PROSPECTIVE STUDIES ON CORONARY HEART DISEASE IN THE ELDERLY

The role of classical and new risk factors

Matty Weijenberg

Stellingen

1. Ouderen vormen voor wat betreft hun risicoprofiel en hun risico op coronaire hartziekten een zeer heterogene groep.
Dit proefschrift.

2. Het aantal witte bloedcellen en serumalbumine, routinematig gemeten klinisch-chemische bloedparameters, kunnen, naast de klassieke risicofactoren, bijdragen aan een betere voorspelling van het voorkomen van klinisch manifeste coronaire hartziekten bij ouderen.
Dit proefschrift.

3. De voedingsmiddelenindustrie vervult een sleutelrol bij populatie-gerichte strategieën met betrekking tot beïnvloeding van risicofactoren. Ook ouderen zouden hiervan kunnen profiteren vanwege het gunstige effect op de bloeddruk, serumlipiden en serumhomocysteïne, en het risico op coronaire hartziekten en sterfte in deze leeftijdsgroep.
Dit proefschrift.

4. Bij ouderen zijn risicofactoren voor coronaire hartziekten over het algemeen belangrijkere voorspellers van sterfte aan de aandoening dan voor de klinische manifestatie ervan.
Dit proefschrift.

5. With respect to the distinctions between aging and disease, from an epidemiologic and particularly an etiologic perspective, the processes of aging and the processes encompassing the pathogenesis of diseases should be regarded as a single category that contributes to ill health. For an etiologic study the distinction between these processes is largely semantic and administrative.
Kaplan GA, Haan MN, Cohen RD. Risk factors and the study of prevention in the elderly: methodological issues. In: Wallace RB, Woolson RF, editors. The epidemiologic study of the eldelry. Ney York: Oxford University Press, 1992:20-36.

6. There is greater variation in physical and mental health in old age than at younger ages; nevertheless, age in itself should not be a barrier to promoting health and preventing or postponing disease, disability and death.
WHO Study Group on Epidemiology and Prevention of Cardiovascular Diseases in the Elderly. Epidemiology and prevention of cardiovascular diseases in elderly people: report of a WHO study group (WHO Technical Report Series, No. 853). Geneva: World Health Organization, 1995.

7. Een grotere en meer langdurige deelname van ouderen aan het maatschappelijk verkeer draagt bij aan een algehele verbetering van het welzijn van zowel ouderen als andere leeftijdsgroepen.

8. The sin comes in believing a causal hypothesis is true because your study came up with a positive result, or believing the opposite because your study was negative.
Greenland S. 1995. In: Taubes G. Epidemiology faces its limits. Science 1995;269:164-169.

9. The essence of knowledge is generalization.
Rothman KJ. Modern Epidemiology. Boston: Little, Brown and Company, 1986.

10. Een investering door de werkgever in een cursus time-management, betrekking hebbend op zowel het werk als het privé-leven van de werknemer, draagt bij aan een optimaal werkrendement.

11. De vrijstelling van houderschapsbelasting van auto's ouder dan 25 jaar kan leiden tot een afname van de verkeersveiligheid.

12. I made up my mind, so don't confuse me with the facts.

13. Maintenant je sais, je sais qu'on ne sait jammais, ... c'est tout ce que je sais, mais ça j'le sais.
Jean Gabin, Maintenant je sais.

Stellingen behorende bij het proefschrift
PROSPECTIVE STUDIES ON CORONARY HEART DISEASE IN THE ELDERLY
The role of classical and new risk factors
van Matty Weijenberg

Wageningen, 12 april 1996

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The role of classical and new risk factors

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PROSPECTIVE STUDIES ON CORONARY HEART DISEASE IN THE ELDERLY

The role of classical and new risk factors

Martje Petronella Weijenberg

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Aan opa en oma

Abstract

PROSPECTIVE STUDIES ON CORONARY HEART DISEASE IN THE ELDERLY
The role of classical and new risk factors

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

Matty P. Weijenberg

In this thesis associations between biological risk factors and the occurrence of coronary heart disease in elderly persons are described. The focus is on classical (i.e. total and high density lipoprotein cholesterol and blood pressure) and some new (i.e. homocysteine, white blood cell count and serum albumin) biological risk factors. The studies are based on two Dutch cohorts. One is a cohort of 292 men and women, aged 64 to 87 years in 1971, from a general practice in Rotterdam, with a mortality follow-up of 17 years. The other is the Dutch cohort of the Seven Countries Study which consists of 939 men aged 64 to 84 years in 1985 from the town of Zutphen. The morbidity and mortality follow-up embraced five years.

In general, the classical risk factors were important predictors of coronary heart disease occurrence in elderly people. Systolic blood pressure was a strong independent long-term predictor of coronary heart disease mortality in elderly women, but the long-term association was less clear in elderly men. Total cholesterol was also significantly associated with long-term mortality from coronary heart disease in elderly women, but in men the association tended to be inverted *U*-shaped, i.e. men with cholesterol levels in the median tertile of the cholesterol distribution had a higher risk than those with levels in the first and third tertile. Regarding the Zutphen study, the short-term associations with incidence of coronary heart disease were usually weaker than with mortality from the disease. Elevated systolic and diastolic blood pressure, and especially isolated systolic hypertension, were important short-term predictors of sudden cardiac death in elderly men. For mortality from coronary heart disease, which was not additionally recorded as sudden, *U*-shaped associations with systolic and diastolic blood pressure levels were observed, i.e. men with the lowest blood pressure levels and those with the highest blood pressure levels and using anti-hypertensive medication had the highest risk. Serum total cholesterol was related to short-term coronary heart disease mortality in elderly men. For incidence of the disease, an association was only observed in a subgroup of the population with serum albumin levels below the median. High density lipoprotein cholesterol was not predictive of mortality from coronary heart disease, but there appeared to be an association with the incidence of the disease.

Concerning the new risk factors, serum homocysteine appeared to be associated with mortality from coronary heart disease in elderly men in the first one-and-a-half years of follow-up only. A strong association with mortality from cerebrovascular disease was observed in normotensive men. In addition, an association with an increased risk of cognitive impairment was suggested. Both white blood cell count and serum albumin were important predictors of coronary heart disease independent of the classical risk factors for coronary heart disease. The association with serum albumin could only partly be explained by baseline health status indicators.

The experience obtained from the studies described in this thesis has made clear that elderly people are a heterogenous group with respect to risk factor levels as well as coronary heart disease risk. It would therefore be desirable to have a measure of susceptibility for coronary heart disease to identify elderly people who are at increased risk of the disease. Our studies suggest that white blood cell count and serum albumin, routine clinical blood chemistry values, may be useful in this respect in addition to the classical risk factors. Future studies should aim at assessing the effectiveness of a multifactorial approach of risk factor management on coronary heart disease and all-cause mortality as well as on quality of life in older men and women.

Contents

Chapter 1
Introduction

Coronary heart disease in the elderly

Since the 1950's, an overwhelming body of knowledge on risk factors for coronary heart disease in middle-aged men has been accumulated.[1-3] Modifications in the major risk factors, e.g. blood cholesterol, blood pressure and cigarette smoking, have proven to contribute to a reduction in the risk of the disease.[4-7] It is only relatively recently that epidemiologic research on coronary heart disease has begun to focus on elderly people. Several reasons underlie this interest.

First, demographic shifts in industrialized countries will lead to striking increases in the number of older people. In the Netherlands, the total number of people is expected to increase by approximately 11 percent from 15.0 to 16.7 million between 1990 and 2010, but this trend will not be equally distributed over different age categories.[8] The largest increase will occur in people over 45 years of age, with the number of people aged 65 years and older forecasted to increase by more than 25 percent from approximately 1.9 to 2.4 million between 1990 and 2010.[8,9] Life expectancy at birth will rise by a mean of 1.5 years in the same period, only half of which will be in good health.[8] If these projections are correct they will be accompanied by growing health care costs.[8]

Second, cardiovascular diseases, of which coronary heart disease is the most common, constitute the major cause of death in the majority of the industrialized countries including the Netherlands.[10] Moreover, 80 percent of all deaths from cardiovascular causes occur in people over 65 years of age.[11] Age-specific mortality rates for cardiovascular diseases as well as coronary heart disease more than double between the age groups 65 to 74 years and 75 to 84 years.[11] Overall, the age-standardized mortality rate from coronary heart disease has been decreasing steadily for the past two decades in most industrialized countries. In the Netherlands a decline of 29 percent among men and 38 percent among women has been observed between 1972 and 1990, corresponding to a decline of 4 per 100,000 persons a year in men as well as women.[8] Part of the decline is a result of improved clinical care of the disease and/or reduced severity of the disease leading to a postponement of death, which in turn accounts for a less marked decline in coronary heart disease mortality rates at older ages.[8] The overall decline may also be due to a reduced incidence of the disease.[11] Accurate population-based information on the prevalence and incidence of the disease is lacking for the elderly. Nonetheless, the effect of demographic changes is expected to be larger than that of the reduced relative prevalence of the disease.

This is expected to result in an increase in the absolute number of patients with myocardial infarction by approximately 34 percent, from 255,500 in 1990 to 343,000 in 2010, in the Netherlands.[8] The elderly are expected to contribute most to this increase and this is estimated to be associated with a considerable rise in the social, health care, and economic burden of the disease in the coming decades.[8]

Finally, although advanced coronary atherosclerosis (i.e. more than 50 percent occlusion of one of the coronary arteries) may be present in approximately half of the elderly population, it is not considered to be an aging phenomenon *per se*.[12] Evidence from angiography studies indicates that progression of atherosclerosis can be stopped and regression can be induced through the management of established risk factors.[13,14] Hence, reduction in coronary heart disease risk in elderly persons can be expected through risk factor management. It is therefore imperative to obtain insight into possible risk factors for coronary heart disease in the elderly.

Risk factors for coronary heart disease in the elderly

Most prospective studies on risk factors for coronary heart disease have been conducted in middle-aged people. Although several studies have addressed the role of conventional risk factors, i.e. blood cholesterol, blood pressure and cigarette smoking, in elderly populations, consensus has not yet been established on the existence or shape of associations in this age group.[15,16] Several factors, specific to epidemiologic research in older persons, may explain the lack of consensus.[17]

Comorbidity and comortality are common in older populations and may obscure the associations between risk factors and the end-point of interest. Health status of an individual at the time of the risk factor measurement can influence the level of the risk factor and thereby alter its relation with coronary heart disease. In addition, underlying or overt disease may interact with the progression or development of coronary heart disease thereby modifying the association between risk factor and outcome. A similar mechanism may underlie the diluting effect of possible competing causes of mortality. Careful consideration of the health status of an individual is therefore imperative in studying coronary heart disease risk in elderly populations. In addition, since death is eventually inevitable and comortality may be considerable in prospective studies in the elderly, it is necessary to address the impact of risk factors on all-cause mortality.

Selective survival of older persons with unfavorable risk factor profiles may also explain discordant results between studies of older and middle-aged people. Indeed,

persons with unfavorable risk factor levels who survived a coronary heart disease event at middle-age and have reached old age may constitute a specific group of people less susceptible to the effect of the specific risk factors.

An additional matter of concern in epidemiologic studies in the elderly is the possibility that risk factors may change with advancing age. The measured level of a risk factor at old age may not be representative of lifetime exposure and misclassification of subjects is possible. However, one must keep in mind the mechanisms that underlie the associations between risk factors and coronary heart disease. If the risk factor is expected to have an acute effect on the disease outcome an assessment of the factor shortly before disease occurrence is most appropriate, whereas if the risk factor is thought to act cumulatively on the progression of coronary heart disease a longer term assessment of the risk factor would be preferable. Unfortunately, the role of risk factors in the pathogenesis of coronary heart disease is complex and not yet resolved, and short- and long term effects may frequently intermingle.

Another issue is the interpretation of the measure of association in studies among elderly persons. Relative risks for coronary heart disease associated with a specific risk factor may decline with advancing age, but absolute excess risks tend to increase with age. From a public health point of view the absolute rates are most important and this should be considered when comparing the impact of a risk factor on disease occurrence between older and younger populations.

Finally there may be other factors, alone or in addition to the conventional risk factors, which become increasingly important in predicting coronary heart disease in elderly people. One such a factor may be homocysteine which has recently been associated with coronary heart disease in middle-aged people.[18,19] Homocysteine is readily modifiable through adequate intakes of or supplementation with folate, vitamin B12 and vitamin B6.[20] Since elderly people generally have low levels of these vitamins,[21] it is important to know whether homocysteine is also associated with coronary heart disease in this age group.

Known markers of underlying disease, inflammation, infection, or a poor nutritional status may also be more effective in predicting coronary heart disease in elderly people than the measurement of the conventional risk factors alone. Some routinely measured blood chemistry values, such as white blood cell count and albumin, may be promising in this respect. Elevated white blood cell count is a marker for underlying inflammation. The epidemiologic evidence for an association between increased white blood cell count and coronary heart disease in middle-aged people is convincing.[22] In addition, plausible mechanisms for the associations have been described[23] suggesting that the count is more than just a marker of possible underlying disease processes. The serum albumin concentration is another marker of general underlying disease and poor nutritional status,

especially in the elderly.[24] Biological mechanisms have also been described for the association between low albumin concentrations and coronary heart disease.[25-27] Since data from elderly populations are lacking, the question arises whether these routine clinical measurements are predictive of the disease in this age group and whether the associations can wholly or partially be accounted for by underlying disease.

Outline of thesis

The prevailing uncertainties regarding the predictive value of risk factors for coronary heart disease in the elderly were the rationale for conducting the studies described in this thesis. Biological risk factors are the main focus of interest. They can be defined as indicators of an acquired physiologic state presumably associated with an increased risk of the disease. They are frequently intermediates between lifestyle factors and disease occurrence. The studies are based on two Dutch cohorts. One is a cohort of 292 men and women, aged 64 to 87 years in 1971, from a general practice in Rotterdam,[28] with a mortality follow-up of 17 years. The other is the Dutch cohort of the Seven Countries Study[3] which consists of 939 men aged 64 to 84 years in 1985 from the town of Zutphen. The morbidity and mortality follow-up embraced five years. Some of the analyses also included other outcome measures than coronary heart disease in view of the known or suspected role of these factors in the etiology of other conditions.

In the first part of the thesis, the classical risk factors for coronary heart disease are addressed. Chapter 2 deals with blood pressure and isolated systolic hypertension as risk factors for coronary heart disease, sudden cardiac death and stroke in elderly men. In chapter 3, the associations between both systolic blood pressure and total cholesterol and 17-year mortality from coronary heart disease were investigated in elderly men and women. The associations of total and high density lipoprotein cholesterol with the five-year risk of coronary heart disease in elderly men is described in chapter 4. Chapter 5 addresses the age related changes in total and high density lipoprotein cholesterol in elderly men during the 1977/1978 - 1993 period.

The second part of the thesis deals with new biological factors which have more recently been associated with coronary heart disease in middle-aged persons and may also be of importance in the elderly. Chapter 6 addresses the role of serum homocysteine, chapter 7 that of white blood cell count and chapter 8 deals with serum albumin and the five-year risk of coronary heart disease in elderly men. Finally, in chapter 9, some methodological issues involved in conducting epidemiologic research in the elderly, the

pathophysiology and etiology of coronary heart disease in this age group and some public health implications are discussed.

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Chapter 2
Blood pressure and isolated systolic hypertension and the five-year risk of coronary heart disease and mortality in elderly men

Abstract

The associations of diastolic (DBP) and systolic (SBP) blood pressure with the risk of coronary heart disease, sudden cardiac and all-cause mortality were investigated in elderly men followed for five years. 885 randomly selected men, aged 64 to 84 years and from the Dutch town of Zutphen participated in the study. Relative risks were adjusted for age, body mass index, total and high density lipoprotein cholesterol, cigarette smoking, alcohol consumption, use of blood pressure lowering medications, and physician who took the blood pressure. Neither DBP nor SBP were significantly associated with the incidence of a first coronary heart disease event, and only DBP showed a U-shaped association with mortality from coronary heart disease (p-quadratic trend = 0.049). However, positive associations with sudden cardiac death were observed (p-linear trend = 0.013 for DBP and 0.002 for SBP). The adjusted relative risk for sudden cardiac death in men with definite isolated systolic hypertension was 9.20 (95% confidence interval: 1.76 - 47.97). DBP and SBP showed a U-shaped relation with coronary heart disease mortality which was not recorded as sudden (p-quadratic trend = 0.007 and 0.018 respectively). DBP and SBP were positively associated with all-cause mortality when possible co-morbidity at the time of the blood pressure measurement was accounted for. Overall, the highest risk for all end-points was observed in men on anti-hypertensive medication, who formed a distinct group with a clustering of cardiovascular risk factors. In conclusion, elevated DBP and SBP, and especially isolated systolic hypertension, are important predictors of sudden cardiac death in elderly men. The highest risk of non-sudden coronary heart disease mortality was found at the lower end of the blood pressure distributions and among men on anti-hypertensive medication.

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Introduction

Diastolic and systolic blood pressure are well established risk factors for coronary heart disease in middle-aged persons.[1] However, among elderly people it is still not clear how diastolic and systolic blood pressure levels are associated with coronary heart disease.[2] Apart from possible selection mechanisms in elderly cohorts,[3] factors related to the physiology of blood pressure may offer an explanation.

Diastolic blood pressure decreases with advancing age after 60 years of age, whereas systolic blood pressure increases continually with age.[4,5] The lower diastolic blood pressure in the elderly has been associated with increased arterial stiffness,[6-8] partly as a result of increased atherosclerosis,[6,9] and with reduced diastolic coronary flow.[8,10] This may explain the inverted, U- or J-shaped associations observed between diastolic blood pressure and coronary heart disease in some studies.[10-15] In line with these findings and the reduced arterial compliance with advancing age,[6] isolated systolic hypertension has been identified as a stronger coronary heart disease risk factor among the elderly than an elevated systolic blood pressure per sé.[9,16-19]

However, U -shaped associations have also been observed between systolic blood pressure and cardiovascular diseases and all-cause mortality.[11,13,14] Since blood pressure levels have a tendency to be low in the presence of certain diseases, comorbidity has been suggested to be responsible for part of the increased morbidity and mortality rates at the lower ends of the blood pressure distributions.[5,11,20-23]

In the present study, we attempted to further clarify these associations in elderly people. Therefore, we investigated the associations of diastolic and systolic blood pressure and isolated systolic hypertension with the risk of coronary heart disease as well as sudden cardiac death in elderly men followed for five years.

Methods

Population

The Zutphen Study is a longitudinal investigation of chronic disease risk factors initiated in 1960 among middle-aged men as the Dutch contribution to the Seven Countries Study.[24] In 1985, 555 men from the 1960 cohort were still alive and were invited for new examinations. In addition, a random sample (two out of three) of all men of the same age living in Zutphen and not part of the 1960 cohort were invited to take part in the study. From then on, the study was continued as the Zutphen Elderly Study. The study was

approved by the Medical Ethics Committee of the University of Leiden, The Netherlands, in 1985. Of the 1266 men approached 939 (74%) agreed to participate. Hundred-and-nine men (9%) could not be examined because of serious illness or death, 62 men (5%) had moved and 156 men (12%) refused to participate or could not be reached. All participants signed an informed consent form. Complete information on risk factors was available for 885 men aged 64 to 84 years.

Examinations

Physical examinations and dietary surveys took place between March and June 1985. Physical examinations were carried out by five trained physicians according to a standardized protocol. Diastolic (fifth Korotkoff phase) and systolic blood pressures were measured in duplicate with a random zero sphygmomanometer (Hawksley) at the right arm while subjects were in supine position. A standard cuff-size was used (12 × 23 cm) which was eventually judged adequate for all men. The measurements were done at the end of the physical examination. The mean of the two blood pressure values was used in the analyses. With the use of a standardized questionnaire, the physicians obtained information on whether men had ever been diagnosed with hypertension. In addition, information was collected on whether these men were currently using anti-hypertensive medication and on the type of medication used, which they had been asked to bring with them.

Categories of diastolic and systolic blood pressures and of isolated systolic hypertension were formed and the lowest category was always regarded as the reference category. Diastolic blood pressure was divided into five categories. The first four categories consisted of men not using anti-hypertensive medication with 75, 85 and 95 mmHg as cut-off values. The highest category consisted of men using anti-hypertensive medication regardless of their blood pressure level. For systolic blood pressure we distinguished between four categories of which the first three consisted of men not using anti-hypertensive medication with 140 and 160 mmHg as cut-off values, and the highest category consisted of men using anti-hypertensive medication. Isolated systolic hypertension was defined excluding men on anti-hypertensive medication. A systolic blood pressure of 160 mmHg or higher and a diastolic blood pressure lower than 90 mmHg constituted the definite category. A systolic blood pressure level between 140 and 160 mmHg with a diastolic blood pressure lower than 90 mmHg was indicative of borderline isolated systolic hypertension. Men with a systolic blood pressure lower than 140 mmHg and a diastolic blood pressure lower than 90 mmHg constituted the reference category ("no isolated systolic hypertension").

Height was measured to the nearest 0.1 cm and body weight was measured to the nearest 0.5 kg while the men were in underwear. Body mass index (weight (kg)/ height

$(m)^2$) was calculated. Non-fasting serum total and high density lipoprotein (HDL) cholesterol were determined enzymatically with the CHOD-PAP mono-testkit from Boehringer Mannheim.[25,26] HDL was isolated after precipitation of apolipoprotein B containing particles by dextran sulphate-Mg^{2+}.[27] The analyses were carried out in the standardized lipid laboratory of the Department of Human Nutrition, Agricultural University, Wageningen, The Netherlands. Information on medication use, prescribed diets and smoking habits (missing for one person) was assessed with a standardized questionnaire. Men using medications known to lower the blood pressure but who had never been diagnosed with hypertension were identified. In the present study smoking was defined as never, former or current cigarette smoking. Information on alcohol consumption was assessed by trained dietitians with a cross-check dietary history,[28] adapted to the Dutch situation.[29] This information was available for 825 men.

Follow-up

Information on the prevalence of coronary heart disease was obtained during the physical examination in 1985 and a similar examination between March and June 1990. For men who did not participate in the 1990 examination, information on major chronic diseases was obtained from a questionnaire for non participants. Information on angina pectoris and myocardial infarction was obtained through the Dutch translation of a questionnaire developed at the London School of Hygiene and Tropical Medicine.[30] The diagnosis of angina pectoris was made when the following symptoms were present:[30] chest pain or discomfort located at the sternum or left chest and left arm due to effort of walking or hurrying, compelling the patient to slow down or take nitroglycerin, and relieved within 10 minutes after stopping the effort. For definite myocardial infarction the final diagnosis was based on whether two of the following three criteria were met: a specific medical history, i.e. severe chest pain lasting for more than 20 minutes and not disappearing in rest, characteristic electrocardiogram changes and specific enzyme elevations. All diagnoses were verified with hospital discharge data and written information from the subjects' general practitioners. All information was eventually coded by a single physician, and the year of first diagnosis was recorded.

Information on the vital status of the participants was obtained till July 1990. One person had moved abroad and was lost to follow-up. The date on which he moved was used as his (censored) end-point date. Information on the causes of death was obtained from the Dutch Central Bureau of Statistics, after verification with hospital discharge data and information from the deceased's general practitioners. The causes of death were coded according to the 9th Revision of the International Classification of Diseases (ICD).[31] Because of the frequency of possible comorbidity and comortality in elderly people the

underlying cause of death is often difficult to establish. Therefore both the primary and secondary cause of death were recorded in defining the end-points. Death due to coronary heart disease was defined by ICD codes 410 through 414 (n = 53), death due to cerebrovascular disease by codes 430 through 438 (n = 29), death due to cardiovascular diseases by codes 390 through 459 (n = 110) and death due to cancer by codes 140 through 208 (n = 69). Sudden cardiac death was recently coded by one trained expert. Because of limitations in the available information sudden cardiac death was regarded to have occurred in the following two situations (n = 44). One was when death was documented to have occurred within two hours after the onset of typical symptoms, and no other causes of death were known. The other was in subjects with a history of heart disease: when "mors subita" was notified by the physician or death occurred unwitnessed (within 12 hours after men had been observed to be well). Men who died of coronary heart disease were also classified according to whether the cause of death was additionally recorded as sudden (n = 30) or non-sudden (n = 23).

The incidence of coronary heart disease was defined by the first occurrence ever of either myocardial infarction, angina pectoris or mortality from coronary heart disease (n = 56).

Statistical methods

Statistical analyses were carried out using the SAS program (SAS Institute Inc., Cary, North Carolina, USA, 1989-1993, version 6.10). All tests were two sided and *p*-values smaller than five percent were considered statistically significant. Differences in risk factor levels between blood pressure categories were assessed using analysis of variance. Cox's proportional-hazard (survival) analysis was carried out to investigate the associations between blood pressure variables and the end-points of interest during five years of follow-up.[32] Dummy variables for the categories of blood pressure and covariates were used in the analyses. Tests for linear and quadratic trends were performed.[32] Associations were adjusted for age, body mass index, total and HDL cholesterol, cigarette smoking, alcohol consumption, use of medications known to lower blood pressure levels without a specific indication for hypertension, and physician who took the blood pressure. Additional adjustment for the use of a sodium-restricted diet (n = 34) did not alter any of the associations, therefore the associations described in the results section were not additionally adjusted for this factor. Interaction terms were evaluated at *p*-values smaller than 10 percent.

Results

At baseline, mean diastolic and systolic blood pressure levels of the cohort were 85.4 mmHg and 151.1 mmHg respectively. Twelve percent (n = 106) of the men were on anti-hypertensive medication and both their diastolic and systolic blood pressures were significantly higher compared to men who were not on anti-hypertensive medication ($p < 0.001$). Only 4.6 percent of the men on anti-hypertensive medication had diastolic and systolic blood pressure levels below 90 and 160 mmHg respectively. The 106 men on anti-hypertensive medication were therefore selected to form a separate systolic and diastolic blood pressure category. The most common anti-hypertensive medications used by the participants were diuretics (59%) and beta-blocking agents (40%), and 22 percent were using both. One hundred and thirty six men were using medications known to lower blood pressure levels without a specific indication for hypertension of which vasodilators (51%), diuretics (43%) and beta-blocking agents (25%) were the most frequent. These men were significantly older (73.6 years versus 71.1 years, $p < 0.001$) and had significantly lower diastolic and systolic blood pressure levels (80.3 mmHg versus 85.2 mmHg and 143.8 mmHg versus 149.7 mmHg respectively, $p < 0.001$ in both cases) compared to men not using this type of medication.

Men with a diastolic blood pressure lower than 75 mmHg were significantly older than men with levels of 85 mmHg or higher, as where men with definite isolated systolic hypertension compared to men without this type of hypertension (table 1). Body mass index increased with increasing category of diastolic and systolic blood pressure. The mean total cholesterol level of men with a diastolic blood pressure between 75 and 94 mmHg was significantly higher than that of men in the lowest diastolic blood pressure category. HDL cholesterol was lowest among men on anti-hypertensive medication compared to the reference blood pressure categories. There were no other significant differences between blood pressure categories.

Diastolic blood pressure was not associated with the incidence of coronary heart disease (p-linear trend = 0.38 and p-quadratic trend = 0.69), but there was a U-shaped association with mortality from this disease (table 2). The adjusted relative risk for sudden cardiac death increased gradually with increasing category of diastolic blood pressure. The highest risk was observed among men using anti-hypertensive medication. Since the associations of diastolic blood pressure with coronary heart disease and with sudden cardiac death were discordant additional analyses were performed. There was a positive association between diastolic blood pressure and mortality from coronary heart disease when sudden cardiac death had also been recorded (figure 1A). For non-sudden coronary heart disease mortality

Table 1. *Mean levels of baseline characteristics by blood pressure category among men from The Zutphen Elderly Study*

Blood pressure category	N	Age (years)	Diastolic blood pressure (mmHg)	Systolic blood pressure (mmHg)	Body mass index (kg/m^2)	Total cholesterol (mmol/L)	HDL cholesterol (mmol/L)
Diastolic blood pressure							
< 75 mmHg	145	73.3 (5.9)*	69.2 (4.5)	133.4 (15.8)	23.9 (3.4)	5.77 (1.04)	1.18 (0.36)
75 - 84 mmHg	265	71.5 (5.2)	79.6 (2.9)	142.2 (15.5)	25.3 (2.8)	6.21 (1.12)	1.11 (0.29)
85 - 94 mmHg	222	70.8 (5.0)	89.1 (2.8)	153.4 (17.3)	25.6 (2.9)	6.22 (1.15)	1.12 (0.26)
≥ 95 mmHg	147	70.8 (5.2)	100.8 (5.5)	168.4 (17.6)	26.3 (3.0)	6.08 (1.11)	1.14 (0.28)
Anti-hypertensive use	106	71.3 (5.5)	93.5 (11.2)	168.5 (22.4)	26.5 (3.8)	6.01 (0.98)	1.05 (0.25)
Systolic blood pressure							
< 140 mmHg	275	71.1 (5.4)	77.6 (8.1)	128.0 (8.1)	24.9 (3.2)	6.06 (1.09)	1.15 (0.32)
140 - 159 mmHg	282	71.4 (5.4)	84.1 (9.5)	149.1 (5.7)	25.4 (3.0)	6.15 (1.10)	1.11 (0.29)
≥ 160 mmHg	222	72.2 (5.2)	93.0 (10.1)	173.9 (12.2)	25.6 (3.0)	6.12 (1.18)	1.14 (0.28)
Anti-hypertensive use	106	71.3 (5.5)	93.5 (11.2)	168.5 (22.4)	26.5 (3.8)	6.01 (0.98)	1.05 (0.25)
Isolated systolic hypertension†							
No‡	252	71.1 (5.4)	76.2 (6.9)	127.5 (8.1)	24.7 (3.2)	6.04 (1.11)	1.15 (0.32)
Borderline§	200	72.2 (5.4)	79.6 (6.8)	148.7 (5.7)	25.1 (2.8)	6.20 (1.15)	1.12 (0.31)
Definite‖	83	73.1 (5.1)	82.8 (5.8)	171.0 (10.8)	25.5 (3.1)	6.12 (1.23)	1.13 (0.25)

*: mean (standard deviation)
†: excluding men on anti-hypertensive medication
‡: systolic blood pressure < 140 mmHg and diastolic blood pressure < 90 mmHg
§: systolic blood pressure between 140 and 159 mmHg and diastolic blood pressure < 90 mmHg
‖: systolic blood pressure ≥ 160 mmHg and diastolic blood pressure < 90 mmHg

Table 2. *Crude rates and adjusted relative risks for mortality from coronary heart disease, sudden cardiac and all-causes according to categories of diastolic blood pressure in elderly men followed for five years (The Zutphen Elderly Study: 1985 - 1990)*

Cause of death		Diastolic blood pressure (mmHg) category					*p*-value linear trend	*p*-value quadratic trend
		< 75 (n = 145)	75 - 84 (n = 265)	85 - 94 (n = 222)	≥ 95 (n = 147)	Anti-hypertensive use (n = 106)		
Coronary heart disease	Rate*	20.8	9.9	12.2	11.7	14.7		
	RR†	1.00	0.52 (0.22 - 1.24)	0.59 (0.24 - 1.47)	0.83 (0.30 - 2.31)	1.40 (0.49 - 4.01)	0.36	0.040
Sudden cardiac	Rate*	8.0	8.2	10.3	14.6	16.8		
	RR†	1.00	1.42 (0.37 - 5.51)	2.03 (0.53 - 7.73)	3.04 (0.75 - 12.37)	4.32 (1.01 - 18.40)	0.013	0.98
All-causes	Rate*	73.6	52.6	35.6	49.7	63.1		
	RR†	1.00	0.98 (0.64 - 1.48)	0.72 (0.44 - 1.16)	1.10 (0.65 - 1.83)	1.47 (0.87 - 2.49)	0.25	0.039

*: /1,000 person years

†: relative risk (95% confidence interval) adjusted for age, body mass index, serum total and HDL cholesterol, cigarette smoking, alcohol consumption, use of medications known to lower blood pressure levels without an indication for hypertension and physician who took blood pressure

a U-shaped association was observed (figure 1A). Concerning all-cause mortality, there was a significant U-shaped association with diastolic blood pressure. Additional analyses did not reveal clear associations between diastolic blood pressure and cancer mortality (p-linear trend = 0.85, p-quadratic trend = 0.27) nor mortality from causes other than cardiovascular diseases or cancer (p-linear trend = 0.32, p-quadratic trend = 0.25).

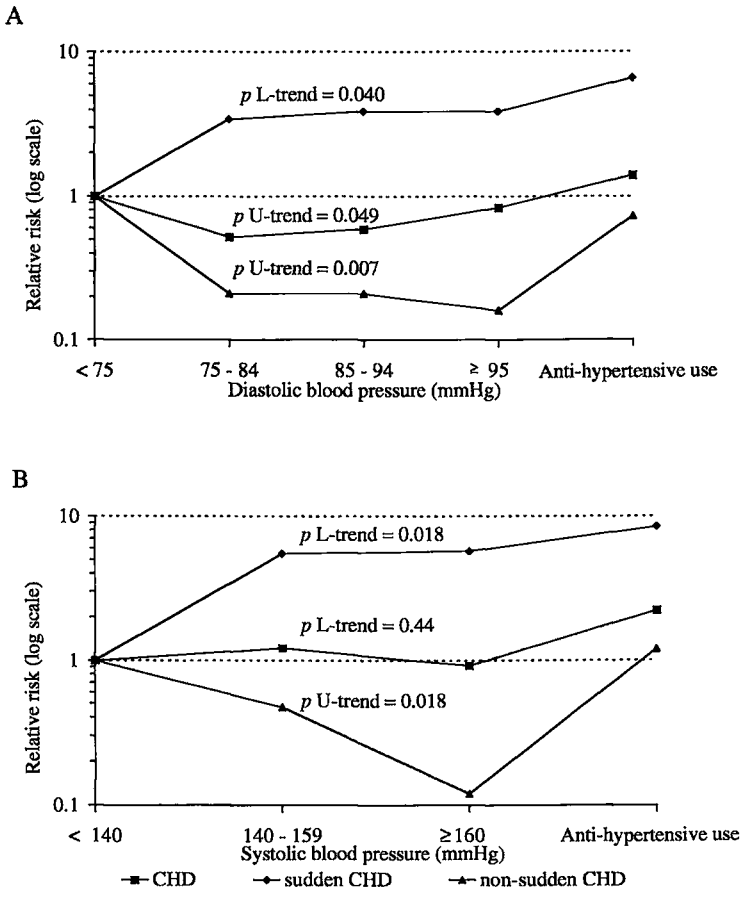

Figure 1. *Adjusted relative risks for mortality from coronary heart disease and from coronary heart disease which was not additionally recorded as sudden according to diastolic (A) and systolic (B) blood pressure categories in elderly men followed for five years (The Zutphen Elderly Study: 1985 - 1990)*

Systolic blood pressure was not significantly associated with the incidence of coronary heart disease (p-linear trend = 0.35, p-quadratic trend = 0.78) nor with mortality due to the disease (table 3). However, a strikingly strong association was observed between systolic blood pressure and sudden cardiac death with the highest adjusted relative risk observed among men using anti-hypertensive medication. Additional analyses revealed a positive association between systolic blood pressure and mortality from coronary heart disease when sudden cardiac death had also been recorded (figure 1B). For non-sudden coronary heart disease mortality however, the association was U-shaped (figure 1B). Systolic blood pressure tended to be positively associated with all-cause mortality and the relative risk reached statistical significance for men on anti-hypertensive medication. Systolic blood pressure was not associated with death due to cancer (p-linear trend = 0.61, p-quadratic trend = 0.57) nor death due to causes other than cardiovascular diseases and cancer (p-linear trend = 0.48, p-quadratic trend = 0.46).

Regarding isolated systolic hypertension, the association with sudden cardiac death was even stronger than that described for systolic blood pressure (table 4). Associations with coronary heart disease and all-cause mortality were lacking.

Age appeared to be an effect modifier in the associations of diastolic and systolic blood pressure with sudden cardiac and all-cause mortality. Additional analyses were therefore performed for younger and older men using the median age of 71 years as a cut-off value (table 5). Regarding sudden cardiac death, the younger men using anti-hypertensive medication had a five-fold higher rate than the older men using this medication. Apart from the men using anti-hypertensive medication, the differences in rates (i.e. the absolute risks) between blood pressure categories were generally larger for the older men, whereas the rate ratios (i.e. the relative risks) were generally smaller. Regarding all-cause mortality, the U-shaped association with diastolic blood pressure was more pronounced in the younger than in the older men when relative risks were compared. However, the absolute risks between categories of diastolic blood pressure were similar for both age groups. For systolic blood pressure the association with all-cause mortality was more pronounced in younger compared to older men and this was observed comparing relative as well as absolute risks.

The use of medication known to lower blood pressure levels without an indication for hypertension was also an effect modifier in the associations of diastolic blood pressure with sudden cardiac death (p = 0.024) and all-cause mortality (p = 0.064) and of systolic blood pressure with all-cause mortality (p = 0.051). Additional analyses revealed that the associations were stronger among non-users of this type of medication. Regarding all-cause mortality, the adjusted association with diastolic blood pressure was linear instead of U-shaped (p-linear trend = 0.038).

Table 3. *Crude rates and adjusted relative risks for mortality from coronary heart disease, sudden cardiac and all-causes according to categories of systolic blood pressure in elderly men followed for five years (The Zutphen Elderly Study: 1985 - 1990)*

Cause of death		Systolic blood pressure (mmHg) category				*p*-value linear trend	*p*-value quadratic trend
		< 140 (n = 275)	140 - 159 (n = 282)	≥ 160 (n = 222)	Anti-hypertensive use (n = 106)		
Coronary heart disease	Rate*	10.9	15.5	11.8	14.7		
	RR†	1.00	1.21 (0.58 - 2.52)	0.92 (0.39 - 2.18)	2.21 (0.83 - 5.94)	0.36	0.28
Sudden cardiac	Rate*	2.3	13.9	14.7	16.8		
	RR†	1.00	4.80 (1.36 - 16.95)	5.56 (1.54 - 20.08)	8.70 (2.11 - 35.90)	0.002	0.17
All-causes	Rate*	45.4	52.6	54.9	63.1		
	RR†	1.00	1.11 (0.76 - 1.61)	1.10 (0.74 - 1.63)	1.70 (1.04 - 2.76)	0.096	0.28

*: /1,000 person years

†: relative risk (95% confidence interval) adjusted for age, body mass index, serum total and HDL cholesterol, cigarette smoking, alcohol consumption, use of medications known to lower blood pressure levels without an indication for hypertension and physician who took blood pressure

Table 4. *Crude rates and adjusted relative risks for mortality from coronary heart disease, sudden cardiac and all-causes according to isolated systolic hypertension category in elderly men followed for five years (The Zutphen Elderly Study: 1985 - 1990)*

Cause of death		Isolated systolic hypertension category*			*p*-value linear trend
		No (n = 252)	Borderline (n = 200)	Definite (n = 83)	
Coronary heart disease	Rate†	10.3	15.3	19.4	
	RR‡	1.00	1.18 (0.50 - 2.78)	1.55 (0.51 - 4.66)	0.45
Sudden cardiac	Rate†	1.7	13.2	22.2	
	RR‡	1.00	4.23 (0.86 - 20.76)	9.20 (1.76 - 47.97)	0.005
All-causes	Rate†	47.2	54.8	74.8	
	RR‡	1.00	1.03 (0.68 - 1.56)	1.24 (0.74 - 2.08)	0.46

*: for definition see footnotes from table 1

†: /1,000 person years

‡: relative risk (95% confidence interval) adjusted for age, body mass index, serum total and HDL cholesterol, cigarette smoking, alcohol consumption, use of medications known to lower blood pressure levels without an indication for hypertension and physician who took blood pressure

Table 5. *Crude rates of sudden cardiac death and all-cause death according to categories of diastolic and systolic blood pressure by age in elderly men followed for five years (The Zutphen Elderly Study: 1985 - 1990)*

	Sudden cardiac death		All-cause death	
	Age < 71 years (n = 330)	Age ≥ 71 years (n = 455)	Age < 71 years (n = 330)	Age ≥ 71 years (n = 455)
Number of cases	17	27	57	155
Diastolic blood pressure				
p-value for interaction*	0.006		0.072	
< 75 mmHg	3.9†	10.8	43.2	94.5
75 - 84 mmHg	1.7	14.6	23.4	81.0
85 - 94 mmHg	6.9	14.4	12.0	63.9
≥ 95 mmHg	10.1	20.9	23.8	73.1
Anti-hypertensive use	25.5	5.0	43.7	89.8
Systolic blood pressure				
p-value for interaction*	0.010		0.072	
< 140 mmHg	0.0	5.0	17.8	76.2
140 - 159 mmHg	8.8	19.7	30.6	77.3
≥ 160 mmHg	8.5	20.0	25.4	80.1
Anti-hypertensive use	25.5	5.0	43.7	89.8

*: *p*-value for interaction of age with blood pressure in age-adjusted model
†: Rate (/ 1,000 person years)

Discussion

The main findings of this study show that diastolic and systolic blood pressures, especially isolated systolic hypertension, are independently associated with the risk of sudden cardiac death in elderly men. Associations with coronary heart disease were less clear. However, for coronary heart disease mortality which was not recorded as sudden cardiac death, the highest risk was observed in men at the lower end of the blood pressure distribution and in men on anti-hypertensive medication. Regarding all-cause mortality, the associations with diastolic and systolic blood pressures were positive among men who were not using medications which may lower blood pressure levels without an indication for hypertension.

Sudden cardiac death and coronary heart disease

Both diastolic and systolic blood pressures were strong predictors of sudden cardiac death in elderly men in this study. To our knowledge, this end-point has not previously been investigated in relation to blood pressure specifically in elderly persons, but the evidence for a positive association is convincing in middle-aged populations.[33] The ascertainment of sudden cardiac death may have been more prone to misclassification than the other end-points in this study since the exact time between the first occurrence of symptoms and death was not always available. However, differential misclassification is unlikely and thus cannot be an explanation for our findings. A possible biologic mechanism underlying the observed association may be an increased sympathetic drive unopposed by parasympathetic stimulation. This change in autonomic control has been described to occur with advancing age. Norepinephrine increases[34] and baroreflex sensitivity declines[7] with advancing age leading to increased sympathetic activity. In addition, sympathetic stimulation increases blood pressure levels. Among the same group of elderly men from Zutphen, a prolonged heart rate adjusted QT interval on the electrocardiogram was shown to be independently associated with the risk of sudden cardiac death.[35] It could be hypothesized that this abnormality in the electrocardiogram results from ventricular electrical instability of the heart which in turn may be a consequence of increased sympathetic activity. Evidence is accumulating for an important role of increased sympathetic activity, and thus of increased blood pressure levels, in predicting sudden cardiac death[36] especially in elderly people.

No clear associations were observed between blood pressure levels and the incidence of coronary heart disease in the present study. Although we included the incidence of angina pectoris in the definition of coronary heart disease, which is rather uncommon, additional analyses including only myocardial infarction and mortality from coronary heart disease (ICD: 410 - 414) as an end-point also did not reveal an association with systolic blood pressure either. Clear linear relations between blood pressure and coronary heart disease have also been lacking in other studies of elderly persons.[2] However, diastolic blood pressure showed a U-shaped relation with mortality from the disease and strong U-shaped associations of both diastolic and systolic blood pressure levels with non-sudden coronary heart disease mortality were observed in this study. These findings may in part be explained by an increase in arterial stiffness[6-8] and a reduction in diastolic coronary flow[8,10] with advancing age. These changes have been associated with both a reduced diastolic blood pressure[6-8] and an increase in atherosclerosis[6,9] and thus coronary heart disease.[6] Concerning systolic blood pressure, we observed the lowest risk of non-sudden coronary heart disease mortality among men with a pressure of 160 mmHg or higher and not on anti-hypertensive medication. An elevated pressure may be necessary in the

presence of stiffer arteries to guarantee adequate blood flow through the arteries[4] and may therefore be protective of non-sudden coronary heart disease mortality.

Both possible underlying mechanisms, increased sympathetic activity and increased arterial stiffness accompanied by a reduced diastolic coronary flow, are probably present in different degrees in older people. The underlying factor or factors determining which mechanism predominates in elderly men is not clear. Although an effort was made to distinguish between these possible mechanisms through the unique definition of the end-points, i.e. sudden and non-sudden coronary heart disease death, it cannot be deducted from this study whether these mechanisms drive the observed associations.

All-cause mortality

Regarding all-cause mortality, there was an indication for a U-shaped association with diastolic blood pressure and a linear positive association with systolic blood pressure, but the relative risks in the different categories did not reach statistical significance. However, the absolute excess risk of death in some of the blood pressure categories is considerable compared to the baseline category, although the relative risk remains small because of a high baseline rate. This phenomenon is often encountered in prospective studies in elderly populations.[37] Bulpitt and Fletcher recently reviewed the epidemiologic evidence for the relationship between blood pressure and mortality in elderly people and observed that the relationship was still not clear among the very old.[2] Very high baseline rates may offer an explanation, as we also observed in the age-stratified analyses. Another possible explanation for the weak association between blood pressure and all-cause mortality, and especially the U-shaped association with diastolic blood pressure, could be increased morbidity with advancing age. The presence of illness may lower the blood pressure[5,11,21-23] and increases the risk of death.[23] However no clear associations were observed between blood pressure levels and mortality due to causes other than cardiovascular diseases. Additional adjustment for self-rated health and the presence of chronic diseases at baseline, such as cardiovascular diseases and cancer, also did not alter any of the associations investigated (results not shown). However, the U-shaped association between diastolic blood pressure and all-cause mortality became a positive linear association when men using blood pressure lowering medication without an indication for hypertension were excluded from the analyses. This type of medication is usually prescribed to treat cardiovascular diseases, and is thus indicative of the presence of these diseases in people while, at the same time, the blood pressure is lowered. Taylor and coworkers therefore postulated that treatment for cardiovascular diseases obscures the association of blood pressure with all-cause mortality.[21] In a recent Norwegian study among people 65 years or older,[22] the exclusion of men using blood pressure lowering medication substantially

reduced the J-shaped association between diastolic blood pressure and all-cause mortality. There may be a third phenomenon involved in our study. Possibly, associations between blood pressure variables and all-cause mortality are weak because of a dilution effect of positive associations observed with specific causes of death, such as sudden cardiac causes and cerebrovascular disease (results not shown). Indeed 141 men died of causes other than sudden cardiac causes or cerebrovascular disease.

Isolated systolic hypertension, and systolic versus diastolic blood pressure

We observed that isolated systolic hypertension was a very strong predictor of sudden cardiac death. This type of hypertension has long been recognized as an important risk factor for cardiovascular diseases in elderly people.[18] The prevalence of isolated systolic hypertension increases with age,[18] and is the result of decreased arterial compliance with advancing age.[6] Apart from isolated systolic hypertension, we also observed systolic blood pressure per sé to be a stronger predictor of sudden cardiac death than diastolic blood pressure. Although the importance of systolic over diastolic blood pressure in predicting cardiovascular diseases increases with age, this predominance of systolic blood pressure has also been observed in middle aged people.[38] Differences in the reproducibility of diastolic versus systolic blood pressure measurements may partly explain the differences in their predictive value.

Age as an effect-modifier

The associations of blood pressure levels with sudden cardiac and all-cause death were stronger in the younger than the older men of this cohort. However, the absolute rates and rate differences tended to be larger in the older men as compared to the younger ones. Therefore, the absolute impact of elevated blood pressure levels in an older population may be substantial especially when compared to middle-aged populations.[37] In contrast, for men on anti-hypertensive medication the rates of sudden cardiac death were five-fold higher in the younger men as compared to the older men. These findings may be explained by selective survival of older men on anti-hypertensive medication.

Anti-hypertensive medication use

Men on anti-hypertensive medication were at the highest risk of coronary heart disease, sudden cardiac death and all-cause death. The untreated blood pressure level of these men is unknown and may be much higher than the level measured during the examination, which was already higher than the blood pressure of the rest of the cohort. In addition, men on anti-hypertensive medication generally had the highest prevalence of other risk factors for cardiovascular diseases. Therefore, these findings do not suggest that the use

of anti-hypertensive medication is causally associated with an increased risk of these end-points. Rather, men on anti-hypertensive medication in this study form a distinct group with a clustering of risk factors for cardiovascular diseases, thus a group at increased risk for cardiovascular diseases. Regarding anti-hypertensive medication use in elderly people, clinical trials have provided evidence for significant reductions in blood pressure levels and in cardiovascular diseases and all-cause mortality in people up to 80 years of age using diuretics and or beta-blockers.[2,39] Whether pharmacological treatment is beneficial after 80 years of age has yet to be evaluated.[2]

The findings from this study suggest that, among elderly men followed for five years, elevated diastolic and systolic blood pressure, and especially isolated systolic hypertension are important predictors of sudden cardiac death. Elderly men on antihypertensive medication may form a distinct group with a clustering of risk factors for coronary heart disease and at the highest risk of coronary heart disease, sudden cardiac and all-cause death. Apart from this group, there is a suggestion for an inverse association between blood pressure levels and coronary heart disease mortality in elderly men. Both diastolic and systolic blood pressure levels are suggested to be positively associated with all-cause mortality when possible comorbidity at the time of blood pressure measurements has been accounted for.

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Chapter 3
Serum total cholesterol, systolic blood pressure and the long-term risk of coronary heart disease mortality in elderly men and women

Abstract

Serum total cholesterol and systolic blood pressure were investigated as risk factors for mortality from ischemic heart disease among 272 elderly men and women during 17 years of follow-up. For men, total cholesterol was not significantly associated with mortality from ischemic heart disease. Among women, a significant positive association was found (p-trend = 0.03 when adjusted for age, body mass index, systolic blood pressure, alcohol consumption, smoking, and the prevalence of myocardial infarction, angina pectoris and diabetes mellitus). Among women a significant positive association was also observed for systolic blood pressure after adjustment for all potential confounders (p-trend = 0.05). Among men, the adjusted association with systolic blood pressure was not statistically significant. The results suggest that total cholesterol and systolic blood pressure are stronger independent risk factors for mortality from ischemic heart disease among elderly women than among elderly men. These differences between gender may be due to selective mortality among middle-aged men and physiological changes in women during menopause.

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Introduction

The total elderly population (65 years and older) in The Netherlands is expected to increase by 100% in the next 50 years. The total number of very old individuals (80 years and older) will increase by 150% in the same period. Despite some improvements in survival over the past ten years, ischemic heart disease continues to be the leading cause of death among the elderly.[1] Therefore prevention of ischemic heart disease in the elderly deserves special attention. However, most studies on risk factors for ischemic heart disease have focussed specifically on middle-aged populations.

In middle-aged men and women, high serum total cholesterol levels, high blood pressure and smoking have been identified as major risk factors for morbidity and mortality due to ischemic heart disease.[2,3] It remains questionable, however, whether these risk factors retain their predictive power among the elderly.[1,4-12] Many studies have shown that the associations of serum total cholesterol, blood pressure and smoking with ischemic heart disease decrease with age.[5,6,8,9,11,12] Several reasons for this progressive decline have been suggested. In the first place, a true picture of mortality from ischemic heart disease among the elderly is difficult to obtain because of comorbidity and comortality.[13] In addition, the predictive force of risk factors may decrease because those most sensitive or more exposed to the risk factor die first, and the remaining population on the average consists of more resistant or less exposed individuals.[1] Furthermore, in many studies the follow-up time, and thus possibly the duration of exposure, may have been too short to detect an association between risk factors and mortality from ischemic heart disease in the elderly.[6,11]

In the present 17-year follow-up study, the predictive power of serum total cholesterol and systolic blood pressure for mortality from ischemic heart disease among elderly men and women was investigated.

Methods

In 1971 a longitudinal health survey was started by one of the authors (CHB) among eligible men and women from his general practice in Rotterdam, The Netherlands. All of his non-institutionalized patients, born before 1907 and able to participate, were invited to take part in the study. Of these 394 subjects 340 (86%) agreed to participate. Due to limited laboratory facilities a total of 292 patients was examined medically in 1971.

During the physical examination, information on the medical history was obtained from the Dutch translation of the questionnaire developed by the London School of Hygiene and Tropical Medicine.[14] Weight and height were measured with patients wearing underwear and socks only. A fasting blood sample was obtained by venipuncture and analyzed for serum total cholesterol by thin layer chromatography.[15] This method was compatible with the Abell-Kendall method. The results were verified by the WHO Regional Lipid Reference Center of the Institute for Clinical and Experimental Medicine in Prague, Czechoslovakia, and were found to be within recommended limits.[16] Blood pressure was measured in the right arm with subjects in the supine position, using a mercury sphygmomanometer. The measurements were carried out in triplicate and the lowest values of the systolic and diastolic (fourth Korotkoff phase) blood pressures were recorded. Hypertension was defined, largely in accordance with the WHO,[17] as a systolic blood pressure greater than or equal to 160 mmHg or a diastolic blood pressure greater than or equal to 95 mmHg or the use of anti-hypertensive medication. Because of possible misclassification of the diastolic blood pressure due to measurement at the fourth Korotkoff sound instead of the fifth, the present study specifically focussed on systolic blood pressure. Information on smoking habits was obtained from a standardized questionnaire. Smoking was defined as smoking cigarettes, pipe or cigars at baseline. Since nearly all men (70.8%) and almost none of the women (18.5%) in this study smoked, the associations between smoking and mortality from ischemic heart disease were not investigated. Information on alcohol consumption was obtained using the cross-check dietary history method,[18] carried out by one trained dietitian. Drug use was determined by studying the patients' records.

Information on the vital status of all 292 participants was obtained in 1988, covering the period January 1=, 1971 through December 31, 1987. Information on the causes of death was obtained from the Dutch Central Bureau of Statistics. The causes of death were coded according to the 9th Revision of the International Classification of Diseases (ICD). Because elderly people often suffer from multiple diseases, making it difficult to determine the underlying cause of death, ischemic heart disease was defined as ICD-codes 410-414 recorded for either the primary or secondary cause of death. Twenty subjects were excluded from the analysis: blood samples were not drawn from seventeen individuals, one person was lost to follow-up and the cause of death of two people was unknown.

Statistical analysis was carried out using the SAS program (SAS Institute Inc., Cary, North Carolina, USA, 1989, version 6.07). Analyses were carried out separately for men and women and all tests were two-tailed. Differences in risk factor levels between the levels of categorical variables were tested using Student's t-tests. In case of skewness of the risk factor distributions, the Mann-Whitney U-test was used. For differences in levels of categorical variables (smoking, alcohol intake, prevalence of myocardial infarction or

angina pectoris, clinical diabetes mellitus and hypertension) the χ^2-test statistic was calculated. Mortality rates for ischemic heart disease were computed per sex-specific tertiles for serum total cholesterol and systolic blood pressure. The proportionality assumption of Cox's proportional-hazard (survival) analysis was tested by including a time-dependent explanatory variable in the models and testing the significance of its regression coefficient.[19] Since the regression coefficients were not significant, the proportionality assumption was regarded to be satisfied and survival analysis was carried out to investigate the association between serum total cholesterol, systolic blood pressure and mortality from ischemic heart disease adjusted for selected confounders. Dummy variables for serum total cholesterol and systolic blood pressure were used in the analysis to compare tertiles separately. *P*-values for trend were derived by testing the significance of the regression coefficients for the categorized cholesterol and blood pressure variables (coded 0,1,2). The difference between men and women in the association of risk factors with mortality from ischemic heart disease was tested by including an interaction variable of gender with the specific risk factor in the model and testing the significance of its regression coefficient. Interactions of selected confounders with each specific risk factor were tested in a similar manner, and no significant interactions were observed.

Results

In 1971, the cohort consisted of 137 men and 135 women between the ages of 64 and 87 years. During the 17-year follow-up period 188 subjects died, 34 men and 24 women died of ischemic heart disease. For eight of these 58 subjects ischemic heart disease was a secondary cause of death. The overall mortality rate for ischemic heart disease was 19.6 per 1,000 person years (py) and was significantly higher for men (25.4 per 1,000 py) than for women (14.8 per 1,000 py) ($p < 0.05$). The baseline mean serum total cholesterol level was significantly lower in men than in women: 6.44 ± 1.32 mmol/L versus 6.98 ± 1.29 mmol/L ($p < 0.001$). Systolic blood pressure was also significantly lower in men than in women: 150.5 ± 22.4 mmHg versus 167.9 ± 24.5 mmol/L ($p < 0.001$). At baseline 44.5% of the men versus 71.1% of the women were classified as hypertensive ($p < 0.001$).

For both men and women, serum total cholesterol was inversely associated with age. Serum total cholesterol levels were significantly lower in participants 70 years or older than in those younger than 70 years ($p < 0.01$ for men and $p < 0.05$ for women). Systolic blood pressure was significantly higher in men aged 70 years or older compared to those younger than 70 years ($p < 0.001$) and in men with diabetes compared to those without diabetes ($p < 0.05$).

Baseline values of selected risk factors according to prevalence of ischemic heart disease at baseline are presented in table 1. Among both men and women there were no significant differences in the selected risk factors between subjects with and without ischemic heart disease at baseline.

Table 1. *Baseline values of selected risk factors for 272 elderly men and women according to the prevalence of ischemic heart disease (IHD) at baseline*

Risk factor	Men		Women	
	IHD* at baseline		IHD* at baseline	
	No (n=106)	Yes (n=31)	No (n=108)	Yes (n=27)
	Mean ± Standard Deviation			
Age (years)	71.6 ± 5.6	69.9 ± 4.2	70.1 ± 4.6	70.9 ± 3.7
BMI (kg/m^2)	24.81 ± 2.95	25.07 ± 2.65	27.53 ± 3.97	26.54 ± 2.30
Serum total cholesterol (mmol/L)	6.38 ± 1.34	6.63 ± 1.27	7.06 ± 1.34	6.67 ± 1.03
Systolic blood pressure (mmHg)	150.0 ± 23.1	152.1 ± 20.1	167.9 ± 24.3	167.8 ± 25.7
Diastolic blood pressure (mmHg)	86.5 ± 11.8	88.7 ± 10.6	93.2 ± 11.7	90.6 ± 11.3
	Percentage			
Smoking	74.5	58.1	20.4	11.1
Alcohol (> 0g/day)	71.7	74.2	38.9	25.9
Diabetes mellitus†	7.6	0.0	10.2	18.5
Hypertension‡	40.6	58.1	70.4	74.1

*: Prevalence of myocardial infarction and/or angina pectoris in 1971
†: Clinical diagnosis
‡: Systolic blood pressure ≥ 160 mmHg and/or diastolic blood pressure ≥ 95 mmHg and/or on anti-hypertensive medication

In table 2 baseline values of selected risk factors according to mortality from ischemic heart disease are shown. Systolic blood pressure and the prevalence of myocardial infarction or angina pectoris were significantly higher among men who died of ischemic heart disease than those who did not. There were no significant differences in serum total cholesterol levels, smoking habits and prevalence of hypertension between the two groups of men. Among women serum total cholesterol levels, systolic blood pressure and prevalence of hypertension were significantly higher for those who died of ischemic heart disease than those who did not.

Table 2. *Baseline values of selected risk factors for 272 elderly men and women according to mortality from ischemic heart disease (IHD) during 17 years of follow-up (1971 - 1987)*

Risk factor	Men		Women	
	Died of IHD		Died of IHD	
	No (n=103)	Yes (n=34)	No (n=111)	Yes (n=24)
	Mean ± Standard Deviation			
Age (years)	70.9 ± 5.4	72.2 ± 5.0	70.0 ± 4.4	71.5 ± 4.5
BMI (kg/m^2)	24.69 ± 2.72	25.40 ± 3.29	27.31 ± 3.79	27.43 ± 3.41
Serum total cholesterol (mmol/L)	6.44 ± 1.39	6.44 ± 1.10	6.89 ± 1.32	7.42 ± 1.07*
Systolic blood pressure (mmHg)	147.7 ± 21.6	159.0 ± 23.0*	164.7 ± 22.8	182.3 ± 27.3†
Diastolic blood pressure (mmHg)	86.6 ± 12.4	88.4 ± 8.4	91.9 ± 11.5	96.3 ± 11.6
	Percentage			
Smoking	70.9	70.6	18.9	16.7
Alcohol (> 0g/day)	70.9	76.5	33.3	50.0
Myocardial infarction and/or angina pectoris	12.6	52.9‡	18.9	25.0
Diabetes mellitus§	6.8	2.9	9.9	20.8
Hypertension‖	40.8	55.9	66.7	91.7*

*: $p \leq 0.05$
†: $p \leq 0.01$
‡: $p \leq 0.001$
§: Clinical diagnosis
‖: Systolic blood pressure ≥ 160 mmHg and/or diastolic blood pressure ≥ 95 mmHg and/or on anti-hypertensive medication

The mortality rate for ischemic heart disease was highest among men in the medium cholesterol tertile, but the difference with respect to the mortality rates for the other tertiles was not statistically significant (table 3). For women, the mortality rate for ischemic heart disease increased with each cholesterol tertile. Regarding systolic blood pressure, mortality rates for ischemic heart disease increased with each tertile for both men and women. The mortality rate for men in the highest tertile of systolic blood pressure was significantly higher than the rate for the lowest tertile ($p < 0.05$). Mortality rates for women in the medium and highest tertiles were significantly higher than the rate for the lowest tertile of systolic blood pressure ($p < 0.05$ in both cases).

Table 3. *Mortality rates (per 1,000 person years) for ischemic heart disease among men and women after 17 years of follow-up (1971 - 1987), listed according to the tertiles of serum total cholesterol and systolic blood pressure*

Risk factor category	Men (n=137)	Women (n=135)
	*Serum total cholesterol**	
Low	20.7 (8)†	7.1 (4)
Medium	36.5 (16)	17.9 (9)
High	19.5 (10)	19.8 (11)
	Systolic blood pressure‡	
Low	13.9 (6)	5.4 (3)
Medium	21.1 (11)	18.4 (11)§
High	44.5 (17)§	21.6 (10)§

*: For men the cut-off points for the tertiles of serum total cholesterol were 5.84 mmol/L and 6.90 mmol/L. For women the cut-off points were 6.34 mmol/L and 7.32 mmol/L

†: In parentheses, the number of deaths due to ischemic heart disease

‡: For men the cut-off points for the tertiles of systolic blood pressure were 135 mmHg and 155 mmHg. For women the cut-off points were 155 mmHg and 175 mmHg

§: $p \leq 0.05$, significant difference with respect to the lowest tertile of systolic blood pressure

For men, serum total cholesterol was not significantly associated with mortality from ischemic heart disease when adjusted for age and also not when additionally adjusted for body mass index, systolic blood pressure, alcohol consumption, smoking, and the prevalence of myocardial infarction, angina pectoris and diabetes mellitus (p-trend = 0.68) (table 4). Among women, serum total cholesterol was significantly associated with mortality from ischemic heart disease when adjusted for age and for the other selected risk factors (p-trend = 0.03). For women in the highest cholesterol tertile (> 7.32 mmol/L) the age-adjusted relative risk for mortality from ischemic heart disease was 3.8 with a 95% confidence interval (CI) excluding unity (95% CI: 1.2-12.2). When adjusted for the other selected risk factors, the relative risk for women in the highest cholesterol tertile remained significantly greater than one (relative risk = 3.9, 95% CI: 1.1-13.7). The adjusted association between serum cholesterol and mortality from ischemic heart disease was significantly different between men and women (p = 0.04).

The age-adjusted relative risk for mortality from ischemic heart disease for men increased with each tertile of systolic blood pressure (table 4). When the model was adjusted for all potential confounders, including anti-hypertensive and/or diuretic medication, there was no significant association between systolic blood pressure and mortality from ischemic heart disease among men (p-trend = 0.34). Among women, systolic blood pressure was positively associated with mortality from ischemic heart disease when adjusted for age (p-trend = 0.08) and for all selected confounders (p-trend

Table 4. *Relative risks and 95% confidence intervals (in parentheses) for mortality from ischemic heart disease for the medium and the highest versus the lowest tertiles of serum total cholesterol and systolic blood pressure for 272 elderly men and women during 17 years of follow-up (1971 - 1987)*

Risk factor category	Men (n=137)		Women (n=135)		Total (n=272)*	
	Adjusted for age	Adjusted for selected risk factors†	Adjusted for age	Adjusted for selected risk factors†	Adjusted for gender and age	Adjusted for gender and selected risk factors†
			Serum total cholesterol‡			
Low	1.0	1.0	1.0	1.0		
Medium	1.9 (0.8-4.4)	2.0 (0.8-5.0)	2.9 (0.9-9.3)	2.2 (0.7-7.7)		
High	1.0 (0.4-2.6)	1.3 (0.5-3.3)	3.8 (1.2-12.2)	3.9 (1.1-13.7)		
p-trend	0.92	0.68	0.03	0.03		
			Systolic blood pressure§			
Low	1.0	1.0	1.0	1.0	1.0	1.0
Medium	1.4 (0.5-3.9)	1.5 (0.6-4.3)	3.1 (0.8-11.3)	3.6 (1.0-13.6)	2.0 (1.0-4.2)	1.5 (0.7-3.1)
High	2.5 (0.9-6.5)	1.7 (0.6-4.9)	3.5 (0.9-13.3)	4.1 (1.0-16.4)	2.3 (1.1-4.9)	1.6 (0.7-3.5)
p-trend	0.05	0.34	0.08	0.05	0.04	0.25

*: Results only shown when the association was not significantly different between men and women
†: The relative risks are adjusted for age, body mass index, alcohol consumption, prevalence of myocardial infarction and/or angina pectoris and diabetes mellitus, smoking, systolic blood pressure or serum total cholesterol and anti-hypertensive and/or diuretic medication
‡: See footnote 1 in table 3 for sex-specific cut-off points for the tertiles of serum total cholesterol
§: See footnote 2 in table 3 for sex-specific cut-off points for the tertiles of systolic blood pressure. For the total population the cut-off points were 145 mmHg and 165 mmHg

= 0.05). For women in the medium and highest tertiles of systolic blood pressure, the relative risk adjusted for all selected risk factors was significantly different from one, i.e. 3.6 (95% CI: 1.0-13.6) and 4.1 (95% CI: 1.0-16.4) respectively. The adjusted association between systolic blood pressure and mortality from ischemic heart disease did not differ significantly between men and women ($p = 0.26$). For the total population the association between systolic blood pressure and mortality from ischemic heart disease was significant when adjusted for gender and age (p-trend = 0.04) but not after additional adjustment for the other risk factors (p-trend = 0.25).

Discussion

The results of the present study suggest that serum total cholesterol and systolic blood pressure are independent risk factors for mortality from ischemic heart disease among elderly women and to lesser extent among elderly men.

Concerning serum total cholesterol, it must be noted that the cut-off points for the tertiles were higher for women than for men. However, when the cholesterol cut-off point (< 6.5 mmol/L) suggested by the Dutch cholesterol consensus was used,[20] the results were essentially the same. Recently at a National Heart, Lung, and Blood Institute workshop, the cholesterol-ischemic heart disease association was re-examined in 25 initially middle-aged populations, from 22 different studies, who had aged.[21] Contrary to the present findings, the strongest association was found among elderly men (pooled relative risk = 1.3). The data on elderly women (16 of the 25 study populations) supported a positive relationship between serum total cholesterol and ischemic heart disease, but there was clearly less consistency in the association (pooled relative risk = 1.1). However, in these studies the National Cholesterol Education Program definitions of a desirable cholesterol level (< 5.17 mmol/L) and a high-risk cholesterol level (≥ 6.20 mmol/L) were used; in the present study, according to these definitions, only 3.7% of the elderly women had a desirable cholesterol level and 71.9% had a high-risk level. This higher serum total cholesterol level could partly explain the higher relative risks found for the elderly women in our study. Of the men in the present study, 16.8% had a desirable cholesterol level and 53.3% had a high-risk level, according to the National Cholesterol Education Program definitions. Possibly these men may have also had relatively higher serum total cholesterol levels at middle-age, suggesting that selective mortality at that age may have played a more important role for these men than for those included in the meta-analysis. This could partly explain the discrepancy in the results for men.

Middle-aged men with high serum total cholesterol levels are indeed at increased risk for mortality from ischemic heart disease.[2,3] In the present study, lower mortality among men in the highest cholesterol tertile versus men in the median tertile may partly be due to selective mortality at middle age. Recently, this effect was observed in the Dutch male cohort of the Seven Countries Study, the Zutphen Study.[22] This phenomenon of 'survival of the fittest' may be less applicable to female populations. In general, middle-aged women are known to have lower serum total cholesterol levels than middle-aged men and the mortality rates for ischemic heart disease are lower for middle-aged women with elevated serum total cholesterol levels than middle-aged men with elevated cholesterol levels.[4,23] Witteman and co-workers observed that the rate of atherosclerosis increases after

ovarian involution[24] which may in part be due to the fact that serum total cholesterol levels have been shown to increase in women around menopause.[25,26] In fact, among the elderly, more women than men have cholesterol levels that exceed the cutoff point of 6.5 mmol/L; for levels above the cutoff point treatment is recommended.[20] Together with the possible absence of selective mortality in the female population of this study, these changes around menopause may offer an explanation for the association found between serum total cholesterol and mortality from ischemic heart disease among the women in this study.

Whether reduction of serum lipid levels in the elderly would have a beneficial effect, as suggested by the observations of this study, should preferably be further investigated in clinical trials. As yet only one trial of a serum cholesterol-lowering diet has been conducted among elderly men[27] and none among elderly women. The results of this trial, which ended in 1968, suggested that a cholesterol-lowering diet can reduce the morbidity and mortality rates of ischemic heart disease. The effect appeared stronger among younger men, aged 50 to 65.5 years, than for men over 65.5 years of age.

Elevated blood pressure is known to be an important risk factor for mortality from ischemic heart disease among middle-aged men and women.[2,3] In the present study, like serum total cholesterol, systolic blood pressure appears to be an independent risk factor for mortality from ischemic heart disease among elderly women but not elderly men. The cut-off points for the tertiles of systolic blood pressure were also higher for women than men. However, when hypertension (yes or no) or a variable indicating whether an individual's systolic blood pressure exceeded the 160 mmHg cut-off value was included in the fully adjusted model, the results were essentially the same. Selective mortality may also have occurred among men with high systolic blood pressure at middle age, leading to fewer but on the whole "stronger" elderly men with an elevated systolic blood pressure. This could explain the lack of association between systolic blood pressure and mortality from ischemic heart disease among the elderly men of this cohort. Nevertheless, the age-adjusted model revealed a significant trend in the association among men and the unadjusted mortality rate for men in the highest tertile of systolic blood pressure was significantly higher than that for those in the lowest tertile. Possibly the suggested lack of association between systolic blood pressure and mortality from ischemic heart disease among men in this study is due to chance. The difference in the association between men and women was not significant and among women systolic blood pressure was significantly associated with mortality from ischemic heart disease. In many studies this association has also been found to persist with age[28-30] and no differences between men and women have been reported.

Several recent trials on the effect of lowering blood pressure in elderly people have shown that clinically relevant reductions in overall mortality and that from ischemic heart disease can be achieved.[30-32] In these trials no differences in reduction of morbidity and mortality between gender have been observed. However, among very old subjects (85 years and older) J and U-shaped associations have been reported[8,9,31] and it remains questionable whether treatment is beneficial in the very old.[9,33]

Some studies have suggested that a long-term accumulated exposure to certain risk factors is needed for the development of ischemic heart disease.[34,35] The association between cholesterol or blood pressure and mortality from ischemic heart disease seems to be strongest in studies among elderly with long follow-up periods,[34,36,37] as in the present study, and weak or absent in studies with short follow-up periods, i.e. 2 years.[6,11] In the Whitehall study (18 years of follow-up) of men aged 40 to 69 years at baseline, for instance, the longer the follow-up time the more predictive the cholesterol concentration was for ischemic heart disease.[34]

As people age, competing causes of mortality increase[38] and this could theoretically have affected the outcome of the present study. To overcome part of this problem ischemic heart disease was defined as the primary or secondary cause of death. Additional survival analysis of all-cause mortality instead of mortality from ischemic heart disease as end-point revealed similar associations with serum total cholesterol or systolic blood pressure for both men and women, but these associations were not statistically significant. This may be due to the fact that only 58 of the 188 subjects who died actually had ischemic heart disease as the primary or secondary cause of death, which is a normal proportion for the Netherlands, and dilution of the effect may have occurred.

In summary, the results of the present study suggest that serum total cholesterol and systolic blood pressure are stronger independent risk factors for mortality from ischemic heart disease among elderly women than elderly men. These differences between men and women may be due to selective mortality among middle-aged men and physiological changes in women during menopause. These associations should preferably be reexamined in larger longitudinal studies of the elderly.

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Chapter 4
Total and high density lipoprotein cholesterol and the five-year risk of coronary heart disease in elderly men

Abstract

The associations of serum total and high density lipoprotein (HDL) cholesterol with coronary heart disease were investigated in men aged 64 to 84 years from the Dutch town of Zutphen during five years of follow-up. In 1985, 885 randomly selected men, 710 of whom did not have a history of clinical coronary heart disease, participated in the study. Associations were adjusted for age, body mass index, systolic blood pressure, cigarette smoking and alcohol consumption. Total cholesterol was not significantly associated with the incidence of coronary heart disease, but for mortality the relative risk (RR) corresponding to a 1.00 mmol/liter increase was 1.40 (95 percent CI 1.07-1.83). HDL cholesterol was not associated with mortality from coronary heart disease. The RR for the incidence of the disease, corresponding to a 0.26 mmol/liter increase, was 0.80 (95 percent CI 0.60-1.08). Regarding the ratio of HDL to total cholesterol, the RR for coronary heart disease incidence corresponding to a 0.05 increase amounted to 0.70 (95 percent CI 0.51-0.95). These results show that in elderly men followed for five years, both total and HDL cholesterol are important in predicting coronary heart disease. Total cholesterol seems to be a stronger risk factor for mortality from the disease, whereas HDL cholesterol is more strongly associated with the incidence of a first coronary heart disease event.

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Introduction

The associations of serum total and high density lipoprotein (HDL) cholesterol levels with the risk of coronary heart disease among middle-aged men are currently well established.[1,2] It is not clear whether these associations pertain to elderly people.[3-10] Several issues should be considered when follow-up studies are conducted among elderly people, which could explain part of this controversy.

When quality of life is considered, it seems more meaningful to investigate the incidence of a first coronary heart disease event rather than death due to this disease. However, most studies among elderly people have focused on mortality from coronary heart disease, and potential risk factors have often been measured in populations that included individuals with the disease at baseline.[4-8] Possible confounding and effect modification of observed associations due to the prevalence of coronary heart disease at baseline may be larger among older persons than among those who are younger because of the higher prevalence of coronary heart disease in the elderly.[11] In addition, due to possible increasing comorbidity and comortality with age, true associations between risk factors and incidence or mortality may be difficult to establish.[12] These problems can possibly be circumvented by conducting prospective studies among apparently disease-free older people during a relatively short follow-up period.

Besides these methodological issues, controversy concerning the predictive value of total cholesterol for coronary heart disease among the elderly may exist because HDL cholesterol has rarely been investigated in this age group. It has been suggested that with increasing age low HDL cholesterol levels may be a more important predictor of coronary heart disease than elevated total cholesterol levels.[13]

We therefore investigated serum total and HDL cholesterol as risk factors for the incidence of and mortality from coronary heart disease among elderly men during five years of follow-up.

Materials and methods

Population

The Zutphen Study is a longitudinal investigation of chronic disease risk factors initiated in 1960 among middle-aged men as the Dutch contribution to the Seven Countries Study.[14] In 1985, 555 men from the 1960 cohort were still alive and were invited for new examinations. In addition, a random sample (two of three) of all men of the same age living in Zutphen who were not part of the 1960 cohort were invited to take part in the

study. From then on, the study was continued as the Zutphen Elderly Study. Of the 1,266 men approached 939 (74 percent) agreed to participate. A total of 109 men (9 percent) could not be examined because of serious illness or death, 62 men (5 percent) had moved and 156 men (12 percent) refused to participate or could not be reached. Complete information on risk factors was available for 885 men aged 64 to 84 years. The two groups of men, those who participated since 1960 and those who did since 1985, did not differ significantly from each other with regard to coronary heart disease risk factors such as age, body mass index, total and HDL cholesterol and blood pressure levels.

Examinations

Physical examinations and dietary surveys took place between March and June 1985. Physical examinations were carried out by five trained physicians according to a standardized protocol. Height was measured to the nearest 0.1 cm and body weight was measured to the nearest 0.5 kg while the men were in underwear. Body mass index (weight (kg)/ height $(m)^2$) was calculated. Systolic and diastolic (fifth Korotkoff phase) blood pressures were measured in duplicate with a random zero sphygmomanometer at the right arm while subjects were in the supine position. These measurements were taken at the end of the physical examination. The mean of the two blood pressure values was used in the analyses. Hypertension was defined as a systolic blood pressure greater than or equal to 160 mmHg or a diastolic blood pressure greater than or equal to 95 mmHg or the use of anti-hypertensive medication regardless of the blood pressure levels.[15] Information on medication use, prescribed diets and smoking habits was assessed with a standardized questionnaire. In our study, smoking was defined as never, former, or current cigarette smoking. Amount of cigarette years (amount (cigarettes/day) × duration (years)) was also computed to investigate its correlation with other baseline variables. Information on alcohol consumption was assessed by trained dietitians with a cross-check dietary history,[16] adapted to the Dutch situation.[17] Dietary information was available for 825 men.

Nonfasting venous blood samples were taken for the analysis of serum total and HDL cholesterol. The analyses were carried out in the standardized lipid laboratory of the Department of Human Nutrition, Agricultural University, Wageningen, the Netherlands. High density lipoprotein was isolated after precipitation of apo B containing particles by dextran sulfate-Mg^{2+}.[18] Total and HDL cholesterol were determined enzymatically with the CHOD-PAP mono-testkit from Boehringer Mannheim.[19,20] Standardization was realized by using calibration sera from the Foundation of Chemical Analysis Quality Control (The Netherlands) for total cholesterol, and calibration sera were prepared according to the method of van der Haar et al.[21] for HDL cholesterol. Control sera were obtained from the Centers for Disease Control, Atlanta, Georgia, USA.

Information on the presence of diabetes mellitus was obtained through a standardized medical questionnaire during the physical examination. Both patients with insulin-dependent and non-insulin-dependent diabetes mellitus were considered in this study.

Follow-up

Information on the prevalence of coronary heart disease was obtained during the physical examination in 1985 and a similar examination between March and June 1990 with the use of the Dutch translation of a questionnaire developed at the London School of Hygiene and Tropical Medicine.[22] For men who did not participate in the 1990 examination, information on major chronic diseases was obtained from a questionnaire for nonparticipants. Coronary heart disease was considered to be present when either myocardial infarction or angina pectoris was diagnosed. For myocardial infarction the final diagnosis was based on whether two of the following three criteria were met: a specific medical history, i.e. severe chest pain lasting for more than 20 minutes and not disappearing in rest, characteristic electrocardiogram changes and specific enzyme elevations. The diagnosis of angina pectoris was based on information obtained from the Rose questionnaire.[22] The diagnoses were additionally verified with hospital discharge data and written information from the subjects' general practitioners. All information was eventually coded by a single physician, and the year of first diagnosis was recorded.

Information on the vital status of the participants was obtained till July 1990. One person had moved abroad and was lost to follow-up. The date on which he moved was used as his (censored) end-point date. Information on the causes of death was obtained from the Dutch Central Bureau of Statistics, after verification with hospital discharge data and information from the deceased's general practitioners. The causes of death were coded according to the 9th Revision of the International Classification of Diseases.[23] Because of the frequency of possible comorbidity and comortality in elderly people the underlying cause of death is often difficult to determine. Death due to coronary heart disease was therefore defined by International Classification of Diseases codes 410-414 recorded for either the primary or secondary cause of death.

The end-points investigated were the incidence of a first fatal or non-fatal coronary heart disease event (i.e. incidence of coronary heart disease), mortality from coronary heart disease, from causes other than coronary heart disease and from all-causes.

Statistical methods

Statistical analyses were carried out using the SAS program (SAS Institute Inc., Cary, North Carolina, USA, 1989, version 6.07). All tests were two sided and p-values smaller than five percent were considered statistically significant. Spearman correlation

coefficients (r) were calculated between total and HDL cholesterol levels and levels of other risk factors for coronary heart disease. Differences in risk factor levels between levels of categorical variables were evaluated using Student's t-tests and Mann-Whitney U-tests in case the risk factor distributions were skewed. For differences in levels of categorical variables the Chi-square test statistic was used. Incidence and mortality rates were computed for tertiles of total and HDL cholesterol.

Cox's proportional-hazard (survival) analysis was carried out to investigate the associations between lipid variables and the end-points of interest during five years of follow-up.[24] Relative risks (RR) for the incidence or mortality end-points are presented according to 1.00 mmol/liter (38.7 mg/dl) increase in total cholesterol and 0.26 mmol/liter (10.0 mg/dl) increase in HDL cholesterol. Associations were adjusted for age, body mass index, systolic blood pressure, cigarette smoking and alcohol consumption. No adjustment was made for the use of cholesterol lowering medication and/or diet because only 25 men were receiving cholesterol lowering treatment (3 percent of the 825 men for whom the information was available) and additional adjustment for this potential confounder did not alter the associations. Total physical activity, as calculated by Caspersen et al.,[25] was also not adjusted for because the inclusion of this variable in the multivariate models did not alter the associations and this information was available for a limited number of men (n = 827).

Interaction terms were evaluated at the 10 percent level. A significant interaction was observed between total cholesterol and body mass index. Therefore, this result is also presented separately for lean and overweight men. The chosen cut-off value was mean body mass index (25 kg/m^2, a cut-off point also often used to indicate overweight[26]).

Results

In 1985, 885 men aged 64 to 84 years (mean age 71.5 years) were examined. The mean baseline serum total cholesterol level was 6.10 (standard deviation (SD) 1.11) mmol/liter and the mean baseline HDL cholesterol level was 1.12 (SD 0.29) mmol/liter. Thirty five percent of the men were hypercholesterolemic (total cholesterol $\geq$ 6.5 mmol/liter),[27] 23 percent of the men had HDL cholesterol levels lower than 0.9 mmol/liter.

Serum total and HDL cholesterol levels were significantly correlated with each other (r = 0.13). Total cholesterol was inversely associated with age (r = -0.21) and positively associated with body mass index (r = 0.18), alcohol intake (r = 0.08), and amount of cigarette smoking years (r = 0.11). HDL cholesterol was inversely associated with body mass index (r = -0.27) and positively associated with alcohol intake (r = 0.31), but did not

vary significantly with age. All these correlation coefficients are statistically significant at the five percent level.

At the initial examination, the prevalence of coronary heart disease was 19.8 percent. Men with coronary heart disease at baseline were significantly older, had lower levels of HDL cholesterol, and a lower mean HDL/total cholesterol level than men without the disease (table 1). Baseline serum total cholesterol levels were higher among men with coronary heart disease at baseline than among men without the disease, although this difference was not statistically significant (p = 0.15). Among men with coronary heart disease in 1985 there were fewer alcohol drinkers, more former smokers, and fewer current smokers than among men without the disease (table 1).

Table 1. *Baseline values of selected risk factors according to prevalence of coronary heart disease in 1985, in 885 men aged 64 to 84 years from The Zutphen Elderly Study*

Risk factors	Prevalence of coronary heart disease*	
	No (n=710)	Yes (n=175)
	Mean (standard deviation)	
Age (years)	71.3 (5.4)	72.2 (5.3)†
Body mass index (kg/m^2)	25.5 (3.2)	25.5 (3.0)
Serum total cholesterol (mmol/liter)	6.06 (1.10)	6.24 (1.11)
HDL cholesterol (mmol/liter)	1.14 (0.30)	1.06 (0.28)‡
HDL/total cholesterol	0.19 (0.06)	0.17 (0.05)§
Systolic blood pressure (mmHg)	151.2 (21.1)	150.6 (22.9)
Diastolic blood pressure (mmHg)	85.6 (11.4)	84.7 (11.7)
	Percentage	
Hypertension	43.2	37.7
Prevalence of diabetes mellitus	6.2	8.0
Alcohol use (> 0 g/day)	75.0	64.0‡
Former cigarette smoker in 1985	48.9	61.1†
Current cigarette smoker in 1985	32.0	22.9†

*: prevalence of myocardial infarction and/or angina pectoris
†: p <0.05
‡: p <0.01
§: p <0.001

Among the 710 men free of coronary heart disease at baseline, a first fatal or non-fatal coronary heart disease event occurred in 56 men during five years of follow-up. The

incidence rate was 17.2 per 1,000 person years. During the follow-up period 212 men from the total population died (52.1 per 1,000 person years), 53 from coronary heart disease (13.0 per 1,000 person years). Four of these men had coronary heart disease coded as a secondary cause of death.

The incidence rate of coronary heart disease did not vary with tertiles of total cholesterol and decreased gradually with increasing tertiles of HDL cholesterol (table 2). The mortality rate from coronary heart disease more than doubled between the first and second tertiles of total cholesterol. For HDL cholesterol the mortality rates did not differ between tertiles of HDL cholesterol.

Table 2. *Incidence and mortality rates for coronary heart disease among elderly men aged 64 to 84 years according to tertiles of total and HDL cholesterol (The Zutphen Elderly Study, 1985)*

Tertiles	Incidence of coronary heart disease*			Mortality from coronary heart disease†		
	Range‡	N	Rate§	Range‡	N	Rate§
Total cholesterol						
Low	2.78-5.55	237	17.2	2.78-5.61	296	7.6
Medium	5.57-6.52	236	16.4	5.62-6.53	293	16.8
High	6.53-13.20	237	18.1	6.54-13.20	296	14.5
HDL cholesterol						
Low	0.56-0.97	234	22.5	0.56-0.95	292	11.2
Medium	0.98-1.22	239	17.3	0.96-1.21	303	16.5
High	1.23-3.34	237	12.0	1.22-3.34	290	11.3

*: based on 710 men free of coronary heart disease at baseline
†: based on 885 men
‡: range in mmol/liter for total and HDL cholesterol
§: rate per 1,000 person years

Survival analysis revealed that HDL cholesterol appeared to be associated with a reduced incidence of coronary heart disease (table 3). The unadjusted association was borderline significant (p for trend = 0.08), and the adjusted relative risk was of the same magnitude but was not significantly different from unity. The relative risk was lower among men without diabetes mellitus at baseline (adjusted RR = 0.74, 95 percent confidence interval (CI) 0.53-1.02, p-interaction = 0.14). HDL/total cholesterol was independently associated with a decreased incidence of coronary heart disease (adjusted RR for a 0.05 increase = 0.70, 95 percent CI 0.51-0.95). HDL cholesterol was not significantly associated with mortality from coronary heart disease.

Table 3. *Relative risks for the incidence of and mortality from coronary heart disease in men aged 64 to 84 years associated with total and HDL cholesterol (The Zutphen Elderly Study, 1985)*

	Incidence of coronary heart disease*		Mortality from coronary heart disease†	
	RR	95% CI	RR	95% CI
Total cholesterol (for 1.00 mmol/liter increase)				
Crude	1.03	0.81-1.32	1.25	0.98-1.59
Adjusted for age	1.11	0.86-1.43	1.41	1.10-1.81
Adjusted for risk factors‡	1.17	0.90-1.52	1.40	1.07-1.83
HDL cholesterol (for 0.26 mmol/liter increase)				
Crude	0.78	0.59-1.03	1.00	0.78-1.28
Adjusted for age	0.80	0.61-1.05	1.02	0.77-1.31
Adjusted for risk factors‡	0.80	0.60-1.08	1.01	0.76-1.34

*: based on 710 men free of coronary heart disease at baseline with 56 new events during follow-up
†: based on 885 men with 53 mortality cases during follow-up
‡: adjusted for age, body mass index, systolic blood pressure, cigarette smoking and alcohol consumption

Serum total cholesterol was not significantly associated with the incidence of coronary heart disease (table 3). No association was observed among men free of diabetes mellitus at baseline (p for trend = 0.79, p-interaction = 0.01). Total cholesterol was positively and independently associated with mortality from coronary heart disease in the total population. The risk factor adjusted relative risk was 1.40 and was reduced to 1.29 (95 percent CI 0.99-1.69) after additional adjustment for the prevalence of coronary heart disease and diabetes mellitus at baseline. In the association with mortality from coronary heart disease there was a significant interaction between total cholesterol and body mass index ($p = 0.005$). Total cholesterol was independently associated with increased mortality from coronary heart disease in overweight men (body mass index ≥ 25 kg/m^2) (adjusted RR = 1.70, 95 percent CI 1.22-2.38), but not in lean men (adjusted RR = 1.06, 95 percent CI 0.67-1.66).

Serum total and HDL cholesterol were not significantly associated with all-cause mortality (adjusted RR = 1.05, 95 percent CI 0.92-1.21 for total cholesterol, and RR = 0.99, 95 percent CI 0.86-1.14 for HDL cholesterol). There were no significant associations between the different lipid variables and mortality from causes other than coronary heart disease. For total cholesterol the adjusted relative risk was 0.96 (95 percent CI 0.81-1.13).

Discussion

The main results of this study show that, in elderly men followed for five years, total cholesterol is an independent risk factor for mortality from coronary heart disease, whereas HDL cholesterol seems to be a stronger risk factor for the incidence of coronary heart disease.

We did not find a convincing relation between total cholesterol and the incidence of coronary heart disease. In a previous investigation among men from the Zutphen Study followed from 1960 until 1985, an inverted U-shaped relation between quartiles of serum total cholesterol and the incidence of myocardial infarction was observed. This was a result of a selective loss of men with serum total cholesterol levels in the highest quartile due to early incidence of, or death from, myocardial infarction.[28] In five studies that were part of a recent National Heart, Lung, and Blood Institute workshop entitled "Cholesterol and Heart Disease in Older People and Women", the association between cholesterol and the incidence of coronary heart disease was also investigated.[29-33] Three of the studies[30-32] found serum total cholesterol to be an independent risk factor for the incidence of coronary heart disease among healthy men aged 65 years or older. In the Framingham Study the association was borderline significant in men aged 65 years or older (p-value for trend = 0.07).[29] In the Italian cohorts of elderly men from the Seven Countries Study, total cholesterol was not an independent risk factor for the incidence of coronary heart disease.[33] A possible explanation for these discrepancies may be the duration of follow-up. A positive and significant, or borderline significant, association was indeed observed among elderly men who were followed for at least 14 years.[29-32] The association was lacking in the Italian studies,[33] in a recent study by Krumholz and colleagues,[9] and in our study, in which the durations of follow-up were only five years or less. Thus, a longer follow-up period may be necessary to reveal an association, whether inverted U-shaped of not, between total cholesterol and the incidence of coronary heart disease in elderly men.

HDL cholesterol was measured in only two of the studies presented during the National Heart, Lung, and Blood Institute workshop in which the incidence of coronary heart disease was investigated.[29,30] Although HDL cholesterol was inversely associated with the incidence of coronary heart disease in both studies, the associations did not reach statistical significance. This is in accordance with our findings. The ratio of HDL to total cholesterol was independently associated with a decreased incidence of coronary heart disease. HDL cholesterol is probably the underlying factor driving this association since there is no evidence from this study for an independent association between total cholesterol and the incidence of coronary heart disease. The inverse association with HDL

cholesterol was especially pronounced among men initially free of diabetes mellitus. In the Bronx Aging Study,[34] a consistently low HDL cholesterol level (≤ 0.8 mmol/liter at two separate assessments) was significantly independently associated with the incidence of cardiovascular disease among men aged 75 to 85 years. In the general male population serum total cholesterol increases with age until 60 years and decreases after 70 years, whereas HDL cholesterol does not change with age.[35] It has been suggested that HDL cholesterol is more important in predicting coronary heart disease with increasing age than total cholesterol is.[13] In the Framingham Study for example, HDL cholesterol was found to be inversely associated with the incidence of coronary heart disease regardless of the total cholesterol level in men and women aged 49 to 82 years.[36] Our study confirms the importance of HDL cholesterol in predicting the incidence of coronary heart disease in elderly men followed for only five years.

For mortality from coronary heart disease, the results of this study are in accordance with those from the review of the National Heart, Lung, and Blood Institute workshop in which the associations between total cholesterol and mortality from coronary heart disease were reinvestigated in 25 initially middle-aged populations that had aged.[5] Caution is warranted in interpreting the results from this review because the relative risks were not adjusted for potential confounders. In our study age and the prevalence of coronary heart disease at baseline were the main confounding factors in the associations between lipid variables and mortality end-points. Adjustment for the prevalence of coronary heart disease and diabetes mellitus at baseline attenuated the association of total cholesterol with mortality from coronary heart disease. The presence of coronary heart disease at baseline may be considered as a confounder because the disease is associated with these mortality end-points and because of acute effects of myocardial infarction on cholesterol levels or the use of cholesterol lowering medication after the diagnosis of coronary heart disease. However, the actual event among men with the disease at baseline may have occurred a long time before baseline cholesterol measurement and treatment for high cholesterol levels was very limited in our cohort. The prevalence of the disease at baseline is, therefore, probably not a confounder but, rather, is an intermediate step in the causal pathway. Thus, additional adjustment for the prevalence of coronary heart disease may have resulted in an over-adjustment of the relative risks. Additional survival analysis among men initially free of coronary heart disease revealed a relative risk (adjusted RR = 1.3) similar to that of the entire population, indicating that the baseline prevalence of disease could not explain our findings.

It should be noted that we observed an interaction of total cholesterol with body mass index. Harris and colleagues[31] found that considerable weight loss in elderly people modified the association between total cholesterol and the incidence of coronary heart

disease, and a significant association was observed only when people who had lost weight were excluded from the analysis. The association between total cholesterol and mortality from coronary heart disease in our cohort was absent among lean men. The mean total cholesterol level of these men was lower than that of overweight men (5.87 (SD 1.09) versus 6.28 (SD 1.09) mmol/liter). A low body mass index and a low total cholesterol level could be a sign of poor health and possible subclinical disease.[37,38] Therefore this older group of lean men could form a heterogenous group including both healthy people who are at a decreased risk of morbidity and mortality and people with clinical or subclinical disease who are thereby at an increased risk of morbidity and mortality.[38] This may explain the lack of association between total cholesterol and coronary heart disease mortality among lean men in our study.

No clear association between HDL cholesterol and mortality from coronary heart disease was seen in contrast to the result for the incidence of the disease. In the Rancho Bernardo Study, which was presented at the National Heart, Lung, and Blood Institute workshop, low HDL cholesterol levels were also not associated with mortality from coronary heart disease among elderly men followed for three years.[39] Our results suggest that in elderly men and during a relatively short period of time, HDL cholesterol is important in predicting less severe coronary heart disease (i.e. not leading to death) whereas total cholesterol predicts fatal coronary heart disease. Additional analyses showed that this is especially the case for acute myocardial infarction (adjusted RR = 1.8). This is supported by the fact that angina pectoris constituted a larger portion of the incidence (39 out of 56) than of mortality from coronary heart disease (3 out of 53). It is also supported by the baseline data: HDL cholesterol was significantly lower in men with coronary heart disease and for total cholesterol the difference between men with or without coronary heart disease did not reach statistical significance. The latter finding could be due to earlier death of men with elevated total cholesterol levels[28] and survival of men with low HDL cholesterol levels. Moreover, a low HDL cholesterol level in itself is not known to be atherogenic.[40] Possibly, the protective effect of HDL cholesterol is strongest in less advanced atherogenesis, thus in men without pre-existing coronary heart disease.

Neither total nor HDL cholesterol appeared to be predictive for all-cause mortality. This would suggest an inverse association between total cholesterol and mortality from non-coronary heart disease.[41] However, such an association was not observed, and neither was an inverse association between total cholesterol and all-cause cancer as suggested by others.[42] The effect of total cholesterol on mortality from coronary heart disease has probably been diluted regarding all-cause mortality as end-point. Indeed, coronary heart disease constituted 25 percent of deaths in this study.

The use of a ratio including both total and HDL cholesterol may not be correct since it remains unknown which component of the ratio is important for the effect under study. However, such a ratio has been strongly suggested for routine use in screening to identify patients with hyperlipidemia[43] and has been identified as most efficiently predicting coronary heart disease at all ages.[44] The Framingham Study was the only one presented at the National, Heart, Lung, and Blood Institute workshop in which such a ratio was investigated and it was the only lipid predictor independently related to the incidence of coronary heart disease in elderly men.[29] This is in accordance with our results.

In summary, evidence from this study indicates that total cholesterol remains an independent predictor of mortality from coronary heart disease in elderly men and that HDL cholesterol appears to be protective of a first coronary heart disease event. Moreover, the associations were observed during a relatively short follow-up time, stressing the short term predictive importance of total and HDL cholesterol for coronary heart disease in older men.

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Chapter 5
Age related changes in total and high density lipoprotein cholesterol in elderly men

Abstract

To investigate changes in total and high density lipoprotein cholesterol concentrations with age and time in elderly men a cohort of men born between 1900 and 1920 from the Dutch town of Zutphen was examined in 1977/78 (n=571), 1985 (n=885), 1990 (n=555) and 1993 (n=345). Linear regression analysis and random effects models were used to assess cross-sectional and longitudinal age and time related changes in cholesterol concentrations. In both cross-sectional and longitudinal analyses total cholesterol decreased by 0.04 mmol/L a year with age. The longitudinal change was observed in the entire population as well as in men who participated in all four examinations (n=135), and in a subgroup of men who were free of common chronic diseases, not on cholesterol lowering medication and/or prescribed diet and rated themselves as being 'healthy' (n=64). High density lipoprotein cholesterol did not change significantly with age on a cross-sectional nor on a longitudinal basis. Among elderly men, total cholesterol diminishes with age both on a cross-sectional and longitudinal basis and high density lipoprotein cholesterol does not vary with age in any way.

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Introduction

The importance of total and high density lipoprotein cholesterol concentrations for coronary heart disease in the elderly is still a subject of debate.[1-9] Part of the controversy may be due to changing cholesterol concentrations with advancing age.[10] Concentrations measured in old age may not be representative of a lifetime exposure and may therefore attenuate associations. Total cholesterol declines with advancing age in elderly men both on a cross-sectional and longitudinal basis.[10-15] It is still unclear whether this observed decline is a consequence of selective survival,[16] clinical or subclinical disease,[12-14] other unknown factors associated with age and cholesterol concentrations,[17 18] or the aging process itself. High density lipoprotein cholesterol concentrations do not seem to vary with age on a cross-sectional basis in elderly men.[19-21] Longitudinal changes in high density lipoprotein cholesterol have not specifically been reported for elderly subjects.

We studied changes in total and high density lipoprotein cholesterol concentrations with age in older Dutch men both in a cross-sectional and longitudinal setting, and investigated whether these were independent of secular trends, selective mortality, loss to follow-up or changes in health status.

Methods

Study cohort

The Zutphen Study is a longitudinal investigation of chronic disease risk factors initiated in 1960 among middle-aged men, born between 1900 and 1919, as the Dutch contribution to the Seven Countries Study.[22] In 1985, 555 men from the 1960 cohort were still alive. In addition to this group, a new random sample (two out of three) of all men born between 1900 and 1920, living in Zutphen and not part of the 1960 cohort were selected to take part in the study. Both total and high density lipoprotein cholesterol concentrations have been measured four times between 1977/78 and 1993 (table 1).

Lipid measurements

Clinical examinations took place between March and June of each examination year. During the 1977/78 examination information from the participants was collected during the same months in one of either years. In all examination years non-fasting venous blood samples were taken for the analysis of total and high density lipoprotein cholesterol. Analyses were carried out in the standardized lipid laboratory of the Department of Human Nutrition, Agricultural University, Wageningen, The Netherlands. High density

Table 1. *Population size and participation rate according to examination year (The Zutphen Study: 1977/78 - 1993)*

	1977/78	1985	1990	1993
Invited (N)	671	1 266	721	544
Participated (N)	611	939	560	390
Participation rate (%)	91	74	78	72
Men with total and high density lipoprotein cholesterol measurements (N)	571*	885	555	345

*: high density lipoprotein cholesterol concentrations available for 570 men

lipoprotein cholesterol was isolated after precipitation of apo-lipoprotein B containing particles by heparin-Mn^{2+} in 1977/78[23] and by dextran sulphate-Mg^{2+} in the other years.[24] In 1977/78, cholesterol was determined using Huang's method,[25] which was calibrated on Abell-Kendall standardized sera. In 1985, 1990 and 1993, cholesterol was determined enzymatically with the CHOD-PAP mono-testkit from Boehringer Mannheim.[26,27] For total cholesterol standardization was realized by using calibration sera from the Lipid Standardization Laboratory from the Center for Disease Control (Atlanta, Georgia, USA) in 1977/78 and from the Foundation of Chemical Analysis Quality Control (The Netherlands) since 1985. For high density lipoprotein cholesterol, calibration sera prepared according to van der Haar and coworkers[28] were used since 1985. In all four examination rounds the deviations of total cholesterol concentrations from the control sera were below the international norm of three percent. For high density lipoprotein cholesterol the deviations from control sera were always lower than 10 percent. The combined within- and between-run coefficient of variation for control sera was lower than three percent for both total and high density cholesterol at the time of analyses in all four examination periods. In 1977/78, 1985 and 1990 cholesterol concentrations were measured in serum and in 1993 in EDTA plasma. Plasma values were multiplied by 1.030[29] to make them comparable to the serum values in the other examination years, since plasma values may be diluted due to the addition of EDTA.[29] Because of the systematic lower concentrations of total cholesterol in 1993 as compared to the concentrations in the other examination years, even after adjustment of the values, plasma of a random sample of 50 men was reanalyzed in July 1994 using the same method as in the 1985, 1990 and 1993 examinations.

Measurement of other variables

In 1985 and 1990 extensive information on cholesterol lowering medication use and prescribed diets for a high cholesterol concentration was obtained through a standardized

questionnaire. In 1993 information was only collected on prescribed diets. In 1977/78, no such information was collected. Note that only very few men were on cholesterol lowering therapy during the examinations (25 in 1985, 10 in 1990 and 8 in 1993).

In 1977/78, 1985 and 1990 information on the presence or a history of angina pectoris or myocardial infarction was obtained through the Dutch translation of the Rose questionnaire[30]. History of diabetes mellitus and any form of cancer was reported using a standardized questionnaire. All the information was verified with hospital discharge data and information from the subjects' general practitioners. In 1993 additional information on the presence or history of these diseases was obtained using a self-administered questionnaire.

Information on self-rated health was collected in 1985, 1990 and 1993 with the question: 'How do you feel?'. The four answer categories were: 'healthy', 'rather healthy', 'moderately healthy' and 'not healthy'. This information was not collected in 1977/78. Low self-rated health has been associated with an increased risk of mortality independent of traditional risk factors and a history of chronic diseases.[31] It is thought to be an indicator of underlying (subclinical) disease which may in turn be accompanied with lowered total cholesterol concentrations.

Statistical methods

In order to account for selective survival or selective participation and the possibility that the total cholesterol concentration is lowered in diseased people,[12] we analyzed three subgroups of participants. The first group comprises the total population of men who participated in at least one of the examination periods (N=1118). Another group consists of those men who participated in each examination (N=135). Finally we selected a 'healthy' group defined as those men from the former group without a history of myocardial infarction, angina pectoris, diabetes mellitus or cancer, never having used cholesterol lowering medication or prescribed diet, and rating themselves as 'healthy' or 'rather healthy' (N=64).

Statistical analyses were carried out using the SAS program (SAS Institute Inc., Cary, North Carolina, USA, 1989, version 6.09). All tests were two sided. A paired-t-test was conducted to evaluate the difference between newly analyzed total cholesterol values and the original values from the random sample of 50 men. Cross-sectional analyses were performed using linear regression analysis of total or high density lipoprotein cholesterol on age in each of the examination years. The assumption of linearity was checked visually using the plots of the studentized residuals by age. To perform the longitudinal analyses the repeated measures of cholesterol were related to age and time effects using a model which allowed for two sources of error - 1) within subjects between occasions and 2)

between subjects. Age and time related changes in total and high density lipoprotein cholesterol were estimated from the model. This was done using the SAS Proc Mixed Procedure, which deals with unbalanced data, by assuming missing observations are missing at random.[32]

Results

Total cholesterol concentrations increased between 1977/78 and 1985 and thereafter decreased with the largest drop observed in the last three years (table 2). This was

Table 2. *Mean values of baseline characteristics by examination period in all men, and in sub-groups of the total study population (The Zutphen Study: 1977/78 - 1993)*

Examination year	Maximum number of men	All men*	Men who participated in each examination (N=135)	'Healthy' men who participated in each examination (N=64)†
		Age (years)		
1977/78	571	66.3 (5.2)‡	63.7 (4.1)	63.5 (4.2)
1985	885	71.5 (5.3)	70.7 (4.1)	70.5 (4.2)
1990	555	75.1 (4.7)	75.7 (4.1)	75.5 (4.2)
1993	345	77.8 (4.4)	78.7 (4.1)	78.5 (4.2)
		Total cholesterol (mmol/L)		
1977/78	571	5.90 (1.06)	5.94 (0.97)	5.96 (1.00)
1985	885	6.10 (1.11)	6.10 (0.99)	6.13 (1.06)
1990	555	6.07 (1.13)	6.08 (1.01)	6.07 (1.05)
1993	345	5.56 (1.06)	5.43 (1.06)	5.47 (1.00)
		High density lipoprotein cholesterol (mmol/L)		
1977/78	570	1.23 (0.31)	1.20 (0.28)	1.26 (0.29)
1985	885	1.12 (0.29)	1.10 (0.27)	1.12 (0.27)
1990	555	1.16 (0.31)	1.10 (0.30)	1.13 (0.31)
1993	345	1.20 (0.35)	1.13 (0.35)	1.18 (0.36)

*: mean values are based on maximum number of men

†: men not having or ever having had angina pectoris, myocardial infarction, diabetes mellitus and cancer, not using or ever having used cholesterol lowering medication or diet, and who rated themselves to be 'healthy' or 'rather healthy'

‡: mean (standard deviation)

observed for all men, as well as for men who participated in each examination and for the 'healthy' men who participated in each examination. The drop in total cholesterol between 1990 and 1993 was not due to measurement error. New analyses for plasma total cholesterol from a random sample of 50 men in 1994 revealed no significant differences in mean concentrations as compared to the total cholesterol analyzed in 1993 (5.54 mmol/L (standard deviation 0.90) as measured in 1994 versus 5.51 mmol/L (standard deviation 0.88) as measured in 1993, p = 0.15). High density lipoprotein cholesterol exhibited a decrease between 1977/78 and 1985 and a slight increase over the later examination years (table 2). These results were similar among the three groups of men. When the birth cohorts were examined separately, the changes of total and high density lipoprotein cholesterol between examination years were generally similar with the exception of an earlier drop in total cholesterol for the oldest birth cohort as compared to the two younger cohorts (figure 1). Whether this is an age related or a secular change with time cannot be deducted from the figure.

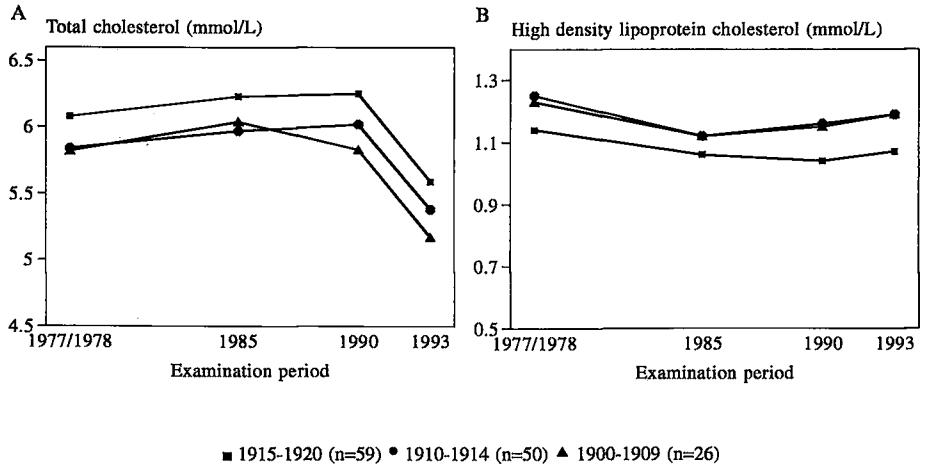

Figure 1. *Cholesterol concentrations by examination year and by birth cohort (n=135)*

On a cross-sectional basis, total cholesterol diminished significantly with age in every examination year (table 3 and figure 2). The decrease tended to be stronger in the more recent examinations, it ranged from a 0.04 mmol/L decrease a year in 1977/78 to a decrease of 0.06 mmol/L a year in 1993. High density lipoprotein cholesterol did not change consistently with age in any of the examination rounds.

Table 3. *Regression coefficients from linear regression of total or high density lipoprotein cholesterol on age and expected concentrations in a man of mean age in each examination year among men aged 58 to 91 years (The Zutphen Study: 1977/78 - 1993)*

	Regression coefficient for age*	Mean age	Expected concentration in a man of mean age (mmol/L)
		Total cholesterol (mmol/L)	
1977/78	-0.038 (-0.054; -0.022)	66.3	5.89
1985	-0.043 (-0.056; -0.029)	71.5	6.07
1990	-0.043 (-0.063; -0.024)	75.1	6.11
1993	-0.058 (-0.083; -0.033)	77.8	5.55
		High density lipoprotein cholesterol (mmol/L)	
1977/78	0.004 (-0.001; 0.009)	66.3	1.24
1985	-0.002 (-0.006; 0.001)	71.5	1.14
1990	0.004 (-0.002; 0.009)	75.1	1.18
1993	-0.002 (-0.010; 0.007)	77.8	1.16

*: regression coefficient (95 percent confidence limits)

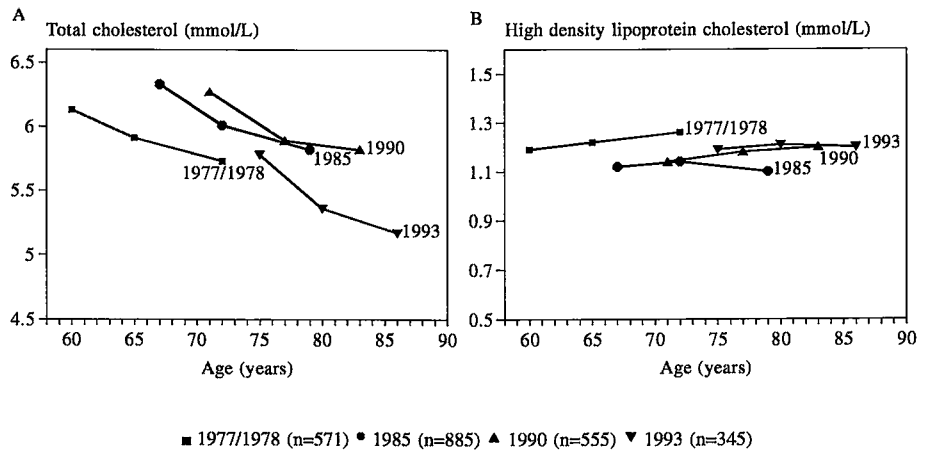

Figure 2. *Cholesterol concentrations by mean age of five-year birth cohorts (the oldest two combined) and by examination*

On a longitudinal basis, total cholesterol decreased by 0.04 mmol/L a year with increasing age of the men (table 4). There was no evidence for a stronger age-related change in the older birth cohorts as compared to the younger ones (not shown). Total cholesterol

increased between 1977/78 and 1985 and between 1985 and 1990. Between 1990 and 1993 total cholesterol decreased by 0.14 mmol/L a year solely due to a period effect. High density lipoprotein cholesterol did not change with age. Between 1977/78 and 1985 high density lipoprotein cholesterol decreased by 0.02 mmol/L a year. These results were essentially the same for the 'healthy' men who participated in every examination period.

Table 4. *Estimated mean changes in total and high density lipoprotein cholesterol per year with age and between examination rounds for all men aged 58 to 91 years and 'healthy' men who participated in each examination (The Zutphen Study: 1977/78 - 1993)*

	All men who participated in at least one examination*	'Healthy' men who participated in each examination†
	Total cholesterol (mmol/L)	
Change with age	-0.043 (-0.054; -0.032)‡	-0.040 (-0.094; 0.014)
Change between 1977/78-1985	0.066 (0.052; 0.081)	0.065 (0.005; 0.124)
Change between 1985-1990	0.029 (0.012; 0.045)	0.028 (-0.037; 0.092)
Change between 1990-1993	-0.135 (-0.164; -0.106)	-0.159 (-0.239;-0.079)
	High density lipoprotein cholesterol (mmol/L)	
Change with age	-0.000 (-0.003; 0.003)	0.010 (-0.007; 0.026)
Change between 1977/78-1985	-0.016 (-0.020; -0.080)	-0.029 (-0.047;-0.011)
Change between 1985-1990	0.007 (0.003; 0.012)	-0.007 (-0.027; 0.190)
Change between 1990-1993	0.011 (0.003; 0.019)	0.007 (-0.018; 0.032)

*: for number of participants see table 2
†: N = 64, for definition see table 2
‡: mean change (95 percent confidence limits)

Discussion

Among men aged 58 to 91 years in our study, total cholesterol decreased with age by 0.04 mmol/L a year, and this was observed in both cross-sectional and longitudinal analyses even after the effect of selective mortality, loss to follow-up or impaired health had been taken into account. A considerable secular drop in total cholesterol was observed between 1990 and 1993. High density lipoprotein cholesterol did not vary with age, but showed a secular drop between the first and second examination periods.

Secular changes in total and high density lipoprotein cholesterol

The change in the method of cholesterol determination between 1977/78 and 1985 has probably not led to systematic differences in cholesterol concentrations between the years.

Huang's method,[25] which was used in 1977/78, was calibrated on Abell-Kendall standardized sera, and the enzymatic determinations with the CHOD-PAP mono-testkit used in 1985 through 1994 were proven to agree rigidly with the method of Abell *et al.*[26,33] However, the change in precipitation method of apo B containing particles between 1977/78 and 1985 may have led to systematic differences in the high density lipoprotein cholesterol concentrations between the examination years. Warnick *et al* found that the heparin-Mn^{2+} method of precipitation generally overestimates the cholesterol concentrations of high density lipoprotein as compared to the dextran sulphate-Mg^{2+} method.[24] Their regression equation can be used to estimate the expected high density lipoprotein cholesterol concentration in 1977/78 if dextran sulphate-Mg^{2+} had been used (0.023 mmol/L + 0.955 × heparin-Mn^{2+} value (mmol/L)). The mean expected high density lipoprotein cholesterol value was 1.17 mmol/L (standard deviation 0.27) for the men who participated in every examination. This value is 2.5 percent lower than the original value and is still significantly higher than the value measured in 1985 ($p < 0.001$). Thus the change in precipitation method cannot fully explain the secular drop in high density lipoprotein cholesterol observed between 1977/78 and 1985.

The drop in total cholesterol between 1990 and 1993 is unexpected. Since the newly analyzed plasma total cholesterol concentrations of the random sample of men in 1994 were not different from the concentration determined in 1993, this cannot be due to measurement error. However, the laboratory analyses were generally higher than the true cholesterol content of the control sera in 1990 (mean 0.9 percent) and were generally lower than the true cholesterol content of the control sera in 1993 (mean 1.7 percent). These deviations from the control sera suggest that total cholesterol may have dropped by 2.6 percent between 1990 and 1993 due to measurement error alone. The cholesterol determinations in the high density lipoproteins were susceptible to the same measurement errors as the total cholesterol determinations. Since there were no changes in high density lipoprotein cholesterol concentrations between 1990 and 1993, this implies that measurement error alone cannot explain the drop in total cholesterol concentrations in the same period. Dietary changes among men of the cohort during 1990 and 1993 may also partly explain the decline in the total cholesterol concentration of the men. In 1991, 1992 and 1993 'Fat Watch' campaigns were carried out on a national level in The Netherlands.[34] Although it remains difficult to establish the effect of such mass media community intervention programs, it has been estimated that it may have caused a drop of 3.5 percent in total cholesterol from 5.7 mmol/L to 5.5 mmol/L in the Dutch population between 1987 and 1992.[35] If we assume that possible changes in diet in the present cohort also resulted in a 3.5 percent decline in total cholesterol, together with the possible difference in measurement of total cholesterol (3.5 + 2.6 = 6.1 percent) this may explain

most of the 6.7 percent (0.135 mmol/L per year) secular drop in the total cholesterol concentration observed in just three years in the present study.

Age related changes in total and high density lipoprotein cholesterol

Our cross-sectional analyses reveal that among men over 58 years of age total cholesterol diminishes with increasing age. The percentage decline in total cholesterol estimated from regression analysis amounted to 20 percent for men aged 72 to 92 years. Newschaffer *et al* observed a 21 percent drop among both men and women between 75 and 95 years of age.[15] An increased age-related drop with advancing age has been observed in other studies among elderly men.[15 19-21 36] In the present study, the effect also tended to be stronger in the later examination periods and possibly with increasing age of the population. These cross-sectional observations of declining total cholesterol concentrations with age may however have been confounded by selective survival of older men with lower cholesterol concentrations.[16]

Our longitudinal analyses also showed that total cholesterol decreases with age. The magnitude of the decline was comparable to the cross-sectional one (0.04 mmol/L per year), and the percentage decline was 15 percent. These results were similar for those men who participated in every examination which shows that selective survival or participation did not affect our results. Newschaffer *et al* reported a longitudinal decline of nine percent among men and women between 75 and 95 years of age.[15]

Total cholesterol concentrations are known to be reduced in people with clinical or subclinical disease and there is evidence that this is a consequence of a host's inflammatory response.[12] Since there is generally increasing morbidity with advancing age in older people[13 37] it is plausible that clinical or subclinical disease has led to the decrease in total cholesterol with age in this study. However, the decline in total cholesterol with age was still of the same magnitude in the subgroup of men who appeared to be and felt healthy, were not on cholesterol lowering medication or prescribed diet, and participated in every examination year. Similar observations have been made in the Honolulu Heart Program cohort of men aged 70 to 90 years.[10] Possibly other factors are involved. Metabolic and hormonal changes with advancing age may play a role by reducing the absorption of dietary determinants of cholesterol, thus leading to reduced cholesterol concentrations in the blood.[18]

Confirming earlier findings from cross-sectional studies,[19-21 36 38] high density lipoprotein cholesterol did not vary with age in the cross-sectional analyses. Wilson and coworkers[39] have recently reported that high density lipoprotein cholesterol declined by 0.07 mmol/L between 1979 and 1983 among men initially aged 65 to 79 years from the Framingham Study. However, they did not take account of possible time related changes in cholesterol

concentrations. Moreover, the aim of their study was to investigate the determinants of change in cholesterol concentrations and not to specifically describe longitudinal changes with advancing age. Our report is the first on longitudinal changes in high density lipoprotein cholesterol concentrations with age in elderly men in which time and age related changes have been disentangled. It shows no longitudinal changes in high density lipoprotein cholesterol with increasing age.

Our study shows that total cholesterol diminishes with age in elderly men both on a cross-sectional and longitudinal basis whereas high density lipoprotein cholesterol does not vary with age in any way. A secular drop in total cholesterol was observed between 1990 and 1993, and this group will be followed to see whether this secular reduction sustains.

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Chapter 6
Serum homocysteine and the five year risk of cardiovascular diseases, cancer and cognitive impairment in elderly men

Abstract

The associations of non-fasting serum homocysteine (Hcy) levels with coronary heart disease (CHD), cerebrovascular disease (CVA), cancer and cognitive impairment was investigated in elderly men followed for five years. 878 men aged 64 to 84 years at baseline from the Dutch town of Zutphen participated in the study. The main outcome measures included the prevalence of, mortality from, and incidence of "first ever" CHD, CVA, and cancer; and the prevalence of cognitive impairment at the follow-up examination as assessed by the Mini Mental State Examination. Thirty-one percent of the men had Hcy levels of 17 μmol/l or higher. After adjustment for major other risk factors, high Hcy levels (upper tertile) at baseline were associated with an increased prevalence of CHD (odds ratio (OR) (95% confidence interval) 1.95 (1.24-3.05)) and of CVA (OR 4.16 (1.99-8.97)), but not of cancer (OR 1.69 (0.83-3.45)). High Hcy levels were associated with increased risks of dying from CHD in the first 1.5 years of observation (crude relative risk (RR) 5.62 (1.23-25.64)), of dying from CVA (2.13 (0.89-5.07)), and of dying from cancer (2.94 (1.32-4.72)). After adjustment, these RR's were reduced for CHD (3.65 (0.76-17.42)) and for cancer (1.87 (0.97-3.59)), and increased for CVA in men without hypertension (17.23 (1.91-154.29)). High Hcy levels were not associated with an increased risk of first ever CHD, CVA, or cancer. Finally, high Hcy levels were associated with an increased risk of cognitive impairment at follow-up (OR 1.73 (1.03-2.92)), which was reduced after adjustment for age and occupation (OR 1.38 (0.79-2.40)). In conclusion, in a general population of elderly men, a high non-fasting Hcy level is common and is strongly associated with the prevalence of CHD and CVA and with fatal CVA in men without hypertension. It may also be a risk factor or marker for cancer and for cognitive dysfunction.

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Introduction

In the elderly, cardiovascular disease, cancer and cognitive impairment are major threats to the ability to lead an independent life. It is therefore important to identify reversible risk factors for these conditions. In this respect, a high homocysteine level may be a unique risk factor because it has been linked to cardiovascular disease,[1] cancer[2] and neurological dysfunction,[3,4] and because it can usually be treated by simple means.[5]

Cross-sectional[1] and prospective[6-9] studies in young and middle-aged subjects have shown that high levels of homocysteine are associated with an increased risk of atherosclerotic cardiovascular disease. How homocysteine affects atherogenesis is not known with certainty, but it is thought to induce endothelial injury and dysfunction[10,11,12] and to stimulate vascular smooth muscle cell proliferation[13] -both important events in the pathogenesis of atherosclerosis.

A high homocysteine level may be linked to cancer because it is a marker of rapid cel growth[2] and of nutritional habits which themselves are associated with an increased risk of cancer.[14]

A very high level of homocysteine, most often the result of certain rare inborn errors of metabolism, is associated with neurological dysfunction. This is thought to be caused by homocysteine-induced vascular damage and by homocysteine-associated neurotoxicity, the basis of which is poorly understood.[3] It is conceivable that a moderately elevated homocysteine level, through similar mechanisms, is also associated with neurological dysfunction, but this has not been investigated. Homocysteine is formed from the demethylation of the essential amino acid methionine, an ubiquitous methyl donor in many biochemical pathways. Homocysteine can be remethylated, which requires vitamin B12 and folic acid, or degraded to cystathionine and cysteine, a process that requires vitamin B6. Deficiencies of these vitamins are common in the elderly and, in cross-sectional studies, are associated with high homocysteine levels[15,16] and with cognitive impairment.[4]

There are no population-based data on homocysteine and clinical cardiovascular disease, cancer or cognitive impairment in the elderly. We therefore wished to investigate these issues in the Zutphen Elderly Study, a population-based prospective investigation in elderly men.

Methods

Population

The Zutphen Elderly Study is a longitudinal investigation of risk factors for chronic diseases in elderly men.[17] It represents a continuation of the Zutphen Study, the Dutch contribution to the Seven Countries Study. In 1985, 555 men of the original cohort, born between 1900 and 1920, were still alive and were invited for new investigations, together with an additional random sample of 711 men of the same age group living in Zutphen and not part of the original cohort. Seventy-four percent (939/1266) of those invited entered the study -from then on called the Zutphen Elderly Study. Complete information was available for 878 men aged 64 to 84 years. In 1990, 551 of the surviving men participated in the examinations; there were 119 non-responders.

Examinations

The baseline examination took place between March and June, 1985. Physical examinations were carried out by five trained physicians according to a standardised protocol. Height was measured to the nearest 0.1 cm, and weight to the nearest 0.5 kg while the men were in their underwear. The body mass index was calculated as weight (kg) / height $(m)^2$. Systolic and diastolic (Korotkoff phase V) blood pressure were measured in duplicate using a random zero sphymomanometer, with the men supine. Hypertension was defined as a systolic blood pressure of 160 mmHg or more, a diastolic blood pressure of 95 mmHg or more, and / or use of antihypertensive drugs. Smoking habits were assessed with a standardised questionnaire; subjects were categorised as never, former or current cigarette smokers. Information on the presence of diabetes was obtained through a standardised medical questionnaire. Information on coronary heart disease was obtained with the Dutch translation of the Rose Questionnaire. Coronary heart disease was considered present when either myocardial infarction or angina pectoris were diagnosed. The diagnosis of myocardial infarction required two or more of the following three criteria: severe chest pain lasting for more than 20 minutes that did not disappear in rest, characteristic changes on electrocardiography, and specific enzyme elevations. Information on cerebrovascular disease (stroke and transient ischaemic attack) and cancer was collected with a standardised questionnaire. All diagnoses were verified with hospital discharge data and written information from the subjects' general practitioners. All information was eventually coded by a single physician.

Venous blood samples were obtained in the non-fasting state. Samples were centrifuged after about 60 minutes, which has been shown to be sufficient to prevent increases in serum homocysteine resulting from *ex vivo* generation of homocysteine by erythrocytes.[18-

[22] Serum was stored at -20°C and assayed in 1995. There is good evidence that total (free plus protein bound) homocysteine levels are stable in serum or plasma stored for 10 years or more.[15,23-25] Serum total homocysteine was measured as previously described in detail.[5,26] The intra- and interassay coefficients of variation are 2.1% and 5.1%, respectively. Because the available amount of serum per subject was limited, we performed duplicate assays in only 65/878 samples (the mean difference in these samples was 6%). Serum homocysteine levels are consequently given as whole numbers. Serum total and high density lipoprotein cholesterol were determined enzymatically with the CHOD-PAP method (Boehringer Mannhein, Mannheim, Germany). High density lipoprotein was isolated by precipitation of apo B-containing particles with dextran sulphate-Mg^{2+}. The lipid analyses were carried out in the standardised lipid laboratory of the Department of Human Nutrition, Agricultural University, Wageningen, the Netherlands.

The intake of alcohol (g per day) and of vitamin B6 (mg per day) was assessed by trained dieticians from a cross check dietary history adapted for the Dutch.[27] Information on the intake or serum levels of folic acid and vitamin B12 was not available. We therefore used anaemia (haematocrit $\leq$ 0.40) and macrocytosis (erythrocyte mean corpuscular volume $\geq$ 100 fl) as proxies for clinical folic acid and vitamin B12 deficiencies.

Follow-up

Information on the vital status on July 1st, 1990 was obtained. Information on the causes of death was obtained from the Dutch Central Bureau of Statistics. The causes of death were coded according to the 9th revision of the International Classification of Diseases (ICD). Because the underlying cause of death in the elderly is often difficult to determine, both the primary and the secondary cause of death were considered in the analyses. Death from coronary heart disease, cerebrovascular disease, and cancer was defined by ICD codes 410-414, 430-438, and 140-239, respectively.

Survivors were invited for a reinvestigation between March and June, 1990. In case of non-response, information on major chronic diseases was obtained from a questionnaire for non-participants. Among the responders, data on cardiovascular disease and cancer were obtained in similar fashion as in 1985 examination. Global cognitive function was tested with the Mini-Mental State Examination,[28] which has been shown to be a reliable and valid indicator of cognitive impairment in epidemiological studies.[29] It was administered by two trained nurses in a hospital setting. When less than four individual items were missing they were rated as errors[30] (n=22), with the exception of those that could not be done because of physical disability, and in that case a weighted total score was given (n=10). When four or more individual items were missing, the entire score was

considered missing (n=5). Complete information on cognitive function and its main determinants was available for 502 of the 551 surviving men. Two men died between the 1990 examination and July 1st, 1990. They were included in both the mortality and the cognitive function analyses. We used a score of 23 or lower as indicative of impaired cognitive function.[29] Lifelong occupation, obtained from a self-administered questionnaire, rather than education level was used as an indicator of intellectual capacity, since these elderly Dutch subjects had had a reduced access to education. Subjects were divided into four categories (class I-IV): professionals, managers and teachers; small business owners; administrative personnel; and manual workers.

Main outcome measures

The main end-points investigated were the prevalence and the mortality from coronary heart disease (52/878), cerebrovascular disease (29/878), or cancer (68/878); mortality from causes other than any cardiovascular disease or cancer (37/878); the incidence of a first ever coronary (56/707) or cerebrovascular event (49/806) and cancer (81/810), *ie*, after exclusion of those who had the relevant disease at baseline; and the prevalence of cognitive impairment at the five year follow-up examination (84/502).

Statistical methods

SAS statistical programmes were used for the analyses (SAS Institute Inc., Cary, North Carolina, USA, 1989-1993, version 6.10). All tests were two-sided. *P* values less than 0.05 were considered statistically significant. The subjects were categorised in tertiles of homocysteine levels. Differences in their baseline characteristics were then evaluated using analysis of variance for normally distributed variables, the Kruskal-Wallis test for variables with a skewed distribution, and an overall chi-square test for categorical variables. Logistic regression analysis was used to investigate the association between tertiles of homocysteine levels and the prevalence of cardiovascular disease and cancer at baseline, and of cognitive impairment at follow-up. Cox's proportional-hazard (survival) analysis was used to investigate the associations between tertiles of homocysteine levels and incidence of cardiovascular disease and cancer. The highest tertile was compared to the two lower tertiles if necessary to avoid empty cells and thus the inability to estimate the relative risk. Interaction terms were investigated at the 0.10 level. One person had moved abroad and was lost to follow-up. The date on which he moved was used as his (censored) endpoint date. Unless stated otherwise, adjusted analyses are those in which the effects of major risk factors are taken into account, *ie*, age, body mass index, systolic blood pressure, total and high density lipoprotein cholesterol, diabetes mellitus and cigarette smoking habits for cardiovascular end-points; age, body mass index, total cholesterol and cigarette

smoking habits for cancer; and age and occupation for cognitive impairment. The analysis of mortality from causes other than cardiovascular disease and cancer was adjusted for age, body mass index, total cholesterol, cigarette smoking habits, and the presence of chronic obstructive pulmonary disease.

Results

The mean (SD) homocysteine level at baseline was 15.8 (8.2) μmol/l. High homocysteine levels were common; 268/878 subjects (31%) had serum levels ≥ 17 μmol/l. Vitamin B6 intake was 0.02 mg per g protein or lower in 736/818 subjects (90%) and 0.016 mg per g protein or lower in 429 subjects (52%). Anaemia and/or macrocytosis were present in 367 subjects (42%). Table 1 shows that higher homocysteine levels were associated with age, lower levels of high density lipoprotein cholesterol, current smoking, lower vitamin B6 intake, a higher prevalence of anaemia and/or macrocytosis, and a history of coronary heart disease, cerebrovascular disease and cancer.

Table 2 shows that higher homocysteine levels were associated with an increased prevalence of coronary heart disease and cerebrovascular disease which remained after adjustment. The association with cerebrovascular disease was stronger in normotensive than in hypertensive subjects. High homocysteine levels were also associated with a higher prevalence of cancer; this was not significant after adjustment.

The mortality rate was 12.9 per 1,000 person-years for coronary heart disease and 7.2 per 1,000 person-years for cerebrovascular disease. The survival curve (figure 1A) shows that coronary heart disease mortality was higher in subjects with homocysteine levels in the third tertile, especially in the first 1.5 years of observation. This was not significant after adjustment (table 3). Mortality from cerebrovascular disease was higher in subjects with homocysteine levels in the third tertile (figure 1B). Further analyses showed that this was significant only in subjects without hypertension (adjusted relative risk 17.23; table 3). Additional adjustment for the presence of cerebrovascular disease at baseline did not appreciably change this risk estimate (relative risk (95% confidence interval) 21.64 (2.21-211.71)). All 29 cases of fatal cerebrovascular disease occurred in patients who had had a previous cerebrovascular event either before the 1985 examination (16 cases) or between the 1985 examination and the time of the fatal event (13 cases).

The mortality rates per 1,000 person-years were 16.9 for cancer and 9.2 for causes other than cardiovascular disease and cancer. High homocysteine levels were associated with an increased risk of dying from cancer (figure 1C). After adjustment, this was of

borderline significance (p for trend =0.056; table 3). Further adjustment for the presence of cancer at baseline resulted in a similar risk estimate (relative risks for the second and third tertiles compared to the first tertile 1.28 (0.66-2.48) and 1.79 (0.92-3.48); p for trend =0.076). High homocysteine levels were also associated with an increased risk of dying from causes other than cardiovascular disease and cancer, which was not significant after adjustment (adjusted relative risks for the second and third tertiles 0.90 (0.35-2.29) and 1.94 (0.83-2.29); p for trend =0.17).

Table 1. *Baseline (1985) characteristics of 878 men according to tertiles of serum homocysteine in the Zutphen Elderly Study*

Characteristic	Homocysteine tertile			p value
	First	Second	Third	
Homocysteine range (μmol/l)	6-12	13-16	17-97	
	Mean (standard deviation)			
Age (years)	70.0 (4.6)	71.5 (2.7)	73.0 (5.7)	< 0.0001
Body mass index (kg/m^2)	25.8 (3.3)	25.3 (2.7)	25.2 (3.6)	0.08
Systolic blood pressure (mmHg)	149.1 (21.4)	151.6 (21.7)	152.7 (21.2)	0.1
Total cholesterol (mmol/L)	6.13 (1.00)	6.13 (1.10)	6.02 (1.21)	0.3
HDL cholesterol (mmol/L)	1.15 (0.31)	1.11 (0.27)	1.09 (0.30)	0.06
Vitamin B6 intake (mg/day)*	1.34 (0.33)	1.30 (0.30)	1.18 (0.30)	< 0.0001
		Percentage		
Current cigarette smokers†	27	30	34	0.02
History of diabetes mellitus	9	6	5	0.1
History of coronary heart disease	15	18	26	0.01
History of cerebrovascular disease	3	7	15	0.001
History of cancer	5	9	20	0.04
Haematocrit ≤ 0.40 and/or macrocytosis	36	43	47	0.02

*: information available for 818 men
†: information available for 877 men

The incidence rates per 1,000 person-years of "first ever" coronary heart disease, cerebrovascular disease and cancer were 17.3, 13.3, and 21.7, respectively. Homocysteine levels were not related to these incidence rates. The adjusted relative risks for the second and third tertiles were 1.13 (0.59-2.14) and 1.15 (0.57-2.32) for coronary heart disease (p

for trend =0.76), 0.80 (0.40-1.57) and 0.80 (0.39-1.65) for cerebrovascular disease (p for trend =0.45), and 0.59 (0.34-1.02) and 0.92 (0.53-1.56) for cancer (p for trend =0.74). The risk estimates for coronary heart disease and cerebrovascular disease did not differ significantly between normotensive and hypertensive subjects.

Table 2. *Odds ratios and 95% confidence intervals for the prevalence of coronary heart disease, cerebrovascular disease, and cancer according to tertiles of serum homocysteine in 878 men in the Zutphen Elderly Study*

Disease		Homocysteine tertiles			p value for trend
		First	Second	Third	
Coronary heart disease	crude	1*	1.28 0.83 - 1.97	2.10 1.37 - 3.22	0.0005
	adjusted†	1*	1.16 0.75 - 1.82	1.95 1.24 - 3.05	0.003
Cerebrovascular disease	crude	1*	1.93 0.89 - 4.16	5.00 2.45 - 10.21	< 0.0001
	adjusted†	1*	1.70 0.78 - 3.73	4.16 1.99 - 8.97	< 0.0001
	normotensive adjusted†	1*	1*	4.51 2.16 - 9.41	
	hypertensive adjusted†	1*	1*	2.04 0.99 - 4.21	
Cancer	crude	1*	2.00 1.02 - 3.94	2.36 1.19 - 4.68	0.02
	adjusted†	1*	1.67 0.84 - 3.34	1.69 0.83 - 3.45	0.18

*: Reference group
†: See Statistical Methods

Table 4 shows that high homocysteine levels in 1985 were associated with an increased risk of cognitive impairment in 1990. Adjustment for age and occupation weakened the association. Further analysis indicated that the risk estimate was not altered appreciably by additional adjustments for body mass index, systolic blood pressure, total and high density lipoprotein cholesterol, cigarette smoking habits, diabetes mellitus, alcohol consumption, presence of cerebrovascular disease at baseline, and vitamin B6 intake. The risk estimates tended to be higher, however, in subjects who had no anaemia and/or macrocytosis (table 4).

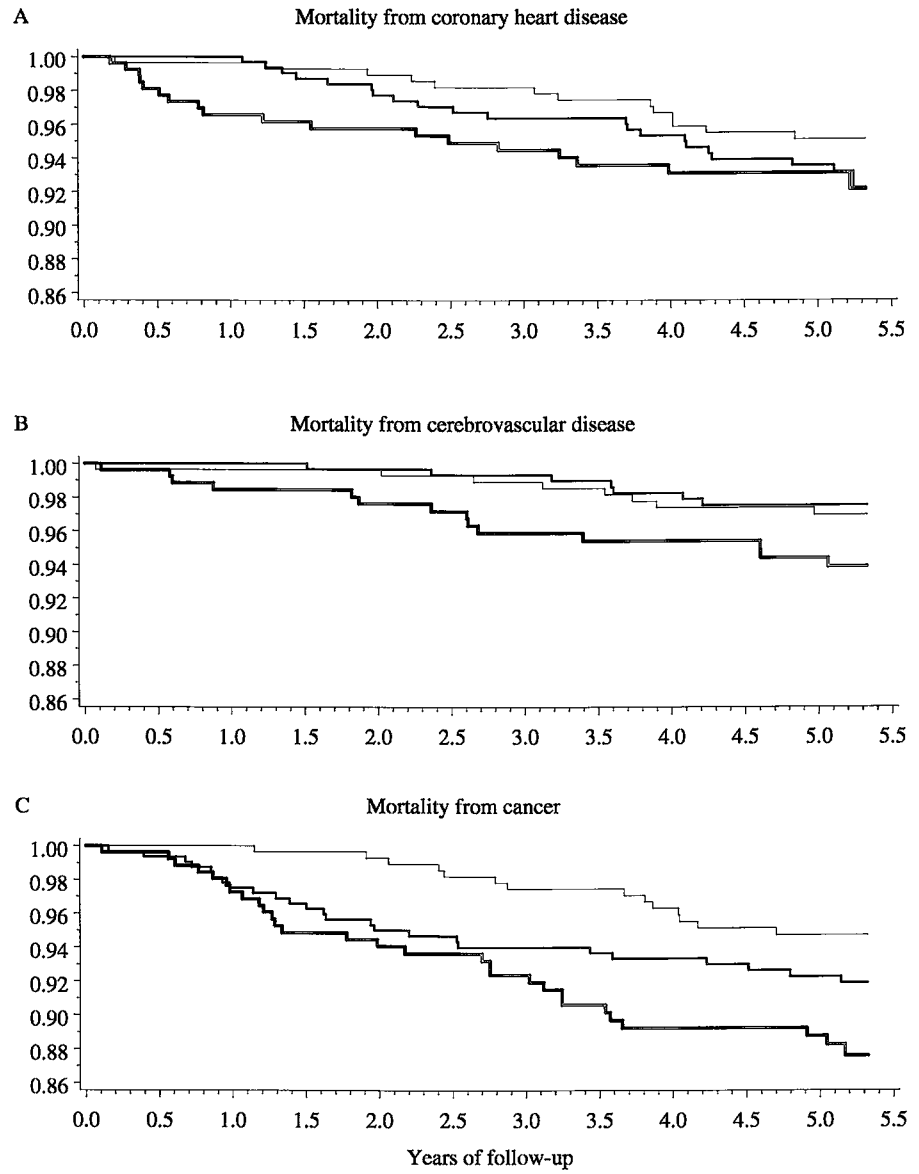

Figure 1. *Mortality (expressed as 1 minus the survival fraction) from coronary heart disease (A), cerebrovascular disease (B), and cancer (C) according to tertiles of serum homocysteine in the Zutphen Elderly Study. The first, second and third tertiles are indicated by the grey, black and heavy black lines, respectively*

Table 3. *Relative risks and 95% confidence intervals for mortality from coronary heart disease, cerebrovascular disease, and cancer acording to tertiles of serum homocysteine in 878 men in the Zutphen Elderly Study*

Disease		Homocysteine tertile			*p* value for trend
		First	Second	Third	
Coronary heart disease	crude	1*	1.49 0.74 - 2.97	1.68 0.82 - 3.43	0.16
	first 1.5 years	1*	1.78 0.33 - 9.71	5.62 1.23 - 25.6	0.01
	adjusted†	1*	1.30 0.64 - 2.63	1.39 0.66 - 2.93	0.51
	first 1.5 years	1*	1.41 0.25 - 7.89	3.65 0.76 - 17.4	0.06
Cerebrovascular disease	crude	1*	0.80 0.29 - 2.21	2.13 0.89 - 5.07	0.07
	adjusted†	1*	0.60 0.21 - 1.71	1.24 0.50 - 3.08	0.47
	normotensive crude	1*	1*	21.35 2.67 - 170.7	
	adjusted†	1*	1*	17.23 1.91 - 154.3	
	hypertensive crude	1*	1*	0.99 0.38 - 2.57	
	adjusted†	1*	1*	0.81 0.30 - 2.16	
Cancer	crude	1*	1.63 0.85 - 3.14	2.94 1.32 - 4.72	0.004
	adjusted†	1*	1.40 0.72 - 2.70	1.87 0.97 - 3.59	0.06

*: Reference group
†: See Statistical Methods

Table 4. *Odds ratios and 95% confidence intervals for the prevalence of cognitive impairment in 1990 according to tertiles of serum homocysteine in 1985 in the Zutphen Elderly Study*

Odds ratios* adjusted for	All subjects (n = 502)	Subjects with haematocrit > 0.40 and erythrocyte mean corpuscular volume < 100 fl (n = 301)
Crude	1.73 1.03 - 2.92	2.13 1.09 - 4.18
Occupation	1.59 0.94 - 2.71	2.00 1.00 - 4.01
Age and occupation	1.38 0.79 - 2.40	1.98 0.96 - 4.08
Age, occupation, and other risk factors†	1.34 0.76 - 2.35	2.22 1.05 - 4.68
Age, occupation, other risk factors†, and vitamin B6 intake	1.25 0.70 - 2.25	1.97 0.91 - 4.24

*: All odds ratios compare the third tertile to the lower two tertiles of serum homocysteine
†: body mass index, systolic blood pressure, total and high density lipoprotein cholesterol, cigarette smoking habits, diabetes mellitus, alcohol consumption and presence of cerebrovascular disease at baseline

Discussion

This is the first prospective, population-based study to examine the role of high homocysteine levels as a risk factor for cardiovascular disease, cancer, and cognitive impairment in the elderly. We found that a high non-fasting serum homocysteine level was common among elderly men, and was strongly associated with the prevalence of coronary heart disease and cerebrovascular disease. It was also a strong risk factor for fatal cerebrovascular disease in men without hypertension. In addition, we found that a high homocysteine level tended to be related to cancer prevalence and mortality, and to the prevalence of cognitive impairment at follow-up. Blood samples were taken in the non-fasting state and serum was not separated immediately. These sampling conditions tend to increase the serum homocysteine level, but relatively more so at low levels.[7,20-22] It is therefore unlikely that the associations between high homocysteine levels and outcome measures were biased by the blood sampling conditions.

Homocysteine levels and cardiovascular disease

Several cross-sectional[1] and two prospective[6,7] studies in middle-aged subjects have shown an increased risk of myocardial infarction with high homocysteine levels. A Finnish population-based study[25] was negative, but the prevalence of high homocysteine levels in

that study was too low to allow definitive conclusions. We found a high homocysteine level to be associated with a history of coronary heart disease at baseline, and, marginally, with mortality from coronary heart disease in the first 1.5 years of observation, but not with the incidence of first ever coronary heart disease. These results suggest that, in men, the coronary risk associated with high homocysteine levels decreases with age. In both previous prospective studies homocysteine was also a stronger risk factor among younger than among older subjects.[6,7]

In cross-sectional studies, a high homocysteine level is strongly associated with cerebrovascular disease in young and middle-aged subjects.[1] Three prospective studies, all in middle-aged subjects, have yielded somewhat inconsistent results. Verhoef *et al* observed an increased risk of stroke that was confined to normotensive men.[8] A relationship between homocysteine levels and stroke risk was absent in the Finnish study[25] but strongly positive among British men.[9] The latter study a synergistic interaction between homocysteine and blood pressure was suggested but the interaction was not statistically significant.[9] In both the cross-sectional and the mortality analyses, we found that the association of a high homocysteine level with cerebrovascular disease was much stronger among normotensive than among hypertensive men. All deaths from cerebrovascular disease occurred in subjects who had had a previous cerebrovascular event; a high homocysteine level was not associated with an increased risk of a first ever cerebrovascular event. A high homocysteine level thus was strongly associated with repetitive and ultimately fatal cerebrovascular disease. Overall, these and previously published data[8,9] suggest that, in men without hypertension, the risk of cerebrovascular disease associated with high homocysteine levels does not decrease with age; on the other hand, there is no indication that a high homocysteine level increases the risk of cerebrovascular disease in elderly men in whom the risk of cerebrovascular disease is already high because of the presence of hypertension.

Homocysteine levels and cancer

The relationship between a high homocysteine level and cancer was apparent mainly for cancer mortality, where it was of borderline statistical significance (table 3). This finding may nevertheless be important, because a high homocysteine level may be linked to an increased risk of cancer through at least three pathways. First, it may be a marker of spillage of homocysteine into the extracellular space by rapidly dividing tumour cells, as observed in acute leukaemia.[2] The increase in cancer mortality associated with a high homocysteine level was not confined to the first years of observation (figure 1), however, which argues against this being the only pathway. Second, a high homocysteine level may be a marker of a marginal vitamin intake which itself may increase the risk of cancer.[14]

More speculatively, homocysteine or a closely related intracellular metabolite might be directly carcinogenic, or a high homocysteine level might be a marker of altered intracellular methylation which may affect the control of cellular growth. These suggestions merit further investigation, because our study clearly raises the possibility that, among elderly men, a high homocysteine level -whether a risk marker or a causal risk factor- may be a clinically useful indicator of an increased risk of dying from cancer.

There was a weak tendency for high homocysteine levels to be associated with mortality from causes other than cardiovascular disease and cancer. Further studies are needed to determine whether this is an artefact or not.

Homocysteine levels and cognitive impairment

High homocysteine levels were associated with a 73% increase in the risk of cognitive impairment, although this decreased to a non-significant 38% after adjustment for age and level of occupation (as an estimate of intellectual capacity). It should be noted that adjusting for age may result in overcorrection if the effect of age on cognitive function is mediated in part through high homocysteine levels. The associations were stronger in subjects without anaemia and/or macrocytosis, which were used as proxies for clinical vitamin B12 and/or folic acid deficiencies, and were not materially affected by the level of vitamin B6 intake (table 4).

Several mechanisms, not mutually exclusive, may be responsible for the relationship between homocysteine and cognitive function. First, hyperhomocysteinaemia increases the risk of atherosclerotic cerebrovascular disease, which, even in the absence of clinical stroke, is associated with cognitive impairment.[31,32] Second, there is some evidence that homocysteine (or a closely related intracellular derivative) is itself directly neurotoxic.[3] Third, hyperhomocysteinaemia may be a marker of a cellular deficiency of vitamin B12, folic acid and/or vitamin B6, all of which may be linked to cognitive impairment through other pathways than high homocysteine levels only. For example, hyperhomocysteinaemia induced by vitamin B12 deficiency indicates a decreased intracellular activity of methionine synthase and thus a decreased formation of the methyl group donor s-adenosylmethionine. Decreased methylation of myelin basic protein plays an important role in the demyelination and the neurological dysfunction observed in vitamin B12 deficiency and in inborn errors that impair the remethylation of homocysteine to methionine.[33] Remethylation is thought to be relatively spared in folic acid deficiency;[33] it is not known to what extent the association between folic acid deficiency and neurological dysfunction[4] can be explained by subtle abnormalities in methyl group tranfer or other mechanisms. We did not measure serum vitamin B12 and folic acid levels and cannot exclude that clinical deficiencies of these vitamins were responsible for cognitive

impairment in some subjects. The association between high homocysteine levels and cognitive impairment were actually *stronger* in subjects without anaemia and/or macrocytosis, either of which is present in 80% or more of patients with clinical vitamin B12 or folic acid deficiency.[19,34] This suggests that clinical deficiencies of these vitamins cannot explain the observed relationships. Vitamin B6, in turn, is involved in the biosynthesis of several neurotransmitters, which might provide a link between vitamin B6 deficiency and cognitive impairment that is separate from its role in homocysteine metabolism. A marginal vitamin B6 intake was common in the study population[35] and is closely related to low serum levels.[16] The relationship between homocysteine levels and cognitive impairment, however, was not clearly affected by adjustment for vitamin B6 intake.

Implications

In our study the mean homocysteine level was 15.8 μmol/l; one third of the subjects had levels of 17 μmol/l or above. Suggested cutoff values for hyperhomocysteinaemia have ranged from 11.4 to 15.8 μmol/l.[6,8,15,16,36] Thus, in our study, high homocysteine levels were common by any standard. Because high homocysteine levels can be reduced by simple treatment with folic acid and vitamin B6 even in the absence of deficiencies of these vitamins,[5] studies are now needed of the effect of treatment of high homocysteine levels on cardiovascular disease, cancer, and cognitive function in the elderly.

Acknowledgments

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Chapter 7
White blood cell count and the five-year risk of coronary heart disease and all-cause mortality in elderly men

Abstract

Since the importance of established risk factors for coronary heart disease is unclear in older people, the associations of white blood cell count with the risk of coronary heart disease and all-cause mortality were investigated in an elderly cohort followed for five years. In 1985, complete information on risk factors of interest was available for 884 randomly selected men, aged 64 to 84 years, from the Dutch town of Zutphen (participation rate 74 percent). Relative risks for a 10^9/L increase in white blood cell count were obtained for the five year incidence of and mortality from coronary heart disease and all-cause mortality. Relative risks were adjusted for age, body mass index, systolic blood pressure, total and high density lipoprotein cholesterol and cigarette smoking habit. Mean white blood cell count was 6.7 (standard deviation 1.8) 10^9/L at baseline. Increased white blood cell count was independently associated mortality from coronary heart disease. The relative risk amounted to 1.32 (95 percent confidence interval (% CI): 1.15 - 1.51). For the incidence of the disease the relative risk was 1.14 (0.98 - 1.32). These associations were observed regardless of cigarette smoking habit. Regarding all-cause mortality, the relative risk amounted to 1.25 (95% CI: 1.17 - 1.35). This association was especially observed among the never and former smokers. In conclusion, white blood cell count predicts coronary heart disease and all-cause mortality in elderly men, during five years of follow-up, independent of conventional risk factors for coronary heart disease.

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Introduction

Epidemiologic evidence is accumulating implicating moderately increased white blood cell count as a risk factor for coronary heart disease.[1-8] Although the observed effect has often, for the largest part, been ascribed to smoking,[2,3,7] biologic mechanisms for the association have also been described.[9,10] Most evidence is based on middle-aged people[1-8] but little is known on the importance of white blood cell count in predicting coronary heart disease in the elderly.[9]

In general, there is currently much debate regarding the importance of conventional risk factors for coronary heart disease in the elderly.[11,12] Likewise, it can be questioned whether white blood cell count continues to predict coronary heart disease with advancing age. On the other hand, since there may be selective survival of elderly people with elevated levels of conventional risk factors,[13] other factors, such as white blood cell count, may become more important. Since atherosclerosis is generally well advanced in the elderly, factors associated with thrombosis may be more significant in predicting coronary heart disease in this age group.[14] In the presence of atherosclerosis, activated monocytes and neutrophils may release products which promote plaque disruption and subsequent thrombus formation and may eventually lead to a coronary event.[10,15]

We investigated whether elevated white blood cell count is associated with coronary heart disease and all-cause mortality in an elderly cohort followed-up for five years.

Methods

Population

The Zutphen Study is a longitudinal investigation of chronic disease risk factors initiated in 1960 among middle-aged men as the Dutch contribution to the Seven Countries Study.[16] In 1985, 555 men from the 1960 cohort were still alive and were invited for new examinations. In addition, a random sample (two out of three) of all men of the same age living in Zutphen and not part of the 1960 cohort were invited to take part in the study. From then on, the study was continued as the Zutphen Elderly Study. Of the 1266 men approached 939 (74 percent) agreed to participate. Hundred-and-nine men (9 percent) could not be examined because of serious illness or death, 62 men (5 percent) had moved and 156 men (12 percent) refused to participate or could not be reached. Complete information on risk factors was available for 884 men aged 64 to 84 years.

Examinations

Physical examinations took place between March and June 1985 and were carried out by five trained physicians according to a standardized protocol. Height was measured to the nearest 0.1 cm and body weight was measured to the nearest 0.5 kg while the men were in underwear. Body mass index (weight (kg)/ height $(m)^2$) was calculated. At the end of the examination, systolic and diastolic (fifth Korotkoff phase) blood pressures were measured in duplicate with a random zero sphygmomanometer at the right arm while subjects were in the supine position. The mean of the two blood pressure values was used in the analyses.

Non-fasting venous blood samples were taken. A Coulter Counter S880 was used to determine white blood cell count and hematocrit. The variation coefficient for the within- and between-run measurement error was less than two percent for both white blood cell count and hematocrit. Serum total and high density lipoprotein cholesterol were determined enzymatically with the CHOD-PAP mono-testkit from Boehringer Mannheim.[17,18] High density lipoprotein was isolated after precipitation of apo B containing particles by dextran sulphate-Mg^{2+}.[19] The lipid analyses were carried out in the standardized lipid laboratory of the Department of Human Nutrition, Agricultural University, Wageningen, The Netherlands. Serum albumin levels were analyzed in an auto-analyzer (SMAC, Technicon, Tarytown, USA) and these analyses were performed in blood samples of 879 men.

Information on smoking habits was assessed with a standardized questionnaire and is missing for one person. Information on the presence of diabetes mellitus, cancer and chronic obstructive pulmonary disease at baseline was obtained through a standardized medical questionnaire during the physical examination and was verified with hospital discharge data and written information from the subjects' general practitioners.

Follow-up

Information on the prevalence and incidence of coronary heart disease was obtained during the physical examination in 1985 and a similar examination in 1990. For men who did not participate in the 1990 examination, information on major chronic diseases was obtained from a questionnaire for non-participants. Coronary heart disease was considered to be present when either myocardial infarction or angina pectoris were diagnosed. For myocardial infarction the final diagnosis was based on whether two of the following three criteria were met: a specific medical history, i.e. severe chest pain lasting for more than 20 minutes and not disappearing in rest, characteristic electrocardiogram changes and specific enzyme elevations. The diagnosis of angina pectoris was based on information obtained from the Dutch translation of the Rose questionnaire.[20] All diagnoses were

additionally verified with information from electrocardiograms, hospital discharge data and written information from the subjects' general practitioners. The information was eventually coded by one physician, and the year of first diagnosis was recorded.

Information on the vital status of participants was obtained till July 1990. One person had moved abroad and was lost to follow-up. The date on which he moved was used as his (censored) end-point date. Information on the causes of death was obtained from the Dutch Central Bureau of Statistics, after verification with hospital discharge data and information from the deceased's general practitioners. The causes of death were coded according to the 9th Revision of the International Classification of Diseases (ICD)[21] and both the primary and secondary cause of death were considered. Death due to coronary heart disease was defined by ICD codes 410-414, death due to cardiovascular disease by codes 410-448, death due to all-cause cancer by codes 140-239, and death due to lung cancer by code 162.

The main end-points investigated were the incidence of a first fatal or non-fatal coronary heart disease event among men without the disease at baseline (i.e. incidence of coronary heart disease), mortality from coronary heart disease and from all-causes.

Statistical methods

Statistical analyses were carried out using the SAS program (SAS Institute Inc., Cary, North Carolina, USA, 1989-1993, version 6.10). All tests were two sided and *p*-values smaller than five percent were considered statistically significant. Cox's proportional-hazard (survival) analysis was carried out to investigate the associations of white blood cell count with end-points during five years of follow-up.[22] The date on which an event occurred was used in the analyses. For coronary heart disease incidence July 1st of the year of diagnosis was used as the date of the event. Relative risks are presented according to a 10^9/L increase in white blood cell count. Interaction terms were evaluated at the 10 percent level. Quintiles of white blood cell count were formed and adjusted relative risks for these quintiles were computed with the lowest category as the reference.

Results

The white blood cell count of the men at baseline ranged between 2.4 and 16.1 10^9/L. White blood cell count was not significantly associated with baseline age nor with most of the conventional risk factors for coronary heart disease (table 1). There was an inverse association with high density lipoprotein cholesterol and serum albumin levels and a positive association with hematocrit. White blood cell count was higher in the 58 men

with diabetes mellitus at baseline compared to men free of the disease (7.1, SD 1.8, 10^9/L versus 6.6, SD 1.8, 10^9/L). This difference was borderline significant.

Table 1. *Population characteristics and associations with white blood cell count in 884 men aged 64 to 84 years (the Zutphen Elderly Study, 1985)*

	Mean (standard deviation)	Spearman correlation coefficient with white blood cell count (10^9/L)
White blood cell count (10^9/L)	6.7 (1.8)	
Age (years)	71.5 (5.3)	- 0.01
Body mass index (kg/m^2)	25.45 (3.20)	0.00
Systolic blood pressure (mmHg)	151.0 (21.5)	0.03
Diastolic blood pressure (mmHg)	85.4 (11.5)	- 0.01
Serum total cholesterol (mmol/L)	6.10 (1.11)	0.01
Serum high density lipoprotein cholesterol (mmol/L)	1.12 (0.29)	- 0.08*
Serum albumin (g/L)	44.1 (2.7)	- 0.14†
Hematocrit	0.45 (0.04)	0.12†
	Number (percentage)	*p*-value‡
Diabetes mellitus	58 (6.6)	0.07

*: $p < 0.05$
†: $p < 0.001$
‡: for Mann-Whitney *U*-test of difference in mean white blood cell count between those with and those without diabetes mellitus

White blood cell count was significantly higher in men who were cigarette smokers at baseline compared to former and never smokers ($p < 0.0001$) (table 2). There was no significant difference in white blood cell count between former smokers who quit more than 10 years ago and those who quit 10 years ago or less nor between the amount of cigarettes smoked among men who smoked in 1985 (table 2).

White blood cell count was not associated with the prevalence of coronary heart disease at baseline (6.7, standard deviation (SD) 1.7, 10^9/L in 175 cases versus 6.6, SD 1.8, 10^9/L in non cases). However, in 21 men who suffered a coronary heart disease event within half a year prior to the blood sampling white blood cell count tended to be higher than that of men without any evidence the disease at baseline (7.0, SD 1.4 10^9/L compared to 6.6, SD 1.8 10^9/L respectively, *p*-value = 0.10).

White blood cell count was positively and independently associated with mortality from coronary heart disease during five years of follow-up (table 3), and the association

Table 2. *Mean white blood cell count according to cigarette smoking habit in elderly men (the Zutphen Elderly Study, 1985)*

Cigarette smoking habit in 1985	N	White blood cell count (10^9/L)
Never smoker	162	6.4 (1.7)*
Former smoker	454	6.3 (1.7)
More than 10 years ago	268	6.2 (1.6)
10 years ago or less	168	6.5 (1.9)
Current smoker	267	7.3 (1.8)
1 - 9 cigarettes/day	63	7.2 (1.9)
10 - 19 cigarettes/day	111	7.3 (1.7)
≥ 20 cigarettes/day	92	7.5 (1.8)

*: mean (standard deviation), overall Kruskal-Wallis test comparing never, former and current smokers: $p < 0.0001$

tended to be exponential (figure 1). Additional adjustment for the presence of coronary heart disease at baseline did not affect the strength of the association (relative risk 1.32, 95 percent confidence interval (% CI) 1.13 - 1.53). The association was of the same magnitude among 793 men with white blood cell count within the normal range of 4.5 to 11 10^9/L (not shown). The association with the incidence of a first coronary heart disease event was also positive, though weaker than with mortality from the disease (adjusted relative risk 1.14, 95% CI 0.98 - 1.32).

Table 3. *Relative risks for mortality from coronary heart disease and all-causes associated with white blood cell count in elderly men (the Zutphen Elderly Study, 1985 - 1990)*

	Coronary heart disease	All-causes
Percentage of deaths (n)	6.0 (53)	23.9 (211)
Crude	1.27 (1.11 - 1.44)*	1.20 (1.12 - 1.28)
Adjusted for age	1.30 (1.14 - 1.49)	1.24 (1.15 - 1.33)
Adjusted for conventional risk factors†	1.32 (1.15 - 1.51)	1.25 (1.17 - 1.35)
Adjusted for additional risk factors‡	1.27 (1.10 - 1.46)	1.21 (1.12 - 1.30)

*: relative risk (95 percent confidence interval) for 10^9/L increase in white blood cell count
†: age, body mass index, systolic blood pressure, total cholesterol, high density lipoprotein cholesterol, cigarette smoking
‡: conventional risk factors and albumin, hematocrit and diabetes mellitus at baseline

White blood cell count was independently associated with all-cause mortality (table 3) in a graded fashion (figure 1). The association persisted even after adjustment for the presence of major chronic diseases at baseline such as cardiovascular diseases, cancer and

chronic obstructive pulmonary disease (relative risk 1.15, 95% CI 1.07 - 1.24). Additional analyses revealed that white blood cell count was associated with increased risk of death from all cardiovascular diseases (risk factor and disease adjusted relative risk 1.21, 95% CI 1.09 - 1.34), cancer (adjusted relative risk 1.10, 95% CI 0.97 - 1.24) and all other causes (adjusted relative risk 1.15, 95% CI 0.98 - 1.35).

The association of white blood cell count with coronary heart disease has often been ascribed to the effect of smoking on coronary heart disease. However, cigarette smoking status in 1985 was not significantly associated with mortality from coronary heart disease (p-trend = 0.64) nor all-causes (p-trend = 0.47) during five years of follow-up. Cigarette smoking was only significantly associated with lung cancer death (p-trend = 0.05). White blood cell count was positively associated with mortality from coronary heart disease regardless of the cigarette smoking status (table 4). The association tended to be stronger among the never smoking men, but was not significantly different from the other smoking categories. Regarding all-cause mortality, there was a significant association with white blood cell count among the non-smokers but not among the current smokers (p-interaction = 0.07).

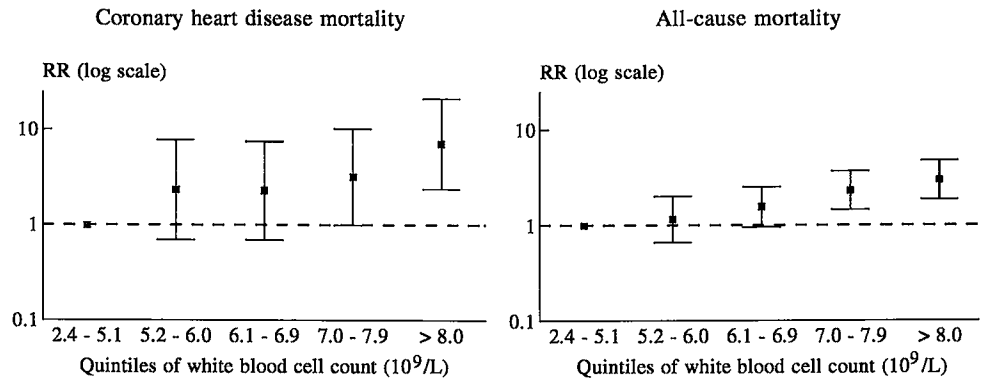

Figure 1. *Relative risks and 95 percent confidence intervals for mortality from coronary heart disease and from all-causes according to quintiles of white blood cell count (The Zutphen Elderly Study, 1985 - 1990)*

Table 4. *Relative risks for mortality from coronary heart disease and all causes associated with white blood cell count in elderly men according to smoking status at baseline (the Zutphen Elderly Study, 1985)*

	Smoking status in 1985		
	Never (n = 162)	Former (n = 454)	Current (n=267)
		Coronary heart disease	
Percentage of deaths (n)	5.6 (9)	7.2 (25)	8.2 (22)
Crude	1.47 (1.10 - 1.96)*	1.28 (1.07 - 1.53)	1.21 (0.94 - 1.56)
Adjusted for age	1.47 (1.10 - 1.95)	1.32 (1.09 - 1.59)	1.26 (0.97 - 1.63)
Adjusted for conventional risk factors†	1.51 (1.10 - 2.08)	1.30 (1.07 - 1.59)	1.20 (0.90 - 1.59)
Adjusted for additional risk factors‡	1.56 (1.13 - 2.16)	1.24 (1.00 - 1.54)	1.27 (0.92 - 1.74)
		All-causes	
Percentage of deaths (n)	24.5 (40)	23.4 (106)	24.3 (65)
Crude	1.28 (1.10 - 1.40)	1.29 (1.18 - 1.41)	1.05 (0.92 - 1.20)
Adjusted for age	1.28 (1.10 - 1.49)	1.33 (1.21 - 1.46)	1.09 (0.95 - 1.25)
Adjusted for conventional risk factors†	1.29 (1.10 - 1.51)	1.33 (1.20 - 1.47)	1.05 (0.91 - 1.22)
Adjusted for additional risk factors‡	1.27 (1.09 - 1.49)	1.26 (1.13 - 1.40)	1.05 (0.91 - 1.22)

*: relative risk (95 percent confidence interval) for 10^9/L increase in white blood cell count
†: age, body mass index, systolic blood pressure, total cholesterol, high density lipoprotein cholesterol, cigarette smoking
‡: conventional risk factors and albumin, hematocrit and diabetes mellitus at baseline

Discussion

This is the first report on the association between white blood cell count and coronary heart disease in an elderly population. An increase of one standard deviation in white blood cell count was associated with a 65 percent increase in the risk of death from coronary heart disease. This effect is considerably higher than the 45 percent increased risk associated with one standard deviation increase in total cholesterol in the same cohort and during the same period.[23] In studies in middle-aged people the effect of white blood cell count on coronary heart disease has been found to be similar[2] to or even smaller[6,8] than that of total cholesterol levels. This suggests that, in advanced age, other factors than the conventional risk factors for coronary heart disease, such as white blood cells, become more important in predicting the disease.

Possible biologic mechanisms underlying the observed associations have been postulated. It is widely recognized that monocytes have a role in the pathogenesis of atherosclerosis.[15] After monocytes are recruited at the site of endothelial injury, they adhere to the vessel wall, enter it and are eventually converted into foam cells. Since the monocyte count constitutes approximately two to ten percent of the total white blood cell count, it is probably not the only fraction of the total white blood cell count responsible for the observed association with coronary heart disease. Indeed, the most common granulocytes, the neutrophils, have also been implicated in the pathogenesis of atherosclerosis.[10] In the presence of atherosclerosis, which can be viewed as an inflammatory disease,[24] white blood cells may become activated. This leads to reduced deformability of especially the neutrophilic cells, to white blood cell aggregation and adherence to the endothelium, and to recruitment of white blood cells, all of which subsequently lead to capillary plugging and tissue ischemia. Activated neutrophils and monocytes may also release products which induce plaque rupture and subsequent thrombus formation which can also result in ischemia.[10,15] The main products released by the cells are oxidants, such as superoxide radicals and nitric oxide,[25] and proteolytic enzymes[10] which can all induce vascular injury. Pathological mechanisms have also been postulated for the association between white blood cell count and diseases other than coronary heart disease. Oxidation metabolites produced by activated white blood cells during inflammation may also be involved in the pathogenesis of cancer and lung disease.[10]

Since white blood cell count is a general indicator of inflammation it may also be a marker of underlying athero-thrombotic disease. The observed association with coronary heart disease is therefore not necessarily causal. Which mechanisms underlie the association cannot be derived from this study.

In our study, the association between white blood cell count and coronary heart disease incidence was less strong than for mortality. Through plaque rupture and thrombus formation, white blood cell count may be more strongly associated with more severe coronary heart disease leading to death.[15] This may also explain the baseline results since there was no clear evidence that men with coronary heart disease at baseline had elevated white blood cell counts as compared to men free of the disease. This was especially observed in those who were first diagnosed with the disease more than half a year prior to the blood sampling. It is possible that the men with elevated white blood cell count have already died. The elevated white blood cell count in men who suffered a coronary heart disease event in the same year as the blood sampling may be the result of an active state of atherosclerosis, i.e. progression.

White blood cell count also predicted all-cause mortality and this has been observed previously in middle-aged populations.[8,26] An elevated white blood cell count may be a

marker for inflammation and is therefore also associated with increased risk of mortality. Although the association remained after adjustment for the clinical diagnosis of major chronic diseases, possible underlying (subclinical) disease may still have driven the observed association. Part of the association may be a consequence of the strong association between white blood cell count with mortality from coronary heart disease.

The association between white blood cell count and coronary heart disease has often, at least in part, been attributed to smoking.[2,3,7] In the present study, the positive association between white blood cell count and coronary heart disease was observed regardless of smoking status. White blood cell count predicted coronary heart disease and all-cause mortality in non-smokers, thus smoking status is does not explain the findings.

Surprisingly, smoking status does not appear to be associated with coronary heart disease in the present study. Since the vast majority of the men in our study have smoked during their lifetime a larger population may be necessary to examine this association.[27] In addition, men who remain smokers in advanced age and survive its detrimental effects may be less susceptible to these effects than those who did not reach old age. The smokers in this study may form a select group of healthy men, regardless of their smoking habit, who are less susceptible to coronary heart disease. In a larger cohort study of older people, cigarette smoking has clearly been associated with increased mortality outcomes and cessation of smoking has been shown to improve survival in the elderly.[27] Therefore caution is warranted in interpreting our findings regarding the risk of cigarette smoking.

In conclusion, white blood cell count appears to predict future coronary heart disease and mortality independent of conventional risk factors. White blood cell count is probably not only an indicator of clinical or subclinical disease, but also a true risk factor for coronary heart disease. Whether a causal pathway is involved should be assessed in future experimental studies. Nevertheless, white blood cell count could be of important public health use since it is routinely measured by clinicians. Moreover, it seems of specific importance in older people since it appears to be more strongly associated to coronary heart disease than, for example, total cholesterol in this age group.

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Chapter 8
Serum albumin and the five-year risk of coronary heart disease and all-cause mortality in elderly men

Abstract

The associations of serum albumin with the incidence of coronary heart disease and mortality were investigated in men from the Zutphen Elderly Study followed for 5 years. In 1985, 820 men randomly selected from a population aged 64 to 84 years, had complete information on risk factors. Relative risks were adjusted for traditional risk factors (age, body mass index, diastolic blood pressure, total and high density lipoprotein cholesterol, smoking and alcohol consumption) and additionally for baseline health status indicators (white blood cell count, physician's health score, self-rated health and history of relevant diseases). Albumin was inversely associated with the incidence of coronary heart disease among men with elevated total cholesterol levels (≥ 6.5 mmol/L) only. The relative risk for one standard deviation increase (2.5 g/L) in albumin was 0.60 (95%-confidence interval: 0.38 - 0.96) and was not altered after additional adjustment for baseline health status. In all men, the relative risk for death due to coronary heart disease was 0.67 (0.49 - 0.92) and reduced to 0.84 (0.61 - 1.15) after adjustment for health status. Comparable health status adjusted relative risks were observed for mortality from cardiovascular diseases (0.83 (0.67 - 1.02)) and all causes (0.86 (0.73 - 0.99)). Independent of traditional risk factors, moderately low serum albumin is predictive of coronary heart disease and all-cause mortality in elderly men. Only part of this could be explained by baseline health status.

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Introduction

Low serum albumin levels, even within the normal range of 35 g/L to 50 g/L, have been associated with all-cause mortality in several free-living older populations.[1-3] Although plausible mechanisms for a causal association have been postulated,[4-7] especially for coronary heart disease, very few studies present cause specific mortality data, or data on the incidence of coronary heart disease. To our knowledge, only the National Health and Nutrition Examination Survey Epidemiologic Follow-up Study provides data on these end-points specifically in elderly people (65 to 74 years old). In this age group, no clear association between serum albumin and coronary heart disease incidence or mortality was observed.[3]

Low albumin levels may be a marker of (mild) inflammation and (underlying) disease. Albumin is a negative acute phase protein[8] and the serum level may be reduced due to a general increased vascular permeability during disease as a result of tissue damage.[9] Some investigators suggest that the relation of low albumin with increased mortality reflects an association of other serum proteins with mortality[10,11] or an association of activated macrophages with cardiovascular diseases.[7] However, low serum albumin is conceivably more than just a marker. Through its specific binding capacity with free fatty acids it may inhibit platelet aggregation and thrombosis.[6] Furthermore, albumin may act as an indirect and "sacrificial" anti-oxidant through its specific binding to metals and scavenging abilities of peroxyl radicals.[4,12]

Hence, it is of interest to investigate whether the association between serum albumin and coronary heart disease and mortality persists after accounting for baseline health status. In addition to a history of disease and a physician's examination of an individual, other factors have proven to be indicative of baseline health status. Low self-rated health has been demonstrated to predict mortality independent of traditional risk factors and a history of chronic diseases.[13] Increased white blood cell count has also been suggested as an indicator of inflammation and has been causally associated with coronary heart disease and other chronic diseases.[14] In earlier reports among elderly people,[1,2,15] the association between serum albumin and mortality was not investigated independent of both traditional risk factors and acknowledged indicators of baseline health status. Therefore, we investigated the associations of serum albumin with the incidence of coronary heart disease and cause specific mortality in elderly men of a population-based cohort and accounted for traditional confounders as well as for baseline health status.

Methods

Population

The Zutphen Study, the Dutch contribution to the Seven Countries Study,[16] is a longitudinal investigation of chronic disease risk factors initiated in 1960 among middle-aged men. In 1985, 555 men from the randomly selected cohort from 1960 were still alive and were invited for new examinations. In addition, a random sample (two out of three) of all men of the same age living in Zutphen and not part of the 1960 cohort were invited to take part in the study. From then on, the study was continued as the Zutphen Elderly Study. Of the 1266 men approached 939 (74 percent) agreed to participate. Hundred-and-nine men (9 percent) could not be examined because of serious illness or death, 62 men (5 percent) had moved and 156 men (12 percent) refused to participate or could not be reached. Complete information on risk factors was available for 820 men aged 64 to 84 years. The study has been approved by the medical ethics committee of the University of Leiden, The Netherlands. Informed consent was obtained from all participants.

Examinations

The examinations took place between March and June 1985. Physical examinations were carried out by five trained physicians according to a standardized protocol and included anthropometric and blood pressure measurements, non-fasting venous blood sampling, and the assessment of the medical history. Height was measured to the nearest 0.1 cm and body weight was measured to the nearest 0.5 kg while the men were in underwear. Body mass index (weight (kg)/ height $(m)^2$) was calculated. At the end of the physical examination, systolic and diastolic (fifth Korotkoff phase) blood pressures were measured in duplicate with a random zero sphygmomanometer at the right arm while subjects were in the supine position. The mean of the two blood pressure values was used in the analyses.

Serum albumin levels were analyzed in an auto-analyzer (SMAC, Technicon, Tarytown, USA) in the Central Laboratory of Clinical Chemistry of the Academic Hospital in Leiden. Serum total and high density lipoprotein (HDL) cholesterol were determined enzymatically with the CHOD-PAP mono-testkit from Boehringer Mannheim.[17,18] HDL was isolated after precipitation of apo B containing particles by dextran sulphate-Mg^{2+}.[19] The lipid analyses were carried out in the standardized lipid laboratory of the Department of Human Nutrition, Agricultural University, Wageningen, The Netherlands. White blood cell count was determined using a Coulter Counter S880 in the Nieuwe Spitaal Hospital in Zutphen.

Information on smoking habits was assessed with a standardized questionnaire and is missing for one person. Life time occupation was used as an indicator of social economic status. It was obtained from a self-administered questionnaire, and divided into four categories: professionals, managers and teachers; small business owners; administrative personnel; and manual workers. Information on alcohol consumption was assessed by trained dietitians with a cross-check dietary history,[20] adapted to the Dutch situation.[21]

Health status indicators and follow-up

At the end of the physical examination, the physicians rated the general health of each participant on a five point scale ranging from excellent health to unhealthy. Participants were also asked to rate their own health on a self administered questionnaire and could choose between: healthy, rather healthy, moderately healthy or not healthy. The association with physicians' rating was moderate (Spearman correlation = 0.37). White blood cell count was also regarded as a health status indicator.[22]

Information on the presence of diabetes mellitus, cancer and chronic obstructive pulmonary disease at baseline was obtained through a standardized medical questionnaire and was verified with hospital discharge data and written information from the subjects' general practitioners.

Information on the presence of coronary heart disease was obtained during the physical examination in 1985 and a similar examination in 1990 using the Dutch translation of the Rose questionnaire.[23] For men who did not participate in the 1990 examination, information on major chronic diseases was obtained from a questionnaire for nonparticipants. Coronary heart disease was considered to be present when either myocardial infarction or angina pectoris was diagnosed. The diagnoses were verified with hospital discharge data and written information from the subjects' general practitioners. For myocardial infarction the final diagnosis was based on whether two of the following three criteria were met: a specific medical history, i.e. severe chest pain lasting for more than 20 minutes and not disappearing in rest, characteristic electrocardiogram changes and specific enzyme elevations. The diagnosis of angina pectoris was based on information obtained from the Rose questionnaire.[23] All information was eventually coded by one physician, and the year of first diagnosis was recorded.

Information on the vital status of participants was obtained till July 1990. Information on the causes of death was obtained from the Dutch Central Bureau of Statistics, after verification with hospital discharge data and information from the deceased's general practitioners. The causes of death were coded according to the 9th Revision of the International Classification of Diseases (ICD)[24] and both the primary and secondary cause of death were considered. Death due to coronary heart disease was defined by ICD codes

410-414, death due to cardiovascular disease by codes 390-459 and death due to all-cause cancer by codes 140-239.

The main end-points investigated were the incidence of a first fatal or non-fatal coronary heart disease event (i.e. incidence of coronary heart disease), mortality from coronary heart disease, cardiovascular diseases, all-cause cancer, all other causes and all causes combined.

Statistical methods

Statistical analyses were carried out using the SAS program (SAS Institute Inc., Cary, North Carolina, USA, 1989-1993, version 6.10). All tests were two sided and *p*-values smaller than five percent were considered statistically significant. Analysis of variance and Kruskall-Wallis tests were carried out to examine differences in continuous variables between albumin tertiles. Chi-square test statistics were used to investigate associations between categorical variables.

Cox's proportional-hazard (survival) analysis was carried out to investigate the associations of serum albumin with end-points during five years of follow-up. Relative risks were adjusted for traditional risk factors and additionally for baseline health status indicators. For coronary heart disease, cardiovascular diseases and all-cause mortality end-points, the traditional confounders adjusted for were: age, body mass index, diastolic blood pressure, total and HDL cholesterol, cigarette smoking (never, former, current) and alcohol consumption (yes or no) at baseline. The same factors were adjusted for in the associations with mortality from cancer and all other causes with the exception of diastolic blood pressure and HDL cholesterol. Regarding the health status indicators, white blood cell count, physician's health score and self-rated health were always considered together with the history of specific diseases depending on the end-point investigated. Interaction terms were evaluated at the 10 percent level.

Results

The mean serum albumin level of men at baseline was 44.1 (SD 2.5) g/L. Three men had an albumin level lower than 35 g/L and thirty had a level lower than 40 g/L. Men with albumin levels in the lowest third of the distribution were significantly older, had lower body mass index, diastolic blood pressure and total cholesterol levels, higher white blood cell counts and were less often alcohol drinkers than men with higher levels of serum albumin (table 1). For diastolic blood pressure, total cholesterol and alcohol consumption the association was graded over the albumin tertiles. For other variables, the differences

were mainly observed between the lowest and the two higher albumin tertiles. Life time occupation was not associated with albumin levels (table 1).

Table 1. *Mean levels of baseline characteristics according to tertiles of serum albumin levels in elderly men (the Zutphen Elderly Study, 1985)*

Characteristics	Albumin tertiles		
	First (n = 311)	Second (n = 274)	Third (n = 235)
Albumin range (g/L)	34.0 - 43.0	44.0 - 45.0	46.0 - 52.0
	Mean (standard deviation)		
Age (years)	72.5 (5.2)	70.9 (5.2)	70.4 (5.1)*
Body mass index (kg/m^2)	24.88 (3.17)	25.86 (3.26)	25.82 (2.86)*
Systolic blood pressure (mmHg)	149.0 (20.8)	151.7 (21.4)	151.6 (21.6)
Diastolic blood pressure (mmHg)	83.7 (12.0)	85.8 (10.3)	87.2 (11.5)†
Total cholesterol (mmol/L)	5.88 (1.12)	6.13 (1.04)	6.38 (1.11)*
HDL cholesterol (mmol/L)	1.11 (0.30)	1.14 (0.30)	1.12 (0.27)
White blood cell count (10^9/L)	6.9 (1.9)	6.5 (1.7)	6.4 (1.8)†
		Percentage	
Alcohol consumption (> 0 g/day)	67.5	73.7	78.7‡
Current cigarette smokers	32.9	29.9	26.4
Former cigarette smokers	50.0	49.6	56.6
Life-time occupation as professionals, managers or teachers	27.1	27.2	20.3

*: $p < 0.001$
†: $p < 0.01$
‡: $p < 0.05$

Serum albumin was associated with baseline health status indicators (table 2). Men with a history of major chronic diseases at baseline were more likely to have lower albumin levels. For men who where rated in excellent health by the physicians and those who rated themselves as healthy, serum albumin levels were less likely to be low (table 2). The differences between these health status indicators were generally most pronounced between the lowest albumin tertile and the two higher tertiles.

Among 657 men free of coronary heart disease at baseline, 51 men became incident of a first fatal or non-fatal coronary heart disease event during the follow-up period. Serum albumin was not associated with the incidence of coronary heart disease (table 3). However, serum total cholesterol was an effect modifier in this association (p-interaction = 0.01), and an inverse association between serum albumin and the incidence of coronary

heart disease was evident in men with serum cholesterol levels of 6.5 mmol/L or higher (table 3). Additional adjustment for baseline health status did not alter the strength of the association. Albumin was not associated with coronary heart disease incidence among men with cholesterol levels lower than 6.5 mmol/L (table 3).

Table 2. *Baseline health status according to tertiles of serum albumin levels in elderly men (the Zutphen Elderly Study, 1985)*

Health status indicators	Albumin tertiles		
	First	Second	Third
History of			
coronary heart disease	25.7 (80)*	16.4 (45)	16.2 (38)†
cardiovascular disease	42.8 (133)	29.9 (82)	29.4 (69)†
cancer	11.3 (35)	5.8 (16)	5.1 (12)‡
chronic obstructive pulmonary disease	20.6 (64)	11.7 (32)	12.8 (30)†
diabetes mellitus	7.1 (22)	6.6 (18)	4.7 (11)
Health score given by physician			
in excellent health	21.4 (66)	35.3 (96)	32.1 (75)†
Self rated health			
healthy	39.7 (120)	50.8 (134)	52.7 (118)†

*: % (n)
†: $p < 0.01$
‡: $p < 0.05$

Table 3. *Relative risks for five-year incidence of coronary heart disease according to one standard deviation (2.5 g/L) in serum albumin level in elderly men overall and by serum total cholesterol level (the Zutphen Elderly Study, 1985 - 1990)*

	Number of incident cases	Relative risk (95% confidence interval)	
		Adjusted for traditional risk factors†	Adjusted for traditional risk factors and health status‡
Overall (n = 656)*	51	0.81 (0.60 - 1.10)	0.86 (0.63 - 1.17)
Serum total cholesterol < 6.5 mmol/L (n = 427)*	30	1.04 (0.69 - 1.57)	1.13 (0.74 - 1.71)
Serum total cholesterol ≥ 6.5 mmol/L (n = 229)*	21	0.60 (0.38 - 0.96)	0.60 (0.35 - 1.02)

*: Number of men free of coronary heart disease at baseline
†: age, body mass index, diastolic blood pressure, total and HDL cholesterol, cigarette smoking and alcohol consumption
‡: white blood cell count, self-rated health, physician's health score and diabetes mellitus

For mortality from coronary heart disease, there was an inverse association with serum albumin independent of traditional coronary heart disease risk factors (table 4). The relationship was attenuated after additional adjustment for baseline health status indicators. For mortality from cardiovascular diseases, the relation with serum albumin was similar and of the same magnitude as that for mortality from coronary heart disease (table 4).

Table 4. *Relative risks for five-year mortality according to one standard deviation (2.5 g/L) increase in serum albumin level in elderly men (the Zutphen Elderly Study, 1985 - 1990)*

Causes of mortality	Number of deaths	Relative risk (95% confidence interval)	
		Adjusted for traditional risk factors*	Adjusted for traditional risk factors and health status†
Coronary heart disease	46	0.67 (0.49 - 0.92)‡	0.84 (0.61 - 1.15)
Cardiovascular diseases	96	0.69 (0.56 - 0.86)	0.83 (0.67 - 1.02)
All-cause cancer	65	0.67 (0.52 - 0.86)	0.98 (0.76 - 1.27)
All other causes	31	0.53 (0.36 - 0.79)	0.61 (0.40 - 0.91)
All causes	189	0.67 (0.56 - 0.76)	0.86 (0.73 - 0.99)

*: age, body mass index, diastolic blood pressure, total cholesterol, cigarette smoking for all end-points, and for HDL cholesterol and alcohol consumption for cardiovascular diseases and all-cause mortality end-points

†: white blood cell count, self-rated health, physician's health score for all endpoints, and diabetes mellitus and a history of coronary heart disease or cardiovascular disease and/or cancer and/or chronic obstructive pulmonary disease depending on mortality outcome

‡: relative risk (95% confidence interval)

For all other major causes of death the risk factor adjusted associations with serum albumin were also inverse (table 4). For cancer mortality the association disappeared after additional adjustment for health status indicators. For all other causes of death the association was only slightly reduced following adjustment for baseline health status. For all-cause mortality additional adjustment for baseline health status attenuated the relation with serum albumin (table 4).

Discussion

In this cohort of elderly men a low serum albumin level, within a normal range, was associated with the occurrence of a first coronary heart disease event in elderly men with elevated cholesterol levels. This association remained when traditional risk factors and

baseline health status were accounted for. A risk factor independent association with mortality from coronary heart disease, cardiovascular diseases and all-causes was observed which could partially be explained by baseline health status.

Traditional risk factors

The association of serum albumin with mortality has often, in part, been explained by its association with other factors associated with mortality[10,11,15,25-27] or by underlying diseases.[8,9,26] However, in many reports no adjustments were made for traditional risk factors for mortality such as body mass index,[1-3,28,29] blood lipids,[1,2,30] blood pressure levels[1,2] and smoking habits.[2] In our study, we adjusted for acknowledged risk factors for cause-specific mortality, and the inverse association persisted for all end-points. The strength of the associations for cause-specific mortality end-points, for a 2.5 g/L increase in serum albumin, were of the same order of magnitude as those observed among middle-aged men in the British Regional Heart Study.[29] Hence, it appears that the predictive importance of serum albumin for cause-specific mortality does not decline with advancing age, as was observed in elderly men from the National Health and Nutrition Examination Survey Epidemiologic Follow-up Study.[3] Within our cohort, there was even an indication that the observed associations were stronger among the older men (71 years or older) than among the younger ones (not shown).

Some investigators ascribed the association of serum albumin with mortality to that of serum globulin with mortality.[10,11] However, as these investigators have done, we estimated serum globulin from the difference between total serum protein and albumin and no association with any of the end-points was observed (not shown). Therefore, it is probably albumin, rather than any of the other serum proteins, that is responsible for the observed associations. This was also reported in the British Regional Heart Study where globulin was directly measured in serum.[31]

Baseline health status

Since it is widely recognized that serum albumin is decreased as a result of infection, inflammation and disease, reported associations between serum albumin and mortality have been adjusted for clinical manifestations of baseline disease.[1-3,29,30] In a cohort of men and women over the age of 71 years, Corti and colleagues additionally adjusted for physical disability, an indication of frailty and high mortality risk, and low albumin levels remained predictive of all-cause mortality. Both self-rated health and white blood cell count are well-recognized indicators of health status in the elderly.[13,14] A physician's rating of the general health status of an individual may prove to be of value in addition to information on the presence of disease and an individual's self-rated health. This is

suggested by our findings since in the fully adjusted model of serum albumin with all-cause mortality, baseline presence of cardiovascular diseases, cancer and chronic obstructive pulmonary disease, self-rated health, the physician's health score, and white blood cell count were all significant confounders. Therefore, by simultaneously adjusting for all these health status indicators, an attempt was made to account for baseline disease or inflammation as much as possible.

After adjustment for baseline health status indicators the strength of the associations of serum albumin with mortality endpoints were of the same magnitude for mortality from coronary heart disease, cardiovascular diseases and all-causes. A low albumin level, even within the normal range, can be viewed as an indicator of inflammation and vascular injury,[9] and possibly of progression of atherosclerosis. Concurrently, plausible mechanisms for the association between serum albumin and atherosclerosis as well as thrombosis have been proposed. Through its specific binding capacities, albumin is conceivably an indirect and "sacrificial" anti-oxidant,[4,12] and an indirect inhibitor of platelet aggregation and thrombosis.[6] Consequently, part of the inverse association between serum albumin and mortality from coronary heart disease and all cardiovascular diseases may be a result of underlying progression of atherosclerosis and thrombosis, as is observed after adjustment for prevalence of related disease at baseline. Another part may be causal. The latter is suggested by the finding that serum albumin was an equally strong predictor of a first coronary heart disease event ever in men with elevated serum total cholesterol levels after adjustment for traditional risk factor levels as well as after additional adjustment for indicators of baseline health.

The effect modification by serum total cholesterol of the association of serum albumin with the incidence of coronary heart disease is somewhat surprising. Possibly a combination of a low serum albumin level, even within the normal range, and an elevated total cholesterol level and thus an elevated low density lipoprotein cholesterol level, promotes the increase of circulating oxidized low density lipoprotein cholesterol. Low serum albumin may thereby indirectly induce the clinical manifestation of athero-thrombotic disease.

The association between serum albumin and mortality from cancer disappeared after adjustment for baseline health status. This suggests that low albumin levels may have been indicative of the presence of cancer at baseline as was observed in the BUPA study.[26] Low serum albumin levels remained predictive of death due to causes other than cardiovascular diseases or cancer. However, we only adjusted for the prevalence of chronic obstructive pulmonary disease, since detailed information on other chronic diseases was not available. If the presence of these diseases could be accounted for, the strength of this association is expected to be reduced,[26] as is the association with all-cause mortality.

In conclusion, low serum albumin, within the normal range, is associated with coronary heart disease and all-cause mortality in elderly men. This may partly be explained by baseline health status, but for coronary heart disease, biological mechanisms associated with progression of atherosclerosis or thrombosis, may also be involved. Since complete information on (underlying) diseases is usually lacking in large prospective studies in elderly populations, serum albumin, a routinely measured blood chemistry parameter, may be a useful risk indicator of overall mortality risk.

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Chapter 9
General discussion

In this thesis prospective studies on the associations between biological risk factors and coronary heart disease occurrence in elderly people have been described. Many of the limitations and strengths of the specific investigations as well as the implications of the specific results have been discussed in the previous chapters. Following a brief recapitulation of the main findings, this chapter focuses on some methodological considerations including the validity of the findings and issues specific to epidemiologic research in older populations. The experience obtained from the studies is discussed in this context. Thereafter, some pathophysiologic and etiologic features of coronary heart disease in the elderly are addressed with respect to the findings. Finally, the overall public health implications of the studies are considered.

Main findings

The major results of the five year follow-up of men from the Zutphen Elderly Study are summarized in the table (based on chapters 4 and 6 through 8). The relative risks are presented for one standard deviation increase in a risk factor and are adjusted for all factors simultaneously, including age, body mass index, cigarette smoking habit and systolic blood pressure. Generally, associations of risk factors with the incidence of coronary heart disease were weaker than those with mortality from the disease. An increased serum total cholesterol concentration was predictive of short-term mortality from coronary heart disease (chapter 4). In the 17-year follow-up study, a similar association was observed among elderly women, but among elderly men the association was inverted *U*-shaped (chapter 3). High density lipoprotein cholesterol tended to be inversely associated with the short-term incidence of coronary heart disease, but was not associated with mortality outcomes (chapter 4). Homocysteine was associated with mortality from coronary heart disease in the first 1.5 years of follow-up only, but an association with the incidence of the disease was lacking (chapter 6). An increased white blood cell count (chapter 7) and a reduced serum albumin concentration (chapter 8) were related to an increased risk of coronary heart disease mortality. Systolic blood pressure was an independent risk factor for long-term mortality from coronary heart disease in elderly women, but not in elderly men (chapter 3). In the five year follow-up study, systolic and diastolic blood pressure were also not linearly related to coronary heart disease, but the

associations tended to be *U*-shaped (chapter 2). However, strong linear associations were observed between blood pressure levels and sudden cardiac death (chapter 2).

Table 1. *Associations of main risk factors* investigated in the five year follow-up of the Zutphen Elderly Study among men aged 64 to 84 years at baseline in 1985*

Risk factor (1 standard deviation)	Relative risks for 1 standard deviation increase in the risk factor (95% confidence interval)†		
	Incidence of CHD 55 events 701 at risk	Mortality from CHD 52 deaths 872 at risk	Mortality from all-causes 209 deaths 872 at risk
Serum total cholesterol (1.10 mmol/L)	1.25 (0.93 - 1.68)	1.53 (1.16 - 2.02)	1.14 (0.98 1.32)
HDL cholesterol (0.29 mmol/L)	0.77 (0.56 - 1.07)	1.12 (0.86 - 1.47)	0.97 (0.85 - 1.11)
Serum homocysteine (8.2 nmol/ml)	0.90 (0.67 - 1.22)	0.99 (0.77 - 1.27)	1.00 (0.89 - 1.12)
White blood cell count (1.8 10^9/L)	1.25 (0.95 - 1.63)	1.55 (1.20 - 1.99)	1.41 (1.23 - 1.61)
Serum albumin (2.7 g/L)	0.84 (0.63 - 1.12)	0.71 (0.55 - 0.92)	0.73 (0.64 - 0.83)

*: except for blood pressure since associations were *U*-shaped (see text)
†: adjusted for all risk factors simultaneously including age, body mass index, cigarette smoking habit and systolic blood pressure

Methodologic considerations

There are several methodologic issues which should be considered in interpreting the results of different studies. The validity of prospective studies is briefly discussed succeeded by some issues specific to epidemiologic research in elderly people.

Validity

Validity deals with the question whether a study measures what it intends to measure. In general, the internal and external validity are considered.[1]

Internal validity With regard to the internal validity, care needs to be taken in avoiding biases and confounding. Selection and recall biases are unlikely to have occurred in our studies since the exposures, risk factor levels, were ascertained before the occurrence of the disease of interest, e.g. coronary heart disease. Loss to follow-up was also not a major concern since there was only one such a case in both cohorts studied. In investigating the

associations between risk factors and end-points, all currently known potential confounders were carefully considered and adjusted for. A disputable confounder is the presence of coronary heart disease at baseline. Within the strict definition of the concept, the presence of the disease at baseline is not a confounder since it is probably an intermediate in the causal pathway between risk factor and end-point. Adjustment for the presence of the disease therefore generally attenuated the observed associations.

External validity The external validity of the studies refers to their generalizability. Regarding the cohort from the general practice in Rotterdam (chapter 3), it must be noted that nearly all people in the Netherlands are registered in a general practice. In addition, all elderly individuals from this general practice were invited to participate in the study with the exception of 30 individuals whom the general practitioner had judged physically or mentally unable to participate. The general practice was located in a working-class area. Therefore, the cohort is not representative of the general Dutch elderly population of that time: it was generally healthier and from a specific socio-economic class. The cohort of the Zutphen Elderly Study consisted of two randomly selected population-based samples (see the methods sections of chapters 2, 4, 6-8). One was based on the middle-aged male population of Zutphen in 1960 who where followed up into old age and the other of the elderly male population of Zutphen in 1985. Women were originally excluded from the study because, at the initiation of the Zutphen Study in 1960 as part of the Seven Countries Study, coronary heart disease was viewed as a major health problem of middle-aged men. In 1985, funding to include elderly women in the Zutphen Study was applied for but not obtained. Although the participation rates of both cohorts were quite high, 86 percent for the Rotterdam cohort and 74 percent for the Zutphen cohort, the participants conceivably had more favorable risk factor levels and a lower risk of coronary heart disease than the rest of the Dutch population of the same age. Results from a non-response survey during the 1993 examination period of the Zutphen Elderly Study confirmed that participants had a lower prevalence of coronary heart disease than non-respondents.[2] However, the possible existing differences between the two cohorts and the general Dutch elderly population do not necessarily imply that the etiologic associations studied in this thesis are different from those in the general population. There is indeed no indication for such a difference with the exception of the gender difference with regard to the Zutphen Elderly Study. The results obtained from this study need verification in elderly women. Overall, the generalizability of the findings does not merely depend on whether the cohorts represent the general population from which they have been sampled or on the sample size of the cohorts. The internal validity, as discussed previously, may be more important.[1]

In conclusion, the findings described in this thesis are judged to be valid and generalizable with respect to the etiologic knowledge that they have provided on risk factors for coronary heart disease in elderly persons.

Issues specific to epidemiologic research in the elderly

Some considerations have to be made when interpreting findings from prospective epidemiologic studies in the elderly. Specific features of elderly populations are discussed with regard to methodology and findings, such as the high prevalence of disease, the high mortality rate, natural selection mechanisms, and age-related changes and individual variations in risk factor levels. Furthermore, special attention is given to the interpretation of the measure of association in epidemiologic studies in the elderly. Finally, some possible methodological explanations for the different findings for incidence and mortality from coronary heart disease are discussed.

Comorbidity and comortality The frequency of comorbidity and comortality increases with advancing age in a population. Since the co-existence of diagnosed or underlying disease or death from causes other than coronary heart disease may modify the association between risk factors and coronary heart disease, this issue is of major concern. The prevalence of diseases at baseline was high in the two elderly cohorts described in this thesis. In the Zutphen Elderly Study for example, 61 percent (542/885) of all men had at least one of the major chronic diseases, i.e. diabetes mellitus, cardiovascular diseases, different types of cancer or chronic non-specific lung diseases. Although this point is widely recognized, there are no satisfying procedures on how to deal with it.

The exclusion of all people with major chronic diseases at baseline does not offer a solution. The remaining group would be a distinct group not representative of a general elderly population. In addition, as was discussed in chapter 8, underlying, undiagnosed disease may be very common in elderly people. This is partly caused by an increased propensity to accumulate illnesses with advancing age. The high prevalence of undiagnosed disease may also be a result of the belief of some elderly people as well as clinicians that a certain degeneration in their health is a normal concomitant of aging. The presence of an actual illness may therefore be disregarded. In our studies, possible indicators of underlying disease, white blood cell count and serum albumin, were investigated in combination with the conventional risk factors for coronary heart disease in order to account for possible underlying illness (chapters 7 and 8). The main conclusions regarding the conventional risk factors were not altered (table 1 on page 130).

However, the results of our studies indicate that it is preferable to investigate interactions between indicators of (underlying) ill-health and risk factors of interest since

certain diseases can alter risk factor levels. In the Zutphen Elderly Study for example, an interaction was observed between total cholesterol and body mass index in the association with mortality from coronary heart disease (chapter 4). The association with total cholesterol was lacking among the lean men. As addressed in chapter 4, a possible explanation could be that men with low body mass index form a heterogenous group. Part of the group may include healthy men with normal total cholesterol levels and at a decreased risk of coronary heart disease mortality, and the other part may include unhealthy men with reduced total cholesterol levels and at an increased risk of coronary heart disease mortality. There was also an interaction between total cholesterol and serum albumin in the association with the incidence of the disease (chapter 8). The association with serum albumin was lacking in men with low total cholesterol levels. Additional analyses revealed a positive association between total cholesterol and the incidence of coronary heart disease in men with low serum albumin concentrations, while the association was lacking in men with high serum albumin concentrations. The latter group may form a select group of healthy men, who remained free of coronary heart disease at middle-age, and in whom an elevated total cholesterol concentration is not associated with an elevated risk of coronary heart disease at this older age. These possible explanations for the observed interactions cannot be verified. However, they suggest pronounced heterogeneity of the cohort complicating the interpretation of the results.

Comortality is partly taken into account in Cox's proportional hazards analysis. This method has the advantage of being able to include individuals who are censored due to death from another cause than coronary heart disease in the analysis.[3] All relative risks presented in this thesis are therefore estimated from Cox's proportional hazards models. However, one must keep in mind that the relative risks for cause-specific mortality endpoints invariably retain the assumption that there are no competing risks.[1] The only endpoint that meets this assumption is all-cause mortality. The association between risk factors and all-cause mortality was always addressed in our studies. In prospective studies of morbidity and specific causes of death, like those described in this thesis, it is not possible to eliminate or to account for competing causes of death. In order to obtain a better notion of the importance of potential competing causes of death, associations of factors with other endpoints than coronary heart disease mortality were additionally investigated if the factors were suspected to be involved in the etiology of these other diseases (chapter 2, 6 and 8).

Selective survival The progressive age related decline in the associations of some risk factors with coronary heart disease may have to do with selective survival. With advancing age, there could be a progressive elimination of susceptible individuals with elevated risk

factor levels from the population. The remaining individuals with elevated risk factor levels may be less susceptible to coronary heart disease and may have a similar risk for the disease than those with normal risk factor levels.[4] This phenomenon possibly explains the inverted *U*-shaped association between total cholesterol and mortality from coronary heart disease among men followed for 17 years (chapter 3). Among middle-aged men of the Zutphen Study followed for 25 years a selective loss of individuals with high cholesterol levels was observed.[5] The inverted *U*-shaped association was not observed between total cholesterol and coronary heart disease incidence or mortality in men from the Zutphen Elderly Study followed for five years (chapter 4). As discussed in chapter 4, differences in length of follow-up may explain this discrepancy.

Changes in risk factor levels with age It is important to acknowledge that the level of a particular risk factor measured at old age may not represent the level of a life-time exposure when interpreting the results.[6] As was discussed previously, elderly people have a high prevalence of (underlying) disease. Many chronic diseases are known to influence the levels of the conventional risk factors, e.g. to lower blood pressure and the total blood cholesterol concentration. Moreover, the frequency of medication use for coronary heart disease, hypertension and hypercholesterolemia increases with age and usually lowers the levels of conventional risk factors. Thus, people with initial elevated levels of these risk factors who are thereby at an increased risk of coronary heart disease, may eventually have lower levels of the risk factors while the ultimate event remains coronary heart disease. This possible misclassification of older people according to their risk factor level attenuates associations between the risk factors and coronary heart disease occurrence. It must be noted that the decline in risk factor levels may be a result of other factors than the presence of (underlying) disease. Indeed, in chapter 5, the total cholesterol concentration of elderly men was shown to decline with age regardless of selective survival, loss to follow-up and the health status at the time of the measurement. Although these age-related physiologic changes may occur independent of health status, they also have the potential to attenuate the total cholesterol-coronary heart disease association. Thus, regardless of the presence of (underlying) illness, it is desirable to know whether coronary heart disease risk factors change with age. Age-related changes in risk factors other than total and high density lipoprotein cholesterol were not addressed in this thesis since the measurement of these risk factors were not available for more than two examination rounds of the Zutphen Elderly Study.

Although the level of a particular risk factor measured at old age may not represent the level of a life-time exposure, it is important to consider its etiologic association with coronary heart disease. The question arises whether a recent short-term exposure or a long-

term exposure to a harmful level of a risk factor is associated with the occurrence of an event.[4] As will be discussed in the next section, the etiology of coronary heart disease is complex, and both short and long term effects of risk factors appear to be of importance. This emphasizes the need for studies investigating age-related changes in risk factor levels.

Within-person variation All biological variables have a tendency for individual variation. Depending on the variable, night and day, daily, weekly or monthly variations exist. A single measurement of a risk factor in individuals of a population will give an accurate mean for that population but the range will be larger than when multiple measurements are made. A single measurement of biological risk factors can thereby lead to a substantial underestimation of the true associations of the usual level of the risk factors with coronary heart disease.[7] This phenomenon is known as regression dilution bias and is not specific to older populations. The amount of underestimation of an association can only be assessed if multiple measurements of biological risk factors from (a subsample of) individuals of the populations under study are available. This information was only available for total and high density lipoprotein cholesterol in the Zutphen Elderly Study (chapter 5) and for total cholesterol and blood pressure in the Rotterdam general practice cohort (chapter 3).[8] Reliability coefficients can be computed from repeated measurements and this coefficient can be used to estimate the relative risks adjusted for regression dilution bias.[8] For serum total cholesterol, for example, the relative risks for mortality from coronary heart disease in the 17-year follow-up study (table 4 of chapter 3) were underestimated by 40 and 15 percent for the second and third tertile respectively. In the Zutphen Elderly Study the relative risk for mortality from coronary heart disease was underestimated by 13 percent per 1 mmol/L increase in total cholesterol (table 3 of chapter 4).

Relative versus absolute risk The prevailing notion that the strength of the association between risk factors and coronary heart disease generally diminishes with age deserves careful attention. Relative risks for coronary heart disease may indeed be lower in the elderly than in middle-aged populations, but absolute risks tend to increase with advancing age. This is best illustrated in chapter 2 where interactions were observed between blood pressure and age in the associations with sudden cardiac death and all-cause mortality. The mortality rates in the reference blood pressure categories were much higher in the older men as compared to the younger men. The risk differences between blood pressure categories were also generally higher in the older versus the younger men, whereas the relative risks were similar or lower. Therefore, the evaluation of the age-related change of an association depends on the measure of effect.

Incidence versus mortality from coronary heart disease The relatively weak association of most risk factors with the first occurrence of a coronary heart disease event as compared to the associations with mortality from the disease in our studies may be due to methodological issues. Associations with incidence were estimated from a population initially free of coronary heart disease at baseline, whereas those with mortality were estimated from a population including people with a history of the disease. Hence, the source populations were different, making comparisons difficult. Excluding people with coronary heart disease at baseline for the mortality analyses would lead to an exceptionally healthy population. Moreover, most people who died of coronary heart disease already had manifestations of the disease. Out of 53 men who eventually died from coronary heart disease in the Zutphen Elderly Study, 34 already had a history of the disease at baseline and another 17 had a non-fatal event during the course of the follow-up period before eventually dying from the disease. Adjusting for a history of coronary heart disease at baseline would be an over-adjustment, because, as discussed previously, it is more likely to be an intermediate than a confounder in the associations studied. A lack of power is probably not a potential explanation for the differential strengths of the associations since the number of events for both coronary heart disease outcomes are similar (56 incident cases and 53 mortality cases).[1,9]

Pathophysiology and etiology of coronary heart disease in elderly people

Apart from methodological issues specific to epidemiologic studies in the elderly, the pathophysiology and etiology of coronary heart disease may be altered in this age group as compared to middle-aged people. These possible changes with advancing age may underlie some of the associations described in the previous chapters and they are briefly addressed in the ensuing paragraphs.

Atherosclerosis and inflammation Coronary atherosclerosis is generally well advanced in the elderly. It is estimated that approximately half of the elderly population of the industrialized countries have more than 50 percent occlusion of one of the coronary arteries.[10] The question rises as to what factors contribute to the manifestation of coronary heart disease in a population with such high prevalence of atherosclerosis. Risk factors associated with the onset of atherosclerosis *per se* may not be sufficient at advanced age to distinguish between those who will and those who will not develop a clinical event. Possibly the identification of factors specifically associated with the progression of the

disease may improve the prediction of clinical events in older populations. Since the introduction of the response to injury hypothesis by Ross and Glomset in 1976,[11,12] the progressive state of atherosclerosis is generally regarded to be an inflammatory disease state.[13] Therefore, in addition to the conventional risk factors for coronary heart disease, factors associated with the inflammatory state of atherosclerosis may enhance the prediction of the occurrence of clinically evident coronary heart disease in elderly people. Indeed, as was observed in our studies, indicators of inflammation, such as an increased white blood cell count (chapter 7) and a reduced serum albumin (chapter 8), proved to predict coronary heart disease occurrence in addition to the conventional risk factors blood cholesterol, blood pressure and cigarette smoking. Since white blood cell count and serum albumin are routinely measured by clinicians, they are potentially useful and promising indicators for the identification of elderly people at increased risk of coronary heart disease.

The role of thrombosis Currently, thrombosis is thought to be the most common precipitating cause of myocardial infarction.[14] In concurrence with the high prevalence of atherosclerosis in the elderly, specific thrombogenic risk factors may become more significant in predicting coronary heart disease with advancing age. Some potentially thrombogenic risk factors were investigated in this thesis. One of them is homocysteine. Findings from in vitro studies suggest that homocysteine can induce thrombosis.[15] As discussed in chapter 6, the thrombotic effect of homocysteine can probably explain only a part of our prospective findings, i.e. the positive associations between homocysteine and fatal coronary heart disease in the first 1.5 years of follow-up, and fatal cerebrovascular disease in men without hypertension.

Activated white blood cells may also enhance the effects of thrombosis since they may have an important hemorheological function by increasing blood viscosity.[16] In addition, as a result of the release of oxidative agents by activated white blood cells, especially the monocytes, vulnerable or unstable plaques may rupture which can lead to thrombus formation and acute occlusion of a coronary artery.[14] These mechanisms may explain the observed association between white blood cell count and coronary heart disease described in chapter 7.

Moreover, the conventional coronary heart disease risk factors, blood pressure and blood cholesterol, may also have a role in promoting thrombosis. As a result of sheer stress on the arterial wall, elevated blood pressure may play a key role in triggering an acute coronary heart disease event.[14] Increased pressure on the arterial wall may cause the disruption of an athererosclerotic plaque resulting in thrombus formation and the occlusion of a coronary artery. This may be one of the mechanisms involved determining the strong

linear association between increased blood pressure levels and sudden cardiac death (chapter 2). The accumulation of cholesterol crystals increases the stiffness of atherosclerotic plaques which, in turn, partially determines its vulnerability to rupture and induce thrombus formation and eventually an acute coronary heart disease event.[14] This may explain part of the association between increased serum total cholesterol and mortality from coronary heart disease (chapter 4).

Altered autonomic function Another mechanism which can lead to the manifestation of acute coronary heart disease, other than arterial occlusion, may be increased electrical instability of the heart.[17] This may lead to arrhythmia, especially ventricular fibrillation, and subsequently to sudden cardiac death. Since there is increased sympathetic stimulation with advancing age, and this may lead to electrical instability of the heart,[18,19] related factors may become increasingly important in predicting acute coronary heart disease in older people. As discussed in chapter 2, this could be one of the explanations for the observed association between elevated blood pressure and sudden cardiac death.

Altered manifestation of coronary heart disease Our findings suggest that, in general, associations of risk factors with the incidence of coronary heart disease were weaker than those with mortality from the disease. As discussed previously, methodological issues may partly explain this difference. Possibly, the manifestation of coronary heart disease is altered with advancing age. Clinical coronary heart disease is more often atypical and asymptomatic in older as compared to younger persons.[20] This is additionally reflected by the strikingly high prevalence of silent myocardial infarction in advanced age.[21,22] Results from a population-based study among people over 55 years of age in Rotterdam, the Netherlands, for example, revealed that for 57.5 percent of the people with an infarction pattern on the electrocardiogram the myocardial infarction had been silent.[22] This may partly be the result of a reduced sensation of pain in some older people or to their conviction that certain symptoms are a normal concomitant of ageing. In these people, a clinical coronary heart disease event may not come to the attention of a general practitioner. In addition, other coronary disorders possibly become relatively more important in elderly people as compared to younger people and might compete with coronary heart disease, such as for example heart failure.[23] These altered manifestations and occurrences of coronary heart disease in the elderly may partly explain the generally weak associations observed between risk factors and the incidence of the disease as described in this thesis.

Altered risk factor levels Apart from the possibly altered pathophysiology of coronary heart disease in elderly people, advanced atherosclerosis in the elderly may also have an impact on risk factor levels and thus on their association with disease occurrence. Reduced diastolic blood pressure associated with isolated systolic hypertension in the elderly is one of the most apparent examples of this phenomenon, and this is extensively discussed in chapters 2 and 3.

In conclusion, the pathogenesis and manifestation of coronary heart disease can change with advancing age and may influence risk factor levels. These age specific features of coronary heart disease should be considered when conducting epidemiologic studies in the elderly and may underlie some of the associations described in this thesis. However, it is not possible to identify which mechanisms ultimately drive the observed associations. Further clinical and experimental research is needed to improve insights herein.

Public health implications

The reasons for conducting prospective studies like the ones described in this thesis are to obtain insight in the causation of coronary heart disease in elderly people and eventually to enhance the knowledge needed to improve the public's health.

Overall, the associations between the conventional risk factors and coronary heart disease incidence tended to be reduced in our studies as compared to studies in middle-aged people. As was discussed previously, this is probably true in terms of relative risks, but the absolute impact of these risk factors on disease occurrence in the elderly may be substantial. The associations with mortality from coronary heart disease as well as all-cause mortality were generally comparable to those observed at middle-age suggesting that the absolute impact of the risk factors on mortality endpoints was larger than that commonly observed at a younger age. In addition, the total number of elderly people afflicted by the disease is considerable.[24] This is expected to influence the prevalence of disability in the elderly and their quality of life and eventually associated health care costs. Therefore, unfavorable levels of risk factors in the elderly can have a considerable impact on public health. Major achievements herein can conceivably be obtained by aiming preventive strategies specifically at elderly people.

Intervention of conventional risk factors The usual way to assess whether prevention is effective in reducing a public health problem is by conducting randomized controlled

trials for the reduction of risk factors. Regarding the conventional risk factors, several successful prevention trials have been conducted specifically in the elderly.

Five randomized trials conducted in hypertensive patients over 60 years of age have provided convincing evidence for the protective effect of antihypertensive treatment on cardiovascular diseases.[25-29] An overall difference of 15/6 mmHg in systolic/diastolic blood pressure levels was achieved between the treatment and the control groups. This was associated with a 19 percent reduction in coronary heart disease incidence and a 34 percent reduction in stroke incidence in treatment versus control groups while non-vascular mortality did not differ between groups.[30] These figures are comparable in size to those observed in younger patients. However, the absolute measures of effect are more than twice as large as those for middle-aged patients.[30] These short-term effects of treatment (4.7 years) are comparable to the expected longer term effects of prolonged blood pressure differences deducted from prospective observational studies.[7] Therefore, most of the effect of blood pressure lowering on coronary heart disease risk reduction can possibly be obtained in the first years of treatment. A recent nutrition intervention in older people with mild to moderate hypertension was successful in reducing blood pressure levels in a short time-period.[31] Replacement of sodium salt by a low sodium, high potassium, high magnesium mineral salt has achieved significant reductions of 7.6/3.3 mmHg in systolic/diastolic blood pressure levels in just 24 weeks. Thus, non-pharmacological treatment of hypertension in the elderly appears to be promising with respect to coronary heart disease prevention.

Recent secondary prevention trials for total cholesterol lowering including elderly persons[32,33] have shown clear benefits in lowering both cardiovascular morbidity and mortality and all-cause mortality. Moreover, a primary prevention trial in middle-aged men without signs of coronary heart disease proved that total and low density lipoprotein cholesterol can be lowered successfully with a statin (HMG-CoA reductase inhibitor).[34] Furthermore, the treatment was successful in reducing the risk of coronary heart disease and all-cause mortality without increasing non-coronary heart disease mortality. In general, the degree of benefit depends on the degree of total cholesterol lowering and not on the type of intervention. However, some cholesterol lowering drugs, namely fibrates and hormones, have adverse effects on coronary heart disease (hormones only), non-coronary heart disease and total mortality independent of their cholesterol lowering effect.[33] Comprehensive lifestyle changes (i.e. a low-fat vegetarian diet, stopping smoking, stress management training, and moderate exercise) in middle-aged patients have shown similar reductions in total cholesterol concentrations as can be achieved by drug treatment.[35] In addition, this intervention has achieved significant regression of atherosclerosis.

A recent pilot study conducted specifically in elderly people suggested a similar cholesterol lowering effect of treatment by statins than has been observed in middle-aged

persons.[36] Therefore, the beneficial effect of cholesterol lowering on coronary heart disease and mortality in the elderly is expected to be similar to observations in younger people. Moreover, both pharmacological and lifestyle interventions are suggested to be successful. In the Zutphen Study a secular decline in serum total cholesterol between 1990 and 1993 was observed in elderly men which could not be explained by laboratory drifts (chapter 5). As discussed in chapter 5, a nationwide "Fat Watch" campaign during the same period may explain part of the decline. The secular change may also be a result of changes over the years in the composition of certain foods. Thus the food industry may have an important role in population-based strategies of lipid management.[37] A recent Finnish trial,[38] for example, suggests that serum total and low density lipoprotein cholesterol can be reduced in subjects with mild hypercholesterolemia by substituting margarine containing sitostanol-ester, which inhibits cholesterol absorption, for part of the daily fat intake. Future primary and secondary prevention trials, conducted specifically in elderly people, are necessary to be certain that total cholesterol lowering in this age-group is beneficial in terms of coronary heart disease incidence, total mortality and quality of life.

With respect to high density lipoprotein cholesterol, the Scandinavian Simvastatin Survival Study (4S) trial showed that treatment with simvastatin significantly increased high density lipoprotein cholesterol levels.[32] A similar increase in high density lipoprotein cholesterol was observed in the pilot phase of the statin-trial conducted specifically in elderly persons.[36] Whether the increase in high density lipoprotein cholesterol can also explain (part of) the reduced risk of the disease cannot be derived from the 4S trial[32] or any other trial up to date. However, several prospective studies have provided evidence for an etiologic association between reduced high density lipoprotein cholesterol and coronary heart disease.[39,40] Moreover, the study described and discussed in chapter 4, suggests that high density lipoprotein cholesterol is also inversely associated with coronary heart disease incidence, but not mortality, in elderly men. Concurrently, increasing this lipoprotein cholesterol level in elderly people with relatively low levels is expected to be associated with a reduced risk of the disease. Future clinical trials, specifically in elderly persons, are necessary to determine the beneficial effect of increasing high density lipoprotein cholesterol levels through treatment or hygienic measures for coronary heart disease incidence, total mortality and quality of life.

Most intervention trials have been carried out specifically in middle-aged persons. Since elderly people tend to have more subclinical coronary heart disease than middle-aged persons, primary prevention of adverse risk factor levels is expected to have an even larger absolute effect on reducing the burden of the disease in this age group than in middle-age. Whether primary prevention trials are effective in reducing the risk of disease and mortality in the elderly remains to be established.

Intervention of other risk factors Regarding the other risk factors addressed in this thesis, homocysteine appears to be the most readily modifiable factor. Indeed, parenteral supplementation of vitamin B12, B6 and folate has been shown to rapidly reduce homocysteine levels in elderly persons.[41] Moreover, there are suggestions that prevention of the progression of atherosclerosis in middle-aged people can be achieved through homocysteine-lowering treatment.[42] Similar effects are to be expected in elderly people since their vitamin B status is generally poor[43] and their serum homocysteine concentrations were elevated (chapter 6). Moreover, since homocysteine may also be involved in other chronic diseases than cardiovascular diseases, such as cancer and cognitive impairment (chapter 6), the treatment of elevated levels in elderly people may have beneficial effects for the general health and quality of life of the elderly population. However, the association between homocysteine and the occurrence of coronary heart disease, as well as these other conditions, is still not established in the elderly (chapter 6). Intervention studies are needed to investigate whether reduction in homocysteine reduces the risk of coronary heart disease and other chronic diseases in this age-group.

Concerning white blood cell count and serum albumin, information on potential determinants is lacking with the exception of cigarette smoking for white blood cell count. Further studies are needed to investigate what lifestyle factors possibly determine these clinical variables. As discussed in the various chapters (chapter 7 and 8), white blood cell count and serum albumin may be useful indicators of progression of atherosclerosis and of general underlying disease and useful factors for identifying elderly persons at increased risk of coronary heart disease and other chronic diseases. In addition, they may be important for monitoring changes in the risk of these chronic conditions.

In general, several factors should be considered with regard to primary prevention of coronary heart disease in the elderly. These include life expectancy, quality of life and the presence of other risk factors.[6] In this respect, the multifactorial etiology of coronary heart disease should be taken into account.[14,44] The evidence obtained in this thesis supports the notion that risk factors act independently in predicting coronary heart disease occurrence in the elderly. Moreover, the lifestyle associated with favorable coronary heart disease risk factor levels is also associated with a lower risk of other major chronic diseases such as other cardiovascular diseases, cancer, cognitive impairment and thus presumably a poor quality of life. Whether multifactorial lifestyle-intervention strategies aimed at elderly persons reduce all-cause mortality and improve quality of life and thereby the public's health needs to be addressed in future trials.

Conclusion

In conclusion, the classical biological risk factors for coronary heart disease (i.e. blood pressure, total and high density lipoprotein cholesterol) are still important in predicting the disease in elderly people. The experience obtained from the studies described in this thesis has made clear that elderly people are a heterogenous group with respect to risk factor levels as well as coronary heart disease risk. It would therefore be desirable to have a measure of susceptibility for coronary heart disease to identify and eventually treat elderly people who are at increased risk of the disease. Our studies suggest that white blood cell count and serum albumin, routine clinical blood chemistry values, may be useful in this respect in addition to the classical risk factors. Future intervention studies should aim at assessing the effectiveness of a multifactorial approach of risk factor management on coronary heart disease and all-cause mortality as well as on quality of life in older men and women.

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Summary

In industrialized countries like the Netherlands there will be a striking increase in the number of older people in the coming decades. This is expected to be accompanied by an increase in the absolute number of persons with coronary heart disease. Therefore, this disease is and will continue to be a major public health problem in the Netherlands especially in the elderly. Although advanced atherosclerosis may be present in approximately half of the elderly persons it is not considered to be an aging phenomenon *per se*. There is evidence that the progression of atherosclerosis can be stopped and regression can be induced. It is therefore imperative to obtain insight into possible risk factors for coronary heart disease in elderly persons. This age group has only recently become a focus of interest in epidemiologic research on the disease. In this thesis the associations between classical biological risk factors (i.e. total and high density lipoprotein blood cholesterol and blood pressure) and some new biological risk factors (i.e. serum homocysteine, white blood cell count and serum albumin) and the occurrence of coronary heart disease in elderly persons are described.

Results are based on data from two Dutch cohorts. One is a cohort of 292 men and women, aged 64 to 87 years in 1971, from a general practice in Rotterdam, with a mortality follow-up of 17 years. The other is the Dutch cohort of the Seven Countries Study which consists of 939 men aged 64 to 84 years in 1985 from the town of Zutphen. The morbidity and mortality follow-up embraced five years.

Systolic and diastolic blood pressure as well as isolated systolic hypertension were investigated in relation to coronary heart disease and sudden cardiac death in elderly men followed for five years (chapter 2). None of these blood pressure variables were significantly associated with the incidence of a first coronary heart disease event, and only diastolic blood pressure showed a *U*-shaped association with mortality from coronary heart disease. Strong positive associations were observed with sudden cardiac death. The adjusted relative risk for men with definite isolated systolic hypertension was 9.20 (95 percent confidence interval: 1.76-47.97). Elevated blood pressure levels and especially isolated systolic hypertension are thus important short-term predictors of sudden cardiac death in elderly men. Evidence from the literature suggests that this strong association may be a result of increased sympathetic activity with advancing age. Diastolic and systolic blood pressure showed a *U*-shaped relation with coronary heart disease mortality which was not recorded as sudden. The literature suggests that this increased risk at the lower end of the blood pressure distributions may, in part, be explained by increased arterial

stiffness with advancing age. The highest risk for both sudden and non-sudden coronary heart disease mortality was observed in men on anti-hypertensive medication, who formed a distinct group with a clustering of cardiovascular risk factors.

Systolic blood pressure was also an independent risk factor for mortality from coronary heart disease in elderly women followed for 17 years (chapter 3). The adjusted relative risk amounted to 4.1 (1.0-16.4) for women with a blood pressure of 175 mmHg or higher compared to women with a level lower than 155 mmHg. Although the difference between men and women was not significant, the association was less clear in men. A possible explanation for this difference between gender may be selective mortality in middle-aged men.

In chapter 3, serum total cholesterol was also an independent risk factor for mortality from coronary heart disease in elderly women followed for 17 years whereas for men the association tended to be inverted *U*-shaped (chapter 3). The adjusted relative risk amounted to 3.9 (1.1-13.7) in women with a total cholesterol level of 7.32 mmol/L or higher compared to those with a level lower than 6.34 mmol/L. Apart from possible selective mortality in middle-aged men as mentioned for blood pressure, these differences between gender could be explained by physiological changes in women as a consequence of menopause.

The short-term predictive value of serum total as well as high density lipoprotein cholesterol for coronary heart disease incidence and mortality was also investigated in elderly men (chapter 4). Total cholesterol was not significantly associated with the incidence of coronary heart disease, but for mortality the relative risk corresponding to a 1.00 mmol/liter increase was 1.40 (1.07-1.83). Additional analyses (chapter 8) revealed that total cholesterol only predicted coronary heart disease incidence in men with serum albumin levels below the median. These men may form a less healthy group more susceptible to the effect of total cholesterol on coronary heart disease incidence. High density lipoprotein cholesterol was not associated with mortality from coronary heart disease. The relative risk for the incidence of the disease, corresponding to a 0.26 mmol/liter increase, was 0.80 (0.60-1.08). The ratio of high density lipoprotein to total cholesterol was independently associated with the incidence of coronary heart disease. These results show that in elderly men followed for a relatively short period of time, both total and high density lipoprotein cholesterol are important in predicting coronary heart disease. Total cholesterol seems to be a stronger risk factor for mortality from the disease, whereas high density lipoprotein cholesterol is more strongly associated with the incidence of a first coronary heart disease event.

Longitudinal data on age-related changes in total and high density cholesterol levels in advanced age are scarce. Since such changes may have implications for the interpretation of their association with coronary heart disease, we investigated age-related changes in cholesterol levels in the Zutphen Study (chapter 5). The total and high density lipoprotein cholesterol concentration of men were determined in 1977/78 (n=571), 1985 (n=885), 1990 (n=555) and 1993 (n=345). Linear regression analysis and random effects models were used to assess cross-sectional and longitudinal age and time related changes in cholesterol concentrations. In both cross-sectional and longitudinal analyses total cholesterol decreased by 0.04 mmol/L per year with age. The longitudinal change was observed in the entire population as well as in men who participated in all four examinations (n=135), and in a subgroup of men who were free of common chronic diseases, not on cholesterol lowering medication and/or prescribed diet and rated themselves as being 'healthy' (n=64). High density lipoprotein cholesterol did not change significantly with age on a cross-sectional nor on a longitudinal basis. These results suggest that caution is warranted in interpreting results of associations between serum total cholesterol measured at old age and different end-points since the measured level of exposure may not be representative of a life-time exposure.

Since, with advancing age, associations of classical risk factors with coronary heart disease may be altered compared to those observed in middle-aged persons, there may be other risk factors of importance in the elderly. Some potentially new risk factors for the disease, i.e. serum homocysteine, white blood cell count and serum albumin, were investigated in the Zutphen Elderly Study.

An elevated serum homocysteine level is thought to be atherogenic and thrombogenic, but a definite mechanism has not been described. Elevated non-fasting homocysteine concentrations were common in elderly Dutch men (chapter 6). Thirty-one percent of the men had homocysteine levels of 17 μmol/l or higher. After adjustment for established major risk factors, high homocysteine levels (upper tertile) at baseline were associated with an increased prevalence of coronary heart disease (odds ratio = 1.95 (1.24-3.05)) and of cerebrovascular disease (odds ratio = 4.16 (1.99-8.97)). High homocysteine levels were associated with an increased risk of dying from coronary heart disease in the first one-and-a-half years of observation (adjusted relative risk 3.65 (0.76-17.42)). For cerebrovascular disease mortality the adjusted relative risk amounted to 17.23 (1.91-154.29) in men without hypertension. High homocysteine levels were not associated with an increased risk of first ever coronary heart disease or cerebrovascular disease. Finally, high homocysteine levels were associated with an increased risk of cognitive impairment at follow-up (odds ratio = 1.73 (1.03-2.92)), which was reduced after adjustment for age and occupation (odds ratio = 1.38 (0.79-2.40)).

White blood cell count, even within the normal range, was a strong predictor of both coronary heart disease incidence and mortality and all-cause mortality independent of the conventional risk factors for coronary heart disease (chapter 7). The adjusted relative risk for a 10^9/L increase in white blood cell count was 1.32 (1.15-1.51) for coronary heart disease mortality and 1.14 (0.98 - 1.32) for the first occurrence of the disease. These associations were observed regardless of cigarette smoking habit. Regarding all-cause mortality, the relative risk amounted to 1.25 (1.17-1.35). This association was strongest among never and former smokers. Apart from being a marker for underlying inflammation, an increased white blood cell count may also directly be involved in both atherogenesis and thrombosis which may explain its strong association with coronary heart disease.

A reduced serum albumin is also a marker of (underlying) illness, but potential mechanisms for an association with coronary heart disease have also been described. Most elderly men had normal serum albumin levels, i.e. 35 g/L or higher. Albumin was inversely related with the incidence of coronary heart disease among men with elevated total cholesterol levels (≥ 6.5 mmol/L) only. The relative risk for one standard deviation increase (2.5 g/L) in albumin was 0.60 (0.38-0.96) and was not altered after additional adjustment for baseline health status. For all men, the relative risk for death due to coronary heart disease was 0.67 (0.49-0.92) and reduced to 0.84 (0.61-1.15) after adjustment for health status. Comparable relative risks, adjusted for health status, were observed for mortality from cardiovascular diseases (relative risk = 0.83 (0.67-1.02)) and all causes (relative risk = 0.86 (0.73-0.99)). Moderately low serum albumin was predictive of coronary heart disease and all-cause mortality in elderly men independent of traditional confounders. Only part of this association could be explained by baseline health status.

In the general discussion (chapter 9) some methodological issues including the validity of the findings and matters specific to epidemiologic research in older populations have been discussed. Several considerations have to be made when interpreting findings from prospective epidemiologic studies in the elderly. Specific features of elderly populations such as the high prevalence of disease, the high mortality rate, natural selection mechanisms, and age-related changes and individual variations in risk factor levels may have attenuated the observed associations. With advancing age, the etiology and pathophysiology of coronary heart disease may be altered as compared to middle-aged persons and this may underlie some of the observed associations. Finally, the overall public health implications of the studies are considered. Although associations with the incidence of coronary heart disease tended to be reduced in terms of relative risks, the absolute impact of these risk factors on disease occurrence in the elderly may be substantial. Therefore, unfavorable levels of risk factors in the elderly can have a considerable impact on public health. Major achievements herein can conceivably be

obtained by aiming preventive strategies specifically at elderly people. The evidence obtained in this thesis supports the notion that risk factors act simultaneously in predicting coronary heart disease occurrence in the elderly. Moreover, the lifestyle associated with favorable coronary heart disease risk factor levels is also associated with a lower risk of other major chronic diseases such as other cardiovascular diseases, cancer, chronic non-specific lung disease and cognitive impairment. Presumably this will lead to an improved quality of life.

In conclusion, the classical biological risk factors for coronary heart disease (i.e. blood pressure, total and high density lipoprotein cholesterol) are still important in predicting the disease in elderly people. The experience obtained from the studies described in this thesis has made clear that elderly people are a heterogenous group with respect to risk factor levels as well as coronary heart disease risk. It would therefore be desirable to have a measure of susceptibility for coronary heart disease to identify and eventually treat elderly people who are at increased risk of the disease. Our studies suggest that white blood cell count and serum albumin, routine clinical blood chemistry values, may be useful in this respect in addition to classical risk factors. Future intervention studies should aim at assessing the effectiveness of a multifactorial approach of risk factor management on coronary heart disease and all-cause mortality as well as on quality of life in older men and women.

Samenvatting

Het aantal ouderen in de bevolking van geïndustrialiseerde landen zoals Nederland zal naar verwachting de komende decennia aanzienlijk stijgen. Dit zal waarschijnlijk gepaard gaan met een toename in het totaal aantal personen met coronaire hartziekten. Deze aandoeningen zullen daarom een nog groter probleem worden voor de volksgezondheid in Nederland. Atherosclerose wordt niet als een normaal verouderingsproces gezien. Toch komt vernauwing van meer dan 50 procent van één van de coronairvaten voor bij meer dan de helft van de ouderen. Progressie van atherosclerose kan worden tegengegaan en regressie kan worden geïnduceerd. Het is daarom van groot belang inzicht te krijgen in mogelijke risicofactoren voor coronaire hartziekten bij ouderen. Pas recentelijk heeft epidemiologisch onderzoek naar deze aandoeningen zich gericht op deze leeftijdsgroep.

In dit proefschrift zijn de verbanden beschreven tussen enerzijds de klassieke (serumtotaal- en hoge-dichtheidslipoproteïne (HDL)-cholesterol en de bloeddruk) en enkele nieuwe (serumhomocysteïne, het aantal witte bloedcellen en serumalbumine) biologische risicofactoren en anderzijds het optreden van coronaire hartziekten bij ouderen. De resultaten zijn gebaseerd op gegevens afkomstig uit twee bevolkingsonderzoeken. De eerste onderzoekspopulatie bestaat uit 292 mannen en vrouwen die in 1971 tussen de 64 en 87 jaar oud waren, afkomstig uit een Rotterdamse huisartsenpraktijk. Na 17 jaar zijn de doodsoorzaken van de overleden deelnemers geregistreerd. De andere onderzoekspopulatie bestaat uit 939 mannen tussen de 64 en 84 jaar in 1985, woonachtig in Zutphen. Na vijf jaar is naast de mortaliteit ook het optreden van niet-fatale coronaire hartziekten geregistreerd. Dit onderzoek staat bekend als de Zutphen Ouderen Studie.

De systolische en diastolische bloeddruk, alsmede geïsoleerde systolische hypertensie zijn onderzocht in relatie tot coronaire hartziekten en plotse hartdood bij oudere mannen uit de Zutphen Ouderen Studie (hoofdstuk 2). Er was geen verband tussen de bloeddruk-variabelen en de incidentie van coronaire hartziekten en alleen de diastolische bloeddruk liet een *U*-vormig verband zien met sterfte aan deze aandoeningen. Er werden echter sterke positieve verbanden waargenomen tussen de bloeddruk-waarden en plotse hartdood. Het gecorrigeerde relatieve risico voor mannen met geïsoleerde systolische hypertensie bedroeg 9.20 (95 procent betrouwbaarheidsinterval: 1.76-47.97) ten opzichte van mannen zonder deze vorm van hypertensie. Een verhoogde bloeddruk en vooral geïsoleerde systolische hypertensie zijn daarom op relatief korte termijn belangrijke voorspellers voor plotse hartdood bij oudere mannen. Uit de literatuur blijkt dat een verhoogde sympatische activiteit met het ouder worden mogelijk een verklaring is voor het sterke verband met

plotse hartdood. De diastolische en systolische bloeddruk toonden een *U*-vormig verband met sterfte aan coronaire hartziekten indien deze niet tevens als plotse hartdood was gecodeerd. Mogelijk is het verhoogde risico op niet-plotse dood aan coronaire hartziekten bij oudere mannen met de laagste bloeddruk-waarden deels het gevolg van een toename in de stijfheid van de arteriën met het toenemen van de leeftijd. Mannen die anti-hypertensiva gebruikten hadden het hoogste risico op zowel plotse als niet-plotse sterfte aan coronaire hartziekten en vormden een specifieke groep waarin een aantal cardiovasculaire risicofactoren frequent samen voorkwam.

Bij oudere vrouwen was de systolische bloeddruk een onafhankelijke risicofactor voor de 17-jaars sterfte aan coronaire hartziekten (hoofdstuk 3). Het gecorrigeerde relatieve risico bedroeg 4.1 (1.0-16.4) voor vrouwen met een bloeddruk van 175 mmHg of hoger ten opzichte van vrouwen met een bloeddruk lager dan 155 mmHg. Dit lange-termijn verband verschilde niet significant tussen vrouwen en mannen, maar bij mannen was het verband minder duidelijk. Selectieve sterfte bij mannen met hypertensie op middelbare leeftijd zou dit verschil mogelijk kunnen verklaren.

Serumtotaalcholesterol bleek eveneens een onafhankelijke risicofactor voor de 17-jaars sterfte aan coronaire hartziekten te zijn bij oudere vrouwen, terwijl bij mannen het lange-termijn verband omgekeerd *U*-vormig leek te zijn (hoofdstuk 3). Het gecorrigeerde relatieve risico bedroeg 3.9 (1.1-13.7) bij vrouwen met een totaalcholesterolgehalte van 7.32 mmol/L of hoger ten opzichte van vrouwen met een waarde lager dan 6.34 mmol/L. Naast de mogelijkheid van selectieve sterfte bij mannen van middelbare leeftijd, zou het waargenomen verschil tussen mannen en vrouwen ook een gevolg kunnen zijn van fysiologische veranderingen bij vrouwen als gevolg van de menopause.

Het korte termijn belang van serumtotaal- en HDL-cholesterol voor de incidentie en sterfte aan coronaire hartziekten is ook onderzocht bij oudere mannen (hoofdstuk 4). Totaalcholesterol was niet significant geassocieerd met de incidentie van de aandoeningen, maar voor sterfte werd wel een duidelijk verband gevonden; een toename in serumcholesterol van 1 mmol/L ging gepaard met een relatief risico van 1.40 (1.07-1.83). Uit additionele analyses (hoofdstuk 8) bleek dat totaalcholesterol wel gerelateerd was aan de incidentie bij mannen met een serumalbuminegehalte lager dan de mediaan. Mogelijk zijn deze mannen minder gezond en daarom meer gevoelig voor het effect van totaalcholesterol op de incidentie van coronaire hartziekten. HDL-cholesterol was niet geassocieerd met sterfte aan coronaire hartziekten. Voor de incidentie bedroeg het gecorrigeerde relatieve risico 0.80 (0.60-1.08) met een toename van 0.26 mmol/L in het HDL-cholesterol. De verhouding tussen HDL- en totaalcholesterol bleek onafhankelijk gerelateerd te zijn aan de incidentie van coronaire hartziekten. Deze resultaten tonen aan dat bij oudere mannen zowel het totaal- als het HDL-cholesterol op relatief korte termijn

nog steeds voorspellend zijn voor coronaire hartziekten. Het totaalcholesterol lijkt een sterkere voorspeller te zijn voor de sterfte aan deze aandoeningen terwijl HDL-cholesterol sterker gerelateerd is aan de incidentie.

Longitudinale gegevens over leeftijdsveranderingen in totaal- en HDL-cholesterol met het ouder-worden zijn schaars. Dergelijke veranderingen zijn van belang voor de interpretatie van verbanden met coronaire hartziekten. Daarom zijn leeftijdsveranderingen in deze cholesterol-variabelen in de Zutphen Studie onderzocht (hoofdstuk 5). Voor deze analyse is gebruik gemaakt van het serumtotaal- en HDL-cholesterolgehalte van mannen die in 1977/78 (n=571), 1985 (n=885), 1990 (n=555) en 1993 (n=345) onderzocht zijn. Lineaire regressie-analyse en 'random-effects models' zijn gebruikt om cross-sectionele en longitudinale veranderingen in cholesterolconcentraties met de leeftijd en de tijd te bestuderen. Uit zowel de cross-sectionele als de longitudinale analyses bleek het totaalcholesterolgehalte van het bloed met 0.04 mmol/L per jaar af te nemen. De longitudinale verandering werd in de totale onderzoeksgroep waargenomen, bij mannen die aan alle onderzoeken hebben deelgenomen (n=135), en tot slot in een subgroep van deze mannen zonder chronische ziekten, mannen die geen cholesterolverlagende medicijnen of dieet gebruikten en een goede ervaren gezondheid hadden (n=64). HDL-cholesterol veranderde niet significant met de leeftijd zowel in de cross-sectionele als de longitudinale analyses. Deze resultaten suggereren dat het totaalcholesterolgehalte gemeten op oudere leeftijd niet representatief is voor een levenslange blootstelling aan een bepaald serumcholesterolgehalte. Bij de interpretatie van verbanden met verschillende eindpunten dient hiermee rekening te worden gehouden.

Omdat verbanden tussen de klassieke risicofactoren en coronaire hartziekten mogelijk veranderen met de leeftijd, zijn andere factoren eventueel van belang bij ouderen. Nieuwe potentiële risicofactoren voor deze aandoeningen, zoals het serumhomocysteïne, het aantal witte bloedcellen en het serumalbumine, zijn in de Zutphen Ouderen Studie onderzocht.

Een verhoogd serumhomocysteïne is mogelijk zowel atherogeen als thrombogeen. Het onderliggende mechanisme is nog niet opgehelderd. Een verhoogde niet-nuchtere homocysteïne concentratie van het bloed komt bij ouderen veelvuldig voor (hoofdstuk 6). Één-en-dertig procent van de mannen had een homocysteïnegehalte van 17 µmol/L of hoger. Na correctie voor mogelijke confounders bleek een hoog homocysteïnegehalte (bovenste tertiel) geassocieerd te zijn met een verhoogde prevalentie van coronaire hartziekten (odds ratio = 1.95 (1.24-3.05)) en cerebrovasculaire accidenten (odds ratio = 4.16 (1.99-8.97)). Een hoog homocysteïnegehalte leek gerelateerd te zijn aan een verhoogd risico op sterfte voor coronaire hartziekten gedurende de eerste anderhalf jaar. Het relatieve risico bedroeg 3.65 (0.76-17.42). Voor sterfte aan cerebrovasculaire accidenten

bedroeg het relatieve risico 17.23 (1.91-154.29) bij mannen zonder hypertensie. Een hoog homocysteïnegehalte was niet geassocieerd met een verhoogd risico op de incidentie van coronaire hartziekten of cerebrovasculaire accidenten. Tenslotte waren hoge homocysteïne niveaus geassocieerd met een verhoogd risico op een verminderd cognitief functioneren (odds ratio = 1.73 (1.03-2.92)). Dit verband werd echter minder sterk na correctie voor leeftijd en beroep (odds ratio = 1.38 (0.79-2.40)).

Het aantal witte bloedcellen, zelfs binnen de normaalwaarden, was een sterke onafhankelijke voorspeller voor zowel de incidentie als sterfte aan coronaire hartziekten alsmede voor totale sterfte (hoofdstuk 7). Het gecorrigeerde relatieve risico, geassocieerd met een 10^9/L toename in het aantal witte bloedcellen, bedroeg 1.32 (1.15-1.51) voor sterfte aan coronaire hartziekten en 1.14 (0.98-1.32) voor de incidentie van deze ziekten. Deze verbanden werden waargenomen ongeacht de rookgewoonte van mannen. Voor de totale sterfte bedroeg het relatieve risico 1.25 (1.17-1.35). Dit verband was het sterkst bij de niet-rokers. Het aantal witte bloedcellen is verhoogd als gevolg van ontstekingen. Er zijn echter aanwijzingen dat het tevens een rol speelt bij zowel atherogenese als thrombogenese. Dit zou een verklaring kunnen zijn voor het sterke verband met coronaire hartziekten.

Een verlaagd serumalbumine is ook een indicator voor (onderliggende) ziekten, maar er zijn tevens biologische mechanismen beschreven voor een verband met coronaire hartziekten. De meeste mannen in de Zutphen Ouderen Studie hadden normale serumalbumine concentraties van 35 g/L of hoger (hoofdstuk 8). Albumine was invers gerelateerd aan de incidentie van coronaire hartziekten bij mannen met een verhoogd serumtotaalcholesterol ($\geq$ 6.5 mmol/L). Het gecorrigeerde relatieve risico bedroeg 0.60 (0.38-0.96) voor één standaard deviatie (2.5 g/L) toename in serumalbumine. Dit veranderde niet na extra correctie voor chronische ziekten, subjectieve gezondheid en het aantal witte bloedcellen aan het begin van het onderzoek. Voor de totale groep bedroeg het relatieve risico voor sterfte aan de ziekten 0.67 (0.49-0.92) en dit werd na correctie voor onderliggende ziekten 0.84 (0.61-1.15). Vergelijkbare relatieve risico's werden gevonden voor sterfte aan cardiovasculaire ziekten (0.83 (0.67-1.02)) en totale sterfte (0.86 (0.73-0.99)). Een matig verlaagd serumalbumine was bij oudere mannen voorspellend voor coronaire hartziekten en totale sterfte onafhankelijk van traditionele confounders. Slechts een deel van dit verband kon door onderliggende ziekten worden verklaard.

In de algemene discussie (hoofdstuk 9) is aandacht besteed aan enkele methodologische aspecten van de onderzoeken zoals de validiteit van de resultaten en aspecten die specifiek betrekking hebben op epidemiologisch onderzoek bij ouderen. Specifieke karakteristieken van oudere populaties zoals de hoge prevalentie van (onderliggende) ziekten, de hoge sterfte, natuurlijke selectie-mechanismen en leeftijdsveranderingen, kunnen de bestudeerde

verbanden hebben afgezwakt. Met het ouder worden verandert mogelijk ook de etiologie en pathofysiologie van coronaire hartziekten en dit kan de bevindingen eveneens deels verklaren. De algemene implicaties van de resultaten voor de volksgezondheid zijn besproken. Ondanks de soms zwakke relatieve risico's voor de incidentie van coronaire hartziekten in vergelijking tot mensen van middelbare leeftijd, is het absolute risico bij ouderen over het algemeen hoger dan op middelbare leeftijd. Een ongunstig risicoprofiel bij ouderen kan daarom grote gevolgen hebben voor de volksgezondheid in het algemeen. Verbeteringen hierin zouden kunnen worden behaald door preventieve maatregelen specifiek op ouderen te richten. De resultaten die beschreven zijn in dit proefschrift hebben verder aangetoond dat risicofactoren onafhankelijk voorspellend zijn voor coronaire hartziekten bij ouderen. Bovendien blijkt een gezonde levensstijl voor coronaire hartziekten ook het risico op andere chronische aandoeningen te verlagen zoals andere cardiovasculaire aandoeningen, kanker, CARA, en een verminderd cognitief functioneren. Dit zal vermoedelijk tevens bijdragen aan een verbeterde kwaliteit van leven.

Concluderend kan gesteld worden dat de klassieke risicofactoren voor coronaire hartziekten (bloeddruk, totaal- en HDL-cholesterol) nog steeds belangrijk zijn voor het voorspellen van deze aandoeningen op oudere leeftijd. Ouderen vormen voor wat betreft hun risicoprofiel en hun risico op coronaire hartziekten een zeer heterogene groep. Het zou daarom wenselijk zijn om factoren te identificeren die een maat zijn voor de gevoeligheid van ouderen om de ziekten te krijgen. Uit de in dit proefschrift beschreven onderzoeken blijkt dat een aantal routinematig gemeten klinisch-chemische bloedparameters, zoals het aantal witte bloedcellen en het serumalbumine, in dit verband zeer bruikbaar is in combinatie met de klassieke risicofactoren. Toekomstig interventie-onderzoek zou gericht moeten zijn op de effecten van een multifactoriële benadering voor het normaliseren van het risicoprofiel op coronaire hartziekten, totale sterfte en kwaliteit van leven bij oudere mannen en vrouwen.

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