colo-rectal and stomach cancers which might be prevented by certain changes in dietary habits. Int J Cancer 1987;40:625:8.

7 Serum total cholesterol and long-term coronary heart disease mortality in the Seven Countries Study

Abstract

Background. The relationship between serum total cholesterol and long-term mortality from coronary heart disease (CHD) was compared in different cultures.

Methods. Total cholesterol was measured at baseline (1958-1964) and at 5- and 10-year follow-up in 12,467 men aged 40-59 years in 16 cohorts located in seven countries in Europe, the United States, and Japan. To increase statistical power six cohorts were formed, based on similarities in culture and cholesterol changes during the first 10 years of follow-up. Relative risks (RRs) were estimated with Cox proportional hazards (survival) analysis, for 25-year CHD mortality for cholesterol quartiles and per 0.50 mmol/l cholesterol increase. Adjustment was made for age, smoking and systolic blood pressure.

Results: The age-adjusted CHD mortality rates in the six cohorts ranged from 3% to 20%. The RR's for the highest compared with the lowest cholesterol quartile ranged from 1.5 to 2.3, except for Japan's RR of 1.1. For a cholesterol level of around 5.45 mmol/l, CHD mortality rates varied from 4% to 5% in Japan and Mediterranean Southern Europe to about 15% in Northern Europe. However, the relative increase in CHD mortality due to a given cholesterol increase was similar in all cultures except Japan. Using a linear approximation, a 0.50 mmol/l increase in total cholesterol corresponded to an increase in CHD mortality risk of 12% (17% after adjustment for regression dilution bias).

Conclusion: Across cultures, cholesterol is linearly related to CHD mortality and the relative increase in CHD mortality rates with a given cholesterol increase is the same. The large difference in absolute CHD mortality rates at a given cholesterol level however, indicates that other factors, such as diet, that are typical for cultures with a low CHD risk are also important with respect to primary prevention.

Introduction

The purpose of the Seven Countries Study was to study associations between risk factors and CHD mortality both at the population and at the individual level. After 5 years of follow-up, Keys et al. had already reported a strong ecological correlation between serum total cholesterol and mortality from CHD in the 16 cohorts of the Seven Countries Study[1]. On the individual level the 5 and 10-year follow-up data suggested strong associations between serum total cholesterol and CHD mortality in the United States and Northern Europe, but much weaker associations in Southern Europe and Japan.[1,2] This could have resulted partly from small numbers of CHD deaths in these cultures.

Recently, the 25-year mortality follow-up of the cohorts of the Seven Countries Study has been completed, and some preliminary data on the extent of CHD differences among cohorts have been reported.[3] These data provided a unique opportunity to examine in detail the relationship between serum total cholesterol level and CHD mortality in different cultures. It is known that a single measurement may not accurately classify individuals with respect to cholesterol levels, which leads to a weakening of the observed relationship often referred to as regression dilution bias.[4] In the current study, total cholesterol was measured not only at baseline but also during follow-up. The relationship between serum total cholesterol level and 25-year mortality from CHD in different cultures will be reported herein, and the effect of regression dilution bias will be discussed.

Materials and methods

Study population
Between 1958 and 1964, 12,763 men aged 40 through 59 years from seven countries were enrolled in the study.[5] A total of 16 cohorts were examined in the following countries: the United States (US Railroad), Finland (East and West), the Netherlands (Zutphen), Italy (Rome Railroad, Crevalcore, and Montegiorgio), Greece (Crete and Corfu), Croatia and Serbia (the former Yugoslavia) (Zrenjanin, Velika Krsna, Belgrade, Dalmatia, and Slavonia), and Japan (Tanushimaru and Ushibuka). In 11 rural cohorts all men aged 40 through 59 from official registries were invited. In the town of Zutphen, for every nine men, four were invited. In the United States and Rome, railroad personnel was recruited.

In Serbia, workers from a large cooperative in Zrenjanin and professors from the University of Belgrade were invited. Overall the participation rate was over 90%, with several cohorts reaching almost 100%.

For the analyses the cohorts were pooled into six groups to obtain more statistical power: Northern Europe (East and West Finland and Zutphen), Inland Southern Europe (Rome, Crevalcore, Slavonia and Belgrade), Mediterranean Southern Europe (Crete, Corfu, Montegiorgio and Dalmatia), Serbia (Velika Krsna and Zrenjanin), the United States and Japan (Ushibuka and Tanushimaru). The criteria for pooling were similarities in baseline cholesterol level, cultural resemblance, and homogeneity with respect to cholesterol changes during the first 10 years of follow-up. In the Northern European cohort, cholesterol remained more or less stable during the first 10 years of follow-up. The American cohort was measured only after 5 years, and cholesterol levels were stable. In the Southern European cohorts, both Inland and Mediterranean, cholesterol levels increased by about 10%. Two Serbian cohorts (Velika Krsna and Zrenjanin) formed a separate group because they showed a dramatic cholesterol increase of about 30%. Cholesterol levels in Japan were more or less stable.

Measurements

In all cohorts the major cardiovascular risk factors were measured in a standardized way at baseline. In most cohorts this examination was repeated after 5 and 10 years, but in Japan cholesterol was not measured 5 years after baseline and in the United States not after 10 years. Details of the methods used have been extensively described elsewhere.[1,5] Total cholesterol was measured in a non-fasting blood sample, according to the Abell-Kendall method, modified by Anderson and Keys, in standardized laboratories.[6] Blood pressure was measured twice with a mercury sphygmomanometer at the end of the physical examination with the subject in supine position, following the methods later described in the World Health Organization (WHO) manual *Cardiovascular Survey Methods*.[7] The mean of the two measurements was used in the analyses. Cigarette smoking was established by means of a standardized questionnaire. For the present analyses smoking was dichotomized into current smoking or non-smoking.

Mortality follow-up

All men were followed up for mortality for 25 years. Only 56 men (0.4%) were lost to follow-up. The underlying cause of death was coded in a standardized way, by two

reviewers (H.B. and A.M.), using the eighth revision of the WHO *International Classification of Diseases*[8]. The final cause of death was adjudicated on the basis of information from the official death certificate, in combination with information from medical and hospital records. The coder of the causes of death was blinded to the risk factor status of the subject. In the case of multiple causes of death, priority was given to unintentional injuries, followed by cancer in advanced stages, CHD, and stroke. For the current analyses CHD was defined as 'fatal coronary events manifested as myocardial infarction or sudden death of probable coronary origin occurring within 2 hours from the onset of symptoms, after the reasonable exclusion of other conditions'. This included ICD-8 codes 410-413, 427.2, 427.6 and 795, the latter code only when the above mentioned definition was met.

Statistical methods

Cohort-specific, age-standardized 25-year CHD mortality rates were computed by weighing cohort-specific CHD mortality rates for 5-year age categories to the age distribution of the total study population. Cox proportional hazards (survival) analysis was used to estimate relative risks (RRs) (SAS statistical package, release 6.07, procedure PHREG)[9]. Cholesterol was analyzed continuously, or as quartiles, with the lowest quartile as the reference category. In all analyses adjustment was made for age (years), cigarette smoking (no/yes) and systolic blood pressure (mmHg). A possible interaction effect of cholesterol with smoking or blood pressure was tested by adding interaction terms to the model and testing (by means of an F-statistic) whether the explained variance was significantly increased.[10] Analyses were carried out with and without respondents with CHD at entry examination. Since results were not different, in this article the analyses based on all respondents are presented.

Respondents were categorized into quartiles based on their baseline cholesterol level. A single baseline cholesterol measurement is subject to random fluctuations, due to both laboratory measurement error and biological day-to-day fluctuations. When subjects are categorized according to their cholesterol level, the bottom category contains disproportionately many persons whose baseline cholesterol was underestimated and the top category has disproportionately many persons whose baseline cholesterol was overestimated[4]. Therefore, the observed cholesterol difference between the bottom and top categories is larger than the 'true' difference. This weakens the estimate of the relationship between cholesterol and mortality. This phenomenon is often referred to as regression

dilution bias[4]. In this study the dilution factor was calculated by dividing the difference in mean cholesterol between the lowest and highest quartiles based on the baseline cholesterol level by the same difference based on the cholesterol level of the survivors at 5-year follow-up. Correction for the regression dilution bias was made by multiplying the coefficients from the survival analysis by the dilution factor and subsequently calculating RRs.

Results

In table 1 mean serum total cholesterol level at baseline and age-standardized 25-year CHD mortality rates are shown, together with some other baseline characteristics. Mean cholesterol levels ranged from around 4.15 to 4.40 mmol/l in the Serbian and Japanese cohorts, to around 5.15 mmol/l in the Southern European cohorts and around 6.20 to 6.70 mmol/l in the American and Northern European cohorts. Average systolic blood pressure ranged from 132.5 mmHg in Serbia to 144.0 mmHg in Northern Europe, and body mass index, expressed as weight in kilograms divided by height in meters, squared, ranged from 22.0 kg/m² in Japan to 25.5 kg/m² in the United States and Inland Southern Europe. The prevalence of smoking varied between 56% in Serbia and 74% in Japan. Prevalence of CHD at baseline ranged from less than 1% in Mediterranean Southern Europe and Japan to 4.6% in the United States. Mortality rates for CHD ranged from 3% in the Japanese cohort to 20% in the Northern European cohort.

In table 2 the cutoff points for the serum cholesterol quartiles are shown together with the mean cholesterol level for each quartile based on the baseline measurements as well as on the cholesterol measurement 5 years after baseline. The ratio of the difference in mean cholesterol between highest and lowest quartiles, baseline value divided by 5 year value, gives an estimate of the magnitude of the regression dilution bias. The regression dilution factor was comparable between the cohorts, and was on average 1.4.

Table 3 shows the RR's per quartile for each of the cohorts, before and after adjustment for age, smoking and systolic blood pressure. In all cohorts except the Japanese one, CHD mortality increased with increasing cholesterol quartile, with an adjusted RR between 1.5 and 2.3 for the highest compared with the lowest cholesterol quartile.

Table 1. *Baseline characteristics of men aged 40-59 at baseline and age-standardized 25-year mortality (%) for the cohorts of the Seven Countries Study*

	N[a]	Age (years)		Cholesterol (mmol/l)		Systolic blood pressure (mmHg)		Body Mass Index (kg/m²)		Cigarette smoking (%)	Prevalence of CHD at baseline (%)	Age-adjusted 25-year CHD mortality rate (%)
		mean	SD	mean	SD	mean	SD	mean	SD			
Northern Europe	2555	49.4	5.5	6.55	1.35	144.0	20.4	23.8	3.1	66.8	2.8	20.3
United States	2571	49.4	5.8	6.20	1.15	139.2	20.8	25.5	3.2	59.0	4.6	16.0
Southern Europe Inland	2993	48.8	5.5	5.25	1.10	141.3	20.7	25.5	3.7	59.3	1.1	9.1
Southern Europe Mediterranean	2605	49.4	5.3	5.15	1.10	137.0	19.4	23.4	3.4	59.2	0.5	4.7
Serbia	1027	49.3	5.8	4.25	0.85	132.5	18.8	23.6	3.5	56.3	1.5	7.7
Japan	1010	49.8	5.7	4.25	0.90	135.0	25.0	22.0	2.4	74.3	0.6	3.2

a: maximum number of valid observations

Table 2. *Cutoff points and mean serum total cholesterol level (mmol/l) (SD) to baseline cholesterol quartile per cohort, and calculated dilution factor*

Cholesterol quartile		Northern Europe	United States	Southern Europe Inland	Southern Europe Mediterranean	Serbia	Japan
1	range	< 5.60	< 5.40	< 4.55	< 4.40	< 3.65	< 3.65
	mean baseline (SD)	5.00 (0.50)	4.85 (0.50)	4.00 (0.45)	3.95 (0.40)	3.30 (0.35)	3.20 (0.35)
	mean 5-yr follow-up (SD)	5.55 (0.95)	5.15 (0.80)	4.75 (0.85)	4.60 (0.80)	4.10 (0.80)	n.a.[a]
2	range	5.60-6.40	5.40-6.05	4.55-5.15	4.40-5.05	3.65-4.15	3.65-4.15
	mean baseline (SD)	6.00 (0.25)	5.75 (0.20)	4.90 (0.20)	4.75 (0.20)	3.95 (0.15)	3.90 (0.15)
	mean 5-yr follow-up (SD)	6.25 (0.75)	5.85 (0.70)	5.45 (0.80)	5.15 (0.70)	4.70 (0.85)	n.a.
3	range	6.45-7.30	6.10-6.90	5.20-5.90	5.10-5.80	4.15-4.75	4.20-4.85
	mean baseline (SD)	6.90 (0.45)	6.50 (0.25)	5.55 (0.20)	5.45 (0.20)	4.45 (0.20)	4.50 (0.20)
	mean 5-yr follow-up (SD)	6.80 (0.85)	6.40 (0.70)	5.90 (0.85)	5.60 (0.75)	5.10 (0.80)	n.a.
4	range	> 7.30	> 6.90	> 5.90	> 5.80	> 4.75	> 4.85
	mean baseline (SD)	8.30 (0.95)	7.75 (0.85)	6.70 (0.70)	6.60 (0.75)	5.35 (0.60)	5.50 (0.55)
	mean 5-yr follow-up (SD)	7.80 (1.15)	7.20 (1.05)	6.70 (1.10)	6.55 (1.05)	5.80 (1.00)	n.a.
Cholesterol difference between the lowest and highest quartiles							
	baseline	3.30	2.90	2.70	2.70	2.05	2.25
	5-yr follow-up	2.25	2.05	2.00	1.95	1.70	n.a.
	Dilution factor	1.45	1.43	1.36	1.39	1.23	n.a.

a: n.a. = not available

Table 3. *Crude and adjusted relative risks (95% confidence interval) per cholesterol quartile for each cohort*

Cohort		Cholesterol quartile				p-value χ^2-trend
		1 (low)	2	3	4 (high)	
Northern Europe	crude	1.0	1.18 (0.90-1.55)	1.45 (1.12-1.89)	2.16 (1.69-2.76)	< 0.001
	adjusted[a]	1.0	1.11 (0.84-1.45)	1.34 (1.03-1.74)	2.03 (1.59-2.59)	< 0.001
United States	crude	1.0	1.22 (0.89-1.68)	1.50 (1.11-2.04)	2.74 (2.07-3.63)	< 0.001
	adjusted	1.0	1.09 (0.79-1.51)	1.39 (1.03-1.89)	2.34 (1.77-3.11)	< 0.001
Southern Europe	crude	1.0	1.24 (0.86-1.79)	1.55 (1.10-2.18)	1.65 (1.17-2.33)	< 0.01
Inland	adjusted	1.0	1.21 (0.84-1.74)	1.50 (1.06-2.12)	1.52 (1.07-2.15)	< 0.01
Southern Europe	crude	1.0	1.12 (0.62-2.00)	1.51 (0.87-2.61)	2.30 (1.38-3.82)	< 0.001
Mediterranean	adjusted	1.0	1.03 (0.57-1.85)	1.35 (0.78-2.33)	1.66 (0.98-2.80)	< 0.05
Serbia	crude	1.0	1.57 (0.76-3.27)	2.17 (1.10-4.30)	2.17 (1.07-4.38)	< 0.05
	adjusted	1.0	1.43 (0.69-2.96)	1.88 (0.94-3.73)	1.86 (0.92-3.76)	≥ 0.05
Japan	crude	1.0	1.46 (0.61-3.50)	0.82 (0.30-2.26)	1.13 (0.45-2.86)	≥ 0.05
	adjusted	1.0	1.51 (0.60-3.84)	0.89 (0.31-2.57)	1.13 (0.42-3.02)	≥ 0.05

a : adjusted for age, systolic blood pressure and cigarette smoking
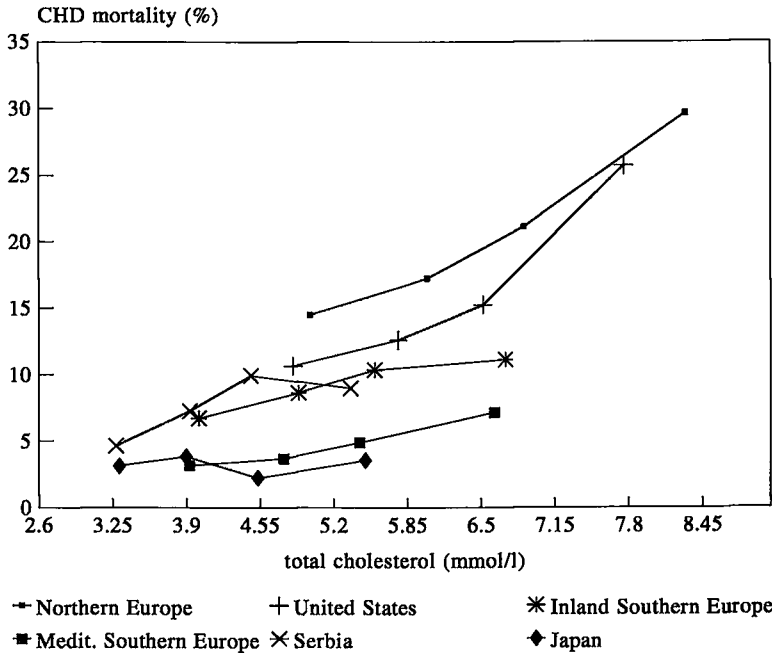
CHD mortality (%)

Figure 1. *25-year CHD mortality rates per baseline cholesterol quartile, adjusted for age, cigarette smoking and systolic blood pressure.*

Table 4. *Relative risks (95% confidence intervals) per 0.50 mmol/l cholesterol increase, before and after correction for regression dilution. Adjusted for age, smoking and systolic blood pressure*

Cohort	Unadjusted for regression dilution		Adjusted for regression dilution
Northern Europe	1.12	(1.08-1.15)	1.18
United States	1.15	(1.10-1.19)	1.21
Southern Europe Inland	1.10	(1.04-1.16)	1.14
Southern Europe Mediterranean	1.12	(1.04-1.22)	1.18
Serbia	1.11	(0.97-1.28)	1.14
Japan	0.96	(0.77-1.18)	not available
Overall	1.12	(1.09-1.16)	1.17[a]

a: Based on the average regression dilution factor of 1.4 (see table 2)

105

In figure 1, mortality rates for all cohorts for each quartile, adjusted for age, smoking and systolic blood pressure, are plotted against the mean cholesterol level of that quartile. From this figure it becomes clear that the absolute level of risk is strikingly different among the cohorts. For example, for a cholesterol level of around 5.45 mmol/l there is a threefold range in mortality rates, varying from 4% to 5% in Japan and the Mediterranean to 10% in Inland Southern Europe, 12% in the United States and 15% in Northern Europe.

A little less than half the CHD mortality occurred in the first 15 years of follow-up, and a little more than half occurred in the last 10 years of follow-up. The relationships of CHD to baseline cholesterol level appeared to be similar in the early and late follow-up periods (data not shown). Death rates were lower in the United States but higher in all other cohorts in the late than in the early follow-up period, with a particularly large increase in Serbia.

Although the absolute levels of CHD mortality are different, the slopes of the curves for the different cohorts are comparable. Based on the approximation of a linear regression analysis, for each of the cohorts the increase in risk was estimated for a 0.50 mmol/l increase in total cholesterol (table 4). An overall estimate for all cohorts combined was also made, adjusting for cohort with dummy variables. Only the RR for Japan did not differ from 1.0; in the other five cohorts the estimated increase for a 0.50 mmol/l cholesterol increase ranged from 1.10 to 1.15. These estimates did not significantly differ from the combined estimate of 1.12. When this combined estimate was corrected for regression dilution bias, it was calculated that a 0.50 mmol/l increase in total serum cholesterol corresponded with an RR of 1.17.

Discussion

No statistically significant differences were observed between the different cohorts with respect to the regression coefficients for the relationship between serum total cholesterol and long-term CHD mortality. For the Japanese cohort the regression coefficient was not statistically significantly different from 1.0. However, for several reasons, power to detect an association in the Japanese cohort was low: the Japanese cohort was relatively small, the number of CHD cases was small (N=22), and cholesterol levels were extremely low. A considerably larger study in China reported a significant positive linear relationship

between total cholesterol and CHD mortality, even at very low cholesterol levels of about 2.95 mmol/l.[11] Baseline cholesterol levels in Serbia were about the same as in Japan, but long-term CHD mortality in this cohort was much higher, probably because total cholesterol increased dramatically (by about 30%) during the first 10 years of follow-up. This and the relatively small size of the Serbian cohort may explain why in this cohort the regression coefficient was positive, although not statistically significant.

It is often reported that the predictive power of a baseline cholesterol measurement decreases over long follow-up periods[12]. A comparison of early and late follow-up in our study, however, showed only a slight reduction in RR's for the late versus early follow-up. From our study it was estimated that an increase in total cholesterol of 0.50 mmol/l corresponds to a 12% increase in long-term CHD mortality and, when corrected for regression dilution bias, even to a 17% increase. Although these estimates are based on an approximate linearity assumption, they correspond with a recent estimate of Law et al.[13] In their analyses the estimated decrease in CHD mortality per 0.6 mmol/l decrease in total cholesterol was 17% for men aged 45 through 54 years (adjusted for regression dilution, 24%) and 12% for men aged 55 through 64 years (adjusted for regression dilution, 17%).

The dilution factors in our study were about 1.4 in Northern Europe, Southern Europe (Inland and Mediterranean) and the United States, and about 1.2 in Serbia. The smaller dilution factor in Serbia can be explained by the fact that during the first 5 years serum cholesterol levels were increasing strongly. The magnitude of the regression dilution bias seems to be universal: apart from similarities in the different cultures of the Seven Countries Study, Law et al.[13] obtained a similar estimate, and also other studies in which cholesterol was remeasured after several years found estimates of around 1.4 (see reference 13). This means that the weakening of observed associations between cholesterol and mortality due to a single cholesterol measurements is the same in different cultures, as long as cholesterol levels are relatively stable. However, the dilution factor, if estimated several years later, would be biased by coexisting time trends.

No interaction was observed between smoking or blood pressure and cholesterol, indicating that RRs were the same in smokers and non-smokers and not dependent on blood pressure levels. However, when an RR of 1.1 is translated into excess mortality, the absolute CHD risk is also important. Since absolute CHD risk is higher in smokers compared with non-smokers, a 10% increase in risk will be translated into a larger number

of excess deaths in smokers than in non-smokers. More generally, with respect to primary prevention, a 10% risk reduction in populations with high cholesterol levels will translate into a larger number of lives saved than a 10% risk reduction in populations with low cholesterol levels.

Our data showed that the relation between serum total cholesterol level and CHD risk was similar in different cultures, but the absolute levels of CHD mortality were strikingly different. Within cultures the RR for the highest cholesterol quartile compared with the lowest was about 1.5 to 2.3. Between cultures, this range was larger, even at the same level of serum total cholesterol. At a cholesterol level of about 5.45 mmol/l, CHD mortality rates varied from 4% to 5% in Japan and Mediterranean Southern Europe to 10% in Inland Southern Europe, 12% in the United States, and 15% in Northern Europe. Because of these differences in mortality rates, the lowest cholesterol quartile for Northern Europe, with a mean level of about 4.90 mmol/l, had about a two-fold higher risk than the highest cholesterol quartile for Mediterranean Southern Europe, with a mean cholesterol level of about 6.45 mmol/l. These differences in risk were not explained by differences in age, smoking, or systolic blood pressure, because the rates were adjusted for these factors. However, because all measurements are subject to measurement error, it is never possible to completely remove the effect of these confounders, so there may be some residual confounding. The public health implication of this difference is important: it may be impossible to achieve a reduction of CHD mortality rates in Northern Europe and the United States to the Mediterranean level by a cholesterol reduction alone; the reduction in cholesterol must be accompanied by changes in other factors to achieve a profile more typical of the 'low-risk' countries.

Other factors that explain these differences have to be considered. First, methodological differences can play a role, such as biases in the coding of the death certificates. However, the study was designed to investigate CHD mortality and its determinants in different cultures, and coding of the causes of death was done by two reviewers using standard criteria to ensure comparability among the countries. Differences in medical care can result in different survival rates that would lead to differences in mortality rates. However, to cause the observed difference in mortality rates, medical care would have to be of lower quality in Northern Europe and the United States than in the Mediterranean countries. It is unlikely that this is the case.

It has been postulated that the oxidized form of low density lipoprotein (ox-LDL) is more atherogenic than native LDL.[14] The amount of ox-LDL is the result of the balance between the amount of oxidative stress (eg, smoking) and the antioxidant capacity.[15] Susceptibility of LDL particles to oxidation is related to their fatty acid composition: polyunsaturated fatty acids increase susceptibility to oxidation compared with monounsaturated fatty acids.[16] Antioxidant capacity is influenced by the amount of antioxidants in the diet (eg, vitamins C and E, ß-carotene and flavonoids).[17] A given level of serum total cholesterol will represent different levels of ox-LDL in different cultures because of differences in the above mentioned factors.

Differences in nutritional factors may play an important role because dietary patterns differ greatly between the cohorts.[1,18] In the present study, dietary information has been collected in a small subsample of men within each cohort, to characterize the dietary pattern of the cohorts. Compared with the Northern European and American diet, the Mediterranean diet contained less meat but more fish, fruits, vegetables, and ethanol.[18] The fatty acids consumed in Northern Europe and the United States were predominantly saturated but in the Mediterranean predominantly mono-unsaturated.[19] Intake of the anti-oxidant vitamins ß-carotene and α-tocopherol was highest in Mediterranean Southern Europe.[20] Flavonoid intake was twice as high in Southern Europe (Inland and Mediterranean) as in Northern Europe and the United States, but was highest in Japan.[21] Intake of flavonoids, polyphenolic substances with antioxidant properties, has been shown to protect against CHD.[22] In an ecological analysis of the Seven Countries Study data, it was shown that, at the cohort level, mean intake of saturated fat and flavonoids and current smoking were strongly correlated (r=0.95) with the CHD mortality rate.[21] This means that *at the cohort level* the occurrence of CHD was almost completely explained by saturated fatty acids, flavonoids, and smoking. However, because of strong correlations between dietary variables, it is possible that dietary variables strongly correlated with saturated fatty acids and flavonoids also play a role.

The effect of a Mediterranean diet was studied in a secondary prevention trial on CHD.[23] Although no difference in total cholesterol levels between the experimental and control group occurred, after 27 months both cardiac and all-cause mortality were significantly lower in the experimental group. The fact that the beneficial effect of the experimental diet was already apparent in the first months suggests an effect of this Mediterranean diet on thrombogenesis.

These results indicate that the relationship between diet and cholesterol explains only a part of the relationship between diet and coronary heart disease. Dietary factors that influence LDL oxidation and thrombotic factors are also of great importance.

Besides differences in diet, differences in biological risk factors (other than total cholesterol) and genetic factors may play a role in explaining differences in absolute risk. Differences in high density lipoprotein (HDL) cholesterol and post-prandial lipoproteins can be present, and are not accounted for by the total cholesterol level.[24] Furthermore, the effect of genetic differences among cultures that would influence atherogenicity of cholesterol (due to differences in LDL-subclasses or HDL cholesterol) can not be ruled out.[25]

In conclusion, our study showed that the relative increase in CHD risk with an increase in serum total cholesterol was comparable in different cultures. However, the absolute increase was quite different from culture to culture, since the absolute level of CHD risk differed substantially among cultures. Therefore, from a public health perspective it is not enough to focus solely on serum cholesterol levels to decrease the burden of CHD in populations. It appears that reductions in serum total cholesterol levels are not likely to bring cultures with a high CHD risk, such as the United States and Northern Europe, back to a CHD mortality level characteristic for the Mediterranean and Japanese cultures unless other factors are also changed. The Mediterranean and Japanese diets, low in saturated fat and rich in antioxidants, may have beneficial effects both on the oxidizability of LDL particles and on thrombogenesis, apart from an effect on LDL levels per se. This stresses the importance of factors other than serum cholesterol, blood pressure and smoking status, such as diet, in CHD prevention.

References.

1. Keys A. (ed.) Coronary heart disease in Seven Countries. Circulation 1970;41:suppl 1.

2. Keys A, Aravanis C, Blackburn H, et al. Seven Countries Study. A multivariate analysis of death and coronary heart disease. Cambridge, England: Harvard University Press 1980;1-381.

3. Menotti A, Keys A, Kromhout D, et al. Inter-cohort differences in coronary heart disease mortality in the 25-year follow-up of the Seven Countries Study. Eur J Epidemiol 1993; 9:527-536.

4. MacMahon, Peto R, Cutler J, et al. Blood pressure, stroke, and coronary heart disease. Lancet 1990;335:765-74.

5. Keys A, Aravanis C, Blackburn H, et al. Epidemiological studies related to coronary heart disease: characteristic of men aged 40-59 in seven countries. Acta Med Scand 1967;Suppl.460:1-392.

6. Anderson JT, Keys A. Cholesterol in serum and lipoprotein fractions: its measurement and stability. Clin Chem 1956;2:145-9.

7. Rose GA, Blackburn H. Cardiovascular survey methods. Geneva WHO, 1968.

8. WHO. International Classification of Diseases. 8th Revision, volume 1 and 2. World Health Organization, Geneva 1967.

9. SAS Institute Inc., SAS Technical Report P-229, SAS/STAT Software: Changes and Enhancements, Release 6.07, Cary, NC: SAS Institute, 1992.

10. Kleinbaum DG, Kupper LL, Muller KE. Applied regression analysis and other multivariable methods. Boston, MA: PWS-KENT Publishing Company 1988;265-79.

11. Chen Z, Peto R, Collins R, MacMahon S, Lu J, Li W. Serum cholesterol concentration and coronary heart disease in a population with low cholesterol concentrations. BMJ 1991;303:276-82.

12. Kromhout D, Bosschieter EB, Drijver M, de Lezenne Coulander C. Serum cholesterol and 25-year incidence of and mortality from myocardial infarction and cancer. The Zutphen Study. Arch Intern Med 1988;8:1031-40.

13. Law MR, Wald NJ, Wu T, Hackshaw A, Bailey A. Systematic underestimation of association between serum cholesterol concentration and ischaemic heart disease in observational studies: data from the BUPA study. BMJ 1994;308:363-6.

14. Steinberg D, Parthasarathy S, Carew TE, Khoo JC, Witztum JL. Modifications of low-density lipoprotein that increase its atherogenicity. N Engl J Med 1989;320:915-24.

15. Witztum JL. The oxidation hypothesis of atherosclerosis. Lancet 1994;344:793-5.

16. Reaven P, Parthasarathy S, Grasse BJ, Miller ER, Steinberg D, Witztum JL. Effects of oleate-rich and linoleate-rich diets on the susceptibility of low density lipoprotein to oxidative modification in mildly hypercholesterolemic subjects. J Clin Invest 1993;91:668-76.

17. Esterbauer H, Striegl G, Puhl H, et al. The role of vitamin E and carotenoids in preventing oxidation of low density lipoproteins. Ann NY Acad Sci 1989;570:254-67.

18. Kromhout D, Keys A, Aravanis C, et al. Food consumption patterns in the 1960s in seven countries. Am J Clin Nutr 1989;49:889-94.

19. Kromhout D, Keys A, Menotti A, et al. Dietary saturated and trans-fatty acids, cholesterol and 25-year mortality from coronary heart disease. The Seven Countries Study. Prev Med 1995;24:308-15.

20. Ocke M, Kromhout D, Menotti A, et al. Average intake of antioxidant (pro)vitamins and subsequent cancer mortality in the 16 cohorts of the Seven Countries Study. Int J Cancer 1995;61:480-4.

21. Hertog MGL, Kromhout D, Keys A, et al. Flavonoid intake and risk of coronary heart disease and cancer mortality in the Seven Countries Study. Arch Intern Med 1995;155:381-6.

22. Hertog MGL, Feskens EJM, Hollman PCH, Katan MB, Kromhout D. Dietary antioxidant flavonoids and risk of coronary heart disease. The Zutphen Elderly Study. Lancet 1993;342:1007-11.

23. Lorgeril M de, Renaud S, Mamelle N, et al. Mediterranean alpha-linolenic acid-rich diet in secondary prevention of coronary heart disease. Lancet 1994;343:1454-9.

24. Slyper AH. A fresh look at the atherogenic remnant hypothesis. Lancet 1992;340:289-91.

25. Austin M, King MC, Vranizan KM, Krauss RM. Atherogenic lipoprotein phenotype. A proposed genetic marker for coronary heart disease risk. Circulation 1990;82:495-506.

8

Serum total cholesterol and long-term non-cardiovascular and all-cause mortality in the Seven Countries Study

Abstract

Background: The association of low total cholesterol levels with non-cardiovascular mortality is still in debate. This led us to investigating the association between serum total cholesterol level (TC) and 25-year non-cardiovascular and all-cause mortality in the Seven Countries Study.

Methods: TC was measured between 1958 and 1964 in 12,467 men aged 40-59 in 16 cohorts located in seven countries in Europe, the USA and Japan. Relative risks (RR) were estimated using Cox proportional hazards (survival) analysis for continuous cholesterol levels and for the TC categories <4.15, 5.16-6.20 and >6.20 mmol/l using the category 4.15-5.15 mmol/l as the reference. Adjustment was made for age, smoking, systolic blood pressure and cohort. Smoking strata were also examined.

Results: Cancer mortality was inversely associated with TC category only in smokers. Non-cardiovascular/non-cancer mortality was inversely associated with TC category in smokers, and elevated in non-smokers only at TC <4.15 mmol/l. For subgroups of diseases (infections and respiratory diseases) inverse associations were observed both in smokers and non-smokers. Total mortality in non-smokers showed a J-shaped relation with TC (lowest mortality for TC of 4.15-5.15 mmol/l), while no association with TC was observed in smokers. Exclusion of deaths during the first 10 years of follow-up did not alter the results. There were indications that alcohol abuse was more prevalent at low TC levels.

Conclusion: The inverse association of TC with non-cardiovascular mortality was most prominent among smokers. The associations observed were, at least partly, explained by confounding. For non-smokers the lowest total mortality rate was observed for TC levels of 4.15-5.15 mmol/l.

Introduction

The strong positive relation between serum total cholesterol and risk of mortality from coronary heart disease has been shown in many prospective studies in the last decades.[1-3] The relation has been shown to be positive and graded, even at lower cholesterol levels, which means that the lower the cholesterol level, the lower the coronary heart disease mortality risk.[4]

However, questions have been raised about an increased mortality risk at the lower end of the cholesterol distribution.[5] A number of studies have reported an increased risk for cancer mortality at a low cholesterol level, thus off-setting the lower cardiovascular mortality risk.[6-16] Apart from an increased cancer risk, a number of studies have also reported an increased mortality risk for non-cardiovascular/non-cancer diseases at lower cholesterol levels.[5,15,17] After 15 years of follow-up in the Seven Countries Study, excess cancer mortality was reported at low cholesterol levels.[18] Recently, the 25-year mortality follow-up of all cohorts of the Seven Countries Study was completed, and we are now able to exclude up to ten years of follow-up in order to exclude early mortality due to pre-existing diseases.

From a public health point of view it is important to know whether the inverse association of total cholesterol and non-cardiovascular mortality is causal. If causal, this would imply that there is an optimum cholesterol level, and that cholesterol lowering below that level would not be desirable. Furthermore, because lipids are important in many body processes, it is important to shed additional light on possible roles of lipids in disease etiology. In this paper, the emphasis will be on the associations of total cholesterol with cancer, non-cardiovascular/non-cancer and all-cause mortality. For comparative purposes also the association between total cholesterol and cardiovascular mortality will be reported.

Materials and methods.

Study population
Between 1958 and 1964, 12,763 men aged 40-59 from seven different countries were enrolled in the study (Keys, 1967). A total of 16 cohorts were examined in the following

countries: USA (US Railroad), Finland (East and West), the Netherlands (Zutphen), Italy (Rome Railroad, Crevalcore and Montegiorgio), Greece (Crete and Corfu), Croatia and Serbia (the former Yugoslavia: Dalmatia, Slavonia, Zrenjanin, Velika Krsna, Belgrade) and Japan (Tanushimaru and Ushibuka). In 11 rural cohorts all men aged 40-59 from official registries were invited. In the town of Zutphen (Netherlands) a four-nineth sample of these men was invited. In the USA and Rome (Italy), railroad personnel was recruited. In Serbia (former Yugoslavia) workers from a large cooperative in Zrenjanin and professors from the University of Belgrade were invited. Overall the participation rate was over 90%, with several cohorts reaching almost 100%.

Measurements

The major cardiovascular risk factors were measured in a standardized way at baseline and, in most cohorts, at 5 and 10 years after baseline. In Japan cholesterol was not measured 5 years after baseline and in the USA not after 10 years. Details of the methods used have been extensively described elsewhere.[19,20] Total cholesterol was measured in a non-fasting blood sample, according to the Abell-Kendall method, modified by Anderson and Keys, in standardized laboratories.[21] Blood pressure was measured twice at the end of the physical examination with the subject in supine position, with a mercury sphygmomanometer following the methodology later described in the WHO manual 'Cardiovascular Survey Methods'.[22] The mean of the two measurements was used in the analyses. Cigarette smoking was established by means of a standardized questionnaire, and, for the present analyses, dichotomized into current smoking or non-smoking.

Mortality follow-up

All men were followed for mortality during 25 years. Only 56 men (0.4%) were lost to follow-up. The underlying cause of death was coded in a standardized way, by two reviewers (H.B. and A.M.) using the 8th revision of the WHO International Classification of Diseases.[23] The final cause of death was adjudicated on the basis of information from the official death certificate, in combination with information from medical and hospital records. The coder of the causes of death was blind to the risk factor status of the subject. In the case of multiple causes of death, priority was given to accidents, followed by cancer in advanced stages, coronary heart disease and stroke. Only the primary cause of death was used in the analyses.

Statistical analyses

For the cohort-specific analyses the cohorts were pooled into six cohorts based on similarities in baseline cholesterol level, cultural resemblance and homogeneity with respect to cholesterol changes during the first ten years of follow-up: Northern Europe (East and West Finland, the Netherlands), the United States, Inland Southern Europe (Rome, Crevalcore, Slavonia, Belgrade), Mediterranean Southern Europe (Dalmatia, Montegorgio, Crete and Corfu), Serbia (Velika Krsna, Zrenjanin), and Japan (Tanushimaru and Ushibuka). In this way, statistical power was increased within each cohort, but adjustment for cultural differences could still be made. In the American, Northern European and Japanese cohorts, cholesterol remained more or less stable during the first 5 respectively 10 years of follow-up. In the Southern European cohorts, both inland and mediterranean, cholesterol levels increased by about 10% while two Serbian cohorts (Zrenjanin and Velika Krsna) showed a dramatic increase of about 30%. A total of 343 men were excluded from the analyses due to missing values of cholesterol, blood pressure or smoking status at baseline, or unknown vital status.

Cox' proportional hazards (survival) analysis was used to estimate relative risks (RR), using the SAS statistical package (release 6.07, procedure PHREG).[24] Cholesterol was entered as a continuous linear term or categorized into four categories (less than 4.15 mmol/l, 4.15-5.15 mmol/l, 5.16-6.20 mmol/l and 6.20 mmol/l or more. Due to small numbers in the lowest category for some cohorts the second cholesterol category (4.15-5.15 mmol/l) was used as the reference category. Adjustment was made for age (years), cigarette smoking (no/yes) and systolic blood pressure (mmHg). Furthermore, to adjust for differences in mortality between the different cohorts, dummy variables for cohort were entered into the model, Northern Europe being the reference. To exclude the effect of early mortality, analyses were repeated with exclusion of the first ten years of follow-up.

Main end-points were cardiovascular diseases, cancer, non-cardiovascular/non-cancer and all-cause mortality. These large, heterogeneous groups of diseases were broken down into smaller, more specific, disease categories if there were 100 cases or more, leading to the following subdivision (for ICD-8 codes see table 2): cardiovascular disease (CVD) was divided into coronary heart disease (CHD), cerebrovascular disease (CVA) and other CVD; cancer was divided into digestive cancer, (which was further subdivided into stomach cancer, colon/rectum cancer, and other digestive cancers (two-thirds of which were liver cancers)), lung cancer, lymphatic/hematopoietic cancers, and other cancers; non-

cardiovascular/non-cancer causes were divided into infections, respiratory diseases (which was subdivided into chronic obstructive pulmonary diseases (COPD) and 'other' respiratory diseases), digestive diseases (subdivided into liver cirrhosis and 'other' digestive diseases), accidents and 'other' non-cardiovascular/non-cancer causes. Mortality from liver cirrhosis and alcohol dependency syndrome combined was defined as 'alcohol related diseases'.

Results

In table 1 some characteristics of the cholesterol distribution in the six cohorts are given. Mean cholesterol levels ranged from about 4.25 mmol/l in Japan and Serbia to about 5.16 mmol/l in the inland and mediterranean Southern European cohorts and about 5.45 mmol/l in Northern Europe and the United-States. The percentage of respondents with a cholesterol level below 4.15 mmol/l ranged from 2% to 48%. During twenty-five years of follow-up 5974 men died, for 5830 of these men a baseline cholesterol measurement was available. About half of all deaths could be attributed to cardiovascular diseases, while cancer and non-cardiovascular/non-cancer causes each contributed about 25% to total mortality (table 2).

Mortality from cardiovascular diseases (CVD) increased with increasing cholesterol category, mainly due to the strong positive relation between total cholesterol and coronary heart disease mortality (table 3). In analyses with linear cholesterol, a 1.0 mmol/l increase in total cholesterol was associated with a RR of 1.16 for CVD mortality. In the cohort-specific analysis a positive relation between total cholesterol and cardiovascular disease mortality was not observed in Southern Europe and Japan (table 4).

Mortality from cancer was elevated in the lowest cholesterol category compared to the second category (4.15-5.15 mmol/l), and decreased further in the highest cholesterol category. The estimated RR per 1.0 mmol/l cholesterol increase was 0.92 (table 3). A significant inverse association with total cholesterol (continuous) was observed for digestive and lung cancer. The association was borderline significant (p<0.10) for lymphatic and hematopoietic cancers. Within the group of digestive cancers, the inverse association was strongest for the group of 'other digestive cancers' compared to stomach and colon/rectum cancers. No association with the remaining cancers was observed.

Table 1. *The serum total cholesterol distribution at baseline in men aged 40 to 59 years, according to cohort. The Seven Countries Study*

Cohort	N	mean	SD	< 4.15 mmol/l		4.15-5.15 mmol/l		5.16-6.20 mmol/l		> 6.20 mmol/l	
				N	%	N	%	N	%	N	%
Northern Europe	2489	6.54	1.34	38	2	295	12	754	30	1402	56
United States	2555	6.21	1.16	61	2	369	14	906	35	1219	48
Southern Europe Inland	2933	5.28	1.09	379	13	1039	35	971	33	544	19
Southern Europe Mediterranean	2548	5.17	1.09	392	15	958	38	778	31	420	16
Serbia	1025	4.25	0.83	475	46	415	40	111	11	24	2
Japan	917	4.27	0.91	441	48	329	36	128	14	19	2
Total	12467	5.53	1.37	1786	14	3405	27	3648	29	3628	29

Table 2. *Frequency of causes of death after 25-years of follow-up[a]*

Cause of death	ICD-8 code(s)	N of cases
Cardiovascular	390-459	2901
Coronary heart disease	410-414, 795	1472
Cerebrovascular disease	430-438	778
Other cardiovascular diseases	390-409, 415-429, 440-459	651
Cancer	140-239	1551
Digestive	150-159	582
Stomach	151	255
Colon and rectum	153,154	158
Other digestive	152,155-159	134
Lung	162	418
Lymphatic/Hematopoietic	200-209	110
Other cancers	140-149, 160, 161, 163-199, 210-239	441
Non-cardiovascular/Non-cancer	0-139, 240-389, 459-794, 796-999	1378
Infections	0-139	140
Respiratory	460-519	366
Chronic obstructive pulmonary disease	490-492	230
Other respiratory	460-489, 493-519	136
Digestive	520-579	267
Liver cirrhosis	571	148
Other digestive	520-570, 575-578	119
Accidents	800-999	297
Other non-cardiovascular/non-cancer	240-389, 580-794, 796-799	308
All causes	0-999	5830

a: based on men with a baseline cholesterol measurement

119

Table 3. *Adjusted[a] relative risks (95% confidence interval) for selected causes of death for different total cholesterol levels using 4.15-5.15 mmol/l as reference and relative risk (95% confidence interval) per 1.0 mmol/l cholesterol increase*

	Cholesterol level (mmol/l)				p-value χ^2-trend	RR per 1.0 mmol/l increase[b]
	≤ 4.15	4.15-5.15	5.16-6.20	> 6.20		
Cardiovascular disease	**0.98 (0.85-1.14)**	**1.0**	**1.12 (1.00-1.26)**	**1.38 (1.23-1.55)**	**<0.001**	**1.16 (1.13-1.21)**
Coronary heart disease	0.91 (0.72-1.17)	1.0	1.19 (1.02-1.40)	1.65 (1.41-1.93)	<0.001	1.26 (1.21-1.31)
Cerebrovascular disease	1.09 (0.87-1.37)	1.0	1.37 (1.13-1.66)	1.22 (0.98-1.52)	0.08	1.03 (0.96-1.10)
Other CVD	0.92 (0.71-1.19)	1.0	0.85 (0.68-1.05)	0.99 (0.80-1.24)	0.87	1.07 (0.99-1.14)
Cancer total	**1.16 (0.98-1.36)**	**1.0**	**1.00 (0.87-1.14)**	**0.89 (0.76-1.03)**	**0.01**	**0.92 (0.88-0.97)**
Digestive	1.25 (0.98-1.61)	1.0	1.09 (0.87-1.36)	0.79 (0.61-1.02)	0.01	0.89 (0.82-0.96)
Stomach	1.01 (0.70-1.44)	1.0	0.77 (0.55-1.08)	0.79 (0.54-1.15)	0.16	0.94 (0.84-1.06)
Colon/rectum	1.23 (0.71-2.13)	1.0	1.50 (0.97-2.32)	0.90 (0.54-1.49)	0.52	0.87 (0.75-1.02)
Other digestive	1.91 (1.16-3.15)	1.0	1.35 (0.84-2.18)	0.70 (0.39-1.25)	0.01	0.80 (0.67-0.95)
Lung	1.16 (0.81-1.66)	1.0	0.85 (0.65-1.12)	0.82 (0.62-1.08)	0.06	0.88 (0.81-0.97)
Lymph/hemato[c]	1.54 (0.81-2.91)	1.0	0.91 (0.55-1.53)	0.76 (0.44-1.32)	0.07	0.88 (0.73-1.05)
Other	0.98 (0.72-1.34)	1.0	1.07 (0.83-1.37)	1.11 (0.84-1.47)	0.40	1.03 (0.94-1.12)
Non-CVD/non-cancer	**1.23 (1.05-1.45)**	**1.0**	**1.00 (0.87-1.16)**	**0.86 (0.73-1.01)**	**0.001**	**0.91 (0.87-0.96)**
Infections	1.72 (1.12-2.64)	1.0	0.81 (0.51-1.28)	0.43 (0.23-0.82)	<0.001	0.68 (0.57-0.82)
Respiratory	0.93 (0.67-1.27)	1.0	0.82 (0.62-1.08)	0.65 (0.48-0.90)	0.03	0.88 (0.79-0.97)
COPD[d]	0.82 (0.54-1.24)	1.0	0.98 (0.70-1.38)	0.70 (0.47-1.05)	0.40	0.93 (0.81-1.05)
Other respiratory	1.13 (0.68-1.85)	1.0	0.61 (0.38-0.97)	0.60 (0.36-0.99)	0.01	0.80 (0.68-0.95)
Digestive	1.87 (1.29-2.69)	1.0	1.26 (0.90-1.76)	1.27 (0.88-1.84)	0.32	0.94 (0.84-1.06)
Liver cirrhosis	1.78 (1.11-2.87)	1.0	1.05 (0.68-1.63)	1.16 (0.72-1.87)	0.25	0.87 (0.74-1.02)
Other digestive	2.01 (1.13-3.58)	1.0	1.60 (0.95-2.71)	1.47 (0.82-2.63)	0.84	1.03 (0.87-1.22)
Accidents	1.05 (0.72-1.53)	1.0	1.21 (0.83-1.68)	1.18 (0.83-1.68)	0.39	1.06 (0.95-1.18)
Other non-CVD/non-cancer	1.09 (0.77-1.56)	1.0	0.99 (0.73-1.33)	0.79 (0.56-1.10)	0.12	0.90 (0.81-1.00)
Total mortality	**1.10 (1.01-1.21)**	**1.0**	**1.06 (0.98-1.14)**	**1.10 (1.02-1.19)**	**0.27**	**1.04 (1.01-1.06)**

a : Adjusted for age, cigarette smoking, systolic blood pressure and cohort
b : Estimated from a separate analysis with cholesterol as a continuous variable
c : Lymphatic/hematopoietic
d : Chronic Obstructive Pulmonary Disease

Table 4. *Relative risk (95% confidence interval) per 1.0 mmol/l total cholesterol increase in the cohorts separately, adjusted for age, cigarette smoking, systolic blood pressure and cohort*

	Cardiovascular	Cancer	Non-cardiovascular Non-cancer	All-cause mortality
Northern Europe	1.21 (1.14-1.28)	0.88 (0.81-0.96)	0.92 (0.82-1.03)	1.06 (1.01-1.10)
United States	1.25 (1.17-1.33)	0.95 (0.85-1.06)	0.89 (0.80-1.02)	1.11 (1.05-1.17)
Southern Europe Inland	1.10 (1.02-1.19)	1.01 (0.92-1.12)	0.89 (0.80-0.99)	1.03 (0.97-1.08)
Southern Europe Mediterranean	1.02 (0.93-1.13)	0.83 (0.74-0.94)	0.89 (0.80-1.00)	0.94 (0.89-1.00)
Serbia	1.19 (1.02-1.39)	0.88 (0.69-1.11)	1.12 (0.92-1.36)	1.07 (0.97-1.20)
Japan	0.88 (0.72-1.07)	0.99 (0.82-1.19)	0.88 (0.71-1.07)	0.92 (0.82-1.02)
Overall[a]	1.16 (1.13-1.21)	0.92 (0.88-0.97)	0.91 (0.87-0.96)	1.04 (1.01-1.06)

a: adjusted for cohort

Table 5. *Adjusted[a] relative risks (95% confidence interval) for smokers (N=7,637) and non-smokers (N=4,781), for different total cholesterol levels, using a cholesterol level of 4.15-5.15 mmol/l as reference, and relative risks (95% confidence interval) per 1.0 mmol/l cholesterol increase[b]*

	Cholesterol level (mmol/l)				RR per 1.0 mmol/l increase[2]
	< 4.15	4.15-5.15	5.16-6.20	> 6.20	
Cancer (total)					
Non-smokers	1.13 (0.80-1.58)	1.0	1.22 (0.94-1.59)	1.08 (0.81-1.44)	0.97 (0.89-1.07)
Smokers	1.16 (0.96-1.40)	1.0	0.93 (0.80-1.10)	0.82 (0.69-0.98)	0.90 (0.85-0.96)
Digestive cancers					
Non-smokers	1.13 (0.69-1.85)	1.0	1.69 (1.14-2.51)	1.07 (0.68-1.69)	0.97 (0.85-1.11)
Smokers	1.29 (0.96-1.72)	1.0	0.87 (0.66-1.15)	0.69 (0.50-0.96)	0.85 (0.76-0.94)
Lung cancer					
Non-smokers	1.34 (0.51-3.50)	1.0	0.99 (0.47-2.08)	0.97 (0.45-2.10)	0.86 (0.67-1.10)
Smokers	1.14 (0.77-1.68)	1.0	0.83 (0.62-1.11)	0.80 (0.59-1.07)	0.89 (0.81-0.98)
Lymphatic/hematopoietic cancers					
Non-smokers	2.17 (0.83-5.68)	1.0	0.89 (0.38-2.11)	1.25 (0.54-2.87)	0.93 (0.71-1.21)
Smokers	1.19 (0.50-2.84)	1.0	0.92 (0.49-1.76)	0.50 (0.24-1.07)	0.84 (0.66-1.07)
Other cancers					
Non-smokers	0.85 (0.45-1.61)	1.0	0.90 (0.56-1.45)	1.09 (0.67-1.78)	1.06 (0.90-1.24)
Smokers	1.02 (0.71-1.46)	1.0	1.14 (0.85-1.54)	1.11 (0.79-1.55)	1.01 (0.91-1.13)

Table 5. *Continued*

	Cholesterol level (mmol/l)				RR per 1.0 mmol/l increase[2]
	< 4.15	4.15-5.15	5.16-6.20	> 6.20	
Non-CVD/Non-Cancer					
Non-smokers	1.35 (1.01-1.80)	1.0	1.14 (0.89-1.47)	1.04 (0.79-1.38)	0.97 (0.89-1.06)
Smokers	1.18 (0.97-1.44)	1.0	0.94 (0.78-1.12)	0.77 (0.63-0.94)	0.88 (0.83-0.94)
Infections					
Non-smokers	2.63 (1.12-6.22)	1.0	0.79 (0.30-2.05)	0.45 (0.13-1.54)	0.69 (0.49-0.97)
Smokers	1.48 (0.90-2.44)	1.0	0.82 (0.48-1.40)	0.43 (0.20-0.92)	0.68 (0.55-0.85)
Respiratory					
Non-smokers	1.20 (0.64-2.25)	1.0	0.76 (0.44-1.31)	0.65 (0.35-1.19)	0.82 (0.67-1.00)
Smokers	0.86 (0.59-1.24)	1.0	0.85 (0.62-1.17)	0.66 (0.45-0.95)	0.90 (0.80-1.01)
Digestive					
Non-smokers	1.58 (0.84-2.98)	1.0	1.23 (0.70-2.18)	1.64 (0.90-2.96)	0.98 (0.81-1.19)
Smokers	2.02 (1.29-3.18)	1.0	1.25 (0.83-1.90)	1.07 (0.67-1.72)	0.91 (0.79-1.06)
Accidents					
Non-smokers	1.08 (0.56-2.08)	1.0	1.57 (0.93-2.65)	1.37 (0.76-2.47)	1.17 (1.00-1.38)
Smokers	1.07 (0.67-1.70)	1.0	1.03 (0.69-1.54)	1.08 (0.70-1.67)	0.98 (0.85-1.13)
Other non-CVD/non-Cancer					
Non-smokers	1.15 (0.63-2.09)	1.0	1.24 (0.78-2.00)	1.04 (0.62-1.77)	0.99 (0.84-1.17)
Smokers	1.05 (0.68-1.64)	1.0	0.84 (0.57-1.24)	0.63 (0.40-0.99)	0.83 (0.72-0.97)
Total mortality					
Non-smokers	1.17 (1.00-1.38)	1.0	1.24 (1.09-1.41)	1.32 (1.16-1.51)	1.09 (1.04-1.13)
Smokers	1.08 (0.97-1.20)	1.0	0.98 (0.90-1.07)	1.01 (0.92-1.11)	1.01 (0.98-1.04)

a : adjusted for age, systolic blood pressure and cohort.
b : estimated from a separate analysis with cholesterol as a continuous variable.

Exclusion of the first ten years of follow-up did not decrease the excess cancer risk at cholesterol levels below 4.15 mmol/l (results not shown). In the cohort-specific analysis, the RR for a 1.0 mmol/l increase was less than one in five of the six cohorts, but statistical significance was reached only in the Northern European and Mediterranean Southern European cohorts (table 4).

Mortality from non-cardiovascular/non-cancer causes was elevated at cholesterol levels of less than 4.15 mmol/l, and reduced at levels above 6.20 mmol/l (table 3). Within this heterogeneous group of diseases, a strong inverse relation was observed for mortality from infections. Mortality from respiratory diseases was not elevated in the lowest cholesterol category, but was reduced in the higher cholesterol categories and was inversely associated with total cholesterol when taken continuously. This inverse association was not so much determined by a relation with COPD, but more by a strong inverse relation with the remainder of respiratory diseases. Mortality from digestive diseases was elevated at cholesterol levels less than 4.15 mmol/l, but did not decrease at higher cholesterol levels. No association with mortality from accidents was observed, and an inverse association with the remainder of non-cardiovascular/non-cancer diseases was observed. Exclusion of the first ten years of follow-up did not decrease the excess risk at cholesterol levels below 4.15 mmol/l. In the cohort-specific analysis, the RR per 1.0 mmol/l cholesterol increase was about 0.9 in all cohorts except Serbia (table 4).

Total mortality was lowest for cholesterol levels of 4.15-5.15 mmol/l, and was statistically significantly elevated at a level below 4.15 mmol/l and at levels of 6.20 mmol/l or above (table 3). The cohort-specific analysis showed that the RR for total mortality for a 1.0 mmol/l cholesterol increase was greater than one in four of the six cohorts (statistically significant in two) (table 4). In cohorts with a low rate of cardiovascular disease mortality (Mediterranean Southern Europe and Japan) the RR was less than one.

Stratification by smoking status revealed that the associations between cholesterol and mortality differed between smokers and non-smokers (table 5, figure 1). The association of cholesterol with CVD mortality was weaker in smokers than in non-smokers (figure 1). The inverse association of cholesterol with cancer mortality was concentrated in smokers, and was observed for lung, digestive and lymphatic/hematopoietic cancers, while no associations with the remainder of cancers was observed.

mortality rate/1,000 person-years

total cholesterol (mmol/l)

-- non-smokers -+- smokers

A *Cardiovascular diseases*

mortality rate/1,000 person-years

total cholesterol (mmol/l)

-- non-smokers -+- smokers

B *Cancer*

mortality rate/1,000 person-years

total cholesterol (mmol/l)

-- non-smokers -+- smokers

C *Non-cardiovascular/non-cancer*

mortality rate/1,000 person-years

total cholesterol (mmol/l)

-- non-smokers -+- smokers

D *All causes*
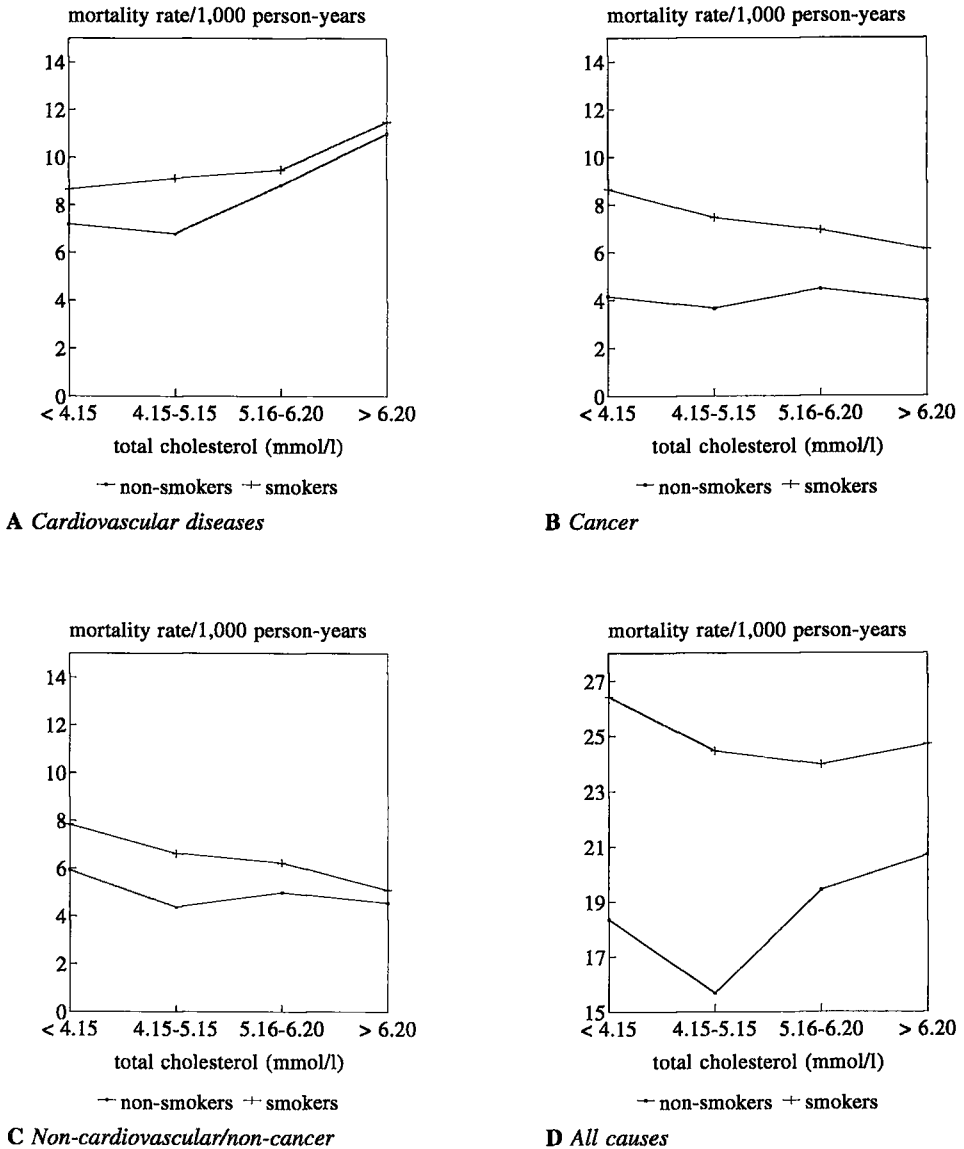
Figure 1. *Mortality rates (per 1,000 person-years) by cholesterol level, adjusted for age, blood pressure and cohort, in smokers and non-smokers.*

Non-CVD/non-cancer mortality was inversely associated with cholesterol in smokers, in non-smokers it was only elevated at cholesterol levels less than 4.15 mmol/l compared to levels of 4.15-5.15 mmol/l, but no further decrease at cholesterol levels above 5.16 mmol/l was observed. For both smokers and non-smokers inverse associations with infections (p<0.01) and respiratory diseases (p<0.10) were present. Mortality from digestive diseases was elevated at cholesterol levels below 4.15 mmol/l in smokers, and accidents seemed to be positively associated with total cholesterol in non-smokers. In smokers, but not in non-smokers, an inverse association with mortality from 'other' non-CVD/non-cancer causes was present. Due to these differential associations, total cholesterol showed a J-shaped association with total mortality in non-smokers, while in smokers total cholesterol was unrelated to total mortality.

Discussion

In smokers, a significant inverse association of total cholesterol with mortality from cancer and non-cardiovascular/non-cancer causes was observed. In non-smokers no association with cancer mortality was observed, while non-CVD/non-cancer mortality was increased at cholesterol levels less than 4.15 mmol/l compared to 4.15-5.15 mmol/l, but did not decrease further with increasing cholesterol levels. Total cholesterol was positively associated with mortality from cardiovascular diseases. Due to the differential associations with non-cardiovascular mortality, total mortality showed a J-shaped association with total cholesterol in non-smokers, while in smokers there was no significant association. Mortality rates for all endpoints were higher in smokers than non-smokers.

A meta-analysis of 19 studies, including the MRFIT Study, showed a significant inverse association between total cholesterol level and cancer mortality, also after excluding the first five years of follow-up.[5] However, it was not concluded that this observation implied causality. In the present study, the inverse association with cancer mortality was observed only in smokers. The inverse association in smokers was due to inverse associations for lung, hematopoietic and digestive cancers. Not all studies have analyzed their data by smoking strata, but a number of studies have observed that the inverse association between total cholesterol and cancer mortality was strongest with smoking-related cancers.[11,15,16] Inverse associations with lung and hematopoietic cancers have been consistently observed in a number of studies.[8,11,12,15] The MRFIT study, a study with over 350,000 study subjects,

is possibly the only one with enough power to analyze a large number of specific cancer sites.[15] They observed inverse associations with lung, digestive, nervous system, lymphatic and hematopoietic cancers.

In the present study, for most subgroups of non-CVD/non-cancer mortality associations were comparable in smokers and non-smokers: infections and respiratory diseases were inversely associated with total cholesterol, while accidents and digestive diseases were unrelated to total cholesterol. The remainder of non-CVD/non-cancer causes was inversely associated with total cholesterol only in smokers. A number of other studies have shown consistent inverse association between cholesterol and mortality from non-CVD/non-cancer diseases[5,12,15], especially respiratory and digestive diseases. In the present study, especially infections (two-thirds of which were tuberculosis) were strongly inversely related with total cholesterol. Most other studies did not analyze this group of diseases separately. It is known that lipid-soluble toxins bind to blood lipoproteins and are transported to the liver for detoxification.[25] Consequently, defence against infections might be impaired when TC is low, leading more often to a fatal outcome. It seems logical, that this effect would be more pronounced in physiologically 'weaker' persons, such as the diseased or elderly.

Accidents were unrelated to low cholesterol levels, as found in other longitudinal studies. The issue of excess mortality from accidents has emerged from intervention trials, and may therefore have more to do with drugs used to lower an initially high cholesterol level, than with 'naturally' low cholesterol levels.[25,26]

The question remains, whether the associations between low cholesterol levels and increased non-cardiovascular mortality are causal. A mechanism by which a low cholesterol would cause excess mortality would be one of relative cholesterol deficiency. It has been shown, however, that cell membranes maintain cholesterol homeostasis exposed to plasma cholesterol levels as low as about 1.5 mmol/l[28], so cholesterol 'deficiency' is unlikely. It has been suggested that persons who have a low cholesterol level at baseline differ in other respects from persons with higher cholesterol levels. They might be genetically different, with a predisposition to non-cardiovascular diseases.

Another explanation could be the presence of preclinical diseases at baseline that cause both a low cholesterol level and an increased risk of non-cardiovascular mortality. In some studies, the inverse association disappeared when early mortality (often defined as deaths

in the first five years of follow-up) was excluded[6,8-10,13], but in a number of other studies this was not the case.[7,11,12,14-16] There is evidence that cholesterol lowering due to pre-existing cancer is manifested from about two to four years prior to diagnosis[29], even up to ten years.[30] In the present study exclusion of the first ten years of follow-up did not abolish the observed excess risk for cancer and non-cancer/non-cardiovascular mortality at cholesterol levels below 4.15 mmol/l. It can not be excluded however, that other diseases such as chronic lung diseases might lower cholesterol many years before death. An indication that pre-existing diseases play a role is the observation that inverse associations with non-cardiovascular mortality were not observed in a 'healthy' cohort, consisting of working persons[31], but were present in a general population cohort in the same region.[32] Also, inverse associations were not observed in cohorts with a relatively young age at baseline.[33-35] In a middle-aged population, the low cholesterol category would not only contain persons with a 'naturally' low cholesterol level, but also persons who have a 'lowered' cholesterol level due to pre-existing diseases.

A number of correlates of low cholesterol levels have been proposed as confounders in the observed associations. One is excessive alcohol consumption which is often associated with poor nutritional status and liver damage, thus influencing cholesterol metabolism and predisposing to a number of diseases. In a recent meta-analysis relations were the same within strata of alcohol consumption[5], suggesting that this does not play a role. In the MRFIT Study a strong inverse association of cholesterol was observed with death attributed to alcohol dependency.[15] In the present study, a total of 168 deaths could be defined as 'alcohol-related' (ICD-8 codes 303 alcoholism and 571 liver cirrhosis). Two-thirds of these occurred among smokers. RR for alcohol-related mortality for the lowest cholesterol category compared to the cholesterol category of 4.15-5.15 mmol/l was 1.9 in non-smokers (p>0.1) and 2.2 in smokers (p<0.01). This indicates that excessive alcohol use was more prevalent in the low cholesterol group, and might explain part of the observed excess risk.

From a public health viewpoint it is important to note that mortality rates in smokers were higher for all disease groups. The inverse associations between serum total cholesterol and mortality from cancer were restricted to smokers, possibly due to a decreased anti-oxidant potential in smokers.[36,37] For non-smokers, total mortality risk was lowest in the cholesterol range 4.15-5.15 mmol/l. There were indications that alcohol abuse was more prevalent in

the low cholesterol group. Therefore, it seems likely that the low cholesterol-mortality association is at least partly explained by confounding factors.

In western countries, few middle-aged persons have blood cholesterol levels below 4.14 mmol/l. Moreover, in the treatment of persons with hypercholesterolemia (mostly defined as cholesterol above 6.19 mmol/l) levels of less than 4.14 mmol/l will seldom be achieved. From a public health viewpoint, excess risk for a cholesterol level of less than 4.14 mmol/l is limited due to the small number of persons with these levels, the relatively low mortality rate for this group and the fact that at least part of the observed associations is due to confounding. Therefore, lowering of relatively high average population cholesterol levels remains a public health need.

References

1. Pooling Project Research Group. Relationship of blood pressure, serum cholesterol, smoking habit, relative weight, and ECG abnormalities to incidence of major coronary events: final report of the Pooling Project. J Chronic Dis 1978;31:201-306.

2. Stamler J, Wentworth D, Neaton JD. Is the relationship between serum cholesterol and risk of premature death from coronary heart disease continuous and graded? JAMA 1986;256:2823-28.

3. Anderson KM, Castelli WP, Levy D. Cholesterol and morbidity: 30 years of follow-up from the Framingham Study. JAMA 1987;257:2176-80.

4. Chen Z, Peto R, Collins R, Mac Mahon S, Lu J, Li W. Serum cholesterol concentration and coronary heart disease in a population with low cholesterol concentrations. BMJ 1991;303:276-82.

5. Jacobs DR, Blackburn H, Higgins M, et al. Report of the Conference on Low Blood Cholesterol: Mortality Associations. Circulation 1992;86:1046-60.

6. Rose G, Shipley MJ. Plasma lipids and mortality: a source of error. Lancet 1980;i:523-6.

7. Kark JD, Smith AH, Hames CG. The relationship of serum cholesterol to the incidence of cancer in Evans County, Georgia. J Chronic Dis 1980;33:311-22.

8. International Collaborative Group. Circulating cholesterol level and risk of death from cancer in men aged 40 to 69 years. JAMA 1982;248:2853-9.

9. Wallace RB, Rost C, Burmeister LF, Pomrehn PR. Cancer incidence in humans: relationship to plasma lipids and relative weight. JNCI 1982;68:915-8.

10. Hiatt RA, Fireman BH. Serum cholesterol and the incidence of cancer in a large cohort. J Chron Dis 1986;39:861-70.

11. Schatzkin A, Hoover RN, Taylor PR, et al. Site-specific analysis of total serum cholesterol and incident cancer in the National Health and Nutrition Examination Survey I Epidemiologic Follow-up Study. Cancer Research 1988;48:452-8.

12. Isles CG, Hole DJ, Gillis CR, et al. Plasma cholesterol, coronary heart disease and cancer in the Renfrew and Paisley survey. BMJ 1989;298:920-4.

13. Törnberg SA, Holm LE, Carstensen JM, Eklund GA. Cancer incidence and cancer mortality in relation to serum cholesterol. JNCI 1989;81:1917-21.

14. Cowan LD, O'Connell DL, Criqui MH, Barrett-Connor E, Bush TL, Wallace RB. Cancer mortality and lipid and lipoprotein levels. The Lipid Research Clinics Program Mortality Follow-up Study. Am J Epidemiol 1990;131:468-82.

15. Neaton JD, Blackburn H, Jacobs D, et al. Serum cholesterol level and mortality findings for men screened in the Multiple Risk Factor Intervention Trial. Arch Intern Med 1992;152:1490-1500.

16. Davey Smith G, Shipley MJ, Marmot MG, Rose G. Plasma cholesterol concentration and mortality. The Whitehall Study. JAMA 1992;267:70-76.

17. Jacobs DR. Why is low blood cholesterol associated with risk of nonatherosclerotic disease death? Annu Rev Publ Health 1993;14:95-114.

18. Keys A, Aravanis C, Blackburn H, et al. Serum cholesterol and cancer mortality in the Seven Countries Study. Am J Epidemiol 1985;121:870-83.

19. Keys A, Aravanis C, Blackburn H, et al. Epidemiological studies related to coronary heart disease: characteristics of men aged 40-59 in seven countries. Acta Med Scand 1967;Suppl.460:1-392.

20. Keys A. (ed.) Coronary heart disease in Seven Countries. Circulation 1970;41:suppl 1.

21. Anderson JT, Keys A. Cholesterol in serum and lipoprotein fractions: its measurement and stability. Clin Chem 1956;2:145-9.

22. Rose G, Blackburn H. Cardiovascular survey methods. Geneva WHO, 1968.

23. WHO. International Classification of Diseases. 8th Revision, volume 1 and 2. World Health Organization, Geneva 1967.

24. SAS Institute Inc., SAS Technical Report P-229, SAS/STAT Software: Changes and Enhancements, Release 6.07, Cary, NC: SAS Institute, 1992.

25. Feingold KR, Grunfeld C. Role of cytokines in inducing hyperlipidemia. Diabetes 1992;41:97-101.

26. Muldoon MF, Manuck SB, Matthews KA. Lowering cholesterol concentrations and mortality: a quantitative review of primary prevention trials. BMJ 1990;301:309-14.

27. Jacobs DR, Muldoon MF, Råstam L. Invited commentary: Low blood cholesterol, non-illness mortality and other nonatherosclerotic disease mortality: a search for causes and confounders. Am J Epidemiol 1995;141:518-22.

28. Marenah CB, Lewis B, Hassall F, et al. Hypocholesterolaemia and non-cardiovascular disease: metabolic studies on subjects with low plasma cholesterol concentrations. BMJ 1983;286:1603-6.

29. Kritchevsky SB, Wilcosky TC, Morris DL, Truong KN, Tyroler HA. Changes in plasma lipid and lipoprotein cholesterol and weight prior to the diagnosis of cancer. Cancer Research 1991;51:3198-203.

30. Winawer SJ, Flehinger BJ, Buchalter J, Herbert E, Shike M. Declining serum cholesterol levels prior to diagnosis of colon cancer. A time-trend, case-control study. JAMA 1990;263:2083-5.

31. Persson B, Johansson BW. The Kockum Study: twenty-two years of follow-up. Acta Med Scand 1984;216:485-93.

32. Petersson B, Trell E, Hood B. Premature death and associated risk factors in urban middle-aged men. Am J Med 1984;77:418-26.

33. Harris T, Feldman JJ, Kleinman JC, Ettinger WH, Makuc FM, Schatzkin AG. The low cholesterol-mortality association in a national cohort. J Clin Epidemiol 1992;45:595-601.

34. Klag MJ, Ford DE, Mead LA, et al. Serum cholesterol in young men and subsequent cardiovascular disease. N Engl J Med 1993;328:313-8.

35. Verschuren WMM, Kromhout D. Total cholesterol and mortality at a relatively young age: do men and women differ? The Consultation Bureau Heart Project. BMJ 1995;(in press)

36. Church DF, Pryor AW. Free radical chemistry of cigarette smoking and its toxicological implications. Environ Health Perspect 1985;64:111-26.

37. Schectmann G. Estimating ascorbic acid requirements for cigarette smokers. Ann NY Acad Sci 1993;686:335-45.

9 General Discussion

Introduction

Results of descriptive and etiological epidemiological studies have been presented: descriptive studies have given insight into current levels of total and HDL cholesterol in the general population of the Netherlands and changes in cholesterol levels over the period 1974-1992. The etiological studies have provided insight into the determinants of total and HDL cholesterol, and the effect of different levels of total cholesterol on mortality from coronary heart disease and all-cause mortality. The effect of cholesterol on mortality was studied in different cultures and in men as opposed to women. Combining the results of these studies, conclusions can be drawn on the public health impact of cholesterol levels in the Netherlands and the reduction in mortality that can theoretically be achieved by lowering cholesterol levels.

Measuring cholesterol

Trends in total cholesterol levels and the relation between total cholesterol levels and subsequent mortality were studied using a single cholesterol measurement for each individual. It is important to realize that a single cholesterol measurement will contain an error component due to biological variation of cholesterol levels in individuals (within-person variation) and within-laboratory variation. The effect of these two sources of measurement error varies with the purposes of the study (e.g. studying trends or studying cholesterol-mortality relations).

Within-person variation
The effect of within-person variation is an important source of error for classification of individuals and for studying cholesterol-mortality relations, however, it is less important when groups are studied and is unimportant for studying trends. Cholesterol levels in humans vary from day to day around a mean level. For individuals a single cholesterol

measurement is not enough to reliably establish their individual mean cholesterol level. A guideline for clinicians is to measure cholesterol on at least three occasions to obtain a reliable mean.[1] On the group level, however, a single measurement can be enough to characterize the group mean accurately because overestimation of the cholesterol level in one subject will be compensated by underestimation of the cholesterol level in another subject. When repeated measurements per individual are used, the group mean will remain similar, but the standard deviation of the distribution will diminish. Therefore, although the mean level will be estimated correctly by a single measurement, the estimated prevalence of the percentage of persons above a given cutoff point will be overestimated.

When studying cholesterol-disease associations prospectively, it is known that a single cholesterol measurement leads to dilution of the observed strength of the association, the so-called regression dilution effect, as described in the methods and discussion sections of Chapter 7. If repeated measurements are available on at least a subsample of the study population, it is possible to correct the observed associations for this dilution effect.[2] In the Seven Countries Study correction for the regression dilution effect increased the relative risk estimates by approximately 40%.

Laboratory drift

Since laboratory drift influences the group mean and the estimated prevalence of hypercholesterolemia over time, it is an important problem in studying trends in cholesterol levels over time. All laboratory measurements have some imprecision. Certified laboratories take part in standardization programs that constantly monitor the accuracy and precision of the measurements. A maximum deviation from the "true" level of plus or minus 5% is allowed.[3] However, within this range drifts are possible. For the individual a small deviation from the true level is not so important, but when studying trends in cholesterol a drift from the maximum allowed overestimation to the maximum allowed underestimation can result in a spurious trend. When the control sera measurements, included in all measurement runs in the laboratory, are available for analysis, small drifts can be detected and trend analyses can be corrected for these.

Table 1 summarizes the effect of within-person variation and laboratory drift for different study purposes.

Table 1. *Importance of within-person and laboratory drift for different study purposes*

Purpose of the study	Within-person variation	Laboratory drift
Individual level	+	-
Group • mean level	-	+
• prevalence of elevated levels	+	+
Trends	-	++
Mortality associations	+	-

++ = of strong influence
+ = of influence
- = not of (much) influence

Trends in plasma cholesterol levels in the Netherlands from 1974-1992

Studying trends in total and HDL cholesterol is important because changes which take place at present will have an impact on morbidity and mortality from coronary heart disease in the near future. Furthermore, monitoring can contribute to evaluating the effectiveness of public health campaigns aimed at reducing cholesterol levels in the general population.

Trends

Drawing a conclusion on the long-term trend in total cholesterol levels in the Netherlands is hampered by the fact that for the period 1974-1986 only information on a limited age range was available and the fact that for women over the period 1981-1986 no information at all was available. In men, a decline in total cholesterol levels was observed in all three study periods, the largest (0.2 mmol/l) in the period 1981-1986. However, data from the Zutphen Study indicated that the decline in 35-year-olds over the period 1981-1986 was not generalizable to older men.[4] For the period 1987-1992 the decline in total cholesterol levels in men aged 20-59 took place mainly in 1991 and remained at this lower level in 1992. Elderly Dutch men showed also a decline in total cholesterol levels between 1990 and 1993[5], so the cholesterol decline in recent years in men does seem to be generalizable to the general population. In women a decline in cholesterol levels was observed in 1991; however, it was not maintained in 1992, resulting in no net change over the period 1987-1992.

In Table 2, the changes that occurred over the three study periods are expressed as a percentage of the cholesterol level at the beginning of the study period. In men, a total decline of about 6.5% over three study periods was observed. It must, however, be noted that the largest decline was observed from 1981-1986 in men aged 33-37 and that this decline was not generalizable to other age groups. In women, no net change in total cholesterol level was observed in the study periods 1974-1980 and 1987-1992.

Table 2. *Changes in mean total cholesterol levels over the three study periods expressed as a percentage of the cholesterol level at the beginning of the study period*

Study	Period	Study population	Change in total cholesterol	
			Men	Women
CB Project	1974-1980	♂ and ♀, 37-43 years	-1.2 %	-0.6 %
RIFOH Project	1981-1986	♂ 33-37 years	-3.5 %	n.a.[a]
Monitoring Project	1987-1992	♂ and ♀, 20-59 years	-1.9 %	-0.1 %

a: n.a. = not available

During the period 1987-1992 not only total cholesterol was measured but also HDL cholesterol. During this period HDL cholesterol levels declined by about 7% in men and did not change significantly in women. Comparing the changes in total and HDL cholesterol by looking at the change in the non-HDL/HDL cholesterol ratio showed that this ratio increased in men and remained unchanged in women. The favorable change in total cholesterol levels in men was thus counteracted by unfavorable changes in HDL cholesterol levels. In the Zutphen Study, however, no change in HDL cholesterol levels was observed in middle-aged and elderly men when five measurements were analyzed over the period 1977-1993.[5] Since it has become increasingly clear that HDL cholesterol is inversely related to CHD mortality, it is important to focus not only on total cholesterol levels but also on the HDL cholesterol fraction.

Prerequisites for monitoring cholesterol levels
In order to accurately monitor cholesterol levels in the general population a number of conditions has to be met. Firstly, the cholesterol measurements have to be carefully standardized. Because the changes that take place in the population are relatively small, measurement error can easily disguise the real changes that are taking place. During the entire period 1974-1992 cholesterol measurements were performed in the Central Clinical Chemistry Laboratory of the University Hospital "Dijkzigt" in Rotterdam, which is the

reference laboratory for cholesterol determinations in the Netherlands. During this whole period the laboratory variation was in the range allowed by the WHO standards for cholesterol measurements. However, a deviation of about 5% (too high or too low) from the "true" level is allowed. In the light of cholesterol changes of a few percent in the population, the range allowed in laboratory measurement error is large. Our analysis of the internal quality control of the laboratory showed that in some periods it was necessary to adjust for these small shifts in laboratory measurements when performing trend analyses.

Secondly, measurements have to be carried out on a continuous basis over a sufficiently long period of time. In all three monitoring projects it was observed that despite the large number of persons examined, mean cholesterol levels (monthly or quarterly) showed fluctuations of as much as 0.1-0.2 mmol/l, even when the same seasons were compared, so that seasonal change could excluded. When two points in time are compared, these fluctuations can lead to spurious conclusions about the changes in cholesterol levels that are taking place. This illustrates the need for continuous measurements.

Thirdly, changes in the study protocol can affect the cholesterol measurements. A puzzling observation was the fact that although the changes in cholesterol levels were relatively small within one project, a slight shift in cholesterol levels became apparent between the projects. At the end of the CB Project in 1980 the mean cholesterol level in men aged 40 was 5.70 mmol/l, while at the start of the RIFOH project in 1981 the mean cholesterol level in men aged 35 was 5.64 mmol/l. This happened despite the fact that the CB and RIFOH projects were carried out in the same locations, by the same personnel and with cholesterol measurements in the same laboratory. Taking into account the 5-year age difference, a larger cholesterol difference between the men in the CB Project and the RIFOH project would be expected (in the order of about 0.2 mmol/l). Apparently, a changing daily routine, with a possible slight impact on procedures pertaining to drawing or handling of the serum samples, can lead to small shifts in cholesterol levels. This further underlines the many pitfalls that comparing cholesterol levels over time has.

On the basis of all these observations it can be concluded that for a good monitoring system it is important to have well standardized measurements with good quality-control data over a sufficiently long period of time without changes in the study protocol. Because this cannot always be attained, it is of great importance to collect quality-control data over

the study period to make it possible to adjust for any changes in the study protocol or laboratory drifts that have taken place during the study period.

Cholesterol changes in relation to mortality changes

Age-standardized CHD mortality in the Netherlands reached its peak in 1972 and has declined since then by 29% in men and 38% in women.[6] The question as to what extent changes in risk factor levels have contributed to this decline is an interesting but difficult one. Firstly, no detailed information on changes in risk factor levels in large samples (with a broad age range) of the general population are available for the 1950s to the early 1980s. Secondly, there is a lag time of 5-10 years between changes in risk factors and changes in mortality. Thirdly, changes in medical care have taken place during the same period. From meta-analyses of a large number of intervention studies it has been estimated that a 1% decline in serum total cholesterol levels results in a 2% to 3% reduction in CHD mortality.[7] Generalizing the changes observed in the three studies described above to the whole population (a "best case" scenario, since there were indications that the decline of 3.5% from 1981-1986 was not generalizable), it could be estimated that the total cholesterol decline of 6.5% in men, would result in a decline in CHD mortality of 13% to 19.5%. In women total cholesterol levels declined by less than 1%, resulting in a predicted CHD mortality decline of at most 2% to 3%. Although for men it seems plausible that cholesterol changes have contributed to the decline in CHD mortality rates, this is not the case for women. With respect to the other major CHD risk factors, blood pressure levels have hardly changed in the last decades.[8] Smoking has decreased substantially in men, from 90% in the 1950s to about 40% at present.[9] Since smoking influences CHD mortality both directly (due to damage to the intima and the influence on thrombogenesis) and indirectly (by increasing levels and atherogenicity of cholesterol), this decline in smoking will have influenced CHD mortality rates. However, in women smoking prevalence increased from about 30% in 1958 to about 40% in 1975, followed by a slight decline in the late 1970s that came to standstill in the late 1980s.[9] These changes in smoking habits of women are much smaller than those in men. Therefore, especially in women it is difficult to explain the observed decline in coronary heart disease mortality by changes in risk factor levels. With respect to improvements in medical care, the introduction of thrombolytic therapy in the mid-1980s has probably contributed substantially to the decline in CHD mortality since that time.

From a public health perspective, it is important to know to what extent the decline in CHD mortality has been caused by primary prevention, and thus by a concomitant decline in CHD incidence, and to what extent it is caused by a better chance of survival after a first event. If caused by a better survival rate only, the prevalence of the disease in the population will increase. In contrast to the national mortality statistics, no data on the incidence of the disease are available on a national level. The regional morbidity registry in the Nijmegen area has not, however, shown a decline in CHD incidence since the 1970s.[6] Hospital statistics showed that hospital admissions at younger ages have decreased, suggesting a decline in the incidence of the disease, while in middle-aged and older persons hospital admission rates have increased. To gain insight into developments that are taking place with respect to the burden of coronary heart disease on the population, a good incidence register for CHD is necessary. The combination of mortality and incidence data provide insight into the prevalence of the disease. This will complement the information from risk factor monitoring and mortality statistics, and yield valuable information on health-care costs and demands in the future.

Plasma total and HDL cholesterol levels in the Netherlands and their determinants

Total cholesterol and its determinants
Most changes in total cholesterol levels that take place during a lifetime occur within the age range of 20 to 60. In men, total cholesterol levels increase greatly between age 20 and 40, after age 40 the increase with age levels off and total cholesterol levels start to decline after age 60. In women total cholesterol levels start to increase around the age of 35 to 40 years, and tend to decline after age 70.[10] Due to this increase in total cholesterol levels with age, prevalence of hypercholesterolemia in the Netherlands increases in men from about 1% at age 20 to 24 to about 30% at age 55 to 59, and in women from about 4% at age 20 to 24 to over 40% at age 55 to 59 (Chapter 3).

In the Netherlands hypercholesterolemia is defined as a total cholesterol level of 6.5 mmol/l or above, according to the Cholesterol Consensus.[11] This cutoff point has been chosen because above this level the relative risk for coronary heart disease tends to increase exponentially. When comparing prevalence figures for hypercholesterolemia, it

must be kept in mind that a small shift in cutoff point can result in a large increase in the prevalence of hypercholesterolemia. Standardized to the age distribution of the general population aged 20-59 in the Netherlands, the prevalence of hypercholesterolemia in 1992 was 14% in both men and women. In comparison, if the United States cutoff point of 6.2 mmol/l is applied, the prevalence of hypercholesterolemia will be 22% in men and 19% in women (figures 1a and 1b). Also for HDL cholesterol a small shift in cutoff point would have considerable impact on the prevalence of low cholesterol levels (figure 1c). The prevalence figures from the present study represent a slight overestimation of the true prevalence, being based on a single measurement.

It can be concluded that large parts of the general population are at increased risk of developing CHD on the basis of their cholesterol levels. Comparing age and gender-specific mean cholesterol levels in the Netherlands to levels in other countries shows that levels in Scandinavian countries (Denmark, Sweden, Finland and Iceland) and some eastern European countries (former Soviet Union, East Germany and Czechoslovakia) are on average about 0.5 mmol/l higher than in the Netherlands. Levels in Spain, Italy, Canada and Australia are comparable to those in the Netherlands and total cholesterol levels in the United States are on average about 0.5 mmol/l lower.[12] This shows that in most industrialized countries (except the United States) hypercholesterolemia is as prevalent or even more prevalent than in the Netherlands.

When identifying subgroups of the general population in which the risk of coronary heart disease is increased due to an elevated total cholesterol level, it is clear that age and gender are the most prominent determinants. However, differences between social strata (defined by educational level) are also observed. Total cholesterol levels are inversely associated with educational level, while HDL cholesterol levels are positively associated with educational level (Chapter 3). More favorable risk profiles for most other risk factors that were measured in the Monitoring Project on Cardiovascular Disease Risk Factors are also observed for higher as opposed to lower educational levels.[13] Therefore, in public health education campaigns special attention should be paid to lower educated groups.
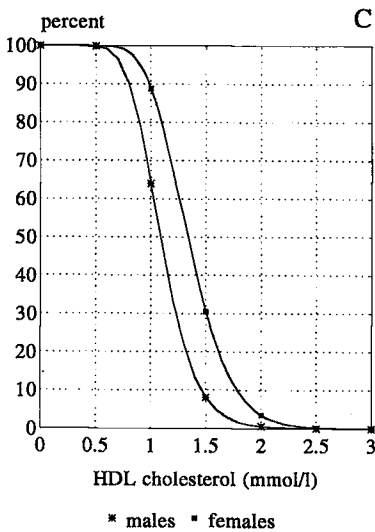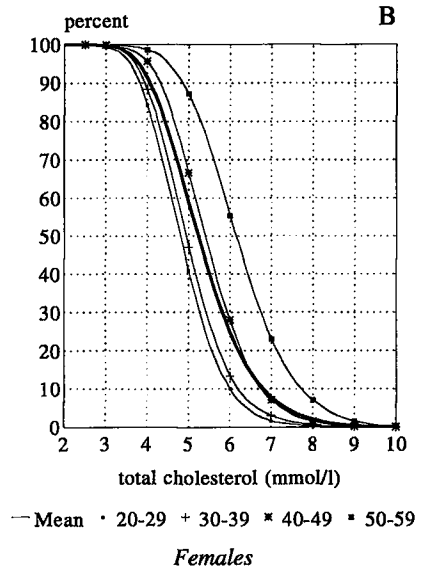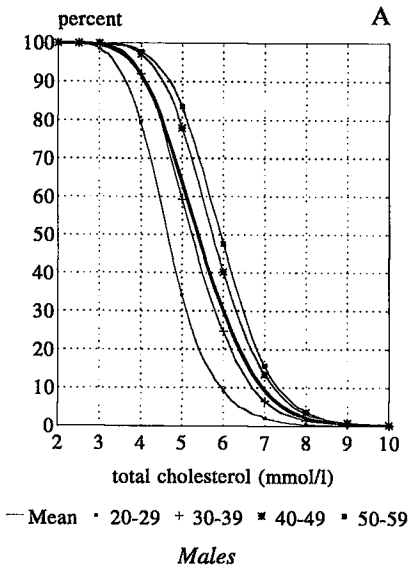
Figure 1. *The percentage of persons below a certain level of total cholesterol in different age categories in males (a) and females (b) and the percentage of persons below a certain level of HDL cholesterol (c) in the Netherlands.*

The question is whether the increase in total cholesterol levels with age is physiological or caused by a "western" lifestyle. In almost all affluent countries an increase with age is observed. One hypothesis is that with aging the activity/turnover rate of the LDL receptors decreases, resulting in a reduced clearance rate of cholesterol from the blood, which leads to an increase in blood cholesterol concentrations. Part of the age-related increase in women is associated with the menopause. It was estimated that due to the menopause cholesterol increases by about 0.45 mmol/l in non-smokers and 0.30 mmol/l in smokers (Chapter 4). Although the menopause itself cannot be prevented, the effects of the menopause can be reduced by postmenopausal use of estrogens. A number of studies have shown that the increase in total cholesterol after the menopause is prevented by hormone replacement therapy.[14] However, side-effects of this therapy are an increased risk for some forms of cancer (endometrium, breast).[14] Therefore wide-scale and long-term use of hormone replacement therapy is still debated. Women have the advantage that the rise in cholesterol levels occurs later in life than in men, which makes their lifetime exposure to cholesterol lower.

Another factor associated with the age-related total cholesterol increase is body mass index. A steady rise in body mass index takes place with increasing age. Each unit increase in body mass index is associated with a cholesterol increase of 0.06 mmol/l in men and 0.03 mmol/l in women. However, it was estimated that the increase in body mass index accounts for only a small part (about 10%) of the cholesterol increase with age.

Other lifestyle factors that influence total cholesterol levels are smoking and diet. Since these factors do not show much change with age, they do not explain the cholesterol increase with age. The data from the Monitoring Project on Cardiovascular Disease Risk Factors showed that total cholesterol levels were higher in smokers than in non-smokers. After adjustment for body mass index, men who smoked had a 0.13 mmol/l higher total cholesterol level than non-smokers. For women this figure was 0.18 mmol/l. Therefore, quitting smoking can contribute to maintaining cholesterol levels at a desirable level.

Although not described in this thesis, dietary factors are important determinants of total cholesterol levels. As early as the 1950s, Keys showed that fat intake was the most important dietary factor with respect to cholesterol levels.[15] Based on a number of studies performed in the 1950s and early 1960s, an equation was developed to predict change in total cholesterol due to changes in dietary fatty acid composition of the diet.[16,17] A meta-

analysis of more recent trials has established the effect of dietary fatty acids on both total and HDL cholesterol.[18] It was estimated that replacing 10% of energy from saturated fat by carbohydrates would lower LDL cholesterol by 0.33 mmol/l but also HDL cholesterol by 0.12 mmol/l, replacement by monounsaturated fat would lower LDL cholesterol by 0.39 mmol/l and HDL cholesterol by 0.03 mmol/l, and replacement by polyunsaturated fat would lower LDL cholesterol by 0.47 mmol/l and HDL cholesterol by 0.05 mmol/l. Due to the higher LDL lowering effect and the lower decrease in HDL cholesterol, it was concluded that replacement of saturated fat by unsaturated fat was more favorable with respect to the lipid profile than replacement by carbohydrate. However, this was only true assuming no effects on body mass index. Furthermore, effects of diet on platelets and oxidizability of LDL were not taken into account due to lack of data.

The influence of genetic factors was not studied within the context of this thesis, but a number of genetic defects are known to lead to elevated cholesterol levels. For example, persons with the monozygous form of familiar hypercholesterolemia (FH) have no LDL cholesterol receptors, leading to extremely high cholesterol levels. This form is rare and only present in about $1:10^6$ persons who have cholesterol levels of 15 mmol/l or more. The more prevalent heterozygous form is estimated to be present in about 1:300-1:500 persons. Persons who are heterozygous for FH have only 50% of the LDL receptors of persons without this genetic trait and usually have cholesterol levels exceeding 8 mmol/l. However, even in these persons strict lifestyle rules can be effective in keeping serum cholesterol levels within an acceptable range.[19] Genetic factors related to levels and atherogenicity of cholesterol are being studied more and more. With increasing technology, the number of genetic polymorphisms and mutations discovered will probably rapidly increase in the near future. This makes it all the more necessary to study gene—environment interactions because expression of genetic traits can be modified by environmental factors. It must, however, be noted that hypercholesterolemia in the general population is to a large extent multifactorial and adverse lifestyle habits play an important role.

An individual's cholesterol level is the outcome of the balance between a great number of interacting factors. Genetics and lifestyle interact: given a genetic trait, the expression of this trait (and therefore the resulting deleterious consequences) can be modified by lifestyle factors. Furthermore, given a certain total cholesterol level, the atherogenicity of that cholesterol level depends on the amount of ox-LDL. This amount of ox-LDL is

dependent on the balance between the oxidizability of the LDL particles (dependent on the amount of polyunsaturated fatty acids), dietary anti-oxidants (e.g. carotenoids, vitamin E and flavonoids) and oxidants (e.g. smoking).

HDL cholesterol and its determinants

HDL cholesterol levels remain remarkably stable with age. The most important determinant of the HDL cholesterol level is gender. In women HDL cholesterol is on average 0.25 mmol/l higher than in men. This gender difference in HDL cholesterol arises from the drop in HDL cholesterol levels in males during puberty due to increasing levels of testosterone.[20] It has been established that a low HDL cholesterol level is an independent risk factor for developing coronary heart disease. It was estimated that an increase of 0.026 mmol/l in HDL cholesterol was associated with a decrease in coronary heart disease mortality of 1.9% to 2.9%.[21] When the internationally used cutoff point of 0.9 mmol/l was used to define low HDL cholesterol[22], prevalence of low HDL cholesterol levels was 22% in men and 6% in women.

Differences in HDL cholesterol between men and women were not explained by differences in factors such as body mass index, smoking, physical activity and alcohol consumption (Chapter 5). Assuming equal levels of these factors, the predicted HDL cholesterol difference between men and women was even slightly greater. Body mass index and cigarette smoking each have an adverse effect on HDL cholesterol levels. Each unit increase in body mass index was associated with a 0.02 mmol/l lower HDL cholesterol in men and women. Cigarette smoking was associated with a 0.10 mmol/l lower HDL cholesterol in men and a 0.13 mmol/l lower level in women. A physically active lifestyle was associated with a 0.04 mmol/l higher HDL cholesterol level than for a physically inactive lifestyle in both men and women. For each glass of alcoholic beverage HDL cholesterol was higher by 0.04 mmol/l in men and 0.07 mmol/l in women.

Thus although HDL cholesterol levels do not change with age and are largely determined by gender, a number of modifiable lifestyle factors are of influence. It can be calculated that a combination of obesity (BMI=30 kg/m²), cigarette smoking (>10 cigarettes/day) and a physically inactive lifestyle leads to a 0.26 mmol/l (men) or 0.29 mmol/l (women) lower HDL cholesterol level compared to a combination of "normal" weight (BMI=25 kg/m²),

no smoking and a physically active lifestyle. The amount of difference due to two different lifestyles is similar to that due to the gender difference.

Total cholesterol and its relation with mortality

Coronary heart disease

When it became clear in the 1950s and 1960s that coronary heart disease mortality was reaching epidemic proportions, a number of large-scale studies were started to elucidate the etiology of this disease. Over the past decades the importance of cholesterol with respect to coronary heart disease mortality has been shown in these studies.[23-26] These studies were mostly done in countries with high CHD mortality rates, such as the United States and Northern Europe, and involved subgroups of those populations that were known to be at high risk: mostly middle-aged men. Although it is known that middle-aged women suffer much less CHD mortality than men, the growing proportion of elderly women in the population makes CHD in women an important public health concern. However, there are few longitudinal studies in women and the question remains whether results from studies in males can be extrapolated to women.

The Consultation Bureau Heart Project (CB Project) provided the opportunity to compare the relation between total cholesterol and coronary heart disease mortality in males and females. Data from the Seven Countries Study were used to compare this relation in countries with different CHD mortality rates.

The most striking observation from these studies was the fact that the relative risk (RR) for the relation between serum total cholesterol and CHD mortality seemed to be more or less universal. For both men and women, the RR for those in the upper 20% of the cholesterol distribution was 3 to 4 fold higher than for those in the lower 20% of the distribution. In different cultures, whether it was one with a low CHD mortality such as Mediterranean Southern Europe or one with a high CHD mortality such as Northern Europe, CHD mortality risk increased by 12% for each 0.5 mmol/l cholesterol increase. However, there were large differences in absolute risk for CHD mortality. In women, CHD mortality rates were five times lower compared to men at any cholesterol level. At a cholesterol level of 5.2 mmol/l, 25-year CHD mortality rates in the Seven Countries

Study, varied from 5% in Mediterranean Southern Europe and Japan, to 10% in Inland Southern Europe and 15% in Northern Europe.

The large difference in CHD mortality rate between men and women (after adjustment for blood pressure, smoking and body mass index) is to a large extent explained by physiological (intrinsic) differences between men and women and not by differences in lifestyle. Women have a higher HDL cholesterol level, which protects them against CHD. It is generally assumed that estrogens protect women against CHD, not only through an effect on cholesterol levels but also through an effect on the susceptibility to CHD.

The large differences in CHD mortality rates (after adjustment for age, blood pressure and smoking) that were observed at a given cholesterol level in the different cultures of the Seven Countries Study were, however, largely determined by differences in lifestyle. It is unlikely that intrinsic (genetic) differences played a role. For example, the Japanese in the Seven Countries Study had very low cholesterol levels, and CHD mortality was extremely low. However, it has been shown that when Japanese change their lifestyle to more American, their cholesterol levels and CHD mortality rates became similar to the American ones.[27] This shows that these differences are unlikely to be explained by genetic differences between the cultures, but that lifestyle factors are the most important in explaining the differences between the cultures with respect to CHD mortality. Analyses of differences in dietary intake between the cultures showed that the Mediterranean and Japanese diets contained a high intake of antioxidant vitamins (e.g. E, C and carotenoids) and flavonoids, combined with a low intake of saturated fat. Since ox-LDL is the atherogenic lipoprotein fraction, a high intake of antioxidants is likely to reduce the amount of ox-LDL. Furthermore, it has been shown that an α-linolenic acid enriched Mediterranean diet has favorable effects on thrombogenesis.[28] These results suggest that the effect of a given total cholesterol level on CHD mortality is modified by lifestyle factors such as diet.

With respect to primary prevention, the difference with respect to relative versus absolute risk is important. For example, the cutoff points of the Netherlands Cholesterol Consensus are based on the observation that above the level of 6.5 mmol/l RR for CHD increases exponentially. However, an exponential increase of a low baseline risk (e.g. in women) may still result in a low absolute risk, while a small increase in a high baseline risk (e.g. in men) may cause a large increase in absolute risk. Furthermore, with the results of the

Seven Countries Study in mind, it is important not to focus solely on total cholesterol levels per se. Attention should also be paid to factors that modify the atherogenicity of cholesterol, such as intake of antioxidants and saturated fat.

Total mortality

Total cholesterol levels show a continuous positive relation with coronary heart disease mortality. In the Netherlands coronary heart disease makes a large contribution to total (all-cause) mortality. If total cholesterol levels are unrelated to other diseases, a positive relation between total cholesterol levels and all-cause mortality is expected that is, however, weaker than the relation with CHD. A number of studies, however, show a J-shaped relation between total cholesterol and all-cause mortality due to higher cancer and non-cardiovascular/non-cancer mortality at low cholesterol levels.[29]

In both the CB Project (Chapter 6) and the Seven Countries Study (Chapter 8) the relation between total cholesterol level and all-cause mortality was studied. All-cause mortality can be divided into three large subgroups of diseases: cardiovascular diseases (including coronary heart disease), cancer and non-cardiovascular/non-cancer diseases. To understand the association between total cholesterol and all-cause mortality, associations between total cholesterol and these subgroups were studied.

In the CB Project, a positive association between total cholesterol and all-cause mortality was observed in both men and women. No associations were observed with non-cardiovascular mortality. Men and women in the upper 20% of the cholesterol distribution had a 60% and 46% higher mortality risk, respectively, than men and women in the lower 20% of the distribution. In the Seven Countries Study however, total cholesterol was unrelated to total mortality due to the fact that inverse associations with cancer (in smokers only) and non-cardiovascular/non-cancer mortality (in both smokers and non-smokers) were observed. Within the subgroups of non-cardiovascular/non-cancer mortality the inverse association was most prominent for infectious diseases.

A recent meta-analysis of a number of longitudinal studies showed an excess all-cause mortality at low cholesterol levels, and a detailed analysis of the MRFIT Study showed inverse associations between total cholesterol and a number of diseases: cancers of different sites, respiratory and digestive diseases. The question on the causality of these findings is, however, still in debate. A number of explanations have been put forward.[30]

First, it is possible that low cholesterol is not the cause but the consequence of a disease (e.g. cancer). However, in many studies the association remained after exclusion of the first 5 to 10 years of follow-up. Also in the Seven Countries Study, excluding the first ten years of follow-up did not alter the results. Secondly, confounding factors or effect modifiers may be associated with low cholesterol levels. It has been shown that excess mortality was not observed in "healthy" cohorts such as young adult men[31] and not in a cohort of employed men, contrary to a general population cohort from the same region.[32] This would indicate that in some studies the group with a low cholesterol level is "polluted" with people who have a low cholesterol level due to adverse health conditions. The effect of age may explain the different findings in the CB Project and the Seven Countries Study: the majority of men and women in the CB Project were between age 35 and 45 at baseline, while the men in the Seven Countries were aged 40-60 at baseline.

Several hypotheses for a causal mechanism by which low cholesterol levels could predispose to diseases have been put forward. One of them is that a low cholesterol concentration leads to unfavorable changes in the composition of cell membranes. A hypothesis on a causal mechanism by which low cholesterol levels increase mortality due to infectious diseases is that lipoproteins bind lipid-soluble toxins and subsequently take them to the liver for detoxification.[33] The Seven Countries Study lends support to this hypothesis because a strong inverse association with mortality from infectious diseases was observed.

From the point of CHD prevention cholesterol lowering is favorable, and in many countries programs have been launched to lower cholesterol levels in the general population. From a broader public health perspective, the relation with all-cause mortality is most important and therefore the issue of a possible higher non-cardiovascular mortality at low cholesterol levels raised a lot of concern. It seems most likely that some of the relations that have been observed between low cholesterol levels and mortality are due to confounding or effect modification by other factors, and that some of the relations observed may be causal. Looking at the cholesterol distribution in the Netherlands, less than 10% of all men and women aged 20-59 years have levels below 4.1 mmol/l and only less than 5% of middle-aged men and women. Low cholesterol levels are not a major public health concern in the Netherlands because the prevalence of low cholesterol in the Netherlands is low and it is probable that the excess mortality at low cholesterol levels is

at the most only partly causal, while the burden of coronary heart disease in the population is high.

Prevention

It has been shown in the longitudinal studies described above that mortality risk differs greatly between persons with different cholesterol levels and different lifestyles. Still the question remains whether persons with a higher cholesterol level can acquire this lower level of risk when they lower their cholesterol levels and change their lifestyles. The final answer to this question can only be given by intervention studies. A number of cholesterol lowering trials has been carried out that have indeed shown that cholesterol-lowering leads to lowering of CHD mortality.[7] However, most trials did not show an effect of cholesterol lowering on total mortality. Several meta-analyses of trials produced conflicting results.[34-37] The most recent meta-analysis showed that all-cause mortality was significantly reduced in secondary prevention trials and that excess mortality in the intervention group was only observed for drug intervention and not for dietary intervention.[37] Furthermore it was concluded that the statistical power to detect an effect of cholesterol reduction on total mortality was small. A recent secondary prevention trial in which total cholesterol was reduced by 25% and HDL cholesterol increased by 8%, showed a reduction in both coronary heart disease mortality and all-cause mortality.[38] It has also been shown that in secondary prevention, regression of the atherosclerotic lesions can be achieved.[38-41] In a number of these trials CHD incidence was reduced, while only a small change in the degree of stenosis was achieved.[39,40] It has been suggested that cholesterol lowering might reduce the cholesterol content in cholesterol-rich lesions.[42] Because cholesterol-rich lesions are most vulnerable to rupture, cholesterol lowering might stabilize vulnerable plaques.[43] Therefore, also in already advanced coronary heart disease, cholesterol lowering can yield health benefits.

The first choice in lowering cholesterol levels is through lifestyle changes that not only decrease the level of total cholesterol, but can also modify its atherogenicity. Furthermore, it will also have a favorable effect on other chronic diseases, such as cancer, because cardiovascular diseases and different types of cancer share a number of common risk factors (e.g. dietary antioxidants, smoking, physical activity). A diet rich in fruits and vegetables (and thus antioxidants and flavonoids), and low in saturated fat, in combination

149

with no smoking, enough physical activity and a desirable weight, will, for many people, lead to a lipid profile that does not need drug intervention.

Main conclusions

Cholesterol levels in the Netherlands are still relatively high, being higher in lower educated than in higher educated persons. Despite a long tradition of health education, a substantial shift towards lower cholesterol levels has, contrary to the United States, not been reached in the Netherlands. That a relatively high proportion of the Dutch population still smokes cigarettes, and the Dutch diet is still relatively high in saturated fat, show that the risk profile with respect to CHD leaves much to be desired. From the CB Project it was estimated that a cholesterol reduction of 0.6 mmol/l was associated with a reduction of about 20% in CHD mortality, 15% in CVD mortality and 6% in all-cause mortality in this population.

The atherogenicity of a given total cholesterol level is dependent on factors that determine the amount of oxidized LDL, such as the antioxidant content of the diet and cigarette smoking. To lower cholesterol levels and the amount of oxidized LDL a multifactorial approach should be followed. A diet low in saturated fat and high in fruits and vegetables and no cigarette smoking are recommended in combination with a physically active lifestyle (e.g. to increase HDL cholesterol levels). Because the proposed lifestyle to lower cholesterol has been proven to be favorable also for the prevention of other diseases, this multifactorial approach goes beyond prevention of CHD and will also lower risk of cancer and other chronic diseases such as diabetes and chronic non-specific lung disease. Given that the causality of the relation between low cholesterol levels and all-cause mortality has not been proven, and is at least partly explained by confounding and effect modification, the health consequences of a low cholesterol is not of public health importance in the Netherlands.

References

1. Binsbergen JJ van, Brouwer A, Drenth BB van, Haverkort AFM, Prins A, Weijden T van der. De NHG-standaard Cholesterol. Hart Bulletin 1992;23:(supplement)27-35.

2. MacMahon S, Peto R, Cutler J, et al. Blood pressure, stroke, and coronary heart disease: part I. Prolonged differences in blood pressure: prospective observational studies corrected for the regression dilution bias. Lancet 1990;335:765-74.

3. Boerma GJM. Welke kwaliteitseisen moeten aan de bepalingen van totaal-cholesterol, HDL-cholesterol en triglyceriden worden gesteld? Hart Bulletin 1987;18:(suppl.1)35-40.

4. Kromhout D, De Lezenne Coulander C, Obermann-de Boer GL, et al. Changes in food and nutrient intake in middle-aged men from 1960 to 1985 (the Zutphen Study). Am J Clin Nutr 1990;51:123-9.

5. Weijenberg MP, Feskens EJM, Kromhout D. Age related changes in total and high density lipoprotein cholesterol in elderly men. The Zutphen Study: 1977/78-1993 (submitted).

6. Jansen J, Kromhout D, Verkleij H. Coronaire hartziekten. In: Ruwaard D, Kramers PGN, eds. Volksgezondheid Toekomst Verkenning. De gezondheidstoestand van de Nederlandse bevolking in de periode 1950-2010. Sdu Uitgeverij: The Hague, 1993; p379-86.

7. Law MR, Wald NJ, Thompson SG. By how much and how quickly does reduction in serum cholesterol concentration lower risk of ischemic heart disease? BMJ 1994;308:367-72.

8. Leer EM van. Blood pressure: trends, determinants and consequences. Wageningen, the Netherlands: Agricultural University, 1995. 131 pp. Thesis.

9. StiVoRo. Annual Report 1992. The Hague: Stichting Volksgezondheid en Roken, 1993. (in Dutch)

10. Netherlands Heart Foundation. Women and cardiovascular diseases. Netherlands Heart Foundation, The Hague, 1994. (in Dutch)

11. Consensus Conference: Lowering blood cholesterol to prevent heart disease. JAMA 1985;253:2080-6.

12. WHO. The WHO MONICA Project: a worldwide monitoring system for cardiovascular diseases. Wld Hlth Statis Annu, 1989.

13. Verschuren WMM, Leer EM van, Blokstra A, et al. Cardiovascular disease risk factors in the Netherlands. Neth J Cardiol 1993;4:205-10.

14. Grady D, Rubin SM, Pettiti DB, et al. Hormone therapy to prevent disease and prolong life in postmenopausal women. Ann Intern Med 1992;117:1016-37.

15. Keys A. The cholesterol problem. Voeding 1952;13:539-555.

16. Keys A, Anderson JT, Grande F. Serum cholesterol response to changes in the diet: IV. Particular saturated fatty acids in the diet. Metabolism 1965;14:776-786.

17. Hegsted DM, McGandy RB, Myers ML, Stare FJ: Quantitative effects of dietary fat on serum cholesterol in man. Am J Clin Nutr 1965;17:281-95.

18. Mensink RP, Katan MB. Effect of dietary fatty acids on serum lipids and lipoproteins. A meta-analysis of 27 trials. Arterioscler Thromb 1992;12:911-9.

19. Williams RR, Hunt SC, Hopkins PN, et al. Evidence for gene-environmental interactions in Utah families with hypertension, dyslipidaemia and early coronary heart disease. Clin Exp Pharmacol Physiol 1992;19(Suppl 20):1-6.

20. Orchard TJ, Rodgers M, Hedley AJ, et al. Changes in blood lipids and blood pressure during adolescence. BMJ 1980;280:1563-7.

21. Gordon DJ, Probstfield JL, Garrison RJ, et al. High-density lipoprotein cholesterol and cardiovascular disease: four prospective American studies. Circulation 1989;79:8-15.

22. European Atherosclerosis Society. Strategies for the prevention of coronary heart disease: a policy statement of the European Atherosclerosis Society. Eur Heart J 1987;8:77-88.

23. The Pooling Project Research Group. Relationship of blood pressure, serum cholesterol, smoking habit, relative weight and ECG abnormalities to incidence of major coronary events: final report of the Pooling Project. J Chron Dis 1978;31:201-306.

24. Stamler J, Wentworth D, Neaton J. Is the relationship between serum cholesterol and risk of premature death from coronary heart disease continuous and graded ? Findings in 356,222 primary screenees of the multiple risk factor intervention trial (MRFIT). JAMA 1986;256:2823-8.

25. Anderson KM, Castelli WP, Levy D. Cholesterol and mortality: 30 years of follow-up from the Framingham Study. JAMA 1987;257:2176-80.

26. Neaton JD, Blackburn H, Jacobs DR, et al. Serum cholesterol level and mortality findings for men screened in the multiple risk factor intervention trial. Arch Intern Med 1992;152:1490-1500.

27. Marmot MG, Syme SL, Kagan A, Kato H, Cohen JB. Studies of coronary heart disease and stroke in Japanese men living in Japan, Hawaii and California: prevalence of coronary and hypertensive heart disease and associated risk factors. Am J Epidemiol 1975;102:514-25.

28. Lorgeril M de, Renaud S, Mamelle N, et al. Mediterranean alpha-linolenic acid-rich diet in secondary prevention of coronary heart disease. Lancet 1994;343:1454-9.

29. Jacobs DR, Blackburn H, Higgins M, et al. Report of the conference on low blood cholesterol:mortality associations. Circulation 1992;86:1046-60.

30. Jacobs DR, Muldoon MF, Råstam L. Low blood cholesterol, non-illness mortality and other nonatherosclerotic disease mortality: a search for causes and confounders. Am J Epidemiol 1995;141:518-22.

31. Klag MJ, Ford DE, Mead LA, et al. Serum cholesterol in young men and subsequent cardiovascular disease. N Engl J Med 1993;328:313-8.

32. Persson B, Johansson BW. The Kockum Study: twenty-two years of follow-up. Acta Med Scand 1984;216:485-93.

33. Harris HW, Grunfeld C, Feingold KR, et al. Human very low density lipoproteins and chylomicrons can protect against endotoxin induced death in mice. J Clin Invest 1990;86:696-702.

34. Ravnskov U. Cholesterol lowering trials in coronary heart disease: frequency of citation and outcome. BMJ 1992;305:15-9.

35. Muldoon MF, Manuck SB, Matthews KA. Lowering cholesterol concentrations and mortality: a quantitative review of primary prevention trials. BMJ 1990;301:309-14.

36. Davey Smith G, Song F, Sheldon TA. Cholesterol lowering and mortality: the importance of considering initial level of risk. BMJ 1993;306:1367-73.

37. Law MR, Thompson SG, Wald NJ. Assessing possible hazards of reducing serum cholesterol. BMJ 1994;308:373-79.

38. Scandinavian Simvastatin Survival Study Group. Randomised trial of cholesterol lowering in 4444 patients with coronary heart disease: the Scandinavian Simvastatin Survival Study (4S). Lancet 1994;344:1383-9.

39. Brown G, Albers JJ, Fisher LD, et al. Regression of coronary artery disease as a result of intensive lipid-lowering therapy in men with high levels of apolipoprotein B. N Engl J Med 1990;323:1289-98.

40. Watts GF, Lewis B, Brunt JNH, et al. Effects on coronary artery disease of lipid-lowering diet, or diet plus cholestyramine, in the St. Thomas' Atherosclerosis Regression Study (STARS). Lancet 1992;339:563-9.

41. Waters D, Higginson L, Gladstone P, et al. Effects of monotherapy with an HMG-CoA reductase inhibitor on the progression of coronary atherosclerosis as assessed by serial quantitative arteriography: the Canadian Coronary Atherosclerosis Intervention Trial. Circulation 1994;264:959-68.

42. Levine GN, Keany JF, Vita JA. Cholesterol reduction in cardiovascular disease: Clinical benefits and possible mechanisms. N Engl J Med 1995;332:512-21.

43. Fuster V. Mechanisms leading to myocardial infarction: insights from studies of vascular biology. Circulation 1994;90:2126-46.

Summary

Coronary heart disease mortality is a major public health problem in the Netherlands. Age-standardized mortality rates for coronary heart disease have declined during the past two decades, but it is not yet clear to what extent this has been caused by primary prevention or better survival rates after a first event. Due to the aging of the population, coronary heart disease will remain an important public health burden. Serum total cholesterol is one of the major risk factors for coronary heart disease. With respect to primary prevention, it is essential to have information on cholesterol levels in the general population and changes that are taking place in these levels. Also information on factors that influence cholesterol levels and information on the strength of the relation between total cholesterol and coronary heart disease mortality is needed. Furthermore, it is important to know whether low cholesterol levels are associated with increased risk for non-cardiovascular diseases.

Changes in total cholesterol levels over the period 1974-1986 were studied using data from the Consultation Bureau Heart Project (CB Project, 1974-1980) and the Risk Factor Project on Coronary Heart Disease (RIFOH Project, 1981-1986) (Chapter 2). Both projects were carried out in five different towns in the Netherlands: Amsterdam, Doetinchem, Maastricht, Tilburg and Leiden. Between 1974 and 1980 about 30,000 men and women aged 37-43 were examined. From 1981-1986 about 80,000 men aged 33-37 were examined; no women were examined during this period. In men and women aged 37-43, a decline in total cholesterol levels was observed between 1974 and the end of 1977, followed by an increase up to the end of 1980. Over the period 1974-1980 this resulted in a net decrease in mean total cholesterol levels of 0.07 mmol/l in men and 0.03 mmol/l in women, while the prevalence of hypercholesterolemia (total cholesterol ≥ 6.5 mmol/l) decreased by 3 percentage points in men and 2 in women. Between 1981 and 1986 a decrease in mean total cholesterol of 0.20 mmol/l was observed in 80,000 men aged 33-37, leading to a decrease in the prevalence of hypercholesterolemia of 4 percentage points.

Over the period 1987-1992 levels of and changes in total and HDL cholesterol levels in over 40,000 men and women aged 20-59 were studied using data from the Monitoring Project on Cardiovascular Disease Risk Factors (Chapter 3). Mean total cholesterol levels

in men increased with age from 4.75 mmol/l at age 20-29 to 5.98 mmol/l at age 50-59 and in women from 4.87 mmol/l at age 20-29 to 6.23 mmol/l at age 50-59. The prevalence of hypercholesterolemia increased in men from 5% at age 20-29 to 29% at age 50-59 and in women from 4% at age 20-29 to 38% at age 50-59. Mean HDL cholesterol levels changed only slightly with age and were on average approximately 1.15 mmol/l in men and 1.40 mmol/l in women. The prevalence of low HDL cholesterol levels ($\leq$ 0.90 mmol/l) increased with age from 15% at age 20-29 to 26% at age 50-59 in men and from 4% to 7% in women. The lipid profile of those with a higher educational level was more favorable than that of those with a lower educational level. Over the period 1987 to 1992, total cholesterol levels in men decreased by 0.12 mmol/l, HDL cholesterol levels decreased by 0.07 mmol/l and the non-HDL/HDL cholesterol ratio increased by 0.22. In women no statistically significant changes were observed. Changes over time did not differ according to age and educational level.

In women a large increase in the prevalence of hypercholesterolemia was observed after age 40. The extent to which this increase is attributable to the menopause was examined comparing about 2,600 premenopausal and 1,700 postmenopausal women in an age range in which most women go through menopause (45 to 54 years) (Chapter 4). After adjustment for age and body mass index (BMI), total cholesterol levels were 0.45 mmol/l higher in non-smoking postmenopausal women than in non-smoking premenopausal women. For smoking women this difference amounted to 0.28 mmol/l. HDL cholesterol levels were 0.04 mmol/l higher in postmenopausal women compared to premenopausal women after adjustment for age, smoking, body mass index, alcohol consumption and physical activity. It was concluded that menopause plays an important role in the total cholesterol increase in women around middle-age.

The effect of lifestyle and biological factors on the difference in total and HDL cholesterol levels in men and women was evaluated in 36,000 men and women aged 20-59 who participated in the Monitoring Project on Cardiovascular Disease Risk Factors between 1987-1991 (Chapter 5). Between age 30 and 50, when total cholesterol is higher in men than in women, 19-38% of the gender difference was explained by differences in body mass index and smoking. After age 50, when total cholesterol is higher in women than in men, the difference was no longer partly explained by differences in BMI and smoking, but was to a large extent due to the cholesterol-increasing effect of menopause in women. The difference in HDL cholesterol between men and women would be about 0.02-0.04

mmol/l larger if the levels of BMI and lifestyle factors (cigarette smoking, alcohol consumption and physical activity) had been similar. The effect of a "low-risk" versus "high-risk" lifestyle on changes of total and HDL cholesterol with age were also estimated. The difference in mean total cholesterol between a "low-risk" (BMI=22.5 kg/m², no smoking) versus a "high-risk" (BMI=30 kg/m², smoking) lifestyle was 0.58 mmol/l in men and 0.40 mmol/l in women. For HDL-C the difference between a "low-risk" (BMI=22.5 kg/m², no smoking, 2 alcoholic drinks per day, physically active) and a "high-risk" (BMI=30 kg/m², smoking, no alcohol, physically inactive) lifestyle was about 0.38 mmol/l in men and about 0.45 mmol/l in women. Between age 20 and 60 total cholesterol levels increase on average by 1.7 mmol/l in men and by 1.6 mmol/l in women. It was estimated that a "low-risk" lifestyle would lead to a 0.3 mmol/l less increase in men and a 0.15 mmol/l less increase in women.

The predictive value of total cholesterol with respect to mortality from coronary heart disease was examined in 50,000 Dutch men and women aged 30 to 54 years at baseline (CB Project) (Chapter 6) and in over 12,000 men from seven different countries who were 40 to 59 years old at baseline (Seven Countries Study) (Chapter 7).

In the CB Project after an average 12 years of follow-up, 818 men and 501 women had died, of whom 194 men and 38 women from coronary heart disease (Chapter 6). After adjustment for age, cigarette smoking, blood pressure and body mass index the relative risk (RR) for CHD mortality was 3.0 (95% confidence interval 1.8-5.1) for men in the highest gender-specific quintile compared to men in the lowest quintile, while for women the relative risk for the highest quintile compared to the lowest was 3.8 (95% confidence interval 1.1-13.1). Although relative risks for CHD mortality were comparable in men and women, the absolute risk was five times higher in men than in women. It was calculated that in this study population a cholesterol reduction of 0.6 mmol/l would be associated with a 20% reduction in CHD mortality in both men and women.

Data from the Seven Countries Study were used to compare the association of cholesterol and CHD mortality in different cultures. Six different groups of cohorts were formed for the analyses on the basis of cholesterol levels, similarities in culture and changes in cholesterol levels during the first ten years of follow-up (Chapter 7). The age-adjusted CHD mortality rates in the six cohorts ranged from 3% to 20%. The relative risks for CHD mortality for the highest compared to the lowest cholesterol quartile ranged from 1.5

to 2.3, except for Japan where it was 1.1. Using a linear approximation, a 0.5 mmol/l increase in total cholesterol corresponded to an increase in CHD mortality risk of 12%. When the regression dilution bias was taken into account this increase in risk was 17%. However, the absolute CHD mortality risk was strikingly different in the different cultures. For a cholesterol level of around 5.4 mmol/l, CHD mortality rates varied from 4% to 5% in Japan and Mediterranean Southern Europe to about 15% in Northern Europe. We concluded that CHD mortality across cultures increases continuously with increasing total cholesterol levels; the relative increase in CHD mortality rates with a given cholesterol increase is similar. The large difference in absolute CHD mortality rates at a given cholesterol level, however, indicates that other factors, such as diet, typical for cultures with a low CHD risk are also important with respect to primary prevention.

The association of (low levels of) total cholesterol with non-cardiovascular and all-cause mortality was examined in the CB Project and the Seven Countries Study. In the CB Project (Chapter 6), no increase of non-cardiovascular mortality (divided into cancer and non-cardiovascular/non-cancer mortality) was observed at low cholesterol levels after an average 12 years of follow-up. Total cholesterol was positively related to all-cause mortality. After adjustment for age, cigarette smoking, blood pressure and body mass index, all-cause mortality was 60^ and 46% higher in the highest compared to the lowest cholesterol quintile in men and women respectively. In the Seven Countries Study (Chapter 8), a possible association of low cholesterol levels and 25-year non-cardiovascular mortality was examined by comparing non-cardiovascular mortality in four cholesterol categories: < 4.15 mmol/l, 4.15-5.15 mmol/l, 5.16-6.20 mmol/l and > 6.20 mmol/l. After adjustment for age, cigarette smoking, blood pressure and cohort, total cancer mortality was inversely associated with cholesterol only in smokers. Non-cardiovascular/non-cancer mortality was inversely associated with cholesterol in smokers, and elevated in non-smokers in the lowest cholesterol category (< 4.15 mmol/l) only. For subgroups of diseases (infection and respiratory diseases) inverse associations were observed both in smokers and non-smokers. Total mortality in non-smokers showed a J-shaped relation with total cholesterol (lowest mortality for the cholesterol category of 4.15-5.15 mmol/l), while no association with cholesterol was observed in smokers. It was investigated to what extent confounding played a role in these associations. Exclusion of deaths occurring in the first ten years of follow-up did not alter the results. To examine the effect of confounding by alcohol consumption, the association of low cholesterol levels with alcohol-related cancers was examined (because there was no information on actual

alcohol consumption). In the lowest cholesterol category, the relative risk of mortality from alcohol-related cancers was about 2. These results suggest that the associations observed were at least partly explained by confounding. For non-smokers the lowest total mortality rate was observed for cholesterol levels of 4.15-5.15 mmol/l.

It was concluded that cholesterol levels in the Netherlands are still relatively high. Despite a long tradition in health education, a substantial shift towards lower cholesterol levels has not been reached in the Netherlands, contrary to, for example, the United States. It was shown that in both men and women total cholesterol is an important predictor of CHD mortality. The strength of the relation between total cholesterol levels and CHD mortality was comparable for different cultures. However, the observation that absolute levels of CHD mortality were strikingly different in these cultures emphasizes the importance of other factors, such as diet, in this context. The fact that the association between low cholesterol levels and non-cardiovascular mortality was not observed in the CB Project (men and women with a relatively young age at baseline) contrary to the Seven Countries Study (men who were middle-age at baseline) provides support to the hypothesis that this association is dependent on age at baseline. This suggests that this association is at least partly explained by pre-existing diseases at baseline. The low prevalence of low cholesterol levels in the Netherlands, together with the probability that the excess mortality at low cholesterol levels is at most partly causal, coupled with the high burden of coronary heart disease in the population, result in low cholesterol not being a major public health concern in the Netherlands. A cholesterol reduction in the general population remains a desirable aim in reducing the burden of coronary heart disease in the population. This reduction should preferably be achieved by means of changes in lifestyle (a diet low in saturated fat and high in fruits and vegetables, no smoking, a desirable body mass index, a physically active lifestyle) because this will lead not only to a reduction of coronary heart disease, but will also be accompanied by a more favorable risk profile with respect to other chronic diseases such as diabetes and cancer.

Samenvatting

De sterfte aan coronaire hartziekte vormt in Nederland - mede door het vergrijzen van de bevolking - een belangrijk volksgezondheidsprobleem. In de afgelopen decennia is de voor leeftijd gestandaardiseerde sterfte aan coronaire hartziekte afgenomen. Of deze daling voornamelijk wordt veroorzaakt door een betere overleving na een hartinfarct, of dat primaire preventie aan deze daling heeft bijgedragen, is nog niet duidelijk. Het is van belang om over gegevens te beschikken over het cholesterolniveau in de algemene bevolking en veranderingen die daarin plaatsvinden. Ook kennis over factoren die het cholesterolgehalte beïnvloeden en informatie over de sterkte van de relatie tussen het cholesterolgehalte en de sterfte aan coronaire hartziekte is in dit kader van belang. Daarnaast is het noodzakelijk om te weten of het sterfte-risico voor niet-cardiovasculaire ziekten mogelijk verhoogd is bij lage cholesterolniveaus. In dit proefschrift wordt op deze onderwerpen ingegaan.

Veranderingen in het totaal cholesterolgehalte in de periode 1974-1986 zijn beschreven op basis van gegevens, verzameld in het kader van het Consultatiebureau Project Hart- en Vaatziekten (CB-Project, 1974-1980) en het Risicofactoren Onderzoek Hart-en Vaatziekten (RIFOH-Project, 1981-1986) (Hoofdstuk 2). Deze projecten werden uitgevoerd in vijf plaatsen in Nederland: Amsterdam, Doetinchem, Maastricht, Tilburg en Leiden. Tussen 1974 en 1980 werden ruim 30.000 mannen en vrouwen in de leeftijd van 37-43 jaar onderzocht. In de periode 1981-1986 werden 80.000 mannen van 33-37 jaar onderzocht; in deze periode werden geen vrouwen onderzocht. Tussen 1974 en eind 1977 werd een daling in het totaal cholesterolgehalte geconstateerd, gevolgd door een stijging tot eind 1980. De netto verandering over de periode 1974-1980 was een daling van 0,07 mmol/l bij mannen en een daling van 0,03 mmol/l bij vrouwen. De prevalentie van hypercholesterolemie (totaal cholesterol ≥ 6,5 mmol/l) daalde 3 procentpunten bij mannen en 2 procentpunten bij vrouwen. In de periode 1981-1986 nam het gemiddelde totaal cholesterolgehalte bij mannen van 33-37 jaar af met 0,20 mmol/l, waardoor de prevalentie van hypercholesterolemie met 4 procentpunten afnam.

Het totaal- en HDL-cholesterolniveau en de veranderingen daarin gedurende de periode 1987-1992 werden bestudeerd met gegevens van ruim 40.000 mannen en vrouwen van 20-59 jaar die werden onderzocht in het kader van het Peilstationsproject Hart- en

161

Vaatziekten (Hoofdstuk 3). Het gemiddelde totaal cholesterolgehalte nam bij mannen toe met de leeftijd van 4,75 mmol/l bij 20-29-jarigen tot 5,98 mmol/l bij 50-59-jarigen, en bij vrouwen van 4,87 mmol/l bij 20-29-jarigen tot 6,23 mmol/l bij 50-59-jarigen. De prevalentie van hypercholesterolemie nam toe met de leeftijd van 5% tot 29% bij mannen en van 4% tot 38% bij vrouwen. Het gemiddelde HDL-cholesterolgehalte veranderde nauwelijks met de leeftijd, en was gemiddeld 1,15 mmol/l bij mannen en 1,40 mmol/l bij vrouwen. De prevalentie van een laag HDL-cholesterolgehalte ($\leq$ 0,90 mmol/l) nam wel enigszins toe met de leeftijd, bij mannen van 15% tot 26% en bij vrouwen van 4% tot 7%. Het lipidenprofiel van de hoog opgeleiden was gunstiger dan dat van de laag opgeleiden. In de periode 1987-1992 nam bij mannen het totaal cholesterolgehalte af met 0,12 mmol/l, het HDL-cholesterolgehalte nam af met 0,07 mmol/l en de non-HDL/HDL-cholesterol ratio nam toe met 0,22. Bij vrouwen werden geen statistisch significante veranderingen gedurende deze periode waargenomen. De verandering door de tijd verschilde niet naar leeftijd of opleiding.

Bij vrouwen neemt de prevalentie van hypercholesterolemie sterk toe na ongeveer het veertigste levensjaar. De invloed van het optreden van de menopauze op deze stijging werd nagegaan door het vergelijken van ongeveer 2.600 premenopauzale vrouwen met 1.700 postmenopauzale vrouwen in de leeftijdsgroep 45-54 jaar, de periode waarin bij de meeste vrouwen de menopauze optreedt (Hoofdstuk 4). Na correctie voor leeftijd en Quetelet index was het totaal cholesterolgehalte in niet-rokende postmenopauzale vrouwen 0,45 mmol/l hoger dan in niet-rokende premenopauzale vrouwen. Bij vrouwen die rookten was dit verschil 0,28 mmol/l. Na correctie voor leeftijd, sigaretten roken, Quetelet index, alcohol-consumptie en lichamelijke activiteit was het HDL cholesterolgehalte in postmenopauzale vrouwen 0,04 mmol/l hoger dan in premenopauzale vrouwen. Geconcludeerd werd dat menopauze een belangrijke rol speelt bij de toename van het totaal cholesterolgehalte bij vrouwen rond de middelbare leeftijd.

De invloed van leefstijlfactoren en biologische risicofactoren op verschillen tussen mannen en vrouwen in het totaal- en HDL-cholesterolgehalte werd onderzocht met gegevens van 36.000 mannen en vrouwen van 20-59 jaar die deelnamen aan het Peilstationsproject Hart- en Vaatziekten (Hoofdstuk 5). In de leeftijd van 30 tot 50 jaar, wanneer het totaal cholesterolgehalte hoger is bij mannen dan bij vrouwen, werd 19-38% van dit verschil verklaard door verschillen in Quetelet index en rookgewoonten tussen mannen en vrouwen. Na het vijftigste levensjaar was het totaal cholesterolgehalte hoger bij vrouwen

dan bij mannen en kon dit verschil niet meer verklaard worden uit verschillen in Quetelet index en rookgewoonten, maar voornamelijk door de toename van het cholesterolgehalte bij vrouwen door het optreden van de menopauze. Het verschil in HDL-cholesterol tussen mannen en vrouwen zou 0,02-0,04 mmol/l groter zijn wanneer er geen verschillen zouden zijn tussen mannen en vrouwen in Quetelet index (QI), rookgewoonten, alcohol-consumptie en lichamelijke activiteit. Ook werd het effect op het cholesterolgehalte van een 'laag risico' leefstijl (QI=22,5 kg/m², niet roken) versus een 'hoog risico' leefstijl (QI=30 kg/m², wel roken) onderzocht. Het verschil in totaal cholesterol tussen de 'laag risico' en 'hoog risico' leefstijl was 0,58 mmol/l voor mannen en 0,40 mmol/l voor vrouwen. Het verschil in HDL-cholesterolgehalte tussen een 'laag risico' leefstijl (QI=22,5 kg/m², niet roken, 2 glazen alcohol per dag en lichamelijk actief) en een 'hoog risico' leefstijl (QI=30 kg/m², wel roken, geen alcohol-gebruik en lichamelijk inactief) was 0,38 mmol/l voor mannen en 0,45 mmol/l voor vrouwen. Tussen het twintigste en zestigste levensjaar neemt het totaal cholesterolgehalte toe met gemiddeld 1,7 mmol/l bij mannen en 1,6 mmol/l bij vrouwen. Geschat werd dat bij een 'laag risico' leefstijl deze toename 0,3 mmol/l minder zou zijn bij mannen en 0,15 mmol/l minder bij vrouwen.

De predictieve waarde van het totaal cholesterolgehalte voor sterfte aan coronaire hartziekte werd onderzocht in een cohort van 50.000 Nederlandse mannen en vrouwen die 30-54 jaar waren bij aanvang van het onderzoek (CB-Project) (Hoofdstuk 6). Dit verband werd ook onderzocht in ruim 12.000 mannen uit zeven landen die 40-59 jaar oud waren bij aanvang van het onderzoek (Zeven Landen Studie) (Hoofdstuk 7).

In het CB-Project waren na gemiddeld 12 jaar follow-up 818 mannen en 501 vrouwen overleden, waarvan 194 mannen en 38 vrouwen aan coronaire hartziekte (Hoofdstuk 6). Na correctie voor leeftijd, roken, bloeddruk en Quetelet index, was het relatieve risico voor sterfte aan coronaire hartziekte 3,0 (95%-betrouwbaarheidsinterval 1,8-5,1) voor mannen in het hoogste geslachts-specifieke cholesterol quintiel vergeleken met mannen in het laagste quintiel. Voor vrouwen bedroeg dit relatieve risico 3,8 (95%-betrouwbaarheidsinterval 1,1-13,1). Alhoewel het relatieve risico voor sterfte aan coronaire hartziekte vergelijkbaar was voor mannen en vrouwen, was het absolute risico voor mannen vijf keer zo groot als voor vrouwen. De geschatte reductie in sterfte aan coronaire hartziekte ten gevolge van een cholesterolverlaging van 0,6 mmol/l in deze populatie was ongeveer 20% voor zowel mannen als vrouwen.

De relatie tussen het totaal cholesterolgehalte en sterfte aan coronaire hartziekte werd vergeleken in verschillende culturen. Hiervoor werd gebruik gemaakt van gegevens van de Zeven Landen Studie. Voor de statistische analyse werden de mannen in 6 cohorten gedeeld, gebaseerd op culturele overeenkomsten en overeenkomsten in cholesterolgehalte en veranderingen daarin tijdens de eerste 10 jaar follow-up (Hoofdstuk 7). De voor leeftijd gestandaardiseerde sterftecijfers varieerden tussen de 6 cohorten van 3% tot 20%. De relatieve risico's voor sterfte aan coronaire hartziekte voor het hoogste cholesterol quartiel ten opzichte van het laagste quartiel waren vergelijkbaar en varieerden van 1,5 tot 2,3, met uitzondering van Japan waar dit relatieve risico 1,1 was. Op grond van een lineaire schatting, nam het risico op coronaire hartziekte bij een toename in het totaal cholesterolgehalte van 0,5 mmol/l toe met 12%. Wanneer werd gecorrigeerd voor 'regression-dilution bias' bedroeg dit percentage 17. Hoewel de relatieve risico's vergelijkbaar waren in de verschillende cohorten, waren er grote verschillen in absolute risico's bij een gegeven cholesterolgehalte. Bij een cholesterolgehalte van 5,4 mmol/l liepen de sterftecijfers uiteen van 4% à 5% in de Japanse en de mediterrane Zuideuropese cohorten, tot ongeveer 15% in het Noordeuropese cohort. Wij concludeerden dat in verschillende culturen het risico op coronaire hartziekte continu toeneemt met het cholesterolgehalte en dat de grootte van deze toename vergelijkbaar is tussen culturen. Er zijn echter grote verschillen in absolute niveaus van sterfte aan coronaire hartziekte, waaruit blijkt dat andere factoren, zoals bijvoorbeeld het voedingspatroon, die kenmerkend zijn voor culturen met een lage sterfte aan coronaire hartziekte, van belang zijn voor primaire preventie van coronaire hartziekte.

De associatie tussen een laag cholesterolgehalte en sterfte aan niet-cardiovasculaire aandoeningen en totale sterfte werd onderzocht in het CB-Project en de Zeven Landen Studie. In het CB-Project (Hoofdstuk 6) werd geen verhoogde sterfte gevonden aan niet-cardiovasculaire oorzaken (verdeeld in de subgroepen kanker en niet-cardiovasculaire/niet-kanker oorzaken) bij lage totaal cholesterolniveaus. Er werd een positief verband gevonden tussen het totaal cholesterolgehalte en totale mortaliteit. De totale sterfte in het hoogste cholesterol quintiel ten opzichte van het laagste cholesterol quintiel was voor mannen en vrouwen verhoogd met respectievelijk 60% en 46%, na correctie voor leeftijd, roken, bloeddruk en Quetelet index. In de Zeven Landen Studie (Hoofdstuk 8) werd de associatie tussen een laag cholesterolgehalte en 25-jaars sterfte aan niet-cardiovasculaire oorzaken onderzocht door het vergelijken van 4 cholesterol categorieën: < 4,15 mmol/l, 4,15-5,15 mmol/l, 5,16-6,20 mmol/l en > 6,20 mmol/l. Na correctie voor leeftijd, roken, bloeddruk

en cohort was alleen bij rokers het totaal cholesterolgehalte invers geassocieerd met sterfte aan kanker. Ook de niet-cardiovasculaire/niet-kanker sterfte was bij rokers invers geassocieerd met het totaal cholesterolgehalte. De sterfte aan deze groep oorzaken was bij niet-rokers in het laagste cholesterol quintiel (< 4,15 mol/l) verhoogd, maar nam niet verder af in de hogere cholesterol quintielen. Voor subgroepen van ziekten uit deze categorie (infecties en luchtwegaandoeningen) werden inverse associaties met het cholesterolgehalte gevonden voor zowel rokers als niet-rokers. Het verband tussen het totaal cholesterolgehalte en totale mortaliteit was bij niet-rokers J-vormig, (de laagste sterfte trad op in de cholesterol categorie 4,15-5,15 mmol/l), terwijl bij rokers geen verband werd gevonden tussen het totaal cholesterolgehalte en totale sterfte. De mogelijke rol van confounders in deze relaties werd nagegaan. Het uitsluiten van de eerste 10 jaar follow-up veranderde de resultaten niet. Een mogelijk verstorend effect van alcoholgebruik (dat niet was gemeten) werd onderzocht door te kijken naar de sterfte aan alcohol-gerelateerde tumoren. In de laagste cholesterol categorie was het risico op sterfte aan deze groep kankers twee maal zo hoog als in de overige cholesterol categorieën. Deze resultaten suggereren dat de gevonden verbanden in ieder geval gedeeltelijk werden verklaard door verstorende factoren waarvoor niet gecorrigeerd kon worden. Voor niet-rokers trad de laagste sterfte op bij cholesterolniveaus van 4,15-5,15 mmol/l.

Geconcludeerd werd dat de totaal cholesterolniveaus in Nederland nog steeds relatief hoog zijn. Ondanks een lange traditie van gezondheidsvoorlichting is er nog geen substantiële verlaging van de cholesterolniveaus opgetreden, in tegenstelling tot bijvoorbeeld de Verenigde Staten. Ons onderzoek toonde aan dat het totaal cholesterolgehalte voor zowel mannen als vrouwen een belangrijke risicofactor is voor sterfte aan coronaire hartziekte. De sterkte van het verband tussen het totaal cholesterolgehalte en de sterfte aan coronaire hartziekte was vergelijkbaar in verschillende culturen. Het grote verschil in het absolute niveau van sterfte aan coronaire hartziekte bij een gegeven cholesterolgehalte tussen culturen, laat het belang zien van andere factoren, zoals voeding, voor deze relatie. Het feit dat in het CB-Project (mannen en vrouwen die relatief jong waren bij aanvang van het onderzoek) geen verhoging van de niet-cardiovasculaire sterfte werd gevonden bij een laag cholesterolgehalte, terwijl dit in de Zeven Landen Studie bij mannen van middelbare leeftijd wel het geval was, ondersteunt de hypothese dat dit verband afhankelijk is van de leeftijd van de onderzochte personen. Dit suggereert dat deze associatie in ieder geval gedeeltelijk verklaard wordt door de aanwezigheid van ongezonde personen in de lage cholesterol categorie. Omdat de prevalentie van lage cholesterolniveaus in Nederland niet

hoog is, de verhoogde sterfte bij lage cholesterolniveaus ten hoogste gedeeltelijk causaal is en de ziektelast ten gevolge van coronaire hartziekte hoog is, wordt geconcludeerd dat lage cholesterolniveaus in Nederland geen belangrijk volksgezondheidsprobleem vormen. Een cholesterolverlaging in de algemene bevolking blijft wenselijk om de ziektelast ten gevolge van coronaire hartziekte in de populatie te verminderen. Deze cholesterolverlaging moet bij voorkeur bereikt worden door veranderingen in leefstijl (een voeding met weinig verzadigd vet, veel groente en fruit, niet roken, geen overgewicht en regelmatige lichamelijk activiteit). Een dergelijke leefstijl leidt namelijk niet alleen tot een vermindering van het optreden van coronaire hartziekte maar ook tot een gunstiger risicoprofiel met betrekking tot andere chronische ziekten zoals diabetes en kanker.

Dankwoord

Het is af! Het is een heerlijk gevoel om op de altijd goed bedoelde vraag "En ..? Is je proefschrift al af?" eindelijk te kunnen zeggen "Ja, het is af!". Maar natuurlijk zijn er velen die eraan hebben bijgedragen dat deze onderneming tot een goed einde kwam.

Allereerst Daan Kromhout. Beste Daan, alle data waarop de artikelen in dit proefschrift zijn gebaseerd zijn mede dankzij jou beschikbaar gekomen of verzameld. Je hebt een fijne neus om 'dode' data tot leven te brengen. Dit geldt niet alleen voor de Zutphen Studie, het Nederlandse deel van de Zeven Landen Studie, maar ook voor de gegevens van het CB-project. Het idee om de hele administratie van dit project, zes jaar na het beëindigen ervan, te laten automatiseren en vervolgens via de gemeenten de vitale status na te zoeken was zeer ambitieus. Het was een enorme onderneming die echter veel vruchten heeft afgeworpen. Ik heb veel van je geleerd. De wijze waarop je ook de -tigste versie van manuscripten weer van A tot Z doorleest, en van nuttig commentaar voorziet (waarbij ook een ontbrekende puntkomma in de literatuurverwijzingen meestal niet aan je aandacht ontsnapt), verbaast mij elke keer weer.

Gatske Obermann-de Boer ben ik dank verschuldigd voor haar bijdrage aan het Peilstationsproject. Gedurende de hele periode heeft zij het veldwerk in goede banen geleid. Gatske, nu ik zelf meer bij dit soort dingen betrokken ben in het MORGEN-Project, zie ik pas echt hoeveel onzichtbaar (en daardoor ondankbaar) werk er moet worden gedaan om een project 'gewoon' te laten lopen. Een eervolle vermelding voor jou is daarom zeker op zijn plaats.

Gegevens van ruim 40.000 Nederlanders komen er niet zomaar. Ik wil daarom graag de peilmedewerkers en locale projectleiders van de GGD-en in Amsterdam, Doetinchem en Maastricht bedanken, die deze gegevens verzameld hebben. En natuurlijk ben ik ook dank verschuldigd aan alle deelnemers die de moeite hebben genomen een lange vragenlijst in te vullen en een bezoek te brengen aan de GGD.

Het werken met gegevens van de Zeven Landen Studie, die rond mijn geboorte gestart is, was fantastisch. Hiervoor ben ik alle medewerkers van het eerste uur (en de daaropvolgende 25 jaar) dankbaar.

David Jacobs, it was a pleasure analyzing data and writing articles together with you. I learned a lot from your experience and I hope our cooperation will continu in the future.

Ellen Röntgen-Pieper, het achterhalen van de vitale status van 50.000 deelnemers aan het CB-Project, bij honderden (vaak niet geautomatiseerde) gemeenten, leek onbegonnen

werk. Je hebt je echter ontpopt als een ware Sherlock Holmes. Dankzij jouw accuratesse en volharding is bijna iedereen teruggevonden, waardoor er nu informatie is over risicofactoren bij een groot Nederlands cohort.

Ik dank de Directie van het RIVM voor de gelegenheid die mij geboden is dit proefschrift af te ronden. Jantien Zoutman wil ik bedanken voor de secretariële ondersteuning en Jan Dorssers en Ruud Romme voor het soepel lopende computernetwerk. Alle niet met name genoemde collega's wil ik bedanken voor de prettige werksfeer op het CCM.

Mijn paranimfen, Jet Smit en Annemarie Jansen, dank ik voor hun bijdrage aan het geheel. Jullie hebben mij niet alleen allerlei klusjes uit handen genomen die bij een promotie komen kijken, maar waren voor mij ook een onmisbaar klankbord.

Ruth de Wijs wil ik bedanken voor de enthousiaste en snelle wijze waarop zij delen van de Engels tekst heeft gecorrigeerd. Mijn lieve zus Louki heeft de Nederlandse delen van de tekst vakkundig aangescherpt en daarnaast (minstens zo belangrijk) wist zij mij door haar komische kijk op dingen uit de onvermijdelijke 'dalletjes' te helpen.

Miriam Slaats ben ik dankbaar voor het enthousiasme waarmee zij de - voor haar vast wat droge - materie heeft vertaald in een sprankelend schilderij, dat de omslag siert van dit proefschrift.

En ten slotte is er natuurlijk Bennie, die voor elk probleem - van welke aard ook - altijd tenminste twee oplossingen heeft. Bedankt voor het lay-outen van dit proefschrift, maar meer nog voor je 'onzichtbare' input (al meer dan 14 jaar).

About the author

Monique Verschuren was born October 21st, 1960 in The Hague, the Netherlands. After completing secondary school (Gymnasium-ß at the Edith Stein College in The Hague), she studied Human Nutrition at the Agricultural University in Wageningen. She graduated in 1986 with majors in nutrition, epidemiology and immunology. From May to November 1986 she worked as an epidemiologist at the Municipal Health Service in Breda. In December 1986 she started her research at the Institute of Social Medicine, University of Leiden on the Monitoring Project on Cardiovascular Disease Risk Factors (project leader prof. D. Kromhout). Since 1988 this project was carried out at the National Institute of Public Health and the Environment in Bilthoven. In 1988 she attended the New England Epidemiology Summer Program, a three week course in Epidemiology and Biostatistics, at Tufts University, Boston, USA. Since 1992 she has a permanent position as researcher at the National Institute of Public Health and the Environment. In 1992 she participated in the 25th Ten Day International Teaching Seminar on Cardiovascular Disease Epidemiology and Prevention in Rio de Janeiro, Brasil. Since 1993 she is a member of the Management Team of the Monitoring Project on Chronic Disease Risk Factors.