CARDIOVASCULAR DISEASE IN WOMEN

An Epidemiological Study of Atherogenic Factors

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CARDIOVASCULAR DISEASE IN WOMEN

An Epidemiological Study of Atherogenic Factors

Hart- en vaatziekten bij vrouwen

Een epidemiologisch onderzoek naar atherogene factoren

Proefschrift

ter verkrijging van de graad van doctor

aan de Erasmus Universiteit Rotterdam

op gezag van de rector magnificus

Prof. Dr C.J. Rijnvos

en volgens besluit van het College van Dekanen.

De openbare verdediging zal plaatsvinden op

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door

Johanna Cornelia Maria Witteman

geboren te Hillegom

PROMOTIE COMMISSIE

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	Prof. K.-T. Khaw
	Prof. Dr M.L. Simoons

Even if, for example, both genders were equally represented in the study experience, the overall result does not necessarily apply to both genders but to people of average gender - which is an empty category.

– Olli S. Miettinen, *Theoretical epidemiology,* 1985

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Publications and manuscripts based on the studies described in this thesis

Chapter 2.1
Witteman JCM, Kok FJ, Saase JLCM van, Valkenburg HA. Aortic calcification as a predictor of cardiovascular mortality. Lancet 1986;ii:1120-2.

Chapter 2.2
Witteman JCM, Kannel WB, Wolf PA, Grobbee DE, Hofman A, D'Agostino RB, Cobb JC. Aortic calcified plaques and cardiovascular disease (The Framingham Study). Am J Cardiol 1990;66:1060-4.

Chapter 3.1
Witteman JCM, Grobbee DE, Valkenburg HA, Hemert AM van, Stijnen Th, Hofman A. Systolic and diastolic blood pressure and progression of atherosclerosis in women. A 9-year population-based follow-up study (submitted).

Chapter 3.2
Witteman JCM, Grobbee DE, Valkenburg HA, Hemert AM van, Stijnen Th, Bruijn AM de, Hofman A. Serum cholesterol and progression of atherosclerosis in women. A 9-year population-based follow-up study (submitted).

Chapter 3.3
Witteman JCM, Grobbee DE, Valkenburg HA, Hemert AM van, Stijnen Th, Hofman A. Cigarette smoking and progression of atherosclerosis in women. A 9-year population-based follow-up study (submitted).

Chapter 3.4
Witteman JCM, Grobbee DE, Kok FJ, Hofman A, Valkenburg HA. Increased risk of atherosclerosis in women after the menopause. Br Med J 1989;298:642-4.

Chapter 4.1
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Chapter 4.2
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Chapter 4.3
Witteman JCM, Grobbee DE. Calcium and magnesium in hypertension: current evidence. In: Laserre B, Durlach J, eds. Magnesium - a relevant ion? London: John Libbey, 1991, p 79-96.

Chapter 4.4
Witteman JCM, Grobbee DE, Derkx FHM, Bouillon R, Bruijn AM de, Hofman A. Reduction of blood pressure with oral magnesium supplementation in women with mild to moderate hypertension (submitted).

CHAPTER 1

INTRODUCTION

1 Introduction

Cardiovascular disease is generally considered to be a disorder of men. One reason for this is the low incidence of the disease in women at younger age. At older age, however, cardiovascular disease also becomes the most important cause of mortality in women: at age 40, 15% of the mortality in women is due to this disease compared to 45% at age 70.[1] Currently, there is an increased recognition of the public health importance of cardiovascular disease in women, but data on cardiovascular risk factors are limited. The investigation of the determinants of cardiovascular disease in women was the main purpose of the work underlying the studies presented in this thesis.

Traditionally, epidemiologists have studied cardiovascular disease by examination of the relation between potential risk factors and the prevalence or incidence of cardiovascular events. One of the disadvantages of these studies is that events reflect a near end-stage of disease which limits the study of risk factors at earlier stages of disease. The major underlying process of cardiovascular disease is atherosclerosis. To study atherosclerosis non-invasively in asymptomatic non-hospitalized subjects it is necessary to rely on vessels other than the coronary or cerebral. In the studies presented in this thesis the presence of radiographically detectable calcified plaques in the aorta was used as a measure of atherosclerosis. Its ability to reflect a generalized process was studied by examination of the association of aortic atherosclerosis with cardiovascular morbidity and mortality using follow-up data of 1,359 men and 1,597 women of the EPOZ study (Epidemiological Preventive Organization Zoetermeer), and of 2,336 men and 2,873 women of the Framingham Study.

Data on aortic atherosclerosis were used to study the determinants of atherosclerosis in women. The research questions were (1) Do the recognized risk factors (elevated blood pressure, elevated serum cholesterol and cigarette smoking) predict progression of atherosclerosis in women? (2) Are characteristics unique to women related to progression of atherosclerosis? The first question was examined among 855 middle-aged women who participated in the EPOZ study in 1975-1978 and were re-examined after an average of 9 years. Of risk factors unique to women, menopause has received much attention but its cardiovascular effects are still unproven. The association between menopausal state and aortic atherosclerosis was examined cross-sectionally among 294 premenopausal and 319 postmenopausal women who participated in the EPOZ study.

Subsequent to the detection of cardiovascular risk factors, research should preferably be directed towards the detection of potentially modifiable determi-

nants of the risk factor, followed by intervention studies to examine whether change in the determinant affects the risk factor level. Using 4-year follow-up data of the Nurses' Health Study, the relation of several nutritional factors and the incidence of hypertension was studied among 58,218 US registered nurses. In a randomized placebo-controlled trial the effect of magnesium supplementation on blood pressure was examined among 91 women with mild to moderate hypertension.

The levels of research are presented in figure 1.

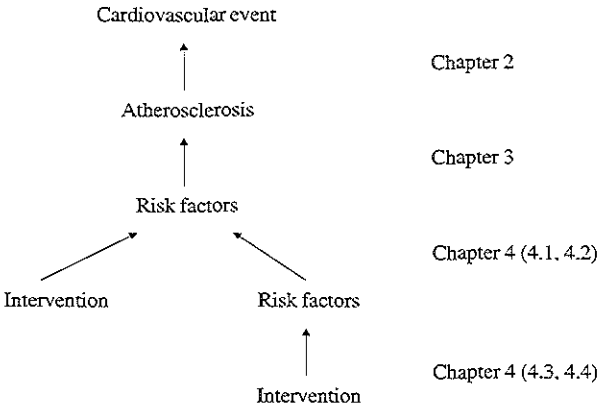

Figure 1 Levels of research

In Chapter 2 the association of aortic calcification with cardiovascular morbidity and mortality is reported. Chapter 3 presents studies on the determinants of atherosclerosis in women. Chapter 4 deals with the relation of nutritional factors and hypertension and reports the results of the magnesium intervention study. In chapter 5 the findings of the presented studies are discussed and new research areas are delineated. In chapter 6 methodological implications of the study of the disease process are discussed.

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CHAPTER 2

AORTIC CALCIFIED PLAQUES AND CARDIOVASCULAR DISEASE

2.1 Aortic Calcification as a Predictor of Cardiovascular Mortality *

Abstract

Since aortic calcification is seen on X-rays of the prelumbar region in many patients, its relation with cardiovascular disease (CVD) was investigated in a prospective study in The Netherlands. X-rays were made in 1,359 men and 1,598 women, in 1975-1978. In the subsequent 9 years, 50 men and 33 women died from CVD. The prevalence of aortic calcification was about 10% in middle-aged subjects, and rose with age to a maximum of 45% in men and 75% in women. Aortic calcification was associated with a six-fold increased risk of CVD death in men aged 45 years, independent of major CVD risk factors. For each year of age over 45, risk associated with the presence of aortic calcification declined by 6%. Death rates in middle-aged women were too small for risk analysis. These results suggest that atherosclerosis in other than coronary or cerebral vessels may have predictive relevance for CVD death: its diagnosis indicates intervention on present CVD risk factors.

Introduction

Information about the extent of arterial atherosclerosis will probably lead to better prediction of cardiovascular disease (CVD) than estimates based solely on traditional risk factors. The clinical significance of coronary and cerebral atherosclerosis is generally accepted, but no method is yet available for non-invasive measurement on a large scale. Since significant correlations have been found between atherosclerosis in different vessel-beds,[1-5] lesions in arteries other than coronary or cerebral arteries may have predictive relevance for CVD death.

At necropsy, atherosclerosis in the abdominal aorta was found to be more common in subjects who had died from CVD than in those who had died from other causes.[1,2,6,7] In one study of atherosclerosis in a general population,[8] manifest CVD was associated with aortic calcification, seen on X-rays of the lumbar spine. Aortic calcification thus diagnosed represents true intimal atherosclerosis.[9] Our aim was to determine the prevalence and predictive value of

* Lancet 1986;ii:1120-2

aortic calcification by X-ray examination in a cohort of 2,957 subjects in The Netherlands.

Subjects and methods

In 1975-1978, the prevalence and determinants of several chronic diseases were studied in Zoetermeer, a suburb of the metropolitan area of The Hague in The Netherlands (EPOZ-study). All inhabitants aged 5 years and over of one rural and one urban district were invited for a medical examination. Details of the initial survey have been reported previously.[10] This study comprised subjects aged 45 years and over. In both sexes the response rate was 72%, resulting in 1,359 male and 1,598 female participants. Data on deaths in the cohort were obtained from the municipal register of Zoetermeer. Vital status could be determined for 95% of the cohort. The cause of death was established from death certificates provided by the general practitioners, hospital records, and from the Dutch Central Bureau of Statistics. The classification was based on the International Classification of Diseases, 9th revision.[11]

For this study, data from 6-9 years of follow-up (up to Dec 31, 1983) were used. 101 subjects died from CVD (ICD codes 390-458). Information on aortic calcification was missing in 5 cases because the prelumbar region was insufficiently visible. No X-rays's were available for 13 cases; 8 of them were living in an old people's home, where no X-ray examination of the lumbar spine was done. The percentage of missing information in the cases was comparable to that in the total group of cohort members. 83 deaths (50 men, 33 women) were available for statistical analysis. Death was caused by coronary heart disease (ICD codes 410-414) in 82% of the male and 58% of the female deaths, and cerebrovascular disease (ICD codes 430-438) in 6% of male and 18% of female deaths. The remaining deaths were mainly caused by pulmonary embolism; no subjects died from aortic aneurysm.

At entry to the baseline study, blood pressure, total serum cholesterol and body-mass index were measured, and respondents were asked about their medical history and smoking habits. X-rays were taken of several joints, including two lateral views of the lumbar spine (HT12-S1). Films were taken at a fixed distance, with subjects in sitting position in maximum anteflextion or retroflexion. Calcification was scored as present if densities were clearly visible in an area parallel and anterior to the lumbar spine. No attempt was made to estimate the extent of calcification.

The prevalence of aortic calcification was based on all subjects aged 45 years and over taking part in the initial survey. Each CVD case was paired with four controls randomly selected from the pool of cohort members and matched for age and sex. The differences in mean values of baseline characteristics were

tested by two-sample t-tests. Relative risks were calculated as odds ratio's, with confidence limits according to Woolf's method.[12] Adjustments for cardiovascular risk factors were made with the logistic regression model. Logistic regression coefficients and their standard errors were estimated by the method of maximum likelihood.[13] Blood pressure, total serum cholesterol, and body-mass index were entered as continuous variables, and smoking (current, past, never) and diabetes as indicator variables. Because it is of interest whether the prediction by aortic calcification of CVD death changes with age, an interaction term for these two variables was entered in the logistic regression model. This interaction term (age multiplied by aortic calification) stands for the joint effect of aortic calcification and years of age over 45; it is zero at age of 45. The risk of CVD death at this age is, therefore, solely reflected by the regression coefficient of aortic calification. For each subsequent year of age the risk will increase or decrease, depending on sign of the regression coefficient of the interaction term.

Results

The prevalence of aortic calcification showed a sharp increase with age. The prevalence was higher in men than in women up to the age of 65 years. After this age the sex ratio reversed with prevalence in men and women reaching a maximum of 45% and 75%, respectively (figure 2.1.1).

Baseline characteristics of cases and controls are shown in table 2.1.1. There were significant differences between cases and controls in total serum cholesterol and systolic blood pressure in both sexes and in smoking in men only. The risk of CVD death assiociated with the presence of aortic calcification

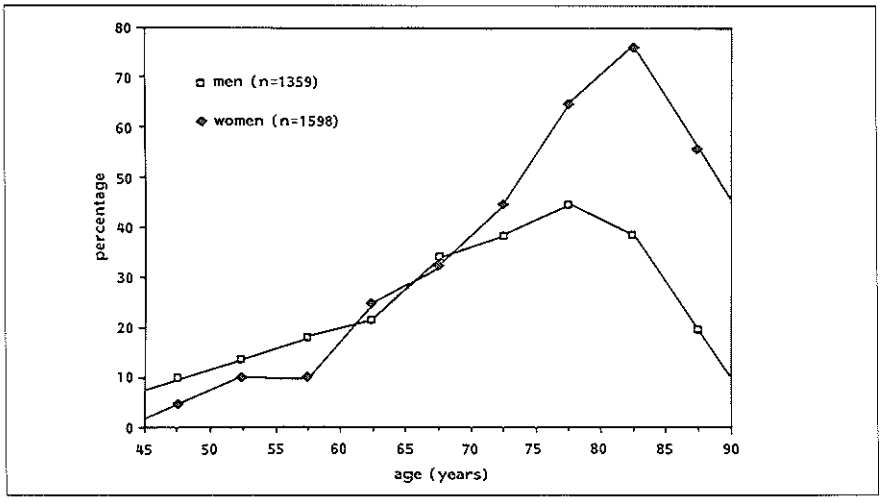

Figure 2.1.1. Prevalence of aortic calcification

in men (relative risk 1.8, 95% confidence interval 0.96-3.5), was largely accounted for by the high risk among middle-aged subjects (table 2.1.2). In women, the association between aortic calcification and CVD death was weak (relative risk 1.6, 95% CI 0.73-4.3). Because only 5 women under 65 years died of CVD, no relative risk was calculated in this group.

Aortic calcification was positively, but not significantly, associated in both sexes with total serum cholesterol and systolic blood pressure, and in men also with diastolic blood pressure and smoking. In both sexes, aortic calcification showed a negative, not significant, association with body-mass index. Aortic calcification and the main CVD risk factors were entered as independent variables in a logistic model. Since the risk assiociated with aortic calcification was reduced by age, an interaction term was added. The adjusted CVD risk for aortic calcification was 6.4 (95% CI 1.2-35.2) for men aged 45 years. The risk

Table 2.1.1. Baseline characteristics of CVD cases and matched controls

	Men		Women	
	Cases n = 50	Controls n = 200	Cases n = 33	Controls n = 132
Mean (SD)				
Age (years)*	64.3 (9.6)	64.7 (9.5)	73.4 (9.0)	73.4 (8.5)
Systolic blood pressure (mmHg)	147.2 (23.4)	139.4 (21.0)†	163.7 (26.4)	151.4 (21.9)†
Diastolic blood pressure (mmHg)	85.0 (15.1)	81.2 (13.2)‡	83.9 (13.9)	83.2 (11.0)
Serum cholesterol (mmol/l)	6.5 (1.0)	6.0 (1.0)†	6.8 (1.4)	6.4 (1.1)†
Body mass index (kg/m^2)	25.4 (3.0)	24.9 (2.7)	26.1 (3.4)	26.4 (3.9)
Percentage				
Current smokers	58	40†	15	12
Past smokers	36	44†	6	10
Diabetes	12	3‡	15	2‡

* matching variable
For differences between cases and controls: †$p<0.05$; ‡$p<0.1$
SD=standard deviation

Table 2.1.2. Crude relative risks of CVD death (RR), associated with the presence of aortic calcification

		No (%) with aortic calcification		
		Cases	Controls	RR (95% CI)
Men	≤ 65 yr	27 (37)	108 (18)	2.8 (1.1 –7.0)
	> 65 yr	23 (39)	92 (34)	1.3 (0.49–3.2)
Women	≤ 65 yr	5 (0)	20 (10)	– *
	> 65 yr	28 (71)	112 (56)	1.9 (0.79–4.8)

* numbers too small for calculation
CI = confidence interval

estimate of the interaction term was 0.94 (95% CI 0.87-1.0), implying a 6% reduction in risk for each year of age over 45. In women, no independent effect of aortic calcification was found. Among the major CVD risk factors, significant effects were found for total serum cholesterol and diabetes in men, and for systolic blood pressure in women (table 2.1.3).

Discussion

The pattern of the prevalence of aortic calcification according to age and sex accords with results of necropsy studies.[1,2,7,14] The decline after the seventh decade may be due to earlier deaths of subjects prone to atherosclerosis. The higher prevalence in older women than older men is not likely to be due to response bias or selective mortality in our population, since studies which show a female predominance for the prevalence of aortic calcification, still showed the usual male predominance of coronary calcification.[1-3] These findings suggest the presence of sex-specific localising features for arterial calcification, although no explanation for this observation is yet available.

Aortic calcification nearly doubled the CVD risk in men, and in women a tendency in the same direction was found. These positive associations confirm the results of necropsy studies.[1,2,6,7] Since aortic calcification is associated with serum cholesterol, hypertension and diabetes[2,3,8] it was interesting to see whether aortic calcification still had predictive value when these risk factors were taken into account. After multivariate adjustment, aortic calcification showed a strong independent effect, even though its presence was positively

Table 2.1.3. Adjusted* relative risks of CVD death (RR) for aortic calcification and CVD risk factors

		RR (95% CI)	
		Men	Women
Variable	Change	(n = 250)	(n = 165)
Aortic calcification	present/absent	6.41 (1.17-35.2)	1.49 (0.03-83.5)
Age	1 yr	1.02 (0.97-1.07)	0.96 (0.89-1.04)
Aortic calcification x age		0.94 (0.87-1.01)	1.00 (0.88-1.14)
Systolic blood pressure	10 mmHg	1.11 (0.90-1.37)	1.33 (1.31-1.36)
Diastolic blood pressure	10 mmHg	1.02 (0.73-1.41)	0.83 (0.58-1.25)
Serum cholesterol	0.3 mmol/l	1.12 (1.02-1.23)	1.06 (0.96-1.16)
Body-mass index	1 kg/m^2	1.05 (0.93-1.19)	0.96 (0.85-1.08)
Smoking	current	3.31 (0.87-12.6)	1.38 (0.38-4.94)
	past	1.66 (0.43-6.45)	0.49 (0.09-2.74)
Diabetes	yes/no	4.18 (1.06-16.6)	4.07 (0.77-21.7)

* Adjusted by logistic regression analysis for effects of all other variables
CI = confidence interval

associated with most of the major cardiovascular risk factors. The risk associated with the presence of aortic calification for men ranged from a six-fold increase at the age of 45 years to no excess risk at age 75. Aortic calcification, being an ageing phenomenon, apparently loses discriminative power in later decades.

Hyman and Epstein[9] have studied the validity of radiological detection of aortic calcification for diagnosis of atherosclerosis through comparison with autopsy material. All patients with visible calcified deposits in the abdominal aorta on X-ray of the lumbar spine (n = 20), were found to have atherosclerosis at necropsy; giving a sensitivity of 100%. Atherosclerosis was in most cases severe, which accords with the generally accepted view that calcified plaques represent an advanced stage of atherosclerosis. In 5 of the 31 cases in which no calcification was diagnosed radiologically, atheroslerotic plaques of varying degrees were found in the aorta at necropsy. False-negative misclassification in our study would mean only that our calculated risk estimate is conservative. The negative association we found between aortic calcification and body mass index might indicate that a larger amount of soft tissue masks the visualisation of calcified streaks. Results of necropsy studies on this association are conflicting.[2,3] However, since we controlled for body-mass index in multivariate analysis, the adjusted risk is unbiased. Nonetheless, the radiological method used for screening purposes, will miss many cases of atherosclerosis. Developments in imaging techniques promise the possibility for detecting less severe atherosclerosis in peripheral arteries.[15] It would be interesting to see whether atherosclerosis detected early in these arteries also has predictive value for CVD later in life.

Since uncomplicated aortic atherosclerosis will not give rise to clinical symptoms causing death, the demonstrated mortality risk is likely to be due to concomitant atherosclerosis in other vessel-beds. In the International Atherosclerosis Project[3] a correlation coefficient of 0.87 was found between aortic and coronary atherosclerosis, by ranking of 19 geographical area's. Within populations, age-adjusted correlation coefficients between lesions in the aorta and in the coronary and cerebral arteries respectively, ranged from 0.24 to 0.48.[1,4] Although statistically significant, these low coefficients do not simply allow prediction of atherosclerosis in one artery from measurement in another. The strong CVD risk found for aortic calcification in our study, however, indicates that its casual finding in middle-aged subjects warrants attention. The risk was found to be additive to that formed by major determinants of CVD, but the factors underlying this independent risk are still unknown. Action should, therefore, best be taken by reducing the overall risk through an evaluation of the risk profile and treatment of present CVD risk factors.

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2.2 Aortic Calcified Plaques and Cardiovascular Disease (the Framingham Study) *

Abstract

The relation between the presence of calcified plaques in the thoracic aorta, as detected on chest X-rays and the development of cardiovascular disease is examined during 12 years of follow-up of the Framingham cohort (n=5,209). The prevalence of aortic calcified plaques approximately doubled with each decade of age with only a trivial male predominance. Its presence was associated with a two-fold increase in risk of cardiovascular death in men and women younger than age 65, even after other risk factors were taken into account. Similar increases in risk were found for coronary artery disease, stroke and intermittent claudication among middle-aged women. In middle-aged men these risks were less marked. The predictive value of aortic calcified plaques generally diminished with age. Risk of sudden coronary death in men with calcified plaques in the thoracic aorta ranged from a sevenfold increase at age 35 to no excess risk at age 70. These results support the view that atherosclerosis is a generalized process. The finding of aortic calcified plaques in a relatively young subject on a routine chest X-ray should be regarded as a sign for potential development of clinically manifest atherosclerotic disease in the cardiac, cerebral, and peripheral arterial circulation.

Introduction

Atherosclerosis has been suggested to be a generalized process.[1] Thus, the presence of an atherosclerotic cardiovascular event can predict the occurence of a subsequent event, located elsewhere.[2,3] From a preventive point of view, it would be desirable to have an indicator of atherosclerosis before the clinical appearance of disease. One of the deep vessels that can be examined with relative ease is the aorta. Calcific densities in the aorta, as detected on roentgenograms, represent an advanced stage of intimal atherosclerosis and can be viewed as calcified plaques.[4] Its presence, as measured in a general population sample, was associated with manifest coronary artery disease.[5] In a recent

* Am J Cardiol, 1990:66:1060-4

prospective follow-up study, the presence of calcified plaques in the abdominal aorta was strongly related to subsequent cardiovascular death.[6] This report presents findings on the predictive value of calcified plaques in the thoracic aorta for the development of cardiovascular disease at various sites.

Subjects and methods

Patient population

The Framingham cohort of 2,336 men and 2,873 women, aged 30 to 62 years at entry, has been examined biennially since 1948. The sampling procedure, design and methods of the study have been presented elsewhere.[7] Roentgenograms of the thorax were routinely taken at biennial exams. The subject was positioned upright in maximal respiration phase with full anterior chest against film cassette. The X-ray tube was placed at the level of the seventh thoracic vertebra, at a distance of 2 m. Calcified plaques were diagnosed as present when typically shaped densities were seen in the aortic arch or in the descending part of the thoracic aorta. The scoring, as either present or absent, was performed by the same roentgenology consultant at exams 5 to 11. Because X-rays at exam 11 were only obtained in a subset of the participants, data on calcified plaques from exams 5 to 10 were used in the analysis. The number of participants ranged from 4,422 subjects at exam 5 to 3,599 subjects at exam 10. Information on aortic calcified plaques at these exams was available for an average of 99% of subjects. Aortic calcified plaques were considered present after the first manifestation, even though the plaques might not be visualized on all of the subsequent exams. Among subjects with a first manifestation of aortic calcified plaques, 18.3% failed to show these plaques on subsequent exams.

Participants were monitored during 12 years of follow-up for development of cardiovascular disease. Loss to follow-up was < 2%. Cardiovascular events considered in this study were coronary artery disease, stroke, intermittent claudication, sudden coronary death and total cardiovascular death. Detailed definitions and measurement criteria have been given elsewhere.[7] Coronary artery disease included myocardial infarction, coronary insufficiency and death from coronary artery disease. About half of the strokes were due to atherothrombotic brain infarction. Other events classified as stroke included cerebral embolus, intracranial hemorrhage, and isolated transient ischemic attacks. Intermittent claudication was diagnosed by a history of cramping discomfort in the calf, which was clearly provoked by walking and relieved by rest. Sudden coronary death was defined as any death occurring within 1 hour of onset of symptoms without other probable cause of death suggested by medical history. The diagnoses were made on the basis of biennial clinical examination, surveillance of hospital discharge records, information obtained from private physici-

ans and review of mortality information. The diagnoses were jugded by a panel of investigators. The risk factors examined in this study were systolic and diastolic blood pressure, serum total cholesterol, smoking, Framingham relative weight and diabetes mellitus, measured as described previously.[7]

Data analysis

The prevalence of aortic calcified plaques was determined by categories of age and sex. The risk of developing aortic calcified plaques was examined in relation to antecedent levels of risk factors, evaluated at biennial exams among subjects free of these plaques (exams 5 to 9). After stratification for age, Mantel-Haenszel odds ratio were calculated to approximate relative risk (RR), with the lowest level of the risk factor as reference category. Age-adjusted trends in relative risk over categories of the risk factor were calculated by the extended Mantel-Haenszel chi-square test.[8] Risks of cardiovascular events were determined by plaque status measured biennially. Analyses were based on 2-year incidence between examinations. The information from exams 5 to 10 was pooled by considering each examination for each participant as an independent observation. The population at risk consisted of subjects free of the event studied. Additional analyses were performed among subjects free of interim overt cardiovascular disease and among subjects with overt cardiovascular disease. Logistic regression analysis was used to adjust for age (in years) and other cardiovascular risk factors. The parameters of the logistic model were estimated by the maximum likelihood method of Walker-Duncan.[9] Based on previous findings of the excess risk being largest among relatively young subjects,[6,10,11] risks were calculated separately for subjects aged < 65 years and for those aged 65 years. To examine further the effect of age on the association between aortic calcified plaques and cardiovascular disease, an interaction term was entered in the logistic model with subjects of all ages included. The term was constructed in such a way, that, when ß is the regression coefficient of the interaction term as derived by the model, the relative risk changes with $-(1 - e^{\beta}) \times 100$ percent each year of age over 35 years.

Results

Prevalence and risk factors for aortic calcified plaques

Calcified plaques in the thoracic aorta were present in 8.5% of men and in 3.9% of women, aged 40 to 44 years. After a low rate of increase up to age 50, the prevalence started to increase at a constant rate, with women catching up to men at age 60. The prevalence reached > 80% in both sexes at age 75 to 80. Among the cardiovascular risk factors examined, the presence of aortic calcified plaques was most strongly related to antecedent levels of systolic and diastolic blood

pressure (figures 2.2.1 and 2.2.2). Associations with other risk factors were generally positive, but rather weak. The trend of risk reached borderline significance for serum cholesterol among women (chi-square=3.2; p=0.07), with the age-adjusted RR of the highest level of cholesterol compared to the lowest being 1.5 (95% confidence interval 0.9 to 1.9).

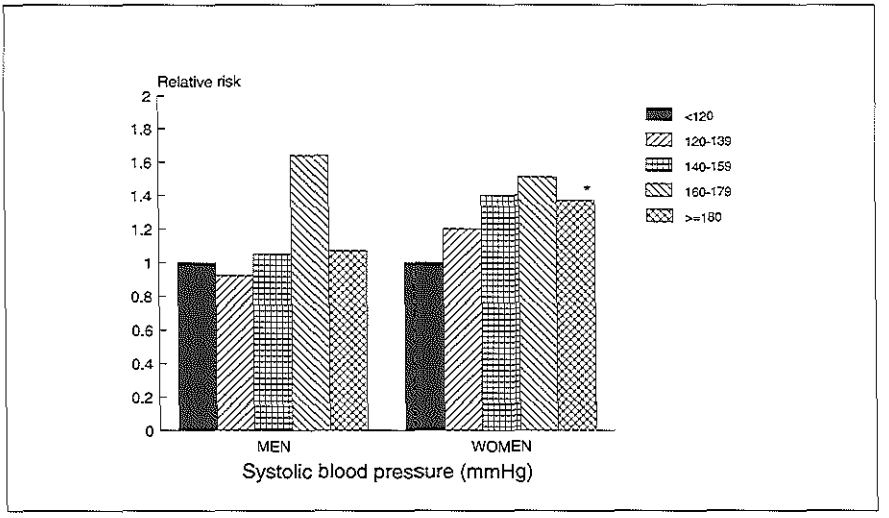

Figure 2.2.1. Age-adjusted relative risk of aortic calcified plaques by level of systolic blood pressure in men and women, aged 38 to 80 years. Framingham Study, 10-year follow-up. Significant trend: * Chi square = 15.7; p<0.001

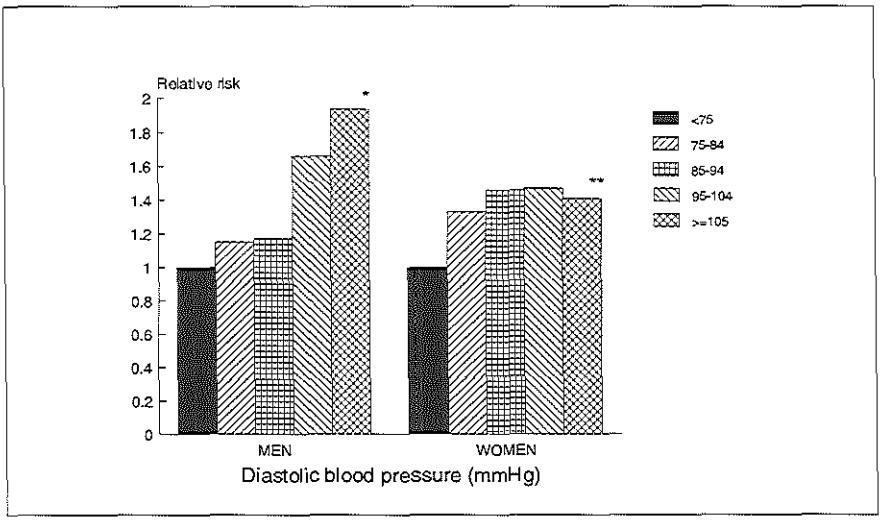

Figure 2.2.2. Age-adjusted relative risk of aortic calcified plaques by level of diastolic blood pressure in men and women, aged 38 to 80 years. Framingham Study, 10-year follow-up. Significant trend: * Chi square = 15.1; p<0.001; ** Chi square = 12.6; p<0.001

Aortic calcified plaques and cardiovascular disease

Relative risks of cardiovascular disease by presence of aortic calcified plaques are presented for men and women in 2 categories of age (tables 2.2.1 and 2.2.2). Age-adjusted relative risks among middle-aged men were significant and substantial for sudden coronary death and total cardiovascular death. Among middle-aged women risks were significant for all examined cardiovascular events, except intermittent claudication. Sudden coronary death occurred only among 9 women in each of the 2 age-categories; therefore, no risk analyses were performed. After adjustment for cardiovascular risk factors the relative risks decreased only slightly (tables 2.2.1 and 2.2.2). Except for risk of stroke among men, no significantly elevated risks were found among subjects aged ≥ 65 years (tables 2.2.1 and 2.2.2).

Examination of the relative risks among subjects free of interim overt cardiovascular disease improved the relative risks slightly for middle-aged men, but apart from risk of cardiovascular death none reached significance. Among middle-aged women, risk of intermittant claudication reached significance (adjusted RR=2.4; 1.1 to 5.6; p=0.04); risk of cardiovascular death, however, lost significance (adjusted RR=1.6; 0.7 to 3.3; p=0.26). Only minor changes in

Table 2.2.1. Relative risks of specified cardiovascular events by presence of aortic calcified plaques among men in two age groups. Framingham study, 12-year follow-up

	Aortic calcified plaques	< 65 years			≥ 65 years		
		No. of events/ observations*	RR† (95% CI)	RR‡ (95% CI)	No. of events/ observations*	RR† (95% CI)	RR‡ (95% CI)
Cardiovascular death	0	45/6,241	1.8¶	1.7§	16/651	1.3	1.1
	+	50/2,520	(1.2-2.8)	(1.1-2.6)	62/1,520	(0.7-2.3)	(0.6-2.0)
Sudden coronary death	0	13/6,241	2.2§	2.1§	5/651	1.0	0.9
	+	18/2,520	(1.0-4.7)	(1.0-4.6)	13/1,520	(0.4-2.8)	(0.3-2.6)
Coronary artery disease	0	131/5,780	1.3	1.2	26/542	0.9	0.8
	+	91/2,244	(1.0-1.8)	(0.9-1.7)	57/1,166	(0.6-1.5)	(0.5-1.4)
Stroke	0	24/6,176	1.4	1.2	4/642	3.6§	3.2§
	+	19/2,475	(0.7-2.6)	(0.6-2.4)	35/1,454	(1.3-10.3)	(1.1-9.2)
Intermittent claudication	0	35/6,150	1.3	1.2	8/625	1.3	1.2
	+	26/2,441	(0.7-2.2)	(0.7-2.0)	21/1,372	(0.6-2.9)	(0.5-2.7)

* This may be viewed as the 2-year cumulative incidence (observations = person-exams); the population at risk consists of subjects free of the specified event

† Adjusted for age

‡ Adjusted for age, systolic blood pressure, serum total cholesterol, cigarette smoking, Framingham relative weight and diabetes mellitus

§ $p<0.05$; ¶ $p<0.01$

CI = confidence interval; RR = relative risk; o = absent; + = present

Table 2.2.2. Relative risks of specified cardiovascular events by presence of aortic calcified

	Aortic calcified plaques	< 65 years			≥ 65 years		
		No. of events/ observations*	RR† (95% CI)	RR‡ (95% CI)	No. of events/ observations*	RR† (95% CI)	RR‡ (95% CI)
Cardiovascular death	0	25/8,123	2.2	1.9§	16/816	1.1	1.0
	+	30/3,143	(1.2-3.9)	(1.1-3.5)	62/2,328	(0.6-1.9)	(0.6-1.8)
Sudden coronary death	0	6/8,123	–	–	1/816	–	–
	+	3/3,143			8/2,328		
Coronary artery disease	0	65/7,948	1.7¶	1.6§	22/738	1.2	1.1
	+	70/2,947	(1.2-3.5)	(1.1-2.3)	75/1,948	(0.7-1.9)	(0.7-1.8)
Stroke	0	16/8,066	2.4§	2.1§	14/787	1.0	1.0
	+	21/3,096	(1.2-4.8)	(1.1-4.3)	43/2,223	(0.7-1.4)	(0.5-1.8)
Intermittent claudication	0	14/8,091	1.9	1.7	4/785	1.9	1.8
	+	19/3,100	(0.9-3.9)	(0.8-3.6)	22/2,223	(0.5-5.4)	(0.6-5.2)

* This may be viewed as the 2-year cumulative incidence (observations = person-exams); the population at risk consists of subjects free of the specified event
† Adjusted for age; ‡ Adjusted for age, systolic blood pressure, serum total cholesterol, cigarette smoking, Framingham relative weight and diabetes mellitus
§ $p<0.05$; ¶ $p<0.01$; CI = confidence interval; RR = relative risk; o = absent; + = present

relative risks of other events were observed among middle-aged women after exclusion of subjects with interim overt cardiovascular disease. The relative risks of cardiovascular death were also examined among subjects with manifest cardiovascular disease, with 52 deaths occurring among middle-aged men and 23 among middle-aged women. The adjusted relative risks were 1.4 (0.8 to 2.5; p=0.28) and 1.8 (0.7 to 4.8; p=0.22), respectively.

Interaction terms for the presence of aortic calcified plaques and age approximated statistical significance for coronary artery disease (ß = -0.036; p=0.04) and intermittent claudication (ß = -0.067; p=0.07) in women, after adjustment for other cardiovascular risk factors: A nearly fourfold increase in risk of coronary artery disease was observed among women at age 35 (adjusted RR = 3.8; 1.4 to 10.0; p=0.008), which decreased with 3.5% per year to no excess risk at age 72. The increase in risk of intermittent claudication in women was over 10-fold at age 35 (adjusted RR = 10.6; 1.4 to 78.9; p=0.02), and decreased with 6.5% per year to no excess risk at age 70. Among men, the age dependency of the relative risks was not as pronounced as in women, except for sudden coronary death, with the increase in risk being nearly sevenfold at age 35 (adjusted RR = 6.7; 0.9 to 49.1; p=0.06), and decreasing with 5.3% per year (ß= -0.054; p=0.14) to no excess risk at age 70.

Discussion

The results of this prospective study demonstrate that the early presence of calcified plaques in the thoracic aorta is independently associated with excess cardiovascular disease. This seems to support the view that atherosclerosis is a generalized process. However, before any interpretation of this kind can be made, the accuracy of our measurements should be addressed.

In comparison to lateral X-rays of the lumbar spine, on which calcified plaques in the abdominal aorta can be easily detected, chest X-rays are more difficult to interpretet. However, Hyman and Epstein,[4], showed that the validity of the roentgenographic examination of calcified plaques was equally good for definite diagnoses in both parts of the aorta. All subjects with definite presence of calcified plaques in the thoracic aorta detected on posteroanterior chest X-rays (n=18), had intimal atherosclerosis at autopsy, usually of an advanced degree. All doubtful cases, however, were negative at autopsy (n=5). Of 49 subjects in whom no aortic calcified plaques were detected, atherosclerosis at autopsy was present in 4, indicating a false-negative rate of nearly 10%. False negative findings after an initial manifestation of aortic calcified plaques, due to a varying penetration of the X-rays over subsequent exams, is a likely cause of the lack of consistency of diagnosis observed in a part of our population. The presence of false positive findings by confusion of aortic calcified plaques with calcium in other stuctures, like the ribs, is also possible. Although the sex-specific pattern and the strong increase in prevalence of calcified plaques in the thoracic aorta from < 10% at age 40 to 44 up to > 80% at age 75 to 80, is in agreement with autopsy data,[11,12] we would have expected to detect less on X-rays in the living. Therefore it is possible that our prevalence data are generally somewhat overestimated. However, misclassification is likely to be random, and if present in our study, would have resulted in an underestimation of the strength of the associations, rather than having introduced the observed effects.

The cardiovascular risk associated with the presence of aortic calcified plaques was generally stronger in younger subjects and in women. Similar results have been observed in autopsy studies.[10,11] Apart from sampling bias in autopsy studies this may also be due to differences in the relative importance of the atherosclerotic lesion among the many factors involved in the development of ischemic myocardial lesions.[10] When compared to women, aortic calcified plaques in men develop at an earlier age, and its presence is found to be associated with cardiovascular disease in younger age groups.[13] It thus may be that men with an early presence of aortic calcified plaques have died before entry into the study. In this respect, it is of interest to note that after an initial difference, the prevalence of aortic calcified plaques no longer prevailed in men. We do not believe, however, that this can be fully ascribed to selective mortality

in men with early atherosclerosis, because autopsy studies that showed that the prevalence of aortic calcified plaques was similar or even higher in females, still showed the usual male predominance of coronary atherosclerosis.[13-15] A possible explanation is that the menopause plays a part in the strong increase in prevalence of aortic calcified plaques in women after middle age, as observed in a recent study.[16]

Associations of aortic calcified plaques with cardiovascular risk factors were except for blood pressure, found to be generally weaker than usually observed for coronary atherosclerosis. The presence of aortic calcified plaques is more closely related with age than any other artery,[13] and this may weaken the association with other factors. Also, the thoracic aorta may react somewhat differently to these risk factors than the coronary arteries. Apart from these possibilities, we may not exclude a possible underestimation of the associations due to misclassification of the presence of calcified plaques. The finding of an independent cardiovascular risk associated with the presence of aortic calcified plaques demonstrates that the coexistence of atherosclerosis in different arterial beds cannot be fully ascribed to shared risk factors.

Chest X-rays are frequently recorded in the clinic and in health screening programs. A careful search for calcified plaques in the thoracic aorta is not always incorporated in the X-ray examination. Our data indicate that its detection may be of clinical value. When calcified plaques in the thoracic aorta are detected in a relatively young person, this should be regarded as a sign for increased cardiovascular risk. Attention should be directed toward the prevention of widespread atherosclerotic disease.

Acknowledgement

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CHAPTER 3

DETERMINANTS OF ATHEROSCLEROSIS IN WOMEN

3.1 Systolic and Diastolic Blood Pressure and Progression of Atherosclerosis in Women*

Abstract

Hypertension has been shown to be an important cardiovascular risk factor in men and women. The assocation is generally ascribed to a role of elevated blood pressure in the initiation and progression of atherosclerosis. Direct prospective data on the relation, however, are sparse.

The association of blood pressure with progression of aortic atherosclerosis was examined in a population-based cohort of 614 women, initially aged 45 to 64 years. All women were examined radiographically for calcified deposits in the abdominal aorta, which have been shown to represent intimal atherosclerosis. After 9 years of follow-up mild and advanced progression of atherosclerosis could be demonstrated in 20 and 15 percent of women, respectively. A graded association was observed between baseline systolic blood pressure and progression of mild and advanced atherosclerosis. Both low and high-normal to high baseline levels of diastolic blood pressure were associated with an excess risk of advanced progression. This J-shaped relation for diastolic blood pressure was also seen when change in diastolic pressure was considered, with women with a strong decrease as well as those with a rise of diastolic pressure having an excess risk of progression, compared to the reference category. The findings did not change after adjustment for other cardiovascular risk factors.

The results of this follow-up study in middle-aged women suggest that elevations of both systolic and diastolic blood pressure prior to the development of sustained hypertension promote the atherosclerotic process. The findings further suggest that low diastolic pressure in middle-aged and older women may indicate vessel wall stiffening, possibly caused in part by atherosclerosis.

Introduction

The incidence of cardiovascular disease shows a strong increase in women after middle-age.[1] Although less data are available for women than for men, there is convincing evidence that high blood pressure is a major contributor to risk of cardiovascular disease in women.[1-8] The increased risk is generally ascribed to a role of elevated blood pressure in the initiation and progression of atherosclerosis.

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* Submitted

Data on the association between hypertension and atherosclerosis have been provided by postmortem studies.[9-11] Determination of the exact nature of the relationship, however, is problematic, because, on the one hand blood pressure may cause vessel wall changes and accelerate atherosclerosis. On the other hand, the presence of atherosclerosis in the aorta and large vessels may reduce the elasticity of the vessel wall, thereby increasing the pulse pressure.[12] Not many studies have tried to clarify the relationship in vivo because direct assessment of atherosclerosis in asymptomatic subjects is difficult. A major artery that can be examined with relative ease is the aorta. Calcific densities in the aorta, as detected on radiographs, represent an advanced stage of atherosclerosis[13], and have been shown to be related to cardiovascular morbidity and mortality.[14,15] In the present study, we investigated the association of level and rise of blood pressure with progression of aortic atherosclerosis during 9 years of follow-up of a population-based cohort of 614 middle-aged women.

Subjects and methods

Between 1975 and 1978, a population study on risk factors for chronic diseases was conducted in the Dutch town of Zoetermeer. The inhabitants of two districts of the town were invited for a medical examination. Details of this study have been published previously.[16-18] In 1985, all female participants aged 45 to 64 years at baseline were invited for a follow-up examination during which information was obtained on risk factors for osteoporosis and some cardiovascular risk factors.[17,18] Of 1167 women invited, 71 had died and 87 had moved away during the follow-up period. Of the remaining women, 855 (85%) were reexamined. The mean duration of follow-up was 8.9 ± 0.8 years.

Measurement of aortic atherosclerosis

Aortic atherosclerosis was diagnosed by radiographic detection of calcific deposits in the abdominal aorta. The technical procedures for X-ray measurements were similar at baseline and follow-up. At each occasion lateral abdominal films (T12-S1) were made at a fixed distance with subjects in sitting position. Baseline and follow-up films were examined in pairs. Calcifications were considered to be present when linear densities were seen in an area parallel and anterior to the lumbar spine (L1-L4). Progression was defined as the occurrence of new calcifications or enlargement of the calcified area present at baseline. The extent of change was scored on a 3-point scale. Approximate indications were given for the 3 grades. Mild progression referred to the occurrence of one or more new small streaks of at least 1 cm in length, or a small increase in length of the involved area (1 to 2 cm). Moderate progression referred to the occurrence of several larger streaks spreaded over the anterior or

posterior wall, or a moderate increase in length of the involved area of up to 5 cm (average distance between midpoints of 2 adjacent vertebra). A notable increase in confluency of the streaks was also scored as moderate progression. Severe progression referred to an increase in length of the involved area of more than 5 cm. In these cases the abdominal aorta was usually outlined with streaks over its entire length at follow-up. In no subject a decrease in extent of atherosclerosis was observed. Examples of the 3 grades of atherosclerotic progression are shown in figure 3.1.1. All films were examined by 2 independent observers without knowlegde of the risk factor status of the subjects. Prior to the scoring a sample of the films was read by the 2 observers simultaneously to reach agreement on interpretation of the grading protocol. When the interobserver difference of independent readings was qualitative (change present versus absent) or when the difference in severity grades was more than 1, films were reviewed by both observers simultaneously to reach consensus. When the difference in severity grades was 1, the highest grade was used in the analysis. The percentage of agreement was 0.75 and the weighted kappa statistic was 0.77, with weights taken as the squared distance between categories. In 59 subjects progression could not be measured because baseline or follow-up films were missing (n=50) or not readable (n=9).

Measurement of risk factors

At baseline and follow-up, blood pressure was measured on the left arm using a random zero sphygmomanometer with subjects in sitting position. The mean of 2 readings was used in the analysis. Height and weight were measured without shoes and with indoor clothing. Quetelet index was calculated as weight devided by height square. Serum total cholesterol was measured by an automated enzymatic method.[19] Information on smoking habits and medical history was obtained by a self-administered questionnaire, which was checked during an interview with a physician. Subjects were asked to bring all drugs used at the time of investigation to the research centre, where they were recorded.

Data analysis

Blood pressure measurements at baseline and at follow-up were missing in 6 and 2 women, respectively. After exclusion of women with missing data on atherosclerotic change (n=59) or baseline blood pressure (n=6) and exclusion of women who reported antihypertensive medication (n=184) or a history of a major cardiovascular event at baseline (n=15), data of 614 women were used in the analysis. In addition, women with missing blood pressure at follow-up (n=2) and those who reported antihypertensive medication at follow-up (n=95) were excluded from the analysis of blood pressure change and considered separately. The risk of atherosclerotic progression was estimated in categories of baseline blood pressure level

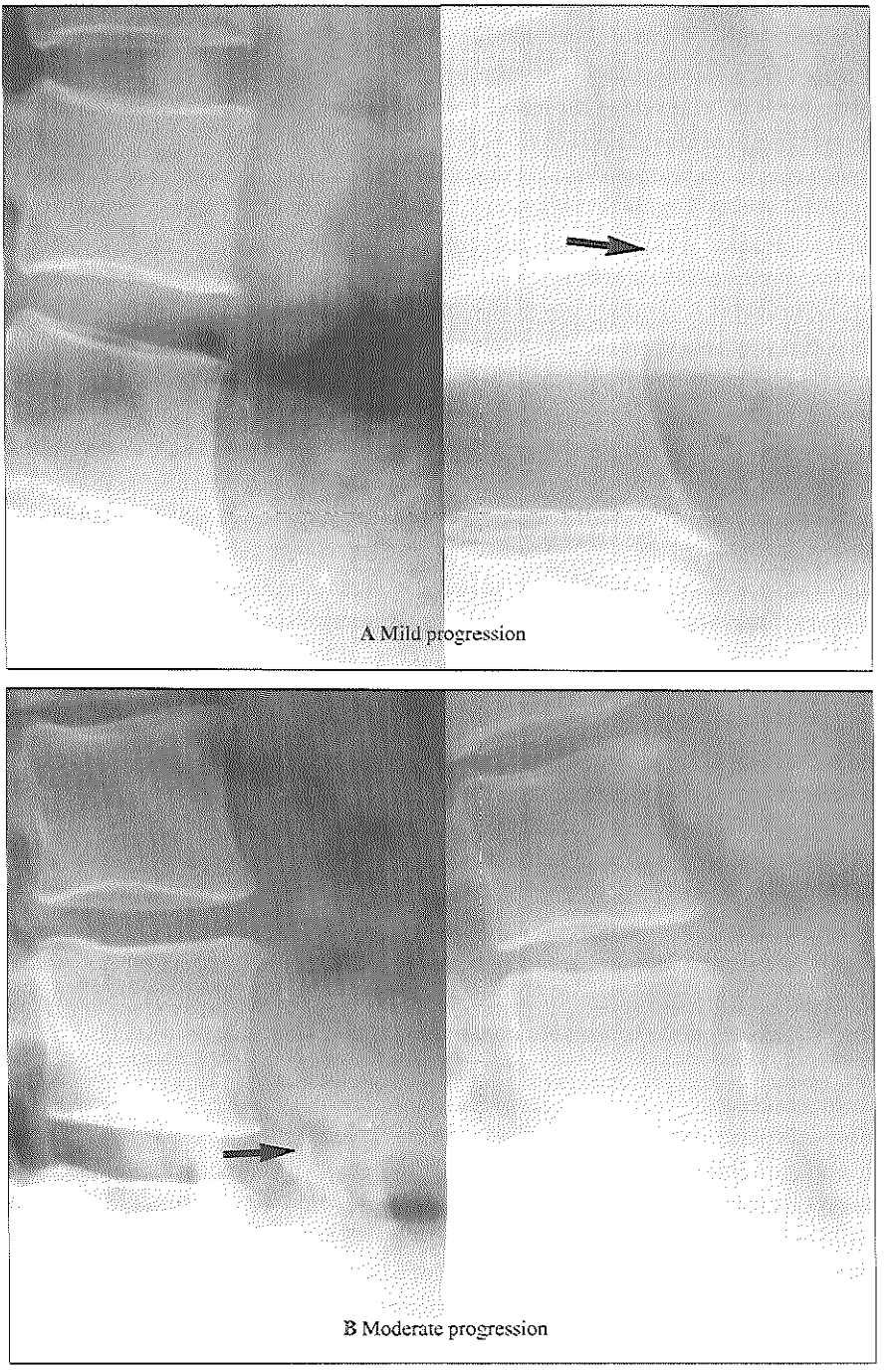

Figure 3.1.1. Gradation of atherosclerotic progression in the abdominal aorta. Left: baseline X-ray; right: follow-up X-ray

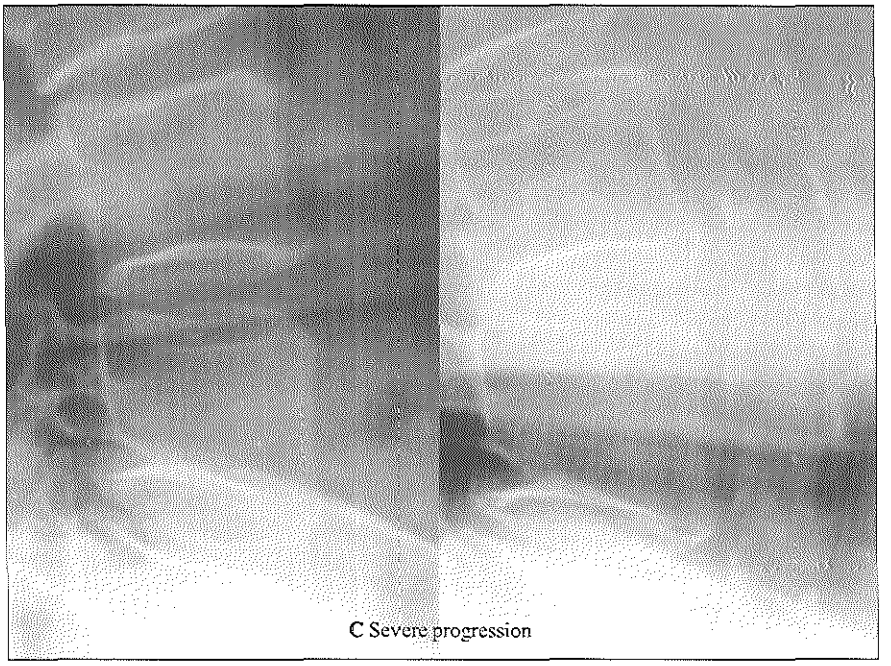

Figure 3.1.1. Continued

and of blood pressure change. For systolic blood pressure the lowest category was used as reference. Inspection of the data showed that the relation of diastolic blood pressure with progression of atherosclerosis was J-shaped; therefore the second category was used as reference. Analyses were performed by polychotomous logistic regression analysis, using the PR module of the BMDP statistical package.[20] The model specified regression coefficients for the effects of blood pressure on risks of distinct grades of progression, neglecting the order of the grades. The odds-ratio's as derived from logistic regression analysis were used as an approximation of relative risk. Some caution with interpreting the estimate is needed, however, in case the percentage of subjects with progression in a particular category is relatively high (i.e. more than 20%).[21] In these instances the presented odds-ratio's will have overestimated the corresponding relative risks. As an example, the odds ratio of advanced progression for the highest category of change in diastolic blood pressure compared to the reference category is 3.5. The corresponding relative risk computed by substituting the mean age of the study population in the logistic model is 2.8.

Results

Table 3.1.1 shows the baseline characteristics of the study population. The mean age of the population was 52.9 ± 5.6 years. Aortic atherosclerosis was present

Table 3.1.1. Baseline characteristics of 614 Dutch women, aged 45 to 64 years

Characteristic		
Mean (SD)		
Age (years)	52.9	(5.6)
Height (m)	1.63	(0.06)
Weight (kg)	66.5	(9.3)
Quetelet index (kg/m^2)	25.0	(3.2)
Systolic blood pressure (mmHg)	132.5	(18.2)
Diastolic blood pressure (mmHg)	81.6	(10.7)
Serum cholesterol (mmol/l)	6.2	(1.1)
Percentage		
Current smokers	36	
Former smokers	16	
Diabetes mellitus	2	
Postmenopausal	62	
Anti-hypertensive treatment	21	
Aortic atherosclerosis	20	

at baseline in 20% of women. Sixty-two percent of women was postmenopausal at baseline, this percentage was 99 at follow-up. At follow-up, mild, moderate and severe progression were observed in 121, 68 and 23 women, respectively. Because the number of women with severe progression was low, moderate and severe progression were combined in the analysis, referred to as advanced progression. The relative risks of progression according to baseline levels of blood pressure are presented in table 3.1.2. and in figure 3.1.2. The risks of mild and advanced progression increased with increasing systolic blood pressure. An excess risk of advanced progression was seen in both women with a diastolic blood pressure below 75 mmHg and women with levels of 85 mmHg and higher, compared to the reference group. No association was observed between diastolic blood pressure and risk of mild progression. In an additional analysis women with a diastolic blood pressure below 75 mmHg were further devided into those with a pulse pressure under and those with a pulse pressure above the median (49 mmHg). The age-adjusted relative risks of advanced progression of these groups were 1.4 (0.7-3.1) and 2.1 (1.0-4.5) respectively, compared to the reference category. Of other cardiovascular risk factors significant associations were found with serum cholesterol and with smoking. Adjustment for cardiovascular risk factors did only slightly alter the risk estimates associated with blood pressure. Table 3.1.3 presents the relative risk among women without atherosclerosis at baseline (n=490). Risk estimates were similar to those of the whole group. The excess risk associated with systolic blood pressure was also present among women with a diastolic blood pressure below 90 mmHg: the

Table 3.1.2. Relative risks of mild and advanced progression of atherosclerosis by categories of blood pressure

	Category			
Systolic BP (mmHg)	<120	120-139	140-159	≥160
Total number	152	273	143	46
Mild progression				
No. of cases	25	53	32	11
RR (95% CI)*	1.0‡	1.0 (0.6-1.8)	1.3 (0.7-2.3)	1.7 (0.7-4.2)
RR (95% CI)†	1.0‡	1.4 (0.8-2.5)	1.5 (0.8-2.9)	2.4 (0.9-6.4)
Advanced progression				
No. of cases	16	34	26	15
RR (95% CI)*	1.0‡	1.0 (0.5-2.0)	1.6 (0.8-3.2)	3.7 (1.5-8.9)
RR (95% CI)†	1.0‡	1.3 (0.6-2.5)	1.8 (0.9-3.9)	4.4 (1.6-12.0)
Diastolic BP (mmHg)	<75	75-84	85-94	≥95
Total number	175	222	137	80
Mild progression				
No. of cases	33	45	27	16
RR (95% CI)*	1.0 (0.6-1.7)	1.0‡	1.0 (0.6-1.7)	1.0 (0.5-2.0)
RR (95% CI)†	0.9 (0.5-1.5)	1.0‡	1.0 (0.5-1.8)	1.0 (0.5-2.1)
Advanced progression				
No. of cases	27	22	28	14
RR (95% CI)*	1.7 (0.9-3.2)	1.0‡	2.1 (1.1-3.9)	1.8 (0.9-3.9)
RR (95% CI)†	1.6 (0.8-3.0)	1.0‡	2.0 (1.0-3.9)	1.8 (0.8-4.1)

* Adjusted for age; † Adjusted for age, serum cholesterol, smoking status, Quetelet index, diabetes mellitus, menopausal status and duration of follow-up; ‡ Reference group; RR = relative risk; CI = confidence interval

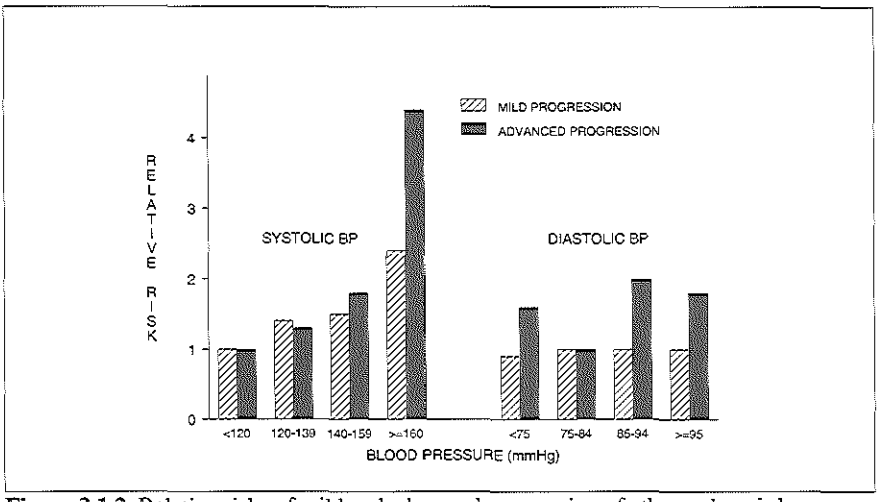

Figure 3.1.2. Relative risks of mild and advanced progression of atherosclerosis by baseline level of systolic and diastolic blood pressure. Relative risks are adjusted for age and other cardiovascular risk factors

age-adjusted relative risks of mild progression of atherosclerosis of atherosclerosis were 1.1 (0.6-1.8), 1.7 (0.8-3.4) and 1.6 (0.3-9.6), respectively, and the relative risks of advanced progression were 1.1 (0.6-2.2), 2.2 (1.0-5.0) and 6.7 (1.6-29.0), respectively.

The average changes in systolic and diastolic blood pressure during follow-up, excluding women using antihypertensive medication, were 10.0 ± 16.1 mmHg and -0.2 ± 9.4 mmHg, respectively. The change in systolic blood pressure was positively though nonsignificantly associated with age (coefficient of linear regression (ß) = 0.19 mmHg/year, SE=0.13; p=0.15) and significantly with change in body weight (ß=0.89 mmHg/kg, SE=0.13; p<0.0001, adjusted for age). The change in diastolic blood pressure was inversely associated with age (ß= -0.26 mmHg/year, SE=0.07; p=0.0004) and positively with change in body weight (ß=0.37 mmHg/kg, SE=0.08; p<0.0001, adjusted for age) and serum cholesterol (ß=0.81 mmHg/mmol/l, SE=0.42; p=0.05, adjusted for age and body weight).

Table 3.1.4 presents the relative risks of progression of atherosclerosis for categories of blood pressure change. No associations were seen with mild

Table 3.1.3. Relative risks of mild and advanced progression of atherosclerosis by categories of blood pressure among women without aortic atherosclerosis at baseline

	Category			
Systolic BP (mmHg)	<120	120-139	140-159	≥160
Total number	132	218	108	32
Mild progression				
No. of cases	13	30	20	6
RR (95% CI)*	1.0‡	1.4 (0.7-2.8)	1.9 (0.9-4.2)	2.4 (0.8-7.3)
RR (95% CI)†	1.0‡	1.7 (0.8-3.6)	2.2 (1.0-5.1)	3.1 (1.0-10.0)
Advanced progression				
No. of cases	10	14	8	7
RR (95% CI)*	1.0‡	0.8 (0.4-2.0)	1.0 (0.4-2.7)	3.6 (1.2-11.0)
RR (95% CI)†	1.0‡	1.0 (0.4-2.4)	1.2 (0.4-3.4)	4.1 (1.2-14.0)
Diastolic BP (mmHg)	<75	75-84	85-94	≥95
Total number	138	190	98	64
Mild progression				
No. of cases	15	31	15	8
RR (95% CI)*	0.7 (0.4-1.3)	1.0‡	0.9 (0.5-1.8)	0.8 (0.3-1.8)
RR (95% CI)†	0.6 (0.3-1.2)	1.0‡	0.8 (0.4-1.7)	0.7 (0.3-1.7)
Advanced progression				
No. of cases	10	13	8	8
RR (95% CI)*	1.8 (0.8-4.3)	1.0‡	1.5 (0.6-3.9)	2.4 (0.9-6.5)
RR (95% CI)†	1.7 (0.7-4.1)	1.0‡	1.6 (0.6-4.6)	2.4 (0.8-6.9)

* Adjusted for age; † Adjusted for age, serum cholesterol, smoking status, Quetelet index, diabetes mellitus, menopausal status and duration of follow-up; ‡ Reference group; RR = relative risk; CI = confidence interval

Table 3.1.4. Relative risks of mild and advanced progression of atherosclerosis by categories of blood pressure change

	Category			
Change in systolic BP (mmHg)	≤0	1-9	10-19	≥20
Total number	144	104	144	126
Mild progression				
No. of cases	28	19	31	20
RR (95% CI)*	1.0‡	1.0 (0.5-2.0)	1.3 (0.7-2.4)	0.8 (0.4-1.6)
RR (95% CI)†	1.0‡	1.1 (0.6-2.3)	1.6 (0.8-3.1)	1.0 (0.5-2.2)
Advanced progression				
No. of cases	12	14	22	21
RR (95% CI)*	1.0‡	1.7 (0.8-4.0)	2.2 (1.0-4.7)	2.0 (0.9-4.3)
RR (95% CI)†	1.0‡	1.7 (0.7-4.1)	2.2 (1.0-5.0)	1.9 (0.8-4.5)
Change in diastolic BP (mmHg)	≤10	−10-0	1-9	≥10
Total number	66	203	175	74
Mild progression				
No. of cases	10	39	35	14
RR (95% CI)*	0.8 (0.4-1.8)	1.0‡	1.3 (0.7-2.1)	1.4 (0.7-2.9)
RR (95% CI)†	0.8 (0.3-1.9)	1.0‡	1.4 (0.8-2.4)	1.7 (0.8-3.6)
Advanced progression				
No. of cases	13	17	25	14
RR (95% CI)*	2.5 (1.3-5.6)	1.0‡	2.2 (1.1-4.3)	3.5 (1.6-8.0)
RR (95% CI)†	3.1 (1.3-7.6)	1.0‡	2.5 (1.2-5.2)	4.1 (1.7-10.0)

* Adjusted for age; † Adjusted for age, change in serum cholesterol, smoking status, change in body weight, diabetes mellitus, menopausal status and duration of follow-up; ‡ Reference group
RR = relative risk; CI = confidence interval

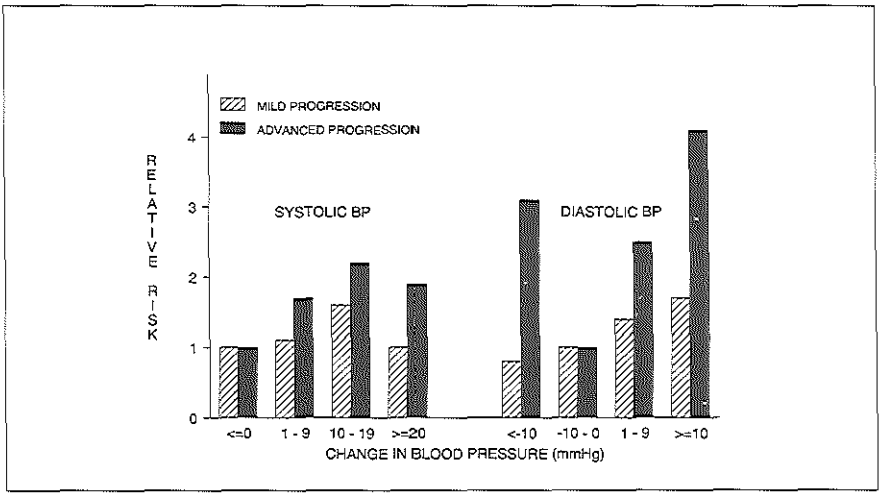

Figure 3.1.3. Relative risks of mild and advanced progression of atherosclerosis by change in systolic and diastolic blood pressure during follow-up. Relative risks are adjusted for age and other cardiovascular risk factors

progression. An excess risk of advanced progression was seen in women with an increase in systolic blood pressure compared to those with no increase, but estimates were of borderline significance and no clear trend was detected. The relation between change in diastolic blood pressure and risk of advanced progression was J-shaped, with an excess risk among both women with a strong decrease and women with an increase in pressure, compared to the reference group. Similar results were seen after adjustment for levels of other risk factors. When women with a decrease in diastolic blood pressure of more than 10 mmHg were further devided into those with a change in pulse pressure under and above the median (9 mmHg), the age-adjusted relative risks of advanced progression were 1.1 (0.3-4.2) and 3.9 (1.5-9.9) respectively, compared to the reference category. At follow-up, use of antihypertensive medication was reported by 95 women. The age-adjusted relative risks associated with antihypertensive medication were 1.6 (0.9-2.7) and 2.1 (1.2-3.7) for mild and advanced progression of atherosclerosis respectively.

Finally, we examined the association of blood pressure with atherosclerotic progression among women with a baseline serum cholesterol below 6.5 mmol/l. The age-adjusted relative risks associated with baseline blood pressure and blood pressure change are presented in table 3.1.5. In general, the trends in relative risk were similar to those among the whole group, though some of the relative risks lost significance because of small numbers in the categories.

Discussion

In this study among middle-aged women, baseline level of systolic blood pressure was independently associated with progression of aortic atherosclerosis during 9 years of follow-up. An excess risk of advanced progression was observed for women with low baseline levels of diastolic blood pressure as well as for those with high-normal and high diastolic blood pressure levels. This J-shaped relation with progression was even more marked when change in diastolic pressure was considered, with both women with a strong decrease and those with an increase in pressure showing an excess risk of progression.

Before conclusions can be drawn from these data, some aspects of the study need to be considered. The present population-based follow-up approach of study does not have the potential selection bias characteristic of autopsy and angiographic studies. The study allowed to evaluate the effects of both baseline blood pressure and blood pressure change during follow-up. However, the significance of aortic calcification needs consideration. The validity of radiographic assessment of aortic calcification for the diagnosis of atherosclerosis has been studied by comparison with material obtained at necropsy. The method was shown to be highly specific and in most cases visible calcification repre-

sented advanced atherosclerosis.[13] The observation that serum cholesterol and smoking were associated with aortic calcification further confirms that it represents intimal atheroslerosis. Quantification of atherosclerotic change was based on visual grading. To improve the measurement all films were read by 2 observers and consensus was sought when the scores were discrepant.

Table 3.1.5. Relative risks of mild and advanced progression of atherosclerosis by categories of blood pressure level and change among women with baseline cholesterol below 6.5 mmol/l

	Category			
SBP (mmHg)	<120	120-139	140-159	≥160
Mild progression				
No. of cases	12	26	16	6
RR (95% CI)*	1.0†	1.1 (0.5-2.3)	1.6 (0.7-3.7)	2.4 (0.7-7.6)
Advanced progression				
No. of cases	7	16	16	8
RR (95% CI)*	1.0†	1.0 (0.4-2.7)	2.3 (0.8-6.1)	4.6 (1.4-16.0)
DBP (mmHg)	<75	75-84	85-94	≥95
Mild progression				
No. of cases	17	23	14	6
RR (95% CI)*	1.0 (0.5-2.1)	1.0†	1.1 (0.5-2.3)	0.9 (0.3-2.4)
Advanced progression				
No. of cases	11	14	13	9
RR (95% CI)*	1.1 (0.5-2.6)	1.0†	1.5 (0.6-3.4)	2.2 (0.9-5.8)
Change in SBP (mmHg)	≤0	1-9	10-19	≥20
Mild progression				
No. of cases	17	11	13	8
RR (95% CI)*	1.0†	0.9 (0.4-2.2)	0.8 (0.4-1.9)	0.6 (0.3-1.6)
Advanced progression				
No. of cases	6	9	12	8
RR (95% CI)*	1.0†	2.1 (0.7-6.5)	2.1 (0.8-6.1)	1.7 (0.6-5.3)
Change in DBP (mmHg)	<–10	–10-0	1-9	≥10
Mild progression				
No. of cases	5	22	16	6
RR (95% CI)*	0.8 (0.3-2.4)	1.0†	1.1 (0.5-2.3)	1.2 (0.4-3.2)
Advanced progression				
No. of cases	6	8	12	9
RR (95% CI)*	2.7 (0.9-8.7)	1.0†	2.5 (0.9-6.5)	6.0 (2.0-18.0)

* Adjusted for age; † Reference group
SBP = systolic blood pressure; DBP = diastolic blood pressure; RR = relative risk; CI = confidence interval

Elevation of systolic blood pressure has long been considered to be without clinical significance.[22] This view started to change when observational studies demonstrated that systolic blood pressure was at least as predictive for the occurrence of cardiovascular disease as was diastolic blood pressure.[2,22] At older age, the prevalence of isolated systolic hypertension increases, which is considered to reflect rigidity of the large arteries. In addition, findings of the Framingham Study suggest that isolated systolic hypertension carries a cardiovascular risk independent of arterial rigidity.[23,24] This is supported by findings of the SHEP trial, which have provided evidence that treatment of isolated systolic hypertension lowers the incidence of stroke and myocardial infarction.[25] In the present longitudinal study, baseline systolic blood pressure was associated with subsequent progression of atherosclerosis, which may indicate that systolic pressure is causally related to the atherosclerotic process. The excess risk was also present among women with a diastolic pressure below 90 mmHg. However, because systolic and diastolic blood pressure are highly correlated and moderately elevated levels of diastolic blood pressure increased the risk of progression, separation of the effects of systolic and diastolic blood pressure is difficult. The major concern is whether elevated systolic blood pressure does not reflect rather than cause vessel wall hardening and atherosclerosis. A causal interpretation is supported by the observation that the relation remained when the analysis was restricted to women without atherosclerosis at baseline, though mild instances of atherosclerosis at baseline may have been missed by measuring calcified plaques. Further support for a causal interpretation is given by the observation that the association of atherosclerotic progression with baseline systolic blood pressure was more pronounced than the association of progression with change of systolic blood pressure during follow-up.

The findings for diastolic pressure indicate that both low and high-normal to high levels of this blood pressure component are associated with an increased risk of atherosclerotic progression. It has been suggested that increased rigidity of the aorta and large arteries tends to lower the diastolic blood pressure.[26,27] The excess risk of progression in women with low diastolic pressure might therefore reflect the presence of a hardened vessel wall, possibly caused in part by atherosclerosis and likely to show further progress during follow-up. The observation of similar risk among those without evidence atherosclerosis at baseline, suggests that the hardening of the vessel wall is present before calcified plaques can be detected radiographically. A J-shaped pattern was more clearly seen when change in diastolic pressure was considered. This may indicate that, if two opposing processes are operating rather late in life, they can be better discriminated by recent changes than by blood pressure levels, which result from both short-term and long-term experiences. The finding of the J-shape curve is of interest in the light of the present discussion on the relation between low diastolic blood pressure and coronary heart disease.[28-30] It is possible that

the association is partly due to the association between low diastolic pressure and hardened arteries and atherosclerosis, as has been suggested.[29] This is supported by the observations in the present study showing that the increased risk of progression among those with a low diastolic blood pressure (or strong decrease in diastolic pressure) was confined to those with a relatively high pulse pressure (or large increase in pulse pressure).

Several mechanisms by which hypertension affects the vessel wall have been put forward, including induction of endothelial changes, enhancement of wall permeability to lipoproteins and stimulation of smooth muscle cell proliferation.[31] Based on the results of animal experiments the view is hold that a critical level of serum cholesterol is required before hypertension exerts its effect.[32] In the present study, we found similar risk estimates when analyses were restricted to women without elevated cholesterol levels at baseline. Thus, an elevated cholesterol level does not seem to be a prequisite for the atherogenic effect of hypertension, at least at cholesterol levels presently observed in women in Western societies.

A question that remains is whether it is justified to make inferences from data of aorta calcification to atherosclerosis in other vessel beds. Aortic atherosclerosis has been found to be a predictor of cardiovascular events at various sites, which supports that aortic atherosclerosis reflects a generalized process. It is possible, however, that the aorta, because of its specific structural and functional characteristics, reacts differently to elevated blood pressure than other arteries. Postmortem studies have shown that atherosclerosis of the coronary arteries varied with hypertension in a way similar to that of the abdominal aorta in both sexes.[9,11] Furthermore, hypertension was found to be associated with all types of lesions, including calcified lesions[9-11], which supports the vascular generalizability of the findings. On the other hand, the aorta is the main determinant of arterial compliance and its impact on blood pressure associated with vessel wall hardening will be larger compared to the coronary arteries.

In summary, in the present follow-up study in middle-aged women a graded, independent association was observed between systolic pressure and progression of atherosclerosis. This suggest that systolic blood pressure promotes atherosclerosis, though even in this longitudinal study interpretation should be cautious. Both low and high-normal to high levels of diastolic blood pressure were associated with an excess risk of atherosclerotic progression; the J-shaped relation was even more clearly seen when change in diastolic pressure was considered. Taken together, the findings suggests that elevations of both systolic and diastolic blood pressure prior to the development of sustained hypertension may promote the atherosclerotic process. The findings further suggest that low diastolic blood pressure in middle-aged and older women may indicate vessel wall stiffening, possibly caused in part by atherosclerosis.

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3.2 Serum Cholesterol and Progression of Atherosclerosis in Women*

Abstract

The relation of serum total cholesterol with cardiovascular disease in men has been shown to be continuous and graded over the whole range of cholesterol values. In women, available data suggest that only relatively high levels of cholesterol are associated with an increased risk of cardiovascular disease. Studies using clinical endpoints, however, may underestimate the relation with atherosclerosis.

The association of serum total cholesterol with progression of aortic atherosclerosis was examined in a population-based cohort of 775 women, initially aged 45 to 64 years. All women were examined radiographically for calcified deposits in the abdominal aorta, which have been shown to represent intimal atherosclerosis. After 9 years of follow-up mild and advanced progression of atherosclerosis could be demonstrated in 20 and 17 percent of women, respectively.

No excess risk was present for baseline cholesterol levels up to 6.0 mmol/l. Above this level, a gradual increase in risk was observed, with the relative risks of mild and advanced progression exceeding 2 for women with a cholesterol level of 7.0 mmol/l or higher compared to women with a cholesterol level below 5 mmol/l, after adjustment for age and other cardiovascular risk factors. During follow-up serum cholesterol increased on average by 0.11 mmol/l per year (SD 0.12). The rise in cholesterol was associated with only a borderline significantly increased risk of mild progression; no association was seen with advanced progression.

These follow-up data suggest that an effect of serum cholesterol on progression of atherosclerosis in women is already present at moderately elevated levels of cholesterol. Whether the rise in cholesterol in women after middle-age contributes to progression of atherosclerosis remains to be proven.

Introduction

A positive association between serum total cholesterol level and cardiovascular morbidity and mortality has been found in both sexes.[1-6] Among men, the

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* Submitted

relationship has been established as continuous and graded over the whole range of cholesterol values.[7] The available data for women suggest a threshold effect with only relatively high levels of cholesterol associated with an increased risk of cardiovascular disease.[3,5,6] Differences in the predictive value of cholesterol in the sexes may be due to the sex differences in the change of cholesterol with age. Cholesterol levels off in men at the age of 30 to 40 years, while in women the increase in cholesterol gets steeper after the age of 40.[8] Considering that the development of a cardiovascular event is likely to be the result of lifelong accumulated exposure, the association of serum cholesterol in middle-aged women with later development of events may not be very accurate. A more direct method for evaluation of the effect of cholesterol is to study its relation with progression of atherosclerosis. To study atherosclerosis noninvasively in asymptomatic non-hospitalized subjects it is necessary to rely on vessels other than the coronary or cerebral. The aorta is a major artery that can be examined with relative ease. Calcific densities in the aorta, as detected on radiographs, represent an advanced stage of atherosclerosis[9], and have been shown to be related to cardiovascular morbidity and mortality.[10,11] In the present study, we investigated the association between level and rise of cholesterol with progression of aortic atherosclerosis during 9 years of follow-up of a population-based cohort of 775 middle-aged women.

Subjects and methods

Between 1975 and 1978, a population study on risk factors for chronic diseases was conducted in the Dutch town of Zoetermeer. The inhabitants of two districts of the town were invited for a medical examination. Details of this study have been published previously.[10,12] In 1985, all female participants aged 45 to 64 years at baseline were invited for a follow-up examination during which information was obtained on risk factors for osteoporosis and some cardiovascular risk factors.[13] Of 1167 women invited, 71 had died and 87 had moved away during the follow-up period. Of the remaining women, 855 (85%) were reexamined. The mean duration of follow-up was 8.9 ± 0.8 years.

Measurement of aortic atherosclerosis

Aortic atherosclerosis was diagnosed by radiographic detection of calcific deposits in the abdominal aorta. The technical procedures for X-ray measurements were similar at baseline and follow-up. At each occasion lateral abdominal films (T12-S1) were made at a fixed distance with subjects in sitting position. Baseline and follow-up films were examined in pairs. Calcifications were considered to be present when linear densities were seen in an area parallel and anterior to the lumbar spine (L1-L4). Progression was defined as the

occurrence of new calcifications or enlargement of the calcified area present at baseline. The extent of change was scored on a 3-point scale. Approximate criteria were given for the 3 grades. Mild progression referrred to the occurrence of one or more new small streaks of at least 1 cm in length, or a small increase in length of the involved area (1 to 2 cm). Moderate progression referred to the occurrence of several larger streaks spreaded over the anterior or posterior wall, or a moderate increase in length of the involved area of up to 5 cm (average distance between midpoints of 2 adjacent vertebra). A notable increase in confluency of the streaks was also scored as moderate progression. Severe progression referred to an increase in length of the involved area of more than 5 cm. In these cases the abdominal aorta was usually outlined with streaks over its entire length at follow-up. In no subject a decrease in extent of atherosclerosis was observed. All films were examined by 2 independent observers without knowlegde of the risk factor status of the subjects. Prior to the scoring, a sample of the films was read by the 2 observers simultaneously to reach agreement on interpretation of the grading protocol. When the interobserver difference of independent readings was qualitative (change present versus absent) or when the difference in severity grades was more than 1, films were reviewed by both observers simultaneously to reach consensus. When the difference in severity grades was 1, the highest grade was used in the analysis. The percentage of agreement was 0.75 and the weighted kappa statistic was 0.77, with weights taken as the squared distance between categories. In 59 subjects progression could not be measured because baseline or follow-up films were missing (n=50) or not readable (n=9).

Measurement of risk factors

Serum total cholesterol at baseline was measured by an automated enzymatic method.[14] During follow-up a modified reagent was used (CHOD/PAP High Performance, Boehringer Mannheim). In the transition period both reagents have been used simultaneously, obtaining an excellent correlation ($r>0.99$). The overall coefficient of variation was 2.5% at baseline and 2.3% at the time of follow-up.

All measurements were carried out in the laboratory of the Department of Epidemiology & Biostatistics (Erasmus University Medical School, Rotterdam), which participates from 1978 in the lipid standardization program of the WHO Regional Lipid Reference Centre in Prague (Dr. D. Grafnetter) and from 1977 in the Dutch National Cholesterol standardization program (KCA foundation), initiated in analogy to the program of the CDC Lipid Standardization Laboratory in Atlanta. In addition, during the baseline period quality control was indirectly checked on the CDC protocol by monthly comparison with cholesterol determination using the Abell-Kendall method (Gaubius Institute

TNO, Leiden). Both accuracy and precision were within the acceptable limits (CDC/WHO) over the whole period. Cholesterol determinations at follow-up were performed on serum samples stored at -20°C for up to 4 years. Repeated measurements performed by our laboratory in frozen serum showed no significant changes up to 4 years after sampling compared to frozen samples measured within one week after venapuncture. The standard deviation of these duplicate measurements did not exceed 3.0% in all instances and did not show a significant drift. Blood pressure was measured on the left arm using a random zero sphygmomanometer with subjects in sitting position. The mean of two readings was used in the analysis. Height and weight were measured without shoes and with indoor clothing. Quetelet index was calculated as weight divided by height square. Information on smoking habits and medical history was obtained by a self-administered questionaire, which was checked during an interview with a physician.

Data analysis

After exclusion of women with missing or non-evaluable data on atherosclerotic change (n=59) or baseline cholesterol (n=6) and exclusion of women who reported a history of major cardiovascular event at baseline (n=15), data of 775 subjects were used in the analysis. Since cholesterol measurements at follow-up were missing for 18 women, analysis of change in cholesterol could be performed in 757 women. The risk of atherosclerotic progression was estimated in categories of baseline cholesterol with the lowest category as reference. Analyses were performed by polychotomous logistic regression analysis, using the PR module of the BMDP statistical package.[15] The model specified regression coefficients for the effects of cholesterol on risks of distinct grades of progression, neglecting the order of the grades. The odds-ratio's as derived from logistic regression analysis were used as an approximation of relative risk. Some caution with interpreting the estimates is necessary, however, when the percentage of subjects with progression in a particular category is relatively high (i.e. more than 20%).[16] In these instances the presented odds-ratio's will have overestimated the corresponding relative risks.

Results

Table 3.2.1 shows the baseline characteristics of the study population. The mean age of the population was 53.3 ± 5.7 years. Aortic atherosclerosis was present at baseline in 22% of women. Sixty-two percent of women was postmenopausal at baseline, this percentage was 99 at follow-up. At follow-up, mild, moderate and severe progression were observed in 158, 101 and 31 women, respectively. Because the number of women with severe progression was low, moderate and

Table 3.2.1. Baseline characteristics of 775 Dutch women, aged 45 to 64 years

Characteristic		
Mean (SD)		
Age (years)	53.3	(5.7)
Height (m)	1.63	(0.06)
Weight (kg)	67.4	(9.5)
Quetelet index (kg/m^2)	25.4	(3.3)
Systolic blood pressure (mmHg)	134.1	(18.7)
Diastolic blood pressure (mmHg)	82.6	(11.0)
Serum cholesterol (mmol/l)	6.2	(1.1)
Percentage		
Current smokers	35	
Former smokers	15	
Diabetes mellitus	2	
Postmenopausal	62	
Anti-hypertensive treatment	21	
Aortic atherosclerosis	22	

severe progression were combined in the analysis, referred to as advanced progression. The relative risks of progression according to categories of baseline cholesterol are presented in table 3.2.2. and in figure 3.2.1. Elevated risks of progression were seen for the 2 upper categories compared to the lowest category. The risk was similar for mild and advanced progression. Adjustment for other cardiovascular risk factors resulted in a slight reduction of the relative risks, but significance remained. When the analysis was restricted to subjects without aortic atherosclerosis at baseline (n=601), the age-adjusted relative risks of progression for the subsequent categories of serum cholesterol were 1.6 (95% confidence interval 0.6-4.1), 3.0 (1.2-7.5) and 3.5 (1.3-9.3) for mild progression and 1.3 (0.5-3.7), 1.5 (0.5-4.3) and 2.1 (0.7-6.5) for advanced progression. Similar increases in risk were seen among women with aortic atherosclerosis at baseline (n=174), though these did not reach statistical significance due to small numbers.

During follow-up serum cholesterol rose on average by 0.11 ± 0.12 mmol/l per year. Body weight rose on average by 1.4 ± 5.5 kg and systolic blood pressure by 10.3 ± 17.8 mmHg. The change in cholesterol with time was inversely associated with age (coefficient of linear regression (ß)= -0.03 mmol/l/yr, SE=0.007; p<0.001) and directly related to change in body weight (ß=0.02 mmol/l/kg, SE=0.007; p=0.001, adjusted for age) and change in systolic blood pressure (ß=0.004 mmol/l/mmHg, SE=0.002; p=0.07, adjusted for age and body weight). The increase in cholesterol was positively associated with the risk of mild progression (relative risk (RR) (per mmol/l) = 1.2; 1.0-1.5), but

Table 3.2.2. Relative risks of mild and advanced progression of atherosclerosis by categories of baseline cholesterol level

		Serum cholesterol (mmol/l)			
		<5.0	5.0-5.9	6.0-6.9	≥7.0
Progression		N=95	N=265	N=251	N=164
Mild	No. of cases	10	41	62	45
	RR (95% CI)*	1.0‡	1.5 (0.7-3.2)	2.8 (1.3-5.8)	3.4 (1.6-7.3)
	RR (95% CI)†	1.0‡	1.4 (0.7-3.0)	2.5 (1.2-5.2)	2.9 (1.3-6.3)
Advanced	No. of cases	10	30	49	43
	RR (95% CI)*	1.0‡	1.1 (0.5-2.3)	2.1 (1.0-4.4)	3.0 (1.4-6.6)
	RR (95% CI)†	1.0‡	1.0 (0.5-2.3)	1.9 (0.9-4.2)	2.4 (1.1-5.5)

* Adjusted for age
† Adjusted for age, systolic blood pressure, smoking status, Quetelet index, diabetes mellitus, anti-hypertensive medication, menopausal status and duration of follow-up
‡ Reference group
RR = relative risk, CI = confidence interval

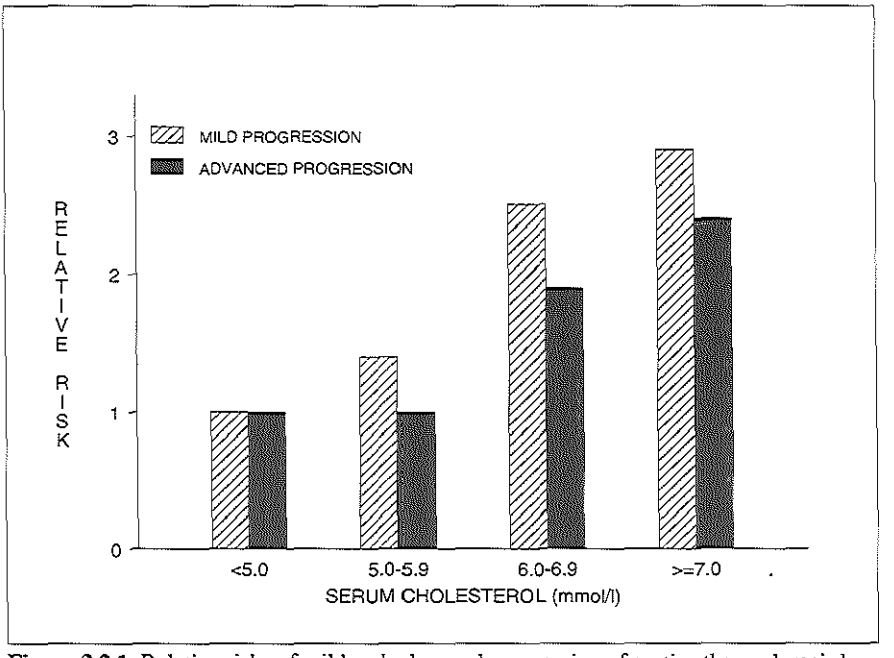

Figure 3.2.1. Relative risks of mild and advanced progression of aortic atherosclerosis by categories of serum cholesterol. Relative risks are adjusted for age and other cardiovascular risk factors

not with the risk of advanced progression (RR = 1.0 (per mmmol/l); 0.8-1.3). Adjustment for other cardiovascular risk factors, including changes in body weight and systolic blood pressure, did not materially affect the risk estimates.

Discussion

In this study among middle-aged women, a positive and independent association was observed between serum cholesterol level and progression of aortic atherosclerosis during 9 years of follow-up. No risk was observed for cholesterol levels up to 6.0 mmol/l. Above this level, a gradual increase in risk was observed, with the relative risks of mild and advanced progression exceeding 2 for women with a serum cholesterol level of 7.0 mmol or higher compared to women with a cholesterol level below 5 mmol/l, after adjustment for age and other cardiovascular risk factors. During follow-up, serum cholesterol rose on average by 0.11 mmol/l per year. The rise in cholesterol was associated with only a borderline significant increased risk of mild progression; no association was seen with advanced progression. Before conclusions can be drawn from these data some aspects of the study need to be considered.

The present population-based follow-up approach of study does not have the potential selection bias characteristic of autopsy and angiographic studies. The study allowed to evaluate the effects of both baseline cholesterol level and cholesterol change during follow-up. However, the significance of aortic calcification as a measure of atherosclerosis needs consideration. The validity of radiographic assessment of aortic calcification for the diagnosis of atherosclerosis has been studied by comparison with material obtained at necropsy. The method was shown to be highly specific and in most cases visible calcification represented advanced atherosclerosis.[9] Quantification of atherosclerotic change was based on visual grading. To improve the measurement all films were read by 2 observers and consensus was sought when the scores were discrepant.

Several prospective studies have examined the relation between total cholesterol and risk of coronary heart disease in women.[2-6,17-20] The results of the studies have been reviewed by Bush et al.[21] The findings have not been consistent, with absent or modest associations in some studies[2,20], or relating to a single endpoint in others.[17] In the Framingham Study and the Donolo - Tel Aviv Study, both comprising relatively large numbers of events, the association was examined at all levels of cholesterol. An elevated risk for coronary heart disease was evident only at levels of cholesterol above 6.9 mmol/l.[3,5] In the present study, we found an increased risk of progression of aortic atherosclerosis at cholesterol levels above 6.0 mmol/l. This suggests that in women also moderate levels of cholesterol may affect the arterial wall. A question that arises is whether the data on aorta atherosclerosis can be applied to coronary athero-

sclerosis. Aortic atherosclerosis has been found to be a predictor of cerebrovascular as well as cardiac events, which supports the view that aortic atherosclerosis reflects a generalized process.[11] Moreover, the results of prospective autopsy studies in men showed comparable associations of coronary and aortic atherosclerosis with cholesterol level.[22,23]

During the 9 years of follow-up serum cholesterol increased on the average by 1 mmol/l. The increase was significantly associated with increases in body weight. Body weight increased during follow-up but the mean change was not large enough to account completely for the observed change in cholesterol. An effect of menopause on serum cholesterol has been demonstrated.[24] In the present study, serum cholesterol increased by an average of 0.14 ± 0.11 mmol/l per year in women who were premenopausal at baseline and passed through menopause during follow-up and by 0.11 ± 0.12 mmol/l per year in women who were postmenopausal at baseline, which suggests that the increase cannot be fully explained by change in menopausal state. The increase is larger than observed in other longitudinal studies performed in a similar period. The Framingham Offspring Study found an 8-year increase in total cholesterol of 0.5 mmol/l in women initially aged 45 to 49 years[25]; the change in women of similar age in the present study was 0.13 mmol/l per year. Since the laboratory participated in a standardization program throughout the follow-up period, laboratorium drift can be excluded. Part of the reason for the strong increase could be the relatively low frequency of postmenopausal estrogen use in the Netherlands. Users of postmenopausal estrogens have been shown to have lower serum total cholesterol levels compared to non-users, the difference ranging from 0.2 to 0.5 mmol/l in women aged 55 to 74 years.[26] At follow-up, 1.3% of women in the present study reported current use of estrogens, while U.S. figures of up to 34% are reported among women aged 55 to 74 years in a similar period (1984-1987).[27]

The rise in cholesterol had only a borderline significant effect on the risk of mild progression, and did not affect the risk of advanced progression. Possibly, lifelong accumulated exposure is a more important determinant of atherosclerotic progression than recent changes. A weak association might have been obscured by the regression towards the mean effect. Adjustment for baseline level is commonly used to control for this effect.[28,29] However, when the outcome measure is related to baseline level, as is often the case, this method of adjustment may lead to overestimation of the true effect.[30,31] Some studies have examined the effects of change in cholesterol on the occurrence of cardiovascular disease, but results have been inconsistent.[28,29,32]

Possible mechanisms by which elevated cholesterol levels accelerate atherosclerosis have been reviewed by Steinberg.[33] Although LDL cholesterol accounts for most of the increase in total cholesterol in women after middle

age[8], HDL cholesterol has been shown to be a better predictor of coronary heart disease in women.[5,34,35] Unfortunately, in the present study, which started in the early seventies, no information is available on HDL cholesterol levels.

In summary, in this 9-year follow-up study among middle-aged women, the risk of atherosclerotic progression increased with increasing serum cholesterol at levels above 6.0 mmol/l. This suggest that even moderately increased levels of serum cholesterol in women are atherogenic. During follow-up, cholesterol rose on average by 0.11 mmol/l per year. In contrast to the effect of baseline cholesterol, a contribution of the rise in cholesterol to the atherogenic process was not clearly seen. Nevertheless, understanding of the causes of the increase in cholesterol is necessary to understand the mechanisms involved in lipid regulation in women after middle-age.

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3.3 Cigarette Smoking and Progression of Atherosclerosis in Women*

Abstract

Cigarette smoking has been recognized as an important risk factor for cardiovascular disease in men and women. Whether atherosclerosis plays a role in the pathofysiological mechanism is still debated. The association between cigarette smoking and progression of aortic atherosclerosis was examined in a population-based cohort of 758 women, initially aged 45 to 64 years. All women were examined radiographically for calcified deposits in the abdominal aorta, which have been shown to represent intimal atherosclerosis. After 9 years of follow-up progression of atherosclerosis could be demonstrated in 37% of women.

Progression of atherosclerosis was positively associated with number of cigarettes smoked per day. Compared to women who had never smoked, the relative risks of those who smoked 1 to 9, 10 to 19 and 20 or more cigarettes per day were 1.4 (95% confidence interval 1.0-1.9), 2.0 (1.6-2.5) and 2.4 (1.8-3.1) respectively, after adjustment for age and other cardiovascular risk factors. Among former smokers the risk decreased with increasing duration of stopping but a significant excess risk was still observed after 5 to 10 years since quitting. Elevated levels of blood pressure and serum cholesterol were no prerequisite for the atherosclerotic effect of smoking.

These prospective follow-up data provide evidence for an effect of cigarette smoking on progression of atherosclerosis. The findings suggest that the rate of progression may be reduced by cessation of smoking, but a residual effect seems to be present for at least 10 years.

Introduction

Cigarette smoking has since long been recognized as one of the major risk factors for cardiovascular disease in men.[1-5] In the past decade, smoking has also been identified as an important cardiovascular risk factor in women.[6-11] In spite of the strong support for a causal relationship, understanding of the pathophysiological basis is incomplete. The observations of a rapid reduction in risk of cardiovascular disease after cessation of smoking suggest that acute

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* Submitted

mechanisms dominate.[12,13] Some of the larger studies, however, found an excess risk up to 10 years after quitting,[2,14,15] which indicates that chronic processes are also involved. An association between smoking and atherosclerosis of the coronary arteries has been observed in autopsy[16-18] and angiographic studies,[19,20] but results have not been consistent. Limitations of these studies are that they usually measure the association at one point in time and that the study population is highly selective, which may cause serious bias. A prospective examination of the relationship in asymptomatic persons would give a clearer picture but data are sparse. A major artery that can be examined with relative ease is the aorta. Calcific densities in the aorta, as detected on radiographs, represent an advanced stage of atherosclerosis,[21] and have been shown to be related to cardiovascular morbidity and mortality.[22,23] In the present follow-up study, we investigated the association between cigarette smoking and progression of aortic atherosclerosis during 9 years of follow-up of a population-based cohort of 758 middle-aged women.

Methods

Between 1975 and 1978, a population study on risk factors for chronic diseases was conducted in the Dutch town of Zoetermeer. The inhabitants of two districts of the town were invited for a medical examination. Details of this study have been published previously.[24] In 1985, all female participants aged 45 to 64 years at baseline were invited for a follow-up examination during which information was obtained on risk factors for osteoporosis and some cardiovascular risk factors. The reponse rate of the women at baseline was 77%. Of 1167 women invited for the follow-up study, 71 had died and 87 had moved away during the follow-up period. Of the remaining women, 855 (85%) were reexamined. The mean duration of follow-up was 8.9 ± 0.8 years.

Measurement of aortic atherosclerosis

Aortic atherosclerosis was diagnosed by radiographic detection of calcific deposits in the abdominal aorta. The technical procedures for X-ray measurements were similar at baseline and follow-up. At each occasion lateral abdominal films (T12-S1) were made aimed at assessment of the lumbar vertebrae. Films were made at a fixed distance with subjects in sitting position. Baseline and follow-up films were examined in pairs. Aortic calcifications were considered to be present when linear densities were seen in an area parallel and anterior to the lumbar spine (L1-L4). Progression was defined as the occurrence of new calcifications or enlargement of the calcified area present at baseline. The extent of progression was graded, but because of relatively small numbers in the categories, severity grades were combined in the analysis of the present

study. In no subject a decrease in aortic atherosclerosis was observed. All films were examined by 2 independent observers without knowlegde of the risk factor status of the subjects. Prior to the scoring a sample of the films was read by the 2 observers simultaneously to reach agreement on interpretation of the scoring protocol. In case of interobserver difference of independent readings, films were reviewed by both observers simultaneously to reach consensus. The percentage of agreement for absence versus presence of progression was 0.88 and the kappa statistic was 0.74. In 59 subjects progression could not be measured because baseline or follow-up films were missing (n=50) or not readable (n=9).

Assessment of risk factors

Information on smoking habits was obtained by a self-administered questionnaire. At baseline, subjects were asked whether they had smoked at least 100 cigarettes up to the time of investigation and if so, the age of starting. Current smokers were asked about the number of cigarettes they smoked per day. Former smokers were asked about the number of cigarettes they had smoked at the time of stopping and at what age they had stopped. Both categories of smokers were asked to indicate whether or not they inhaled the smoke. At follow-up, information was recorded on whether the women were currently smoking and if so, the number of cigarettes they smoked per day. Information on smoking habits was missing for 9 women.

For the present analysis, current smokers were categorized according to the number of cigarettes smoked per day as reported at baseline (1-9, 10-19 and 20 or more). These will be referred to as light, moderate and heavy smokers. Women who reported current smoking at baseline but did not report smoking at follow-up were allocated to a separate group, referred to as quitters. Former smokers were considered to be those who had smoked at least 100 cigarettes but were not smoking at baseline. The remaining women were considered to be never smokers. Nine former smokers and 7 women who reported at baseline to have never smoked, reported smoking at follow-up. These 16 women were excluded from the analysis.

Blood pressure was measured on the left arm using a random zero sphygmomanometer. The mean of two readings was used in the analysis. Height and weight were measured without shoes and with indoor clothing. Quetelet index was calculated as weight devided by height square. Serum total cholesterol was measured by an automated enzymatic method.

Data analysis

After exclusion of women with missing or non-evaluable data on atherosclerotic change and smoking habits and exclusion of women who reported a history of major cardiovascular event at baseline (n=15), data of 758 subjects were used

in the analysis. Characteristics of smoking categories were adjusted for age by analysis of covariance (continuous variables) or standardized by the direct method, using the age-distribution of the whole study population as standard (dichotomous variables). The risk of progression was evaluated for all smoking categories relative to those who had never smoked. Because progression was observed in a relatively high percentage of subjects, the odds ratio's as derived from logistic regression analysis would be overestimates of the corresponding relative risks. Therefore, a simple model was used, which is directly in terms of relative risk.[26] The model equation is $Prob\ (progression) = e^{\alpha + \beta_k + \tau x}$ in which e^{β_k} represents the relative risk of progression for smoking category k relative to non-smokers and τx represents the confounders. The model parameters were estimated by maximization of the likelihood function, using the LE module of BMDP statistical package.[27] Age was included in the models after logarithmic transformation. Standardized estimates of the probability of progression were computed by substituting the mean age of the study population in the model. Attributable risks, defined as the excess risk of progresssion among smoking categories compared to non-smokers, were computed as the difference between age-standardized probabilities. A limitation of the applied model is that it does not prevent individual predicted probabilities from exceeding 1. In all age-adjusted analyses predicted probabilities were below 1.0. The multivariate model including other risk factors as continuous variables gave probabilities which exceeded 1.0 for 7 subjects. Probabilities of these subjects were set to 1.

Results

Table 3.3.1 shows the baseline characteristics of the study population. The mean age of the population was 53.3 ± 5.7 years. Thirtyfive percent were current smokers and 15% were former smokers. Aortic atherosclerosis was present at baseline in 22% of women. Baseline smoking habits are presented in table 3.3.2. During follow-up 84 women quit smoking. After adjustment for age, quitters had smoked less cigarettes per day at baseline and had a lower percentage of inhalers compared to continuing smokers, with former smokers inbetween. Ninetynine percent of subjects were postmenopausal at follow-up.

Smoking and progression of atherosclerosis

During follow-up, progression of atherosclerosis was observed in 284 women (37%). The relative risks of progression according to smoking status are presented in table 3.3.3 and figure 3.3.1. The risks increased with number of cigarettes smoked per day. The risk of former smokers was comparable to that of light smokers. Women who quit smoking during follow-up had only a slightly increased risk. However, when those who had smoked less than 10 cigarettes

Table 3.3.1. Baseline characteristics of 758 Dutch women, aged 45 to 64 years

Characteristic		
Mean (SD)		
Age (years)	53.3	(5.6)
Height (m)	1.63	(0.06)
Weight (kg)	67.5	(9.6)
Quetelet index (kg/m^2)	25.4	(3.4)
Systolic blood pressure (mmHg)	134.3	(18.8)
Diastolic blood pressure (mmHg)	82.6	(11.1)
Serum total cholesterol (mmol/l)	6.2	(1.1)
Percentage		
Current smokers	35	
Former smokers	15	
Diabetes mellitus	2	
Postmenopausal	63	
Anti-hypertensive treatment	21	
Aortic atherosclerosis	22	

Table 3.3.2. Baseline smoking habits in groups of continuing smokers, quitters and former smokers

	Continuing smokers N=182	Quitters* N=84	Former smokers† N=111
No. cigarettes/day	13.2 (0.6)	6.7 (0.9)¶	10.4 (0.8)§
Age of starting (yrs)	22.9 (0.6)	24.5 (0.8)	21.5 (0.8)
Duration of smoking (yrs)‡	29.4 (0.6)	27.8 (0.9)	19.5 (0.8)¶
Inhaling (%)	80.0 (3.8)	39.1 (13.4)¶	68.8 (6.9)

Values are age-adjusted means or percentages and standard error
* Subjects who stopped smoking during follow-up
† Subjects who stopped smoking before the baseline examination
‡ Before the baseline examination
§ $p < 0.005$; ¶ $p < 0.0001$, relative to continuing smokers

per day and had not inhaled the smoke were excluded (n=44), the relative risk of quitters raised to 1.7 (1.3-2.3). This suggests that the low risk of those who quit during follow-up can be ascribed to their light smoking habits. When analyses were restricted to subjects without aortic atherosclerosis at baseline (n=605), the age-adjusted relative risks of progression were 1.8 (95% confidence interval 1.1-2.9), 2.8 (2.0-4.1) and 3.0 (2.0-4.7) for light, moderate and heavy smokers, respectively, and 1.1 (0.6-1.8) and 1.7 (1.1-2.6) respectively for quitters and former smokers.

Though positive associations were found, duration of smoking and inhalation habit were not significantly associated with atherosclerotic progression in

continuing smokers after number of cigarettes was taken into account in multivariate analysis. The relative risk among former smokers decreased with increasing duration of stopping but a borderline significant excess risk was still seen after 10 years since quitting (table 3.3.4). The risk estimates did not materially change when analyses were performed among subjects without aortic atherosclerosis at baseline. Numbers were too small to differentiate the risk according to smoking habits.

Table 3.3.3. Relative risks of atherosclerotic progression by smoking status

	Never smoked	Continuing smokers (cig/day)			Quitters*	Former smokers†
		1-9	10-19	≥20		
Progression	N=381	N=57	N=76	N=49	N=84	N=111
No. of cases	112	23	44	32	28	45
RR (95% CI)‡	1.0§	1.5 (1.1-2.0)	2.0 (1.6-2.5)	2.3 (1.8-2.8)	1.2 (0.9-1.7)	1.6 (1.2-2.0)
RR (95% CI)¶	1.0§	1.4 (1.0-1.9)	2.0 (1.6-2.5)	2.4 (2.8-3.1)	1.1 (0.7-1.5)	1.5 (1.1-1.9)

* Subjects who stopped smoking during follow-up
† Subjects who stopped smoking before the baseline examination
‡ Adjusted for age
¶ Adjusted for age, systolic blood pressure, serum total cholesterol, Quetelet index, diabetes mellitus, menopausal status and duration of follow-up
§ Reference group
CI = confidence interval; RR = relative risk

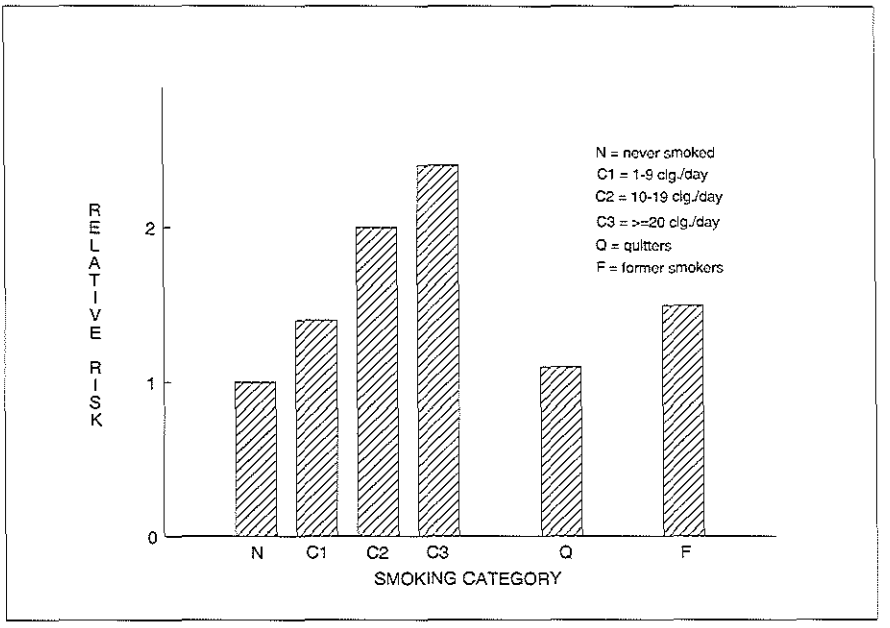

Figure 3.3.1. Relative risks of progression of atherosclerosis for smoking categories. Relative risks are adjusted for age and other cardiovascular risk factors

Table 3.3.4. Relative risks of atherosclerotic progression for former smokers by number of years since quitting

	Never smoked	Years stopped		
		< 5	5-9	≥ 10
	N=381	N=24	N=34	N=48
No. of cases	112	13	14	16
Relative risk*	1.0†	2.3	1.6	1.5
95 % confidence interval	–	1.6-3.3	1.1-2.2	1.0-2.2

* Adjusted for age
† Reference category

Other risk factors

Mean (SE) ages of continuing smokers, quitters and former smokers (52.1 (0.4), 53.0 (0.6) and 52.1 (0.5) years, respectively) were significantly lower compared to never smokers (54.3 (0.3) years) ($p< 0.05$). Continuing smokers had a higher baseline level of serum total cholesterol compared to never smokers (6.3 (0.1) versus 6.1 (0.05) mmol/l, $p=0.06$), after adjustment for age. Cholesterol levels of quitters and former smokers were similar to those of continuing smokers. No baseline differences were seen in blood pressure levels. Age-adjusted baseline levels of Quetelet index of continuing smokers (25.0 (0.2) kg/m^2) and former smoker (24.9 (0.3) kg/m^2) were significantly lower compared to those of never smokers and quitters (25.7 (0.2) and 25.7 (0.4) kg/m^2, respectively) ($p< 0.05$). Progression of atherosclerosis was positively associated with baseline levels of systolic blood pressure (ß=0.01 per mmHg, SE = 0.003, $p=0.0005$) and serum total cholesterol (ß=0.14 per mmol/l, SE = 0.02, $p< 0.0001$). After adjustment for cardiovascular risk factors the relative risks of progression associated with smoking hardly changed (table 3.3.3).

The risks of progression associated with smoking were examined within categories of other risk factors (table 3.3.5). The relative risks were modified by age with younger women having the highest relative risks. To obtain age-standardized relative risks of progression within categories of other risk factors, models included next to the interaction term for the risk factor and smoking also interaction terms for the risk factor and age and for smoking and age. Relative risks were generally higher among women with lower levels of other risk factors. Attributable risks were comparable for subgroups of serum cholesterol and were somewhat more variable for subgroups of systolic blood pressure. Attributable risks were generally higher in the subgroup with a lower Quetelet index compared to those with a higher Quetelet index (table 3.3.5).

Relative risks were most pronounced when smoking women with an elevated level of systolic blood pressure or serum cholesterol were compared to never smokers without the risk factor. This was especially the case among women under the age of 55 years. For smoking women of this age with either an elevated

Table 3.3.5. Relative risks of atherosclerotic progression by smoking status within categories of other risk factors

		Never smoked	Continuing smokers (cig/day)			Quitters*	Former smokers†
			1-9	10-19	≥ 20		
Age (years)							
< 55	Progression (%) (N)	15 (205)	37 (40)	51 (56)	58 (34)	25 (51)	32 (80)
	Relative risk [95% CI]	1.0§	2.5 [1.5-4.1]	3.4 [2.3-5.0]	3.9 [2.6-5.7]	1.7 [1.0-2.9]	2.1 [1.4-3.2]
	Attributable risk	0	22	36	43	10	17
≥55	Progression (%) (N)	43 (176)	47 (17)	65 (20)	79 (15)	48 (33)	59 (31)
	Relative risk [95% CI]	1.0§	1.1 [0.6-1.8]	1.5 [1.1-2.0]	1.8 [1.4-2.4]	1.1 [0.7-1.7]	1.4 [1.0-1.8]
	Attributable risk	0	4	22	36	5	16
Systolic blood pressure (mmHg)							
< 140	Progression (%)‡ (N)	26 (247)	35 (37)	65 (48)	70 (33)	40 (61)	39 (68)
	Relative risk [95% CI]‡	1.0§	1.3 [0.8-2.3]	2.5 [1.8-3.5]	2.7 [2.0-3.7]	1.5 [1.0-2.3]	1.5 [1.0-2.3]
	Attributable risk‡	0	9	39	44	14	13
≥ 140	Progression (%)‡ (N)	34 (132)	63 (20)	63 (28)	75 (15)	36 (23)	59 (43)
	Relative risk [95% CI]‡	1.0§	1.9 [1.2-3.0]	1.9 [1.2-2.8]	2.3 [1.4-3.7]	1.0 [0.6-1.9]	1.8 [1.2-2.5]
	Attributable risk‡	0	29	29	41	2	25
Serum total cholesterol (mmol/l)							
< 6.5	Progression (%)‡ (N)	24 (253)	40 (38)	57 (48)	64 (29)	25 (44)	41 (64)
	Relative risk [95% CI]‡	1.0§	1.7 [1.0-2.8]	2.4 [1.6-3.5]	2.7 [1.9-3.8]	1.1 [0.6-2.0]	1.7 [1.2-2.6]
	Attributable risk‡	0	16	33	40	1	17
≥ 6.5	Progression (%)‡ (N)	38 (124)	53 (19)	70 (27)	85 (20)	47 (40)	56 (46)
	Relative risk [95% CI]‡	1.0§	1.4 [0.9-2.3]	1.8 [1.3-2.5]	2.3 [1.3-4.0]	1.3 [0.8-1.9]	1.5 [1.0-2.1]
	Attributable risk‡	0	15	32	47	9	18
Quetelet index (kg/m^2)							
< 25	Progression (%)‡ (N)	23 (167)	55 (32)	68 (45)	71 (32)	42 (42)	61 (61)
	Relative risk [95% CI]‡	1.0§	2.4 [1.5-3.9]	3.0 [2.1-4.3]	3.2 [2.2-4.5]	1.9 [1.1-3.1]	2.7 [1.8-3.9]
	Attributable risk‡	0	32	45	48	19	38
≥ 25	Progression (%)‡ (N)	33 (212)	37 (25)	58 (30)	73 (16)	36 (41)	36 (50)
	Relative risk [95% CI]‡	1.0§	1.1 [0.6-2.0]	1.8 [1.2-2.6]	2.2 [1.5-3.4]	1.1 [0.7-1.8]	1.1 [0.7-1.7]
	Attributable risk‡	0	4	25	40	3	3

Numbers do not always add up to the total number due to missing values
* Subjects who stopped smoking during follow-up; † Subjects who stopped smoking before the baseline examination
‡ Age-standardized; § Reference group; CI = confidence interval

systolic blood pressure (n=28) or an elevated level of serum cholesterol (n=25) the relative risks of progression were 5.1 (3.0-8.8) and 5.9 (3.6-9.9), respectively. A similar increase in risk was not observed for the combination of smoking and high Quetelet index. Among never smokers the risk of progression was higher in overweight compared to lower weight women (p=0.10). Among current and former smokers, however, progression was similar or less frequent in overweight women, which reached significance among former smokers (p=0.04). This could not be ascribed to less intensive smoking in overweight compared to lower weight smokers because the mean number of cigarettes smoked per day in each smoking category did not differ between the 2 subgroups of Quetelet index.

Discussion

In this study, cigarette smoking was a strong independent predictor of progression of aortic calcification in middle-aged women during 9 years of follow-up. Before conclusions can be drawn from these data, some methodologic issues need to be considered. The first concerns the design of the study. The present population-based follow-up study does not have the potential biases due to selectivity of the study population that are inherent to autopsy and angiographic studies. The study gives estimates of baseline smoking status that are not influenced by the disease, and allows to take changes in smoking behavior during follow-up into account. Second, we need to consider the significance of aortic calcification. The validity of radiographic assessment of aortic calcification for the diagnosis of atherosclerosis has been studied by comparison with assessments made on necropsy material. The method was shown to be highly specific and in most cases visible calcification represented advanced atherosclerosis.[21] The observation that systolic blood pressure and serum cholesterol were associated with aortic calcification further confirms that the measure represents intimal atheroslerosis. To improve the assessment all films were read by 2 observers and consensus was sought when the scores were discrepant.

A question is whether it is justified to make inferences from data of aorta calcification to other vessels. Aortic atherosclerosis has been found to be a predictor of cardiovascular events at various sites,[22,23] and this supports the concept of generalized atherosclerosis. It is possible, however, that the aorta is more vulnerable to the effects of smoking than other arteries. Autopsy studies have shown a relatively strong relation of smoking with aortic atherosclerosis,[17,28-30] which was generally more pronounced than the association with coronary atherosclerosis.[17,28,29] However, we should be aware of potential bias in making inferences from comparisons among deaths[29]. Results of angiographic studies examining the association of smoking with coronary athero-

sclerosis have been inconsistent.[19,20,31] Also in these studies potential bias should be considered. As smoking may have acute effects on the myocardium, it may be that smokers develop clinical symptoms that require coronary angiography with less significant coronary artery disease than nonsmokers.[32]

The reduced cardiovascular risk in past smokers compared to current smokers is a consistent finding among studies.[5,14] Those who stop smoking are not a representative selection of smokers and concern has been expressed about possible differences in baseline levels of other risk factors. Only some studies have been able to measure risk factors of smokers before they quit.[33,34] In agreement with the observations in these studies, the reduced risk of progression that was observed among quitters compared to continuing smokers in the present study could not be ascribed to differences in baseline levels of blood pressure and serum cholesterol. A careful interpretation is needed, however, because of differences in smoking habits. Fiftytwo percent of quitters were light, noninhaling smokers, compared to only 10% among continuing smokers. Exclusion of these light smokers resulted in a notable increase of the relative risk of quitters.

In contrast to the rapid disappearance of the excess risk of myocardial infarction after cessation of smoking, as observed in men and women by Rosenberg et al,[12,13] other studies have found some remaining excess risk of cardiovascular disease up to 10 to 15 years after quitting.[2,14,15] Compared to never smokers, we observed a bordeline significant excess risk of atherosclerotic progression after 10 years since quitting. This may be compatible with a cascade of the atherosclerotic proces caused by early vessel-wall damage. The occurrence of endothelial injury after nicotine exposure has been suggested by results of animal experiments,[35] but studies in experimental animals have generally failed to demonstrate atherosclerotic effects similar to that observed in humans.[36]

We found that the estimates of relative risk were generally higher among younger women and among those with lower levels of additional risk factors. These results agree with observations of studies on smoking and risk of cardiovascular events.[8,9,37] This suggests that elevated levels of blood pressure or serum cholesterol are no prerequisite for the atherosclerotic effect of smoking. The findings in high-risk subgroups, however, may have been affected by the high probabilities of progression which might have limited a further increase. This may also be the reason why attributable risks were not elevated in high-risk subgroups, as observed for risk of cardiovascular disease in a large prospective study in women.[7]

The risks were most pronounced when smoking women with elevated levels of systolic blood pressure or serum cholesterol were compared to never smokers without the risk factor. This was especially so among relatively younger women, in which the combination of risk factors elevated the risk 5 to 6 times. With

respect to Quetelet index results are more difficult to interpret. Overweight increased the risk of progression among those who had never smoked. Among past and current smokers, however, progression was similar or less frequent in overweight compared to leaner women. A high level of body fat may hamper the radiographic detection of calcifications and therefore the risk of progression associated with overweight may be underestimated in our study. However, this would not have affected the results differentially across smoking categories and the observed interaction remains valid. After menopause fatty tissue becomes the major determinant of endogenous estrogen activity. Possibly body fat protects postmenopausal women from the cardiovascular consequenses of an estrogen lowering[38] or pro-androgenic[39] effect of cigarette smoking, which needs further investigation.

In summary, the present study in women provides evidence for an effect of smoking on progression of atherosclerosis. The risk of progression decreased with increasing time since quitting, but a residual effect seemed to be present for at least 10 years. This observation supports the view that cigarette smoking, besides acute effects, may also have prolonged effects on the vessel wall. This finding underlines the need to stop smoking at an early age. In the last decades, there has been a strong decline in the prevalence of smoking in men, but not in women.[5] This points to the need of directing special attention to smoking behaviour in women.[40]

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3.4 Increased Risk of Atherosclerosis in Women after the Menopause*

Abstract

An increase in the incidence of cardiovascular disease has generally been observed in postmenopausal women, but there have been few studies of the association between menopausal state and atherosclerosis. In this study 294 premenopausal and 319 postmenopausal women aged 45 to 55 years were examined radiographically for calcified deposits in the abdominal aorta, which have been shown to represent intimal atherosclerosis. Aortic atherosclerosis was present in eight (3%) of the premenopausal women and in 38 (12%) of the postmenopausal women. After adjustments for age and other indicators of cardiovascular risk women with a natural menopause had a 3.4 times greater risk of atherosclerosis than premenopausal women (95% confidence interval 1.2 to 9.7; $p<0.05$); women who had had a bilateral oophorectomy had a 5.5 times greater risk (1.9 to 15.8; $p<0.005$). No excess risk of atherosclerosis was observed among women who had had a hysterectomy without removal of both ovaries. These results suggest that when oestrogen production stops, either naturally or after surgery, the risk of atherosclerosis is increased.

Introduction

An increase in the incidence of cardiovascular disease after the menopause has generally been observed, though not all studies agree with this finding.[1,2] Non-invasive assessment of atherosclerosis may help to clarify this issue. Information on the extent of atherosclerosis in premenopausal and postmenopausal women has been gained mainly from necropsy studies. Compared to women with intact ovaries, women who had had bilateral oophorectomy had an exess of coronary atherosclerosis, which approached that in men.[3,4] We investigated the association between menopausal state and the presence of calcified deposits in the abdominal aorta as seen on lateral radiographs of the lumbar spine. The presence of such deposits represents true intimal atherosclerosis,[5] and is a strong predictor of death from cardiovascular causes.[6]

* Br Med J 1989:298:642-4

Subjects and methods

A population study on chronic diseases was conducted in Zoetermeer, a suburb of The Hague in The Netherlands, between 1975 and 1978 (EPOZ study). All inhabitants of one rural and one urban district who were aged 5 years and over were invited for a medical examination. The response rate among middle-aged women was 77%. Details of the initial study have been reported.[7] All women aged 45 to 55 years, without a history of cardiovascular disease (n=676) were eligible for our study. The lower age limit was 45 because radiography of the lumbar spine was performed from this age onwards. The upper age limit of 55 years was chosen to include sufficient premenopausal and postmenopausal women of similar ages.

We diagnosed atherosclerosis by radiographic detection of calcified deposits in the abdominal aorta.[6] Lateral radiographs of the lumbar spine were available for 638 (94%) of the women. Calcification was scored as definitely present when linear densities were clearly visible in an area parallel and anterior to the lumbar spine. No attempt was made to estimate the extent of atherosclerosis. Menopausal state was assessed by a self administered questionnaire. It asked whether the menses had stopped; if so, at what age; and the reason for them stopping (natural of artificial). The type of artificial menopause was ascertained during an interview by a doctor. No differentiation was made between hysterectomy alone and hysterectomy with unilateral oophorectomy. Menopause was defined as no menstruation for at least one year.

Twenty five women had last menstruated within one year before the examination, and these women were excluded from the analysis. This left 613 women, of whom 294 were premenopausal and 319 postmenopausal. In 178 women the menopause had occurred spontaneously, 73 had had a bilateral oophorectomy and 55 had had a hysterectomy without removal of both ovaries. Other reasons for the menopause were reported by five women. Information on the type of artificial menopause was missing for eight women. Information on current use of postmenopausal estrogens was obtained by a questionnaire and was checked during an interview. (Subjects were asked to bring all current medication on a visit to the research centre.) At the time of the study no information was collected on past use of postmenopausal oestrogens.

Blood pressure was measured in sitting position with a random-zero sphygmomanometer. Serum total cholesterol was measured by an automated enzymatic method.[7] Body mass index was calculated as weight divided by height squared. Smoking behaviour was assessed by a self administered questionnaire.

Data analyses

Characteristics of the premenopausal and postmenopausal women were adjusted for age by direct standardization (categorical variables) and by analysis

of covariance (continuous variables). Differences in group means were tested by two tailed *t*-tests. Odds ratios with confidence intervals derived according to Woolf's method[8] were used as an approximation of relative risk for comparison of premenopausal and postmenopausal women and of types of menopause. After stratification by age Mantel-Haenszel odds ratios were calculated with Miettinen's test based confidence intervals.[8] Multivariate analysis was used to adjust for age (one year categories) and other indicators of cardiovascular risk. Logistic regression coefficients and their standard errors were estimated by the method of maximum likelihood,[8] from which adjusted estimates of relative odds with 95% confidence intervals were calculated to approximate relative risk. If the 95% confidence interval did not include the value one the risk estimate was regarded as significant. Trends in relative risk for categories of postmenopausal years were tested by Mantel test for trend.[8]

Results

The postmenopausal women were significantly older than the premenopausal women (table 3.4.1). There was, however, a reasonable overlap in age between the two groups: below the first quintile of age 147 (24%) of the women were postmenopausal; above the fourth quintile 55 (9%) were premenopausal. When the characteristics of the women were adjusted for age significant differences between the two groups were found only for serum total cholesterol concentration (table 3.4.1). The characteristics of the women who had had a natural menopause, women who had had a bilateral oophorectomy, and women who had had a hysterectomy without removal of both ovaries were not significantly

Table 3.4.1. Indicators of cardiovascular risk (adjusted for age) in premenopausal and postmenopausal women

	Premenopausal women N = 294	Postmenopausal women N = 319
Mean (SE)		
Age (years)	49.0 (0.1)	51.9 (0.2)*
Systolic blood pressure (mmHg)	132.9 (1.1)	131.5 (1.1)
Diastolic blood pressure (mmHg)	82.3 (0.7)	82.6 (0.7)
Serum total cholesterol (mmol/l)	5.9 (0.1)	6.3 (0.1)*
Body mass index (kg/m^2)	25.1 (0.2)	25.3 (0.2)
Percentage		
Current smokers	38	45
Past smokers	28	24
Diabetes mellitus	3	1

* $p < 0.001$

different, except for mean age (52.9, 50.7, 50.5 years respectively) and mean number of postmenopausal years or years after operation (4.6, 7.2, 7.6 respectively). Eight (3%) of the premenopausal and 38 (12%) of the postmenopausal women had aortic calcification. Table 3.4.2 gives the numbers of women with aortic calcification according to the type of menopause for those aged 45-50 and those aged 51-55. Estimates of relative risks did not differ significantly between the age groups. Although significantly raised risks were found for women after natural menopause or bilateral oophorectomy, no excess risk was observed for women after a hysterectomy without removal of both ovaries. Women with other reasons for menopause or missing information (n=13) were excluded from the analysis.

Beause age categories of five years may be too broad to adjust adequately for age effects logistic regression analysis was used, with age as a continuous variable. Simultaneous adjustment for age and other indicators of cardiovascular risk, however, resulted in only small changes in the estimated risk: women with a natural menopause had a 3.4 times greater risk of aortic calcification than premenopausal women (95% confidence interval 1.2 to 9.7; $p<0.05$); and women who had had a bilateral oophorectomy had a 5.5 times greater risk (1.9 to 15.8; $p<0.005$). The relative risk for women who had had a bilateral oophorectomy was 1.7 times the risk for women who had had a natural menopause, but this was not significant (95% confidence interval 0.7 to 4.4).

Table 3.4.2. Relative risks of aortic calcification in premenopausal and postmenopausal women by type of menopause for two age groups and for both age groups combined

		Aortic calcification			
		absent	present	RR	95% CI
45 to 50 years	Premenopausal[†]	229	6	1.0	
	Natural menopause	30	3	3.8	(0.9-16.1)
	Bilateral oophorectomy	37	6	6.2	(1.9-20.3)**
	Hysterectomy[‡]	31	0	–	
51 to 55 years	Premenopausal[†]	57	2	1.0	
	Natural menopause	125	20	4.5	(1.0-20.2)[¶]
	Bilateral oophorectomy	24	6	7.1	(1.3-37.8)[#]
	Hysterectomy[‡]	23	1	1.2	(0.1-14.3)
All women[§]	Premenopausal[†]	286	8	1.0	
	Natural menopause	155	23	4.3	(1.4-12.9)[#]
	Bilateral oophorectomy	61	12	6.6	(2.5-17.1)**
	Hysterectomy[‡]	54	1	0.6	(0.1-5.4)

CI = Confidence interval; RR = Relative risk; [†] Reference group
[‡] Includes women with unilateral oophorectomy
[§] Relative risks adjusted for strata of age by method of Mantel-Haenszel; [¶] $p<0.05$; [#] $p<0.01$; ** $p<0.001$

Other variables that were significantly associated with risk of aortic calcification were smoking and serum total cholesterol concentration. Current smokers had a 4.8 times greater risk of aortic calcification than women who had never smoked (1.9 to 11.7; p<0.001). An increase in serum cholesterol concentration of 1 mmol/l was associated with a 1.8 times greater risk of aortic calcification (1.3 to 2.4; p<0.001). An increase in systolic blood pressure of 20 mmHg was associated with a 1.5 times greater risk, but this was not significant (0.9 to 2.5). No associations with risk of aortic calcification were found for diastolic blood pressure, body mass index, or diabetes mellitus.

Among women with natural menopause the risk of aortic calcification adjusted for age and other indicators of cardiovascular risk showed a highly significant trend (p<0.001) with increasing years after the menopause. The findings among women who had had a bilateral oophorectomy were less clear (figure 3.4.1).

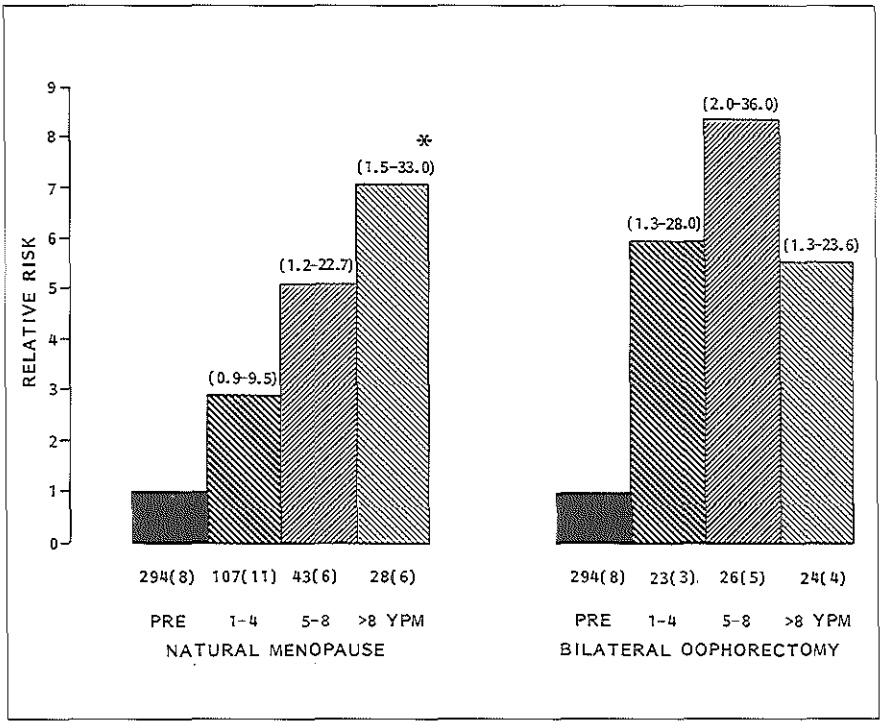

Figure 3.4.1. Relative risks of aortic calcification by number of postmenopausal years, for natural menopause and bilateral oophorectomy. Relative risks are adjusted for age (years) and other cardiovascular risk-indicators. 95% confidence intervals are given on top of the bars. Numbers of subjects in the categories are given with numbers of subjects with aortic calcification in parentheses.
PRE = premenopausal (reference group); YPM = years postmenopausal
* Mantel test for trend: $X^2 = 17.9$; p<0.001

Postmenopausal oestrogens were being taken by three of 25 women (12%) who had last menstruated within one year before the study, and by 14 (4%) who had last menstruated more than one year before the study. Aortic calcification was observed in one of these 17 women.

Discussion

We found a strong association between the menopause, induced either naturally or surgically, and the presence of calcified deposits in the abdominal aorta. Before any inferences can be drawn from our findings the importance of calification in the aorta needs to be considered. The validity of radiographic assessment of aortic calcification in the diagnosis of atherosclerosis has been studied by comparison with assessments made on necropsy material.[5] The method was shown to be highly specific, and in most cases visible calcification represented advanced atherosclerosis. Thus in some of our subjects minor and intermediate stages of atherosclerosis may have been present but not seen; if they had been seen the relative risks would have been even higher. Our observations that serum cholesterol concentration and smoking were strongly associated with aortic calcification further indicate that we were measuring intimal atherosclerosis.

Arteries may vary in their susceptibility to menopausal influences on atherosclerosis, and our results may thus not be extended to the risk of coronary atherosclerosis. Aortic calcification, however, is strongly associated with cardiovascular disease[6,9] and this association, whether mediated by concominant atherosclerosis in other vessel beds or not, emphasises the importance of our findings.

Whether hysterectomy without removal of both ovaries leads to an increased risk of cardiovascular disease is disputable.[1,2] Our results do not confirm an atherosclerotic effect of hysterectomy and suggest that the acceleration of atherosclerosis with the menopause occurs after oestrogen production stops. Some of our subjects may not have been able to report the exact nature of their surgery. If, however, some misclassification did take place the true difference in atherosclerotic risk between women who had had hysterectomy and women who had had bilateral oophorectomy would be even more pronounced.

The trend in risk of atherosclerosis with the number of postmenopausal years, which was seen among women with natural menopause, suggests a causal relation. The findings among women with bilateral oophorectomy, however, were less clear. The number of women taking postmenopausal oestrogens was too small for analysis. Use of replacement oestrogens has always been fairly low in The Netherlands. At the time of study replacement estrogens were used

by about 10% of Dutch perimenopausal women (International Health Foundation, Brussels, unpublished observations).

Serum total cholesterol concentration was significantly higher among postmenopausal women, which agrees with previous findings.[1,10] The effect of the menopause on atherosclerosis, however, could only partly be explained by changes in serum cholesterol concentration or other indicators of cardiovascular risk. Possibly, therefore, oestrogens have a direct effect on the vessel wall, as has been seen in animals.[11]

Our findings suggest a strongly increased rate of atherosclerosis after ovarian involution. Whether this increased rate can be limited to some extent by use of postmenopausal oestrogens remains to be ascertained.

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CHAPTER 4

NUTRITIONAL FACTORS AND HYPERTENSION IN WOMEN

4.1 A Prospective Study of Nutritional Factors and Hypertension among US Women *

Abstract

The relation of various nutritional factors with hypertension was examined prospectively among 58,218 predominantly white US female registered nurses, aged 34-59 years. In 1980, all women completed an independently validated dietary questionnaire. During 4 years of follow-up, 3,275 women reported a diagnosis of hypertension; the validity of the self-report was shown in a subsample. Age, relative weight, and alcohol consumption were the strongest predictors for the development of hypertension. Dietary calcium and magnesium had independent and significant inverse associations with hypertension. For women with a calcium intake of at least 800 mg/day, the relative risk of hypertension was 0.78 (95% confidence interval, 0.69-0.88) when compared with an intake of less than 400 mg/day. The relative risk for magnesium intake of 300 mg/day or more compared with an intake of less than 200 mg/day was 0.77 (95% confidence interval, 0.67-0.88). For women with high intakes of both calcium and magnesium compared with those having low intakes of both, the relative risk of hypertension was 0.65 (95% confidence interval, 0.53-0.80). No independent associations with hypertension were observed for intakes of potassium, fiber, and saturated and poly-unsaturated fatty acids. These prospective findings add to the growing evidence for a protective role of dietary calcium and magnesium in the regulation of blood pressure.

Introduction

The search for nonpharmacologic approaches to prevent and control hypertension has led to interest in the effects of diet. For several decades, research has mainly focused on potentially adverse effects of excess sodium intake.[1] Dietary potassium has been inversely related to blood pressure in some,[2-4] but not all,[5,6] cross-sectional studies, and supplementation has yielded conflicting results in clinical trials.[7,8] Among other dietary constituents of protective effects, calcium,[3,4,6] fiber,[9] and poly unsaturated fatty acids[10] have been suggested. Positive associations have been observed between alcohol intake and blood pres-

* Circulation 1989;80:1320-7

sure.[11] The relation of dietary magnesium with blood pressure has received much less attention.

With few exceptions,[12,13] epidemiologic studies of diet and blood pressure have been cross-sectional[2-6,9,14] rather than prospective. Although most studies excluded subjects on a prescribed diet, these studies still might have included hypertensive subjects who changed their diet after the diagnosis. Furthermore, most studies used a 24-hour recall to measure diet,[3-5,9,14] which is generally a poor reflection of the usual intake of an individual.[15]

In 1980, information on dietary intake over the preceding year was collected from a large cohort of US women. In the present study, we examined nutrient intake in relation to the development of hypertension during 4 years of follow-up. In addition to providing a very large study population, the potential for dietary change secondary to the diagnosis of high blood pressure is minimized in this study because of the prospective design and exclusion of persons with known hypertension at baseline.

Methods

Nurses' Health Study

The Nurses' Health Study is a prospective investigation of major diseases among a cohort of female registered nurses living in 11 states in the United States. In 1976, 121,700 women (98% white) aged 30-55 years completed mailed questionnaires regarding risk factors for cardiovascular diseases and cancer[16] as wel as other major health conditions. Follow-up questionnaires are sent biennially to update information.

Measurement of dietary intake

In 1980, the mailing to participants included a semiquantitative food frequency questionnaire that contained 61 food items, including alcoholic beverages. For each food, a commonly used portion size was specified, and participants were asked about their average frequency of use during the last year. Nine possible responses were provided, ranging from never to six or more times a day. Intake scores for different nutrients were computed by multiplying the reported frequency of each food by the nutrient content of the specified portion; the frequency of any food with a missing value was counted as zero. The nutrients examined in this study were total energy, dietary fiber, total fat, saturated fat, linoleic acid, trans-unsaturated fatty acids, calcium, magnesium, potassium, and alcohol. Calcium and magnesium intake were calculated only from food sources because information on their supplementation was not collected in 1980. We did not attempt to measure sodium intake in this study because of the relatively large and variable contribution of salt added to processed food, in cooking, and

at the table. Extensive data on the reproducibility and validity of the food frequency questionnaire have been published elsewhere.[17-19] Nutrient intakes assessed by the questionnaire are reasonably reproducible, with correlations for intervals of 3-9 months being in the range of 0.5-0.7 for most nutrients. When compared with multiple weeks of dietary records, correlations have ranged between 0.5 and 0.6 for most nutrients when adjusted for total caloric intake (see below).

Diagnosis of hypertension

Blood pressure status was defined by self-reported responses to the questionnaires. In 1976, participants were asked whether or not they ever had a diagnosis of high blood pressure (excluding during pregnancy). On subsequent biennial questionnaires, we inquired whether subjects had been diagnosed as having high blood pressure during the previous 2 years and, if so, the date of diagnosis. The validity of self-reported diagnosis of hypertension was assessed in a separate study.[20] Briefly, a random sample of 100 nurses reporting a diagnosis of high blood pressure on the 1982 questionnaire were contacted again in 1983 to obtain permission for review of their medical records. Records were obtained for 51 subjects. All had recorded values of blood pressure greater than 140/90 mmHg, and for 39 women (77%), blood pressure was greater than 160/95 mmHg. To investigate the likehood of false-negative responses, blood pressure was measured in an age-stratified sample of 194 nurses living in the greater Boston area. Among the 161 women without a previous self-report of high blood pressure, 7% had a blood pressure level higher than 140/90 mmHg, but none of these women had a blood pressure greater than 160/95 mmHg. Thus, the self-reported diagnosis of elevated blood pressure appeared to be a valid measure in this population of registered nurses.

Population for analysis

A total of 98,462 women returned the 1980 Nurses' Health Study dietary questionnaire. Participants with 10 or more blank food items (4%) or with an implausibly high or low total food score (2.7%) were excluded. From the remaining women, we excluded those who reported one or more of the following diagnoses on the 1980 or previous questionnaires: high blood pressure (18,818), myocardial infarction (611), angina pectoris (1,545), diabetes mellitus (2,202) and all cancers except nonmelanoma skin cancer (3,646). Participants indicating on the 1980 questionnaire that they currently used antihypertensive medication (10,433), were on a special diet (15,006), or had been pregnant for at least 6 months since 1978 (1,578) were also excluded. The total number of participants excluded for one or more reasons was 38,262 so that the baseline population consisted of 60,200 women.

Follow-up questionnaires were sent in 1982 and in 1984 to all study participants. The 1,982 nonrespondents to both follow-up questionnaires (3.3%) were excluded, leaving 58,218 women for analysis. There were 3,275 incident cases of hypertension reported on the 1982 or 1984 questionnaire.

Data analysis

Exposure status for all variables was defined by responses to the 1980 questionnaire. Nutrients were adjusted for total caloric intake as described elsewhere,[17] by computing the difference between the observed nutrient intake and expected nutrient intake predicted by regressing the nutrient intakes for the whole population on their total energy intakes. Analyses of hypertension risk were based on 4-year cumulative incidence rates. For each calorie-adjusted nutrient, we specified five categories. Cutoff points were chosen before examining the association of diet with hypertension to provide similar increments in nutrient intake from one category to the next and to include a reasonable number of subjects in each category. The use of even-interval cutoff points has appeal in evaluating linear trends and in comparing different studies. Age-adjusted partial correlation coefficients were calculated between pairs of exposure variables to evaluate potential confounding effects (this correlation matrix is available on request from the author). Multiple logistic regression analysis with categorical variables was used to control for confounding. Relative risks of hypertension with 95% confidence intervals were calculated by comparing each category of intake to the lowest category. Linear trends in risks over successive categories were evaluated by entering indicators for each categorical level of exposure, using the median values of each category. Relative risks were calculated within strata of other variables by combining the second with the third and the fourth with the fifth category of the exposure to obtain more statistically stable associations.

Results

The 4-year cumulative incidence of hypertension rose sharply with age from 2.8% for women aged 34-39 years to 8.9% for women aged 55-59 years (figure 4.1.1). Quetelet's index and alcohol consumption were also strongly associated with an increasing risk of hypertension (table 4.1.1). Nutrients intakes were only weakly correlated with Quetelet's index (all age-adjusted correlation coefficients less than 0.05) but were correlated more strongly with alcohol consumption (r up to 0.30). Therefore, we controlled for age, Quetelet's index, and alcohol consumption in all further analyses. Adjusted relative risks were only significant for intakes of calcium, magnesium, potassium, and fiber (table 4.1.2). No effects were found for total and saturated fat, linoleic acid, and

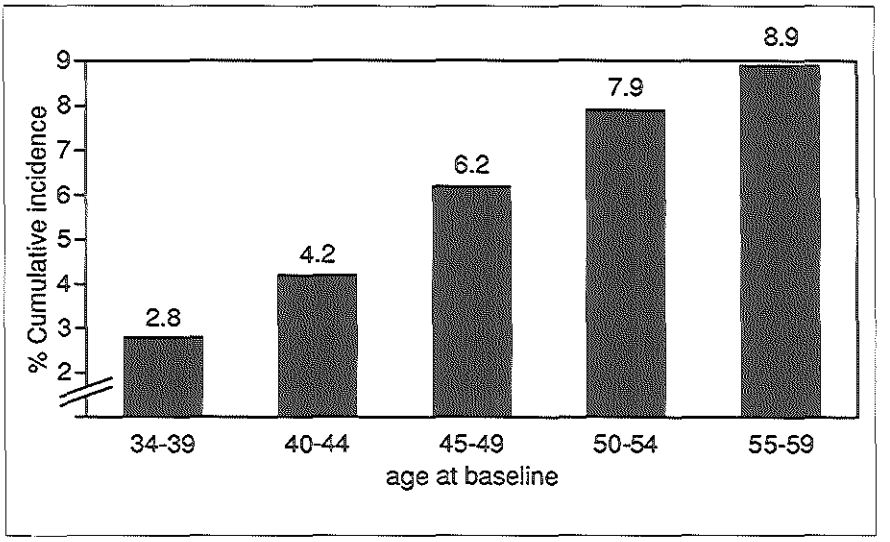

Figure 4.1.1. Bar graph of 4-year cumulative incidence of hypertension by age, among the Nurses' Health Study cohort of 58,218 US women, 1980-1984

trans-unsaturated fatty acids (table 4.1.3). Adjustment for smoking, menopausal status, and postmenopausal estrogen use did not alter the observed associations.

Relatively high correlations were observed between a number of different nutrients. Age-adjusted correlation coefficients for the nutrients presented in table 4.1.2 varied widely and were 0.17 for calcium and fiber, 0.27 for magnesium and fiber, 0.53 for magnesium and calcium, and 0.69 for magnesium and potassium. When entered simultaneously in a multiple logistic model, only dietary calcium and magnesium were independently associated with risk of hypertension (table 4.1.2). The associations of dietary potassium and fiber with risks of hypertension were practically eliminated after controlling for dietary calcium and magnesium (table 4.1.3). The study was sufficiently large so that, even in the multivariate analyses, the confidence interval for potassium did not include any major association; for high dietary fiber intake (>25 g/day), the data were still compatible with a modest protective effect, because the lower bound confidence interval was 0.71.

We next examined dietary intakes of calcium and magnesium from different food sources. Dairy products accounted for 61% of the calcium intake; the main contributors to magnesium intake were fruits and vegetables (27%), cereals (17%), and dairy products (13%). Calcium from both dairy and non-dairy sources and magnesium intake from cereals and dairy foods, as well as from the remaining foods, were each independently associated with lower risks of hypertension (table 4.1.4).

Associations of calcium and magnesium intake with hypertension were examined within categories of other variables that could modify the relation (see

Table 4.1.1. Relative risks of hypertension by level of Quetelet's index and daily intake of alcohol

			Category				
Quetelet's index (kg/m^2)	<23	23-25	26-28	29-31	≥32	χ for trend*	p
Cases (n)	1,002	939	579	391	364		
Total (n)	30,660	16,102	6,081	3,167	2,208		
Relative risk†	1.0	1.67	2.80	3.86	5.70	32	<0.001
95% Confidence Interval		(1.52-1.83)	(2.50-3.13)	(3.42-4.38)	(4.99-6.49)		
Alcohol (g/day)	0	0.1-9	10-19	20-29	≥30		
Cases (n)	1,062	1,291	516	151	255		
Total (n)	17,488	26,560	9,174	2,124	2,872		
Relative risk†	1.0	0.90	1.08	1.47	1.75	8.7	<0.001
Relative risk‡	1.0	0.92	1.13	1.47	1.70	8.0	<0.001
95% Confidence Interval		(0.84-1.00)	(1.01-1.26)	(1.23-1.77)	(1.46-1.98)		

* χ for test of trend
† model includes age, Quetelet's index, and alcohol consumption
‡ model includes age, Quetelet's index, alcohol consumption, and intakes of calcium, magnesium, potassium, and fiber

Table 4.1.2. Relative risks of hypertension by level of energy-adjusted daily intake of calcium, magnesium, potassium, and fiber

			Category				
Calcium (mg/day)	<400	400-599	600-799	800-999	≥1,000	χ for trend*	p
Cases (n)	436	1,047	911	458	423		
Total (n)	6,671	17,664	16,522	9,134	8,227		
Relative risk†	1.0	0.90	0.83	0.77	0.79	3.8	<0.001
Relative risk‡	1.0	0.92	0.86	0.81	0.84	2.7	0.007
95% Confidence interval		(0.82-1.05)	(0.76-0.98)	(0.70-0.94)	(0.71-0.99)		
Magnesium (mg/day)	<200	200-249	250-299	300-349	≥350		
Cases (n)	287	695	971	731	591		
Total (n)	4,494	12,154	16,412	13,324	11,834		
Relative risk†	1.0	0.86	0.88	0.81	0.72	4.5	<0.001
Relative risk‡	1.0	0.91	0.93	0.85	0.78	2.2	0.03
95% Confidence interval		(0.77-1.09)	(0.77-1.12)	(0.69-1.04)	(0.62-0.98)		
Potassium (mg/day)	<2,000	2,000-2,399	2,400-2,799	2,800-3,199	≥3,200		
Cases (n)	395	704	945	705	526		
Total (n)	6,190	12,672	16,466	12,624	10,266		
Relative risk†	1.0	0.86	0.89	0.85	0.77	3.3	<0.001
Relative risk‡	1.0	0.93	1.02	1.05	1.05	1.1	0.26
95% Confidence interval		(0.80-1.00)	(0.86-1.21)	(0.87-1.27)	(0.85-1.30)		
Fiber (g/day)	<10	10-14	15-19	20-24	≥25		
Cases (n)	341	1,133	1,060	504	237		
Total (n)	5,529	19,864	18,829	8,983	5,013		
Relative risk†	1.0	0.94	0.92	0.90	0.76	3.1	0.002
Relative risk‡	1.0	0.97	0.98	0.99	0.87	1.5	0.14
95% Confidence interval		(0.85-1.11)	(0.85-1.13)	(0.84-1.16)	(0.71-1.05)		

* χ for test of trend
† model includes age, Quetelet's index, and alcohol consumption in addition to the specified nutrient
‡ model includes age, Quetelet's index, alcohol consumption, and intakes of calcium, magnesium, potassium, and fiber

Table 4.1.3. Relative risks of hypertension by level of daily intake of energy-adjusted fatty acids

	Category						
Saturated fatty acids (g/day)	<20	20-23	24-27	28-31	≥32	χ for trend*	p
Relative risk†	1.0	1.03	1.05	1.04	1.06	0.8	0.5
Relative risk‡	1.0	1.01	1.02	0.99	0.99	0.4	0.7
95% Confidence interval		(0.88-1.18)	(0.89-1.18)	(0.86-1.14)	(0.86-1.16)		
Linoleic acid (g/day)	<5.0	5.0-7.4	7.5-9.9	10.0-12.4	≥12.5		
Relative risk†	1.0	1.0	0.99	1.05	1.03	0.4	0.7
Relative risk‡	1.0	1.0	0.97	1.02	0.99	0.1	1.0
95% Confidence interval		(0.89-1.11)	(0.86-1.08)	(0.89-1.18)	(0.92-1.20)		
Trans-unsaturated fatty acids (g/day)	<2.5	2.5-3.4	3.5-4.4	4.5-5.4	≥5.5		
Relative risk†	1.0	1.22	1.20	1.16	1.24	1.4	0.2
Relative risk‡	1.0	1.18	1.14	1.08	1.14	0.1	1.0
95% Confidence interval		(1.02-1.36)	(0.99-1.31)	(0.92-1.25)	(0.96-1.34)		
Total fat (g/day)	<55	55-64	65-74	75-84	≥85		
Relative risk†	1.0	1.04	1.04	1.03	1.05	0.4	0.7
Relative risk‡	1.0	1.02	0.99	0.95	0.93	1.4	0.2
95% Confidence interval		(0.90-1.15)	(0.88-1.11)	(0.83-1.08)	(0.80-1.08)		

* χ for test of trend
† model includes age, Quetelet's index, and alcohol consumption
‡ model includes intakes of calcium and magnesium in addition to age, Quetelet's index, and alcohol consumption

Table 4.1.4. Relative risks of hypertension by level of daily calcium and magnesium intakes from different food sources*

			Category				
Calcium (mg/day)	<200	200-299	300-399	400-499	≥500	χ for trend†	p
Dairy	1.0	0.96	0.95	0.91	0.85	3.3	0.001
		(0.85-1.07)	(0.85-1.06)	(0.81-1.03)	(0.77-0.93)		
Nondairy	1.0	0.95	0.94	0.85	0.80	2.6	0.009
		(0.86-1.05)	(0.84-1.06)	(0.73-0.98)	(0.64-0.98)		
Magnesium (mg/day)	<30	30-39	40-49	50-59	≥60		
Fruits and vegetables	1.0	0.98	0.87	0.81	0.88	1.0	0.33
		(0.82-1.25)	(0.72-1.06)	(0.66-0.98)	(0.74-1.05)		
Cereals	1.0	0.91	0.91	0.96	0.88	2.5	0.011
		(0.82-1.01)	(0.80-1.02)	(0.83-1.11)	(0.80-0.96)		
Dairy	1.0	1.0	0.95	0.87	0.86	3.1	0.002
		(0.90-1.11)	(0.85-1.08)	(0.75-1.00)	(0.78-0.95)		
Magnesium other (mg/day)	<100	100-124	125-149	150-174	≥175		
	1.0	1.0	0.92	0.96	0.82	3.2	0.002
		(0.90-1.10)	(0.83-1.03)	(0.85-1.08)	(0.72-0.92)		

* model includes age, Quetelet's index, alcohol consumption, and intakes of calcium and magnesium from other food sources
† χ values for test of trend
95% Confidence intervals are in parentheses

table 4.1.5). Similar relations were found among younger and older women. Weaker associations, however, were observed among women with high relative weight. Menopausal status did not substantially alter the relations with dietary calcium and magnesium. The inverse association of calcium with hypertension was consistently seen among postmenopausal women, regardless of their use of estrogen supplements. An association of magnesium with hypertension, however, was not found among past or current users of postmenopausal estrogens. The relative risk of hypertension for women with high magnesium intake was lower among those who consumed over 20 g alcohol/day than among those with lower intakes.

We also examined associations of hypertension with calcium and magnesium intake expressed in relation to the Recommended Dietary Allowance (RDA) of these nutrients. For women with a calcium intake of at least the RDA of 800 mg/day, the relative risk of hypertension was 0.78 (95% confidence interval [CI] 0.69-0.88) compared with those in the lowest intake category (less than 400 mg/day) after adjustment for age, Quetelet's index, and alcohol consumption. The adjusted relative risk for women above the RDA for magnesium intake of 300 mg/day was 0.77 (95% CI, 0.67-0.88) compared with those in the lowest intake category of magnesium (<200 mg/day). The adjusted relative risk of hypertension for women above the RDA for both calcium and magnesium intake (n=11,248) compared with those in the lowest category for both nutrients (n=2,003) was 0.65 (95% CI, 0.53-0.80).

Discussion

Within this large cohort of women of whom 98% were white, we observed independent inverse associations for dietary calcium and magnesium with the incidence of hypertension. These associations remained after adjusting for age, relative weight, and alcohol consumption, each significantly contributing to the development of hypertension. Adjusting for calcium and magnesium intake eliminated the observed crude inverse associations of dietary potassium and fiber with risk of hypertension. No associations were observed for intakes of saturated fat and linoleic acid.

In this study, we relied on self-reported diagnoses of hypertension. Although a direct measurement is more objective, a single high blood pressure reading may not always represent hypertension because of the large within-person variability in measurements over time.[21] The validity of diagnosis of hypertension in this study is supported by several lines of evidence. First, the accuracy of the measure was shown in a subsample.[20] Second, the age-specific indicence rates of hypertension were nearly identical to those observed in the Framingham study.[22] Third, a strong association between self-reported hypertension and risk

Table 4.1.5. Relative risks of hypertension by level of daily calcium and magnesium intake, within strata of other variables*

Risk		n	Calcium (mg/day)			Magnesium (mg/day)		
			<400	400-799	≥800	<200	200-299	≥300
Age (yr)	≤44	27,038	1.0	0.88	0.79†§	1.0	0.86	0.81
	>45	31,180	1.0	0.86	0.78†§	1.0	0.88	0.75†§
Quetelet's index (kg/m^2)	<23	30,660	1.0	0.84	0.77†¶	1.0	0.67¶	0.62†§
	23-28	22,183	1.0	0.88	0.72†¶	1.0	0.90	0.78†¶
	≥29	5,375	1.0	0.87	0.94	1.0	1.15	0.92
Menopausal status‡	Before	33,995	1.0	0.89	0.75†¶	1.0	0.84¶	0.77†§
	After	23,607	1.0	0.84	0.80†¶	1.0	0.92	0.77†¶
Postmenopausal hormone use	Never	13,859	1.0	0.81	0.80	1.0	0.78	0.66†¶
	Past	5,320	1.0	0.82	0.80	1.0	1.20	0.99
	Current	4,428	1.0	0.94	0.78	1.0	1.13	0.96
Alcohol use (g/day)	<0.1	17,488	1.0	0.94	0.78†¶	1.0	0.92	0.81†§¶
	0.1-19	35,488	1.0	0.82¶	0.76†¶	1.0	0.91	0.77†¶
	≥20	4,996	1.0	0.81	0.79	1.0	0.60¶	0.62¶

* Model includes age, Quetelet's index, and alcohol consumption (except for data stratified by alcohol intake)
† significant trend ($p \leq 0.05$)
‡ missing data on menopause for 616 women
§ $p < 0.01$ for relative risk
¶ $p < 0.05$ for relative risk

of myocardial infarction has been observed prospectively in the Nurses' Health Study.[23] Furthermore, detection bias is likely to be minimal in this analysis because women who subsequently developed hypertension and those who remained normotensive had a similar frequency of doctor visits as reported on the 1978 questionnaire.

Although comparison of the questionnaire data with those from dietary records indicated a reasonable level of validity,[17-19] our measure of diet was certainly not perfect. Because diet was measured before diagnosis, it is reasonable to assume that associations were relatively unbiased with respect to hypertension status; in the event that knowledge of borderline hypertension might have caused women to change their diets, the direction of any change would have been more likely to obscure relations with calcium and magnesium than to create them. For these reasons, the strength of observed associations probably represents an underestimation of the true effects of diet. We did not attempt to measure salt intake in this study; however, a relation between salt intake and hypertension has usually not been found in studies within populations as reviewed elsewhere.[1] In a recent report from the Intersalt study,[24] no significant association was seen between diastolic blood pressure and urinary sodium excretion, and only a 1.6-mmHg change in systolic pressure per 100 mmol Na was found (100 mmol approximately corresponding to the difference between the average US daily excretion and that of isolated primitive populations in Africa and South America). Because the associations between sodium intake and calcium and magnesium intakes are also not likely to be strong (these correlations were both less than 0.05 after adjustment for total caloric intake based on the mean of four 1-week diet records provided by a sample of 173 women in our population), sodium intake could not seriously distort the relations we observed.

Several cross-sectional studies of the relation between calcium intake and blood pressure have recently been published. McCarron et al[3] reported an inverse association of dietary calcium with blood pressure in the National Health and Nutrition Examination Survey (NHANES) I study, but a reanalysis of the NHANES I and the NHANES II investigations did not find such an association[25]; a major limitation of the NHANES studies is that diet was assessed with a single 24-hour recall. Other various large-scale epidemiologic studies support the existence of an association of blood pressure with either total dietary calcium[6,12,13] or calcium from dairy products.[4,26,27] In trials of calcium supplement administration, blood pressure was reduced in subjects with mild[28] or more severe hypertension,[29,30] although in some studies this was observed for only a subset of the population.[28] No effect of calcium supplementation on blood pressure has been found among normotensive subjects in several studies;[29-31]

however, Lyle et al[32] have reported a modest reduction among normotensive men supplemented with 1,500 mg Ca/day.

The relation of dietary magnesium with hypertension has been examined in only a few studies. In a recent analysis of data from the Honolulu Heart Study,[14] a low magnesium intake was found to be the dietary factor most strongly associated with high blood pressure. In two small case-control studies, no associations were observed,[33-34] although an inverse trend was seen in one.[33] The response to short-term supplementation of magnesium, however, may be different from that related to long-term dietary intake, which may inhibit a gradual rise in blood pressure over many years. A blood pressure lowering effect of magnesium supplementation in hypertensive subjects receiving diuretic treatment has been observed,[35] but this could not be confirmed in another intervention study[36] or in a short-term trial among untreated hypertensive subjects.[37]

A protective effect of potassium has been found in several cross-sectional studies,[2-4,24] but no association has been observed in some others.[5,6] A reduction in blood pressure has been shown in one trial of potassium administration[7] but not in another [8]. An association between fiber and blood pressure could not be shown in several intervention studies testing different types of fiber [38-40]. In contrast, an effect has been suggested by another intervention study[41] and one observational study [14]. None of these studies, however, controlled for the intake of minerals.

An inverse relation of blood pressure with a high ratio of dietary poly unsaturated fatty acids to saturated fatty acids has been found in some intervention trials[10,42] and in one observational study[12] but not in others.[3,11,14] No effect was observed in all double-blind trials[43-45] and in most of the unblinded studies.[38,46] To our knowledge, no studies have been reported that examined the relation of trans-unsaturated fatty acids with blood pressure in humans.

We examined the possibility that other variables might modify the observed associations of calcium and magnesium intake with hypertension. The effects of both nutrients were less among women in the highest category of Quetelet's index. No effect was shown for magnesium among past or present users of postmenopausal hormones, which might be due to oestrogen-induced magnesium retention.[47] A particularly strong effect of magnesium was observed among subjects in the highest category of alcohol intake, perhaps because alcohol consumption increases urinary excretion of magnesium.[48] The finding of independent associations of calcium and magnesium from different food sources provides support that the observed effects are due to these nutrients themselves, rather than being mediated by some other factor in specific foods.

In summary, high relative weight and alcohol consumption were the strongest predictors of hypertension, and our data support the existing public

health recommendations for control of these factors.[49] Compared with the adverse impact of overweight and alcohol intake, the protective effects of dietary calcium and magnesium were modest. However, as pointed out by Rose[50], even a small reduction in the average blood pressure of a population can result in major reduction in complications. The presence of significant trends and the existence of plausible mechanisms of action[28,32,51,52] indicate the need for further clinical trials to determine whether or not the observed associations between intakes of calcium and magnesium and blood pressure are causal. Such trials should also address previous suggestions that other metabolic factors may influence susceptibility to the effects of low calcium and magnesium intake. A large portion of our study population consumed less than the RDA of calcium and magnesium and thus could potentially benefit from a higher intake of these nutrients. However, the relative advantages of increasing intake by changes in diet or by supplementation need to be carefully considered as would the possible consequences for other important health-related outcomes.

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4.2 Relation of Moderate Alcohol Consumption and Risk of Systemic Hypertension in Women *

Abstract

The relation between alcohol consumption and the risk of development of hypertension was studied among 58,218 US female registered nurses aged 39 to 59 years who were free of diagnosed systemic hypertension and other major diseases. In 1980, all of these women completed an independently validated dietary questionnaire, which included use of alcoholic beverages. During 4 years of follow-up, 3,275 women reported an initial diagnosis of hypertension; validity of the self-report measure was demonstrated in a subsample. When compared to nondrinkers, women drinking 20 to 34 g of alcohol per day (about 2 or 3 drinks) had a significantly elevated relative risk of 1.4; the 95% confidence interval (CI) was 1.2 to 1.7 after adjustment for age and Quetelet's index. For women consuming ≥35 g/day, the relative risk was 1.9 (95% CI 1.6 to 2.2). Adjustment for smoking and dietary variables did not alter these results. Independent significant associations were observed for the consumption of beer, wine and liquor. These prospective data suggest that alcohol intake of up to about 20 g/day does not increase the risk of hypertension among women, but beyond this level, the risk increases progressively.

Introduction

A positive association between heavy alcohol consumption and elevated blood pressure has been observed in several large observational studies.[1-6] The effects of light and moderate drinking, however, are less clear. Among men, linear relations either with or without a low threshold level, are reported.[1,2,4,7,8] Among women, U- and J-shaped curves are the dominant findings.[2-4,9] Most population studies, especially in women, are cross-sectional and few included information on other dietary variables that might account for the effects of alcohol.[10,11] An acute pressor effect of alcohol intake has been observed in short-term intervention studies,[12,13] but it is not clear whether these effects are

* Am J Cardiol 1990:65:633-7

sustained. We prospectively examined the relation between alcohol consumption and risk of hypertension among a large cohort of US women.

Methods

Study Protocol

The Nurses' Health Study is a prospective investigation of determinants of major diseases among a cohort of female registered nurses from 11 large states. In 1976, 121,700 women (approximately 98% white), aged 30 to 55 years, completed mailed questionnaires that included items regarding risk factors for cardiovascular diseases and cancer as well as a variety of other health conditions. Follow-up questionnaires are sent biennially to update information.

Measurement of Alcohol Consumption

In 1980, the mailing included a semiquantitative food frequency questionnaire that contained 61 foods and beverages, including beer, wine and liquor.[14] For each item, participants were asked about their average frequency of use over the last year. Nine possible responses were provided, ranging from never to ≥6 times a day. The alcohol content was estimated as 13.2 gm for a bottle or can of beer (12 ounces), 10.8 gm for a glass of wine (4 ounces) and 15.1 gm for a drink of liquor (1.5 ounces).[15] Total alcohol intake for each individual was computed as the sum of the contribution from beer, wine and liquor. Past alcohol users were defined as women who reported no alcohol intake during the previous year but recorded that intake of beer, wine or liquor had greatly decreased during the past 10 years.

The validity of the questionnaire measure of alcohol was evaluated in a subsample of the participants.[12] Alcohol consumption calculated from the questionnaires was highly correlated with intake estimated from four 1-week dietary records (Spearman r= 0.90) and was reproducible over a 1-year interval (Spearman r = 0.90). The mean alcohol intake as assessed by the diet records (9.0 g/day) was virtually identical to the value derived on he same participants from the questionnaire.[15] Furthermore, alcohol assessment by questionnaire was significantly correlated with plasma high-density lipoprotein cholesterol (Spearman r = 0.40),[15] which is increased by alcohol intake.[7]

Diagnosis of hypertension

Blood pressure status was defined by self-reported responses to the questionnaires. In 1976, participants were asked whether they have ever had a diagnosis of high blood pressure (excluding during pregnancy). On subsequent questionnaires we inquired whether subjects had been diagnosed as having high blood pressure during the previous 2 years, and if so, the date of the diagnosis.

The validity of self-reported diagnosis of high blood pressure was assessed in a separate study.[16] Briefly, a random sample of 100 nurses reporting a diagnosis of high blood pressure on the 1982 questionnaire were contacted again in 1983 to obtain permission to review their medical record. Of 85 women who had reported elevated blood pressure and responded to the validation questionnaire, 1 denied having elevated blood pressure; the remainder confirmed their prior self-report and 62 gave written permission for review of their medical records. Records were obtained for 51 subjects. All had recorded values of blood pressure >140/90 mm Hg and for 39 women (77%), blood pressure was >160/95 mm Hg. To investigate the likehood of false-negative responses, blood pressure was measured in an age-stratified sample of 194 nurses living in the greater Boston area. Among the 161 women without a previous self-report of high blood pressure, 7% had a blood pressure >140/90 mm Hg, but none had a blood pressure >160/95 mm Hg. Thus, the self-reported diagnosis of elevated blood pressure appeared to be a valid measure in this population of registered nurses.

Population for analysis

A total of 98,462 women returned the 1980 Nurses' Health Study diet questionnaire. Participants with 10 or more blank food items or with an impausibly high or low total food score were excluded. In addition, we excluded women who reported one or more of the following diagnoses on the 1980 questionnaire or any previous questionnaire: high blood pressure; myocardial infarction; angina pectoris; diabetes mellitus; and all cancers except nonmelanoma skin cancer. Participants indicating on the 1980 questionnaire that they currently used antihypertensive medication, were on a special diet or had been pregnant for at least 6 months since 1978 were also excluded. Thus the total number of participants excluded was 38,262 so that the baseline population of this study consisted of 60,200 women.

Follow-up questionnaires were sent in 1982 and 1984 to all participants. Nonresponders to both follow-up questionnaires (n = 1,982) were excluded, leaving 58,218 women for analysis. Cases of hypertension were defined as women who reported a diagnosis of hypertension on the 1982 or 1984 questionnaire (n = 3,275).

Data analysis

We specified 5 categories of alcohol consumption, with cutpoints comparable to those in other studies. Exposure status for all potential confounders was defined by responses to the 1980 questionnaire. Analyses were based on 4-year cumulative incidence rates of hypertension. Multiple logistic regression was used to control potential confounding effects, with the independent variables entered as categorical variables. Relative risks of hypertension with 95%

confidence intervals (CI) were calculated by comparing each category of alcohol intake to the nondrinkers. Linear trends in risk were evaluated by entering ordered categorical variables using the median values of each category. Relative risks were calculated within strata of age, Quetelet's index (kg/m^2) and smoking, and combining the third and fourth category of alcohol consumption to obtain more stable estimates. The percentage of hypertension incidence among exposed subjects attributable to alcohol consumption was calculated using the formula of attributable proportion.[17]

Results

Nondrinkers comprised 30% of the study population; 46% consumed 0.1 to 9 g of alcohol per day; 16% consumed 10 to 19 g, 5% consumed 20 to 34 g; and 4% consumed ≥35 g of alcohol per day. The mean daily intake of the highest intake category was 46 g of alcohol. The 4-year cumulative incidence of hypertension rose progressively over 5-year age categories, from 2.8% for women aged 35 to 39 years to 8.9% for women aged 55 to 59 years.

When compared to nondrinkers, the age-adjusted risk of hypertension was slightly reduced among women with an intake <10 g of alcohol per day (table 4.2.1). The relative risk increased slightly for the 20 to 34 g/day category but increased sharply for consumption of ≥35 g/day. After adjustment for Quetelet's index, the relative risks relating alcohol intake to hypertension increased due to a strong positive association of hypertension with Quetelet's index and an inverse association of Quetelet's index with alcohol consumption. Among women who consumed 20 to 34 g of alcohol per day (about 2 or 3 drinks), the adjusted relative risk of hypertension was 1.4 (95% CI 1.2-1.7); consumption of ≥35 g of alcohol per day was associated with an adjusted relative risk of 1.9 (95% CI 1.6-2.2) (table 4.2.1). When the highest intake category was restricted to women consuming at least 50 g of alcohol per day (n = 468), the relative risk

Table 4.2.1. Relative risks of hypertension by alcohol consumption level

	Alcohol intake (g/day)						
	0	0.1-9	10-19	20-34	≥35	χ for trend*	p
Cases (n)	1062	1291	516	194	212		
Total	17488	26560	9174	2860	2136		
RR†	1.0	0.8	0.9	1.1	1.6	6.4	<0.001
RR‡	1.0	0.9	1.1	1.4	1.9	9.9	<0.001
95% CI		(0.8-1.0)	(1.0-1.3)	(1.2-1.7)	(1.6-2.2)		

* χ values of test for trend
† adjusted for age;
‡ adjusted for age and Quetelet's index
CI = confidence interval; RR = relative risk

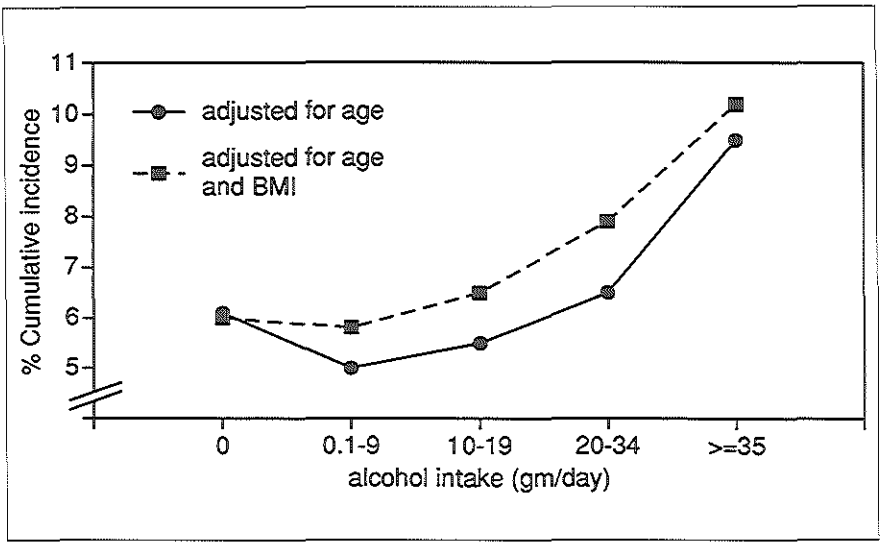

Figure 4.2.1. Four-year cumulative incidence of hypertension by level of alcohol consumption, standardized for age and Quetelet's index to the distribution among nonsmokers. BMI = body mass index

of hypertension was 2.7 (95% CI 2.0-3.5). The 4-year cumulative incidence of hypertension by alcohol consumption level is shown in figure 4.2.1.

We then considered the possibility that other factors may confound the association between alcohol intake and blood pressure. In comparison to nondrinkers, the group of women with an intake of ≥10 g of alcohol per day had a higher caffeine intake (436 vs 369 mg/day) and a higher percentage of current smokers (39 vs 23%), after adjustment for age. However, caffeine intake and smoking were not significantly associated with hypertension and adjustment for these variables did not change the association of alcohol consumption with hypertension. We examined the effects of 31 nutrients, including calcium, potassium, magnesium, fiber and saturated and polyunsaturated fatty acids. None of these affected the relation between alcohol and hypertension when included in a logistic model.

We compared the incidence of hypertension among past drinkers (n = 6,202) with the incidence among nondrinkers. The risk of hypertension among past drinkers was not significantly increased (relative risk = 1.2; 95% CI 0.9 to 1.5), and exclusion of past drinkers did not alter the observed relative risks for drinkers compared to nondrinkers.

The association between alcohol intake and hypertension was examined within specific age categories (table 4.2.2). The relation was significantly stronger among younger women, as indicated by a negative interaction term for age and alcohol intake when it was included in the logistic model ($p = 0.004$). Comparison of the risks of hypertension over strata of Quetelet's index showed

Table 4.2.2. Relative risks of hypertension by alcohol intake within strata of age and

	0	0.1-9	10-34	≥35	χ for trend*	p
Age (yrs)†						
34-39	1.0	0.9 (0.7-1.1)	1.4 (1.1-1.9)	3.4 (2.1-5.4)	5.9	<0.001
40-49	1.0	0.9 (0.8-1.0)	1.2 (1.0-1.4)	1.9 (1.5-2.5)	6.2	<0.001
50-59	1.0	0.9 (0.8-1.1)	1.1 (1.0-1.3)	1.7 (1.4-2.1)	5.3	<0.001
QI (kg/m^2)†‡						
<23	1.0	0.9 (0.8-1.0)	1.2 (1.0-1.4)	2.0 (1.6-2.6)	6.6	<0.001
23-28	1.0	0.9 (0.8-1.1)	1.2 (1.0-1.4)	2.0 (1.6-2.5)	6.7	<0.001
≥29	1.0	0.9 (0.9-1.1)	1.1 (0.9-1.5)	1.4 (0.9-2.1)	1.9	0.053

* χ of test for linear trend
† adjusted for Quetelet's index
‡ adjusted for age
95% Confidence intervals in parentheses

a weaker association among women with a high relative weight (table 4.2.2). No differences in risks of hypertension were observed among smokers and nonsmokers when examined separately.

We next calculated alcohol intakes from beer, wine and liquor separately. Wine was consumed by 58% of the population and liquor by 46%, while beer was consumed by only 24%. Since the consumption of beer, wine and liquor tended to be correlated, we investigated the independent effects of each of the beverages by entering them simultaneously in the logistic model. Each beverage was independently associated with the risk of hypertension; however, the effect of liquor was stronger than that of wine or beer (table 4.2.3). Nutrient intakes were slightly different for beer, wine and liquor drinkers, but this did not explain the differences in risks of hypertension.

We calculated that among women drinking >20 g of alcohol per day, 32% of the hypertension incidence was attributable to alcohol consumption (adjusted for age and Quetelet's index).

Table 4.2.3. Relative risks of hypertension by alcohol intake from beer, wine and liquor

	Alcohol intake (g/day)						
	0	0.1-4	5-9	10-14	≥15	χ for trend*	p
Beer†	1.0	1.0 (0.9-1.1)	1.1 (0.9-1.3)	1.1 (0.9-1.4)	1.5 (1.1-1.9)	3.0	0.002
Wine†	1.0	0.9 (0.8-1.0)	0.9 (0.8-1.2)	1.0 (0.8-1.2)	1.3 (1.0-1.6)	2.4	0.017
Liquor†	1.0	1.0 (0.9-1.1)	1.2 (1.0-1.3)	1.6 (1.3-2.0)	1.8 (1.5-2.0)	9.1	<0.001

* χ of test for trend
† model includes ages, Quetelet's index, and intake of other alcoholic beverages
95% Confidence interval in parentheses

Discussion

In this prospective study among women, 20 to 34 g of alcohol per day (about 2 or 3 drinks) was associated with a 40% increase in risk of hypertension compared with nondrinkers. For women consuming ≥35 g/day the risk was increased by 90%. These associations were independent of age, Quetelet's index, smoking and other dietary factors.

In this study we relied on self-reported diagnosis of hypertension. Although a direct measurement is more objective, a high blood pressure level at a single sitting may not always represent hypertension due to the large within-person variability in measurements over time.[18] The validity of the diagnosis of hypertension in this study is supported by several lines of evidence. First, the accuracy of self-reported hypertension was demonstrated in a subsample, reflecting a strong awareness of blood pressure status in this population of nurses.[14] Second, the trend in cumulative incidence of hypertension with age is comparable with other prospective data,[19] and is consistent with a progressively increasing systolic blood pressure among women in this age range.[20] Detection bias is likely to be minimal in this study since women who subsequently developed hypertension and those who remained normotensive had a similar frequency of doctor visits, as reported in the 1978 questionnaire. Alcohol consumption assessed by our questionnaire data was very similar to that measured on four 1-week dietary records. Random measurement error, if present in our study, is likely to have resulted in an underestimation of the strength of the association rather than having introduced the observed effects. The presence of elevated blood pressure should not have influenced alcohol consumption or its reporting since intake was assessed before the diagnosis of hypertension.

The findings of this study are supported by cross-sectional observations of elevated blood pressures in subjects with heavy alcohol consumption.[1-4] Few of these studies reported the effect of alcohol use on prevalence of hypertension in women. In cross-sectional data from the Kaiser Permanente Study[4] the prevalence of hypertension for women consuming ≥6 drinks/day was increased by 80%. However, only 3% of our highest consumption group (n = 61) reported such a high intake. In a cross-sectional study in France,[8] consumption of 3 to 5 drinks/day was associated with a 70% increase in prevalence of hypertension among women. For women with lower alcohol intake levels ranging from 2 drinks/week[2,4,9] to approximately 2 drinks/day,[3] a decrease in blood pressure has been reported. In our data, women drinking up to 9 g of alcohol per day had a slightly lower risk of hypertension, but this was of marginal statistical significance. We did not confirm previous suggestions that the effects of alcohol on blood pressure are restricted to women above 45 or 50 years of age.[21,22] In contrast, in our data the association was stronger for younger women.

The independent effects of alcohol from beer, wine and liquor suggest that the observed associations are due to alcohol itself, rather than to other substances present in particular beverages. As reported by others[1] the effect was strongest for liquor consumption. We were not able to identify dietary factors or other factors to explain this difference. A causal interpretation of the association between alcohol intake and hypertension is supported by 2 recent intervention trials, demonstrating a direct effect of alcohol consumption on blood pressure.[10,11] However, a biologic mechanism has not been established, and it is unclear whether these short-term increases are sustained.

Our prospective findings suggest that consumption of up to about 20 g of alcohol per day does not increase the risk of hypertension among women. Higher consumption levels, however, were found to have a dose-related association with hypertension. Among women with an intake of ≥20 g/day, 32% of the hypertension incidence was attributable to alcohol consumption. In these women, hypertension may potentially be prevented or reversed by decreasing their alcohol intake.

Acknowledgment

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4.3 Calcium and Magnesium in Hypertension: Current Evidence*

Abstract

Attention is growing for potential roles of calcium and magnesium in the regulation of blood pressure. Results of a small study in 1982 indicated that reduced intake of dietary calcium is related to hypertension. Since then, a large number of reports of an inverse association between dietary calcium and blood pressure appeared in the literature. Data on magnesium intake are limited; an inverse association with blood pressure has been observed in some studies. Most epidemiologic studies on diet and blood pressure, however, have been cross-sectional and are therefore hard to interpret. Recently, independent associations of calcium and magnesium intake with blood pressure were confirmed by a large prospective study among U.S. nurses. For both dietary calcium and dietary magnesium, the risk of developing hypertension was reduced by 20% among those in the highest intake category compared to those in the lowest category.

The final proof of a causal relationship is to be given by intervention studies. To date, at least 18 double-blind placebo controlled calcium intervention studies and at least 8 double-blind placebo controlled magnesium intervention studies have been published. The results of these trials do not unanimously support a contributing role for calcium, nor for magnesium, in the regulation of blood pressure in all subjects. The inconsistency in results may be due to the number of studies that were small or of short duration. It may also partly be due to a possible heterogeneity in response, as observed in some studies. There is clearly a need, especially with respect to magnesium, for larger, well-controlled studies. Further investigation should also try to identify subgroups of susceptible persons, accompanied by developments in knowledge of pathophysiologic mechanisms.

Introduction

A number of nutritional factors have been implicated in the development and treatment of hypertension. For a long time, research has mainly focussed on the potential effects of sodium and potassium intake. Recently, attention has been directed towards a possible role of the divalent cations calcium and magnesium.

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* Calcium and magnesium in hypertension: current evidence. In: Laserre B, Durlach J, eds. Magnesium - A relevant ion? London: John Libbey, 1991, p 79-96.

A variety of disorders of calcium and magnesium metabolism have been described in subjects with hypertension and in animal models. Whether intake of these minerals may exert an effect on blood pressure, however, is still debated. In this review, we will discuss the present evidence for a such relationship provided by results from observational and intervention studies. Subsequently, we will discuss disturbances of calcium and magnesium metabolism in hypertension, followed by an examination of the literature on possible predictors of response to supplementation.

Observational studies

The hypothesis that intake of minerals may influence cardiovascular disease and hypertension was raised at an early time in studies of drinking water. Kobayashi in 1957, was the first to report lower death rates form cerebrovascular disease in area's with hard drinking water compared to area's with soft drinking water.[1] Stitt et al, among 12 towns in England and Wales, found a positive association between the town's hardness of drinking water and the mean blood pressure levels in the populations of these towns.[2] These findings, however, were not confirmed by some other studies and remained subject to much debate.[3]

Another type of epidemiological evidence supportive of the relationship between calcium and magnesium intake and blood pressure came from studies of gestational hypertension. The existence of a link between low calcium intake and pregnancy- induced hypertension was postulated by some investigators as early as 1930. Belizan and Villar found less pregnancy-induced hypertension in areas of increased calcium intake.[4] Comparable data relating dietary magnesium intake and pregnancy-induced hypertension are not available to date. That magnesium deficiency contributes to hypertension in pregnancy is suggested by the succesful use of parenteral magnesium sulphate in the treatment of preeclampsia.

Great interest in the subject has been shown only recently. McCarron in 1982 was the first to report a low calcium intake in hypertensive compared to normotensive subjects.[5] Subsequently, an inverse association between calcium intake and blood pressure has been reported in a large number of population studies (table 4.3.1). Most studies have been based on samples of US populations, but associations were also found in some European studies and among Japanese men living in Hawaii. The inverse association appears to be independent of age, sex, and race. The relationship was found both for total dietary calcium intake and for calcium intake from dairy products. Most studies controlled for potential confounding factors like body mass index and alcohol consumption. Some studies also controlled for intake of other nutrients, though it is doubted whether these can be fully accounted for.[19]

Not all data are consistent however. In the first National Nutrition and Health Examination Study in the USA (NHANES I, 1971-1975) McCarron et al reported an inverse association of dietary calcium and blood pressure among a sample of 10,372 subjects.[17] In re-analysis of the NHANES I data, and analysis of the NHANES II data (1976-1980), however, the association was either not found,[23] or observed in subgroups only.[10,11] A part of this discrepancy may be due to the varying inclusion of confounding and intermediary variables in the statistical models.

For magnesium intake fewer data are available. This is partly because interest in the hypothesis is of even more recent origin than for calcium and partly because of the more restricted availability of data on magnesium content in food composition tables. Population studies that did investigate the relationship with magnesium are listed in table 4.3.1. In a population sample in Belgium of 8,058 men and women, an inverse association was observed between dietary magnesium intake and systolic blood pressure in women.[13] In a study among Japanese men living in Hawaii, a low magnesium intake was found to be the dietary factor most strongly associated with hypertension.[12] McCarron observed that among U.S. adults magnesium intake was lower in hypertensive compared to normotensive subjects,[24] but no such relationship was observed among Scandinavian women.[25]

With few exceptions, epidemiological studies have been cross-sectional. Although most studies excluded subjects on a prescribed diet, these studies still might have included hypertensive subjects who changed their diet after the diagnosis. In 3 prospective analyses, no relationship could be observed between calcium intake and change in blood pressure during a follow-up period of some years.[7,15,18] The only prospective analysis that also presented data on the relationship between magnesium intake and blood pressure was performed among 58,216 female U.S. nurses (Nurses' Health Study).[21] In this study, dietary intake was measured by a mailed food frequency questionnaire. New cases of hypertension were ascertained during four years of follow-up by self-report of a physician's diagnosis of hypertension. After control for the effects of age, Quetelet's index, alcohol consumption and energy intake, significant inverse associations were found for dietary calcium and magnesium. Women with a calcium intake of at least 1000 mg/day had a 20% reduction in risk of hypertension when compared with an intake of less than 400 mg/day. A comparable reduction in risk of hypertension was observed for women with a magnesium intake of 300 mg/day or more compared with an intake of less than 200 mg/day. These prospective data support the hypothesis that calcium and magnesium intake are related to blood pressure. Definite evidence of whether a change in intake of calcium and magnesium can induce a change in blood pressure, however, has to be provided by intervention studies.

Table 4.3.1. Population studies of calcium and magnesium and blood pressure

References	Year	Population	Design	No.	Sex	Age	Dietary data	Ca	Mg
Ackley et al.	1983	USA (CA)	C	5,050	M+F	30-79	ques	milk	–
Caggiula et al.	1986	USA, MRFIT	C,L	–	M	–	24-hr	diet	–
Folsom et al.	1986	USA (MN)	C	1,687	M+F	25-74	24-hr	diet	–
Garcia-Palmieri et al.	1984	Puerto Rico	C	7,932	M	45-64	24-hr	milk	–
Gruchow et al.	1985	USA, NHANES I	C	9,553	M+F	18-74	24-hr	diet	–
Harlan et al.	1985	USA, NHANES I	C	3,854	M+F	25-74	24-hr	diet	–
Joffres et al.	1987	Hawaii	C	615	M	61-82	24-hr	diet	diet
Kesteloot & Joossens	1988	Belgium	C	8,058	M+F	25-74	24-hr	diet	diet
Kok et al.	1986	Netherlands	C	2,291	M+F	40-65	history	diet	–
Kromhout et al.	1985	Netherlands	C,L	605	M	45-64	history	diet	–
Liebman et al.	1986	USA (SE)	C	532	F	14-16	24-hr	diet	–
McCarron et al.	1984	USA, NHANES I	C	10,372	M+F	18-74	24-hr	diet	–
Nichaman et al.	1984	USA (IL)	C,L	1,976	M	40-56	history	diet	–
Reed et al.	1985	Hawaii	C	6,496	M	46-68	24-hr	dairy foods	–
Trevisan et al.	1988	Italy	C	5,049	M+F	20-59	foodfr	milk	–
Witteman et al.	1989	USA, Nurses Study	L	58,218	F	34-59	foodfr	diet	diet
Yamamoto & Kuller	1985	USA	C	1,939	M+F	34-56	foodfr	diet	–

C = cross-sectional; L = longitudinal; M = male; F = female; 24-hr = 24-hour recall; ques = questionnaire; history = dietary history; foodfr = food frequency questionnaire; Ca = calcium; Mg = magnesium

Studies of calcium supplementation

A number of calcium intervention studies have been published that were not reported to be double-blind placebo controlled.[26-32] In addition, 18 reports of double-blind placebo controlled studies have been published, covering 24 hypertensive and nonhypertensive study-populations. For this review, only double-blind placebo controlled studies that presented data of baseline and final blood pressure measurements are included, thereby excluding two of the controlled trials.[33,34] Table 4.3.2 summarizes the main features of the studies. The duration of the trials varied from 1 week to 4 years. The number of subjects varied from 15 to 90. Thirteen studies were performed in mild to moderately hypertensive subjects, and 9 in normotensives, amongst 1 in pregnant women. In 3 studies subjects were on anti-hypertensive medication.[38,45,49] The dose of elemental calcium ranged from 400 mg/day to 2160 mg/day.

The changes in blood pressure are shown in figure 4.3.1. Among the 22 studies presented, 4 showed a significant inverse effect on systolic blood pressure. The largest fall in systolic blood pressure, 21 mmHg, was found by Johnson et al., at the end of 4 years of calcium supplementation in patients who were on antihypertensive medication.[38] In the study of Saito et al., the rise in systolic blood pressure with the initiation of a high sodium diet was 10 mmHg less in subjects who concurrently received a high dose of calcium for 1 week compared to those who received placebo.[43] Villar et al observed a significant fall in systolic blood pressure of 4 mmHg in pregnant women supplemented from the 26th week of gestation onwards.[47] A significant fall in systolic blood pressure of 3 mmHg was found by McCarron and Morris among hypertensive subjects.[40] In this study, a significant fall in diastolic blood pressure of 3 mmHg was observed among normotensive subjects.

The response to calcium supplementation in the presented studies is not clearly related age or sex of the subjects. In the study of Lyle et al, white and black subjects were studied separately but effects on blood pressure were observed in neither group.[39] The response does not seem to be related to initial blood pressure status, nor to concomitant administration of anti-hypertensive medication. Given that high blood pressure may represent a chronic disturbance of calcium homeostasis in some subjects, it might take at least several weeks before an effect of calcium supplementation becomes appearent. McCarron and Morris found that the antihypertensive effect was significant only after 8 weeks.[40] This finding warrants a careful interpretation of 'negative' findings in trials that are of short duration. Nowson and Morgan[42] found no effect from supplementation of either 400 mg of elemental calcium or 800 mg, but most studies used higher doses. The results do not seem to be related to the type of salt used. Meese et al. found no difference in response between supplementation with calcium nitrate versus calcium carbonate.[41] Finally, the use of a low

Table 4.3.2. The effect of calcium supplements on blood pressure; a summary of double-blind placebo controlled studies

Reference	Year		Design	Duration	No.	Sex	Age (yrs)		Baseline BP (mmHg)	Condition	Ca salt	mg Ca/day
							mean	(range)	mean			
Beresteyn et al.	1986		P	6 wks	58	F	21	(20-23)	115/65L	–	carb	1500*
Cappuccio et al.	1987		C	4 wks	18	M+F	49	(28-65)	153/103L	–	lact	1600
Grobbee & Hofman	1986		P	12 wks	90	M+F	25	(16-19)	143/83S	–	citr	1000
Johnson et al.	1985	a	P	4 yrs	81	F	–	(35-65)	123/81S	–	carb	1500
		b		4 yrs	34	F	53	(35-65)	141/90S	treatment	carb	1500
Lyle et al.	1987	a	P	12 wks	54	M	33	(19-52)	117/73L	white	carb	1500
		b		12 wks	21	M	28	(19-52)	118/71L	black	carb	1500
McCarron et al.	1985	a	C	8 wks	32	M+F	48	(21-70)	121/75L	–	carb/citr	1000
		b		8 wks	48	M+F	52	(21-70)	152/94L	–	carb/citr	1000
Meese et al.	1987	a	C	8 wks	26	M+F	49	(22-73)	142/96S	–	citr	800
		b									carb	800
Nowson & Morgan	1986	a	P	8 wks	47	M+F	55	(22-77)	154/91S	–	carb	400
		b									carb	800
Saito et al.	1989		P	1 wk	27	M+F	51	(39-67)	126/81L	low Na diet	glub	2160
Siani et al.	1988		C	4 wks	15	M+F	41	–	139/91L	–	carb/lact	1000*
Strazzullo et al.	1986		C	15 wks	18	M+F	43	–	153/96L	treatment	carb/lact	1000
Thomsen et al.	1987		P	1 yr	28	F	50	–	124/76L	–	carb/lact	2000
Villar et al.	1987		P	12 wks	52	F	21	(18-30)	106/67L	pregnant	carb	1500
Vinson et al.	1987	a	P	7 wks	15	M	–	(19-24)	116/75L	–	gluc	500
		b									yeast	500
Waal-Manning et al.	1988		P	9 mos	52	M+F	61	(20-69)	143/84L	treatment	–	1000
Zoccali et al.	1988		C	8 wks	23	M+F	43	(27-59)	142/88S	–	carb/lact	1000

P = parallel group; C = crossover; wks = weeks, mos = months; yrs = years; M = male; F = female; BP = blood pressure. L = lying; S = sitting; Ca = calcium; carb = carbonate; lact = lactogluconate; citr = citrate; glub = glubionate; gluc = gluconate; * = versus low calcium diet
Lying blood pressure is presented when information was available

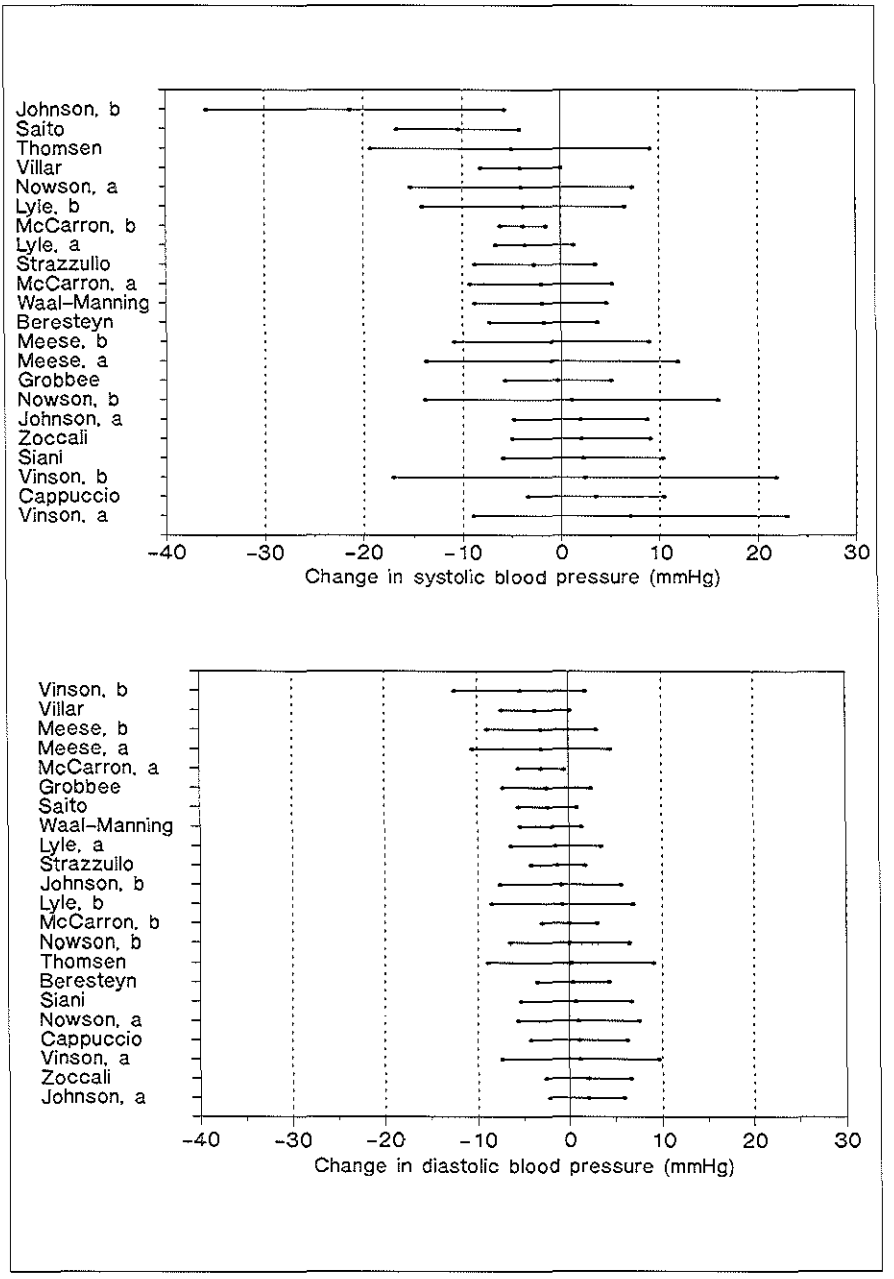

Figure 4.3.1. Effect of calcium supplements on systolic and diastolic blood pressure (mean and 90% confidence interval) in double-blind placebo controlled studies. The figures are based on the difference in blood pressure change from baseline to final measurement between the placebo group and those receiving calcium supplements. The use of this strategy for data presentation may result in discrepancies between numbers and inferences in the original papers and those presented here.

calcium diet for comparison[35,44] does not discriminate between studies that showed an effect and those that did not. In general, the studies differed in many variables and therefore it is hard to distinquish trial features that might be related to a favourable response.

Studies of magnesium supplementation

The studies using magnesium supplementation are less in number compared to those of calcium supplementation. There have been 5 studies published that were not placebo-controlled.[51-55] All studies were performed in mild to moderately hypertensive subjects. In 3, subjects were on anti-hypertensive medication, predominantly diuretics.[51,52,55] The salts used were either sulphate, oxide or aspartate hydrochloride in a dosis of 365 to 600 mg/day. Karppanen et al had used a potassium and magnesium enriched salt mixture with either 10% or 20% of magnesium sulphate, to be used instead of the subjects' usual table salt.[52] All studies observed a significant fall in systolic blood pressure. The largest fall in systolic blood pressure, 12 mmHg, was observed in the study of Dyckner en Wester after 6 months of supplementation in 20 hypertensive subjects on diuretic therapy.[51] Significant inverse associations were also observed for diastolic blood pressure, except for a significant increase in diastolic blood pressure among subjects with low plasma renin activity.[54]

Since these studies were not placebo-controlled, we should be careful with the interpretation. There have been 8 double-blind placebo-controlled studies on magnesium supplementation and blood pressure published to date. The main features of these studies are presented in table 4.3.3. The duration of supplementation varied from 3 weeks to 6 months. Numbers of subjects involved were generally small, except in the study of Sibai et al., who investigated 374 pregnant women.[63] With the exception of the latter study, all were performed among adult hypertensive subjects; in 2 studies subjects were on diuretics.[58,61] The dose of elemental magnesium varied from 243 to 500 mg/day. The results of the studies are presented in figure 4.3.2. In none of the studies was a significant fall in systolic or diastolic blood pressure observed.

Generally the effects shown in the controlled studies of magnesium intervention are far less convincing compared to those in the studies that were not placebo-controlled. Although it is possible that the placebo-controlled magnesium studies did not have enough power to detect an effect on blood pressure, we may conclude that the results of both the calcium and the magnesium supplementation studies performed to date do not provide convincing evidence for an effect of these minerals on blood pressure. How is the discrepancy with the results of population studies be explained? Blood pressure is affected by many genetic and environmental factors and their complex interactions. It is

Table 4.3.3. The effect of magnesium supplements on blood pressure; a summary of double-blind placebo controlled studies

Reference	Year	Design	Duration	No.	Sex	Age (yrs)		Baseline BP (mmHg)	Condition	Mg salt	mg Mg/day
						mean	(range)	mean			
Cappuccio et al.	1985	C	4 wks	17	M+F	52	(33-66)	154/100L	–	asp	365
Harris et al.	1984	P	12 wks	40	–	–	–	≥140/≥90	–	oxide	500
Henderson et al.	1986	P	6 mos	41	M+F	62	–	156/90	diuretics	oxide	301
Nowson & Morgan	1989	P	8 wks	25	M+F	63	(50-77)	151/90L	low Na diet	asp	243
Olhaberry et al.	1987	P	4 wks	14	F	46	(24-64)	152/101L	low Na diet	chlor	385
Reyes et al.	1984	P	3 wks	21	M+F	57	(42-82)	158/110L	diuretics	chlor	385
Rüddel et al.	1989	P	12 wks	14	M	42	–	132/84S	low Mg i.c.	asp-HCl	365
Sibai et al.	1989	P	16-27 wks	374	F	18	(13-25)	110/60	pregnant	asp-HCl	365

P = parallel group; C = crossover; wks = weeks; mos = months; yrs = years; M = male; F = female; BP = blood pressure; L = lying; S = sitting; i.c. = intracellular; asp = aspartate; chlor = chloride; Mg = magnesium. Lying blood pressure is presented when information was available

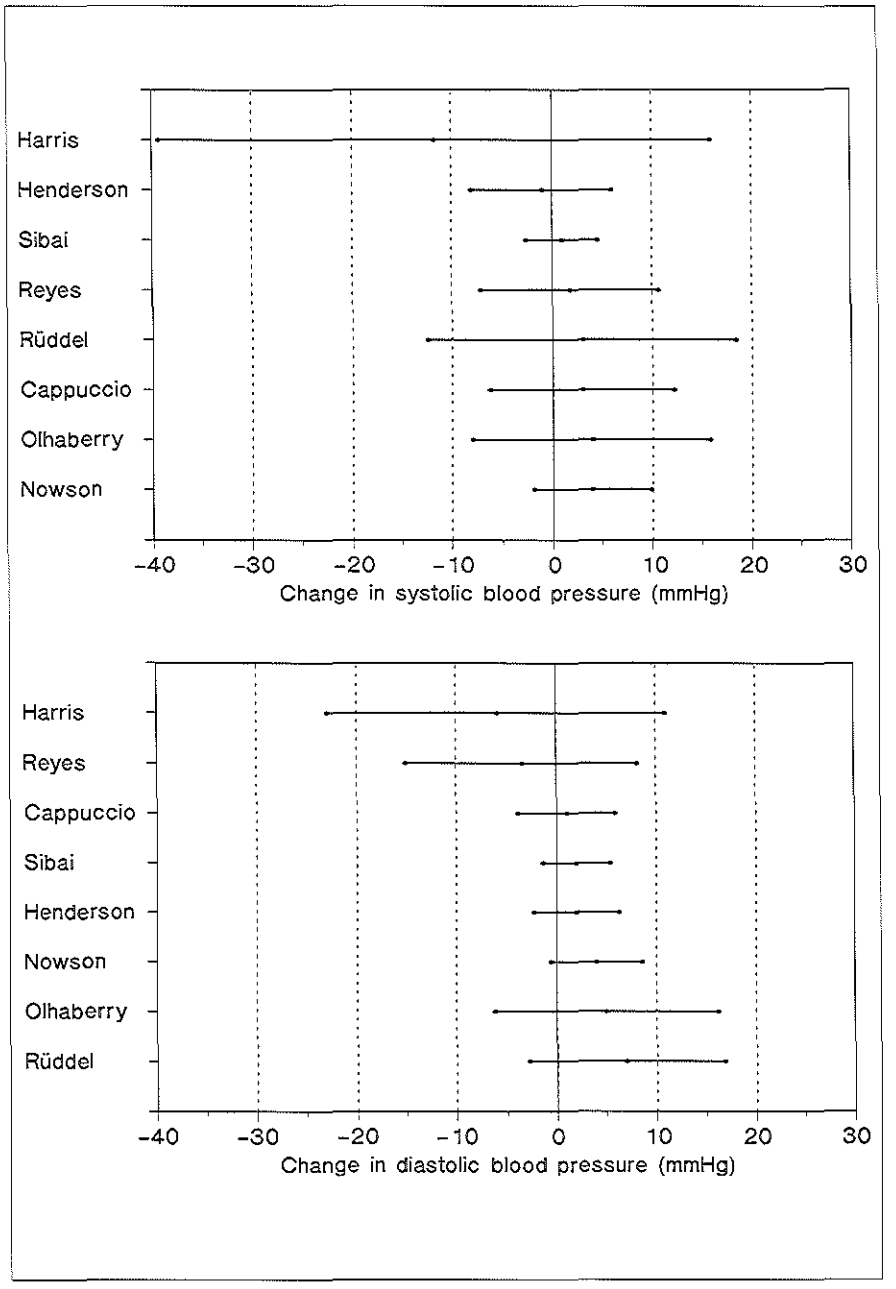

Figure 4.3.2. Effect of magnesium supplements on systolic and diastolic blood pressure (mean and 90% confidence interval) in double-blind placebo controlled studies. The figures are based on the difference in blood pressure change from baseline to final measurement between the placebo group and those receiving magnesium supplements. The use of this strategy for data presentation may result in discrepancies between numbers and inferences in the original papers and those presented here.

conceivable that calcium and magnesium supplements exert an effect differentially according to the specific underlying disturbances in metabolism of these minerals.

Calcium and magnesium homeostasis in hypertension

Parameters of calcium and magnesium homeostasis

URINARY EXCRETION OF CALCIUM AND MAGNESIUM

Results of studies of calcium loading in hypertensive and normotensive subjects have suggested that increased urinary calcium excretion reflects renal calcium leak rather than high dietary intake of calcium.[64] A positive association between urinary calcium excretion and blood pressure has been reported in several large population studies: in 9,321 men on active duty in Belgian military forces,[65] in 528 adults living in two Belgian towns,[66] in 415 adult Bantu of Zaire[67] and among male farmers of three communities in China.[68] The relationship was also observed by studies comparing hypertensive and normotensive subjects.[64,69] In contrast to the studies among adults, no relation of blood pressure with urinary calcium excretion was observed in young subjects.[70]

In steady state, urinary magnesium excretion usually reflects dietary intake. An inverse association between urine magnesium and blood pressure has been found among 3,363 men and 1,262 women in Belgian Army troops,[71] among 8,9 year old boys from 19 European centres,[70] and among male farmers of three communities in China.[68] No relationship, however, was found in other large population studies.[66,67,72]

SERUM LEVELS OF CALCIUM AND MAGNESIUM

A positive association of total serum calcium with blood pressure has been observed among 4,167 men and 3,891 women of a Belgian population sample[73] and among 9,321 men on active duty in military forces.[65] In studies comparing hypertensive and normotensive subjects, however, no such relationship could be found.[8,64,74] Levels of serum ionized calcium were either reduced in hypertensives[8,74] or were not different from normotensives,[64] a weak positive association between serum ionized calcium and blood pressure has been reported.[75]

An observation of low serum magnesium levels in subjects with hypertension compared to normotensive subjects was reported as early as 1958.[76] The relationship was confirmed in a study of 73 Danish men and women.[77] No relationship, however, was observed in later studies that compared hypertensive and normotensive subjects,[78] or examined the relationship in an adult population.[79]

INTRACELLULAR LEVELS OF CALCIUM AND MAGNESIUM

Intracellular levels of the divalent cations may be of more importance than serum or urine levels. It is well established that the intracellular free calcium level plays a central role in smooth muscle cell contraction.[80] Increased levels of free intracellulair calcium in platelets[81] and of calcium activity in erytrocytes[82] have been observed in hypertensive subjects. Also depletion of intracellular free magnesium has been found in erytrocytes of subjects with essential hypertension, which has been suggested to be associated with increased intracellular free calcium levels.[83] It is an attractive hypothesis that increased intracellular concentration of free calcium in the vascular smooth muscle cell is the intermediary factor in the association of low intake of calcium as well as of magnesium with elevated blood pressure. Turlapaty and Altura[84] demonstrated a increase in total exchangeable and intracellular calcium in rat aortic tissue after withdrawal of extracellular magnesium. This finding agrees with the observation of an elevation of smooth muscle cell tension after acute withdrawal of extracellular magnesium in animal experiments[85], performed in vitro.

It is possible that systems that are involved in the regulation of intracellular free calcium also modulate the effect of calcium and magnesium intake on blood pressure.

Systems involved in the regulation of intracellular calcium

IONIC MEMBRANE TRANSPORT

Extracellular free magnesium may alter ionic transport, as reviewed by Altura and Altura[85]: magnesium possibly competes with calcium for binding sites on the membrane of the vascular smooth muscle cell, magnesium may be involved in binding of calcium to intracellular organelles, and it may affect cell membrane permeability and stability. In addition to increasing vascular tone, a decrease in extracellular free magnesium has been shown to potentiate the contractile response of the vessel to vasoactive hormones; the latter may also be due to an enhanced influx of calcium into the cell.[86] Magnesium is known to be a important cofactor for activation of Na^+-K^+-ATPase activity, which plays an important role in determining vascular tone and reactivity. It is possible that a decrease in extracellular free magnesium may decrease Na^+-K^+-ATPase activity, finally resulting in an increase in intracellular free calcium.[85] Alternative contributing mechanisms could be a decreased formation of cyclic AMP within the cells or an inhibition of Ca^{2+}-ATPase activity at the membrane, which is also magnesium-dependent.[85] Low calcium intake is also suggested to decrease activity of Ca^{2+}-ATPase and Na^+-K^+-ATPase[87] but few evidence exist to date.

PARATHYROID HORMONE

The acute and long term actions of parathyroid hormone (PTH) differ to a great extent. Acute infusions of PTH in supraphysiological quantities induce vasodilation in several vascular beds.[88] Long term supplementation with PTH may elevate peripheral resistance and blood pressure in humans and in rats.[89] Several studies have shown elevated levels of circulating PTH in hypertensive subjects.[64,69,90,91] PTH may act by increasing the intracellular free calcium levels in a variety of cells.[92] Belizan et al found a positive association in pregnant women[32] and Grobbee and Hofman in young mildly hypertensive subjects[37] between the change in plasma PTH and the change in diastolic blood pressure associated with calcium supplementation.

Although calcium is the principal regulator of PTH, magnesium has also been observed to play a role. While extreme hypomagnesemia may inhibit the secretion of PTH, the regultion of PTH by magnesium generally seems to be similar to that of calcium, though its potency is much less. Both Cholst et al in pregnant women[93] and Resnick and Laragh in hypertensive subjects[54] found a significant decrease in plasma PTH level with magnesium administration.

RENIN-ALDOSTERON SYSTEM

Resnick[94] described two subgroups of hypertension according to the presence of a low or high plasma renin activity. In low renin hypertension there is an increased level of intracellular free calcium resulting from an influx of calcium from the extracellular calcium pool. Subjects are characterized by low plasma ionized calcium levels and high levels of plasma magnesium. In this condition, calcium supplementation may be beneficial. In high-renin hypertension, the increase in intracellular free calcium results from intracellular disposition of calcium between the bound and free state. Subjects are characterized by high levels of plasma ionized calcium and low levels of plasma magnesium. In this condition, magnesium supplementation may be beneficial.

Predictors of response to calcium and magnesium supplementation

Biochemical indicators

Urinary and serum levels of calcium and magnesium have only incidentically been found to be related to the reponse to supplementation. A high urinary calcium excretion,[45] and a low serum total calcium,[37,95] have been reported to favour responsiveness to calcium supplementation, but these could not be confirmed as predictors of response by other studies.[36,40,50] Rüddel et al. found low erythrocyte magnesium to be a prerequisite for an attenuated blood pressure response during stress following magnesium supplementation in labile hypertensives, but small subgroups were involved.[62] In this study no effect was

observed on causual blood pressure; it thus may be that blood pressure reactivity is especially sensitive to magnesium supplementation. Motoyama et al. observed that subjects with the lowest sodium efflux rate and the highest concentration of intracellular free sodium had the greatest benefit from magnesium supplementation.[53] In the study of Grobbee and Hofman, subjects with higher than median PTH levels had the greatest fall in blood pressure after calcium supplementation.[37] Accordingly, Lyle et al. found that subjects who responded to calcium supplementation had higher PTH levels compared to those that did not respond.[95] Resnick and Laragh observed that subjects with low-renin hypertension benefit preferentially from calcium supplementation and subjects with high-renin hypertension benefit from magnesium supplementation.[31,54] These findings could not be reproduced by several other studies,[36,41,56] but in most of these studies very small subgroups were compared. Among other biochemical indicators of response, serum oestrogen levels may be considered. Evidence from different sources indicate that oestrogens may be related to tissue retention of magnesium.[96] Postmenopausal women have been shown to have an increase in urinary magnesium excretion, which could be attenuated by use of substitution hormones.[97] In the Nurses' Health Study, the risk of hypertension associated with low dietary magnesium was highest among postmenopausal women who had never used substitution hormones. No such effect was shown for dietary calcium in this study.[21]

Intake of nutrients

Studies that evaluated the influence of baseline dietary intake of calcium or magnesium on blood pressure response to supplementation found no evidence for a modifying effect.[40,54] However, the estimates of dietary intake are crude which might obscure real relationships. A possible interaction between calcium and magnesium intake on blood pressure has been investigated in animals,[98] but no data are available from human studies. Resnick and Laragh found that subjects with high urinary sodium excretion tended to have the greatest decline in blood pressure after calcium supplementation.[31] Assuming that 24-hr urinary sodium extretion in part reflects sodium intake, the finding agrees with Resnick's postulate that high sodium diet suppresses renin activity, resulting in an increased intracellular calcium. Other calcium trials, however, observed no difference in effect according to level of baseline sodium excretion[36,42,50] or rather found the greatest blood pressure fall with calcium supplementation amongst subjects with a low sodium intake.[40] With respect to magnesium it is hard to predict whether the response to supplementation is conditional on salt intake. Two of the placebo controlled studies were performed in subjects on low salt intake.[59,60] Although these studies showed rather an increase than a fall in blood pressure with supplementation, power is lacking to discriminate the

results of these studies from those of other studies. Magnesium and potassium have been suggested to interact in many ways to control vascular tone and reactivity,[99] but no data on a possible modifying effect of potassium intake are availabe to date. In an open uncontrolled study, co-administration of 1,25-dihydroxycholecalciferol prevented, instead of supported, a blood pressure lowering effect of calcium supplementation.[100] Amongst other nutrients, alcohol consumption may be important. A high level of alcohol consumption has been shown to increase the urine excretion of magnesium.[101] Results of the Nurses' Health Study showed that the risk of hypertension associated with a low magnesium intake was greatest in subjects with the highest level of alcohol consumption.[21]

Conclusions

Although large population studies suggest a role of calcium and magnesium intake in hypertension, this is not supported by the majority of placebo controlled intervention studies performed to date. This may partly be due, especially in the case of magnesium, to the number of studies that were small or of short duration. The heterogeneity of hypertension suggests that there may be subgroups that are susceptible to supplementation. The results of some of the intervention studies seem to confirm this, but in most studies numbers were too small for a powerful evaluation of predictors of response. Future studies should be large enough to examine possible subceptibility of subgroups defined a priori. The choice of subgroups should be based on insights in pathofysiological mechanisms and should also take into account other factors, like intake of nutrients known to interact with calcium and magnesium metabolism.

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4.4 Reduction of Blood Pressure with Oral Magnesium Supplementation in Women with Mild to Moderate Hypertension*

Abstract

In a double-blind controlled trial, 91 middle-aged and elderly women with mild to moderate hypertension who were not on anti-hypertensive medication were randomly assigned to treatment with magnesium aspartate-HCl (20 mmol magnesium per day) or placebo for 6 months. Magnesium aspartate-HCl in the given dose was well-tolerated and was not associated with an increased frequency of diarrhoea compared to placebo. At the end of the study, systolic blood pressure had fallen by 2.7 mmHg (95% confidence interval -1.2 to 6.7) and diastolic blood pressure by 3.4 mmHg (1.3 to 5.6) more in the magnesium group than in the placebo group. The presence of a low plasma renin or a high plasma parathyroid hormone level at baseline enlarged the effect on systolic but not on diastolic blood pressure. Urinary magnesium excretion significantly increased in the magnesium compared to the placebo group. No changes were seen in other biochemical parameters, including serum levels of total and high density lipoprotein cholesterol. The findings suggest that oral supplementation with magnesium aspartate-HCl may be a safe and effective measure for lowering blood pressure in subjects with mild to moderate hypertension, who are not receiving anti-hypertensive treatment.

Introduction

Magnesium homeostasis has been related to blood pressure regulation on the basis of the importance of magnesium in cellular cation metabolism.[1] Defects in magnesium metabolism have repeatedly been demonstrated in animal and human hypertension.[2,3] An inverse association between magnesium intake and blood pressure has been reported in recent cross-sectional and longitudinal population studies.[4-6] It has not been determined, however, whether magnesium supplementation lowers blood pressure in hypertensive subjects. In an uncontrolled trial Dyckner and Wester found a decrease in blood pressure with magnesium supplementation in hypertensive patients on diurectic treatment.[7]

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* Submitted

Results of later studies are conflicting and the placebo controlled trials have generally not been supportive of an effect.[8-12] Studies published to date, however, were small and most were of short duration which precludes definite conclusions. We conducted a double-blind randomized trial to study the effects of 6 months of magnesium supplementation compared to matched placebo among 91 women with mild to moderate hypertension. The data were also examined for determination of characteristics that might predict blood pressure response to supplementation.

Subjects and methods

Population

Subjects were selected from women living in the Dutch town of Zoetermeer who had participated in several population-based studies on risk indicators for osteoporosis and cardiovascular disease.[13-15] All subjects had their blood pressure measured during a visit at the research center between 1985 and 1988. Those with a blood pressure of 140 and/or 90 mmHg or over and not receiving anti-hypertensive medication at the time of investigation were invited for remeasurement of blood pressure between September 1989 and Februari 1990. Blood pressure was measured at 2 successive visits at least 2 weeks apart. To be eligible for the trial, the average of the 2 measurements of systolic and diastolic blood pressure had to be 140 and/or 90 mmHg or over, but below 185 and 105 mmHg. None of the following conditions were allowed: a known secondary cause of hypertension, a history of major cardiovascular disease, insuline-dependent diabetes mellitus or chronic diarrhoea, major changes in dietary pattern in the recent past, use of anti-hypertensive drugs within the last 6 months and use of drugs known to interfere with mineral metabolism or platelet activity. Of 315 women who had their blood pressure remeasured, 119 did not fulfill blood pressure criteria, 60 met other exclusion criteria and 45 refused participation in the trial. This left 91 women, aged 35 to 77 years, who were randomized.

Trial design

After a 2-week placebo period subjects were assigned to 1 of 2 groups, receiving either 20 mmol magnesium per day as magnesium aspartate-HCl (Magnesiocard®, Verla-Pharm) or matched placebo in a double blind fashion for 6 months. Randomization was carried out within strata defined by original study population and blood pressure level at the last screening visit. All participants gave written consent to the protocol. Magnesium and placebo were provided as 4 sachetts of water-soluble powder per day to be taken with meals. Tablets with identical magnesium content were offered to some subjects on request mainly

for reasons of convenience. Subjects were asked to keep to their customary diet during the study period. All participants were contacted every 6 weeks to monitor the occurrence of possible side effect and to promote compliance with the protocol. Visits at the research center were made at 3 and 6 months.

Measurements

Blood pressure was measured after 3 and 6 months. To obtain a more stable estimate at the end of the trial, blood pressure was measured at 2 visits 1 week apart and the average of these readings was taken as the final estimate. Measurements were performed on the left arm with a random zero sphygmomanometer with subjects in sitting position after 10 minutes of seated rest. At every occasion a series of 3 readings was averaged. All measurements were performed by 1 trained paramedical assistant. Subjects were weighted at each visit wearing indoor clothing without shoes. Quetelet index was computed as weight devided by height square. Intake of nutrients was estimated at baseline by a validated food frequency questionnaire containing 175 food items.[16] The answers were checked during an interview with a dietician. Compliance with intervention was measured by count of sachetts and tablets returned at each visit. Non-compliance was defined as use of less than 80% of the sachetts or tablets. A venous blood sample and a 24-h urine collection was obtained at baseline and after 3 and 6 months. Serum and urinary electrolytes were measured by standard laboratory methods. Plasma intact parathyroid hormone (PTH 1-84) was determined using an immunoradiometric assay based on a combination of a polyclonal amino-terminal and a monoclonal mid-region antibody (IRMA).[17] Renin was measured by its capacity to generate angiotensin I from purified sheep renin substrate[18] and angiotensin I was quantitated by radioimmunoassay. Renin concentration is expressed as milliunits of the MRC human kidney renin standard. Laboratory analyses of all biochemical variables, except total and HDL-cholesterol, were performed at one point in time to exclude laboratory drift.

Thirteen subjects were noncompliant according to the present criteria, 6 on magnesium and 7 on placebo. Measurements after 6 months were performed in all subjects. Measurements at 3 months were not performed in 5 of the noncompliers.

Data analysis

The effect of magnesium supplementation on blood pressure and biochemical variables was examined by comparing the change from baseline levels between the magnesium and placebo group. The data were analysed first on an intention-to-treat basis and subsequently an analysis restricted to compliers was performed. In addition, analyses were performed in subgroups according to selected parameters of calcium and magnesium metabolism. Median values of the variables were

used as classification criteria. Intake of nutrients was adjusted for energy intake by regression of the nutrient intake on energy intake, as described previously.[6,19] For comparison between groups, means and the 95% confidence interval of the difference between groups is given. The relation between baseline variables was studied by linear regression analysis.

Results

Compliance and side effects

In the magnesium group an average of 91% of the sachetts or tablets was taken compared to 88% in the placebo group. Reasons for non-compliance (n=13) were initiation of anti-hypertensive drug treatment (1 women in the magnesium group), intercurrent illness not related to intervention (1 magnesium and 2 placebo), diarrhoea (1 magnesium and 2 placebo), and personal reasons not related to health (3 magnesium and 3 placebo). Among compliers, short episodes of diarrhoea were reported by 3 subjects on magnesium. Softer stools were mentioned by 21 (45%) subjects on magnesium and by 7 (16%) on placebo, but were not experienced as distressing. Side-effects perceived as beneficial and reported by subjects on magnesium were reduction of nervous tension (4 subjects) and alleviations of migraine (1 subject), of restless leg (1 subject) and of calf cramps (1 subject). None of the subjects on placebo reported clear beneficial side effects. Reports of minor symptoms were equally distributed over subjects on magnesium and placebo.

Baseline and follow-up measurements

The 2 groups did not differ significantly in age, Quetelet index, blood pressure and intake of nutrients at baseline (table 4.4.1). In addition, no significant differences were observed for baseline biochemical measurements (table 4.4.2). Baseline blood pressure level was not significantly associated with any of the biochemical variables after adjustment for age. No associations were seen of plasma PTH and renin with serum levels, urinary excretion and dietary intake of calcium and magnesium, except for a borderline significant association between renin and serum total calcium (ß = 0.0037 mmol/l/mU/l, SE = 0.0022, p = 0.10). Body weight, serum electrolytes, PTH, renine and total and HDL-cholesterol levels did not differ between the magnesium and placebo group after 6 months of intervention (table 4.4.2). Urinary magnesium was significantly higher in the magnesium compared to the placebo group at 3 and 6 months (figure 4.4.1).

Blood pressure response

After 6 months of intervention systolic blood pressure had fallen by 2.7 mmHg (95% confidence interval -1.2 to 6.7) and diastolic blood pressure by 3.4 mmHg

(1.3 to 5.6) more in the magnesium group than in the placebo group. No significant differences between the groups were seen after 3 months (table 4.4.3 and figure 4.4.1). When compliers were analysed separately (n=78), the difference in fall of systolic blood pressure was 2.6 mmHg (-1.6 to 6.8) and the difference in fall of diastolic blood pressure was 2.9 mmHg (0.6 to 5.2) after 6 months.

Table 4.4.1. Baseline characteristics by treatment group

	Placebo (n=44) mean (SD)	Magnesium (n=47) mean (SD)
Age (years)	57.1 (11.7)	57.4 (11.9)
Body weight (kg)	70.9 (10.5)	69.2 (11.0)
Quetelet index (kg/m^2)	26.4 (4.0)	26.3 (4.0)
Systolic BP (mmHg)	146.4 (11.2)	146.2 (13.6)
Diastolic BP (mmHg)	90.0 (7.0)	89.4 (6.7)
Pulse rate (beats/min)	73.4 (6.8)	73.4 (9.4)
Dietary intake		
Potassium (mg/day)	3623 (521)	3563 (616)
Calcium (mg/day)	1050 (295)	943 (299)
Magnesium (mg/day)	330 (54)	333 (80)
Energy (kcal/day)	1837 (350)	1865 (491)

Table 4.4.2. Body weight and biochemical variables at baseline and after 6 months

	Placebo (n=44)		Magnesium (n=47)	
	Baseline mean (SE)	6 months mean (SE)	Baseline mean (SE)	6 months mean (SE)
Body weight (kg)	70.9 (1.6)	70.7 (1.6)	69.2 (1.6)	69.1 (1.5)
Pulse rate	73.4 (1.0)	73.0 (1.2)	73.4 (1.4)	72.6 (1.2)
Serum (mmol/l)				
Calcium*	2.43 (0.01)	2.47 (0.02)	2.43 (0.01)	2.46 (0.01)
Magnesium*	0.85 (0.01)	0.84 (0.01)	0.86 (0.01)	0.88 (0.01)
Total cholesterol	6.53 (0.21)	6.42 (0.23)	6.62 (0.22)	6.55 (0.21)
HDL cholesterol	1.37 (0.04)	1.43 (0.06)	1.41 (0.04)	1.46 (0.05)
Plasma PTH (ng/l)	19.6 (0.9)	19.3 (1.2)	22.2 (1.4)	21.1 (1.4)
Plasma renin (mU/l)	9.0 (0.8)	8.7 (0.8)	9.4 (0.8)	9.7 (0.7)
Urine (mmol/24h)				
Sodium	136 (9)	124 (6)	123 (7)	115 (6)
Potassium	62 (3)	57 (3)	56 (2)	63 (3)
Calcium	3.9 (0.2)	4.7 (0.4)	4.2 (0.3)	4.9 (0.3)
Magnesium	3.3 (0.2)	3.4 (0.2)	3.5 (0.2)	5.2 (0.2)†
Creatinine	9.4 (0.3)	9.2 (0.3)	9.0 (0.3)	9.1 (0.2)

* Adjusted for total protein
† $p < 0.0001$, for change from baseline in magnesium compared to placebo group

Table 4.4.4 presents the changes in blood pressure in the magnesium group compared to the placebo group after 6 months within subgroups of renin and PTH. The effect on systolic blood pressure was most pronounced among women with a lower than median plasma renin, either when absolute renin level was used or when renin levels were expressed per mmol sodium excreted per

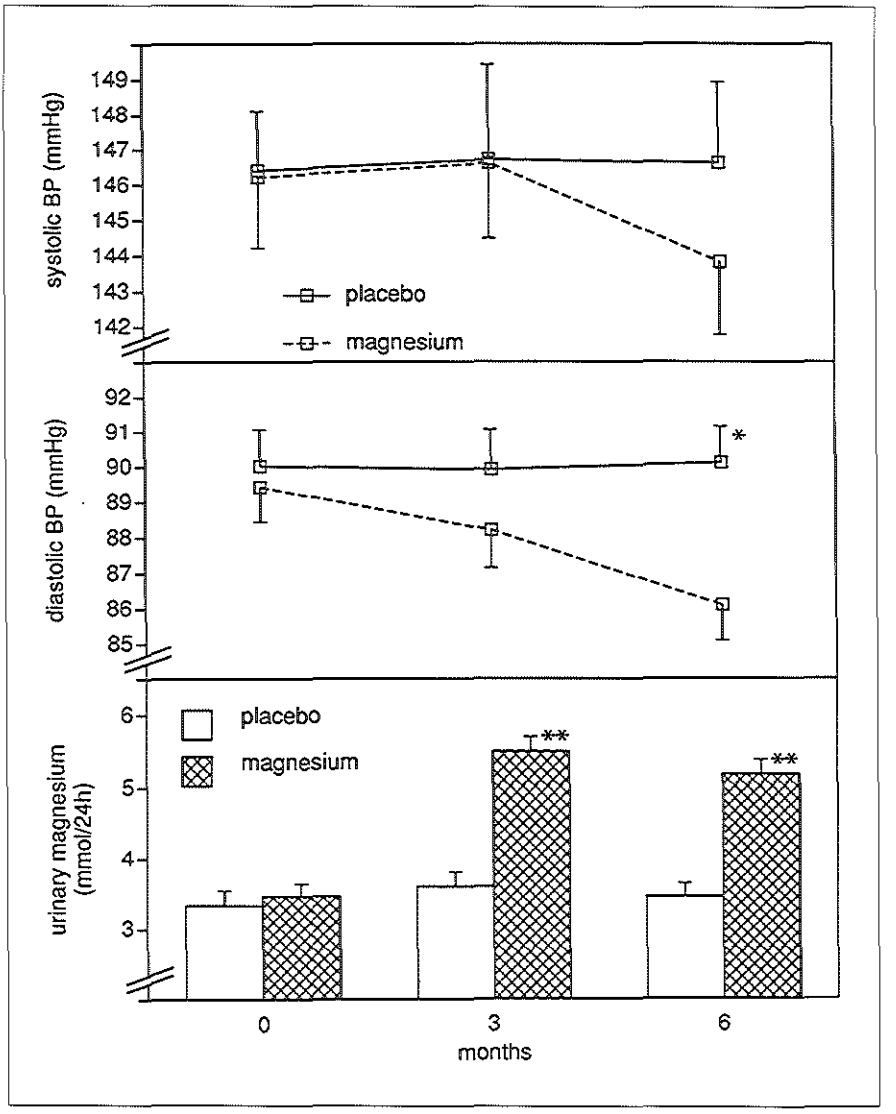

Figure 4.4.1. Means and standard error of systolic and diastolic blood pressure and 24-hour urinary magnesium excretion at baseline and during treatment for subjects on placebo (n=44) and magnesium (n=47).
Values at 3 months are missing for 5 noncompliers
* p = 0.003; ** p< 0.0001 for differences from baseline between groups

Table 4.4.3. Blood pressure at baseline and after 3 and 6 months

	Baseline mean (SD)	3 months* mean (SD)	6 months mean (SD)
Systolic BP (mmHg)			
Placebo (n=44)	146.4 (11.2)	146.7 (13.3)	146.6 (13.5)
Magnesium (n=47)	146.2 (13.6)	146.6 (14.4)	143.8 (14.0)
Difference (95% CI)†		0 (−4.8, 4.9)	−2.7 (−6.7, 1.2)
Diastolic BP (mmHg)			
Placebo	90.0 (7.0)	89.8 (8.0)	90.1 (6.9)
Magnesium	89.4 (6.7)	88.2 (7.1)	86.1 (7.0)
Difference (95% CI)†		−0.9 (−3.4, 1.6)	−3.4 (−5.6, −1.3)

* Values are missing for 5 noncompliers
† Differences in change from baseline between magnesium and placebo group
CI = confidence interval

Table 4.4.4. Change in blood pressure from baseline to 6 months within strata of plasma renin and plasma PTH

	Systolic blood pressure			Diastolic blood pressure		
	Placebo	Magnesium	Δ (95% CI)*	Placebo	Magnesium	Δ (95% CI)*
Renin (mU/l)						
< 8.3	2.8 (1.6)	−5.9 (2.3)	−8.7 (−14.2, −3.2)	0.9 (1.0)	−4.1 (1.5)	−5.0 (−8.5, −1.5)
≥ 8.3	−2.6 (1.6)	0.3 (2.1)	2.9 (−2.3, 8.1)	−0.7 (1.1)	−2.7 (0.9)	−2.0 (−4.8, 0.8)
PTH (ng/l)						
< 20.5	−0.2 (1.6)	0.3 (1.9)	0.5 (−4.4, 5.4)	1.0 (1.3)	−3.4 (1.1)	−4.4 (−7.7, −1.1)
≥ 20.5	0.7 (1.8)	−5.2 (2.4)	−5.9 (−11.8, 0)	−0.7 (0.8)	−3.2 (1.2)	−2.5 (−5.3, 0.3)

Values are means with standard error between brackets
Median values of renin and PTH are used to define strata
* Δ Difference in change from baseline between magnesium and placebo group
CI = confidence interval

24-hour period (change of -4.8 mmHg in magnesium compared to 0.9 mmHg in placebo, difference -5.7 mmHg, -11.2 to -0.2). The effect on systolic blood pressure was also more pronounced among women with a higher than median plasma PTH. Diastolic blood pressure reponse to intervention tended to be more stable across subgroups. Blood pressure responses were not clearly related to age and baseline levels of serum, urine and dietary magnesium. A borderline inverse association was seen between change in diastolic blood pressure and change in renin concentration in the magnesium group (ß = -0.39 mmHG/mU/l, SE = 0.23, p=0.10), but this could not significantly be discriminated from the association found in the placebo group. No associations were seen between change in blood pressure and change in PTH.

Discussion

In this study we observed a decrease in blood pressure after 6 months of oral magnesium supplementation in untreated women with mild to moderate hypertension. The effect was most pronounced for diastolic blood pressure, but a tendency for lowering of systolic pressure was present.

Before conclusions can be drawn, some issues need to be addressed. Participants were selected on the basis of blood pressure levels measured a few years apart and can therefore be considered to have persistent blood pressure elevation. Supplementation of 20 mmol of magnesium per day resulted in a more than 2-fold increase in daily intake which gave good contrast between the groups. The persistent elevation of urinary magnesium in the magnesium group indicates that magnesium was absorbed during the whole observation period. Magnesium supplementation was associated with an increased frequency of soft stools but not of prolonged diarrhoea, when compared to the placebo group. Compliance by count of sachetts and tablets was satisfactory. Analyses were based on the intention to treat principle, but results did not differ substantially when noncompliers were excluded.

A gradual decline in diastolic blood pressure with time was observed. Possibly, cells take up magnesium continuously during longer periods of supplementation, as has been reported for erythrocyte magnesium in a placebo-controlled study.[20] This could be due to a slow cellular uptake of magnesium, as has been observed for several tissues in animal experiments, combined with rapid urinary excretion.[21] The finding suggest that a suboptimal magnesium status was present at the start of the study. The magnesium status of an individual is hard to assess directly because serum and intraerythrocyte values do not adequately reflect the body pool of magnesium.[22] Thirty percent of study subjects had a magnesium intake below the recommended dietary allowance of 300 mg/day, but even at or above this level of intake healthy subjects have been found to be in a negative balance.[23-25]

A blood pressure lowering effect of magnesium supplementation has been reported in several uncontrolled studies,[7,26-28] but the result could not be confirmed by the majority of placebo controlled studies.[8-12] Most of these studies, however, were small and of short duration. In one controlled study among 40 untreated subjects with mild hypertension magnesium suppletion was accompanied by a gradual reduction of blood pressure which reached significance after 12 weeks.[29] This supports the view that a longer period of supplementation may be needed before an effect can be detected. The importance of baseline magnesium status as a predictor of blood pressure response to supplementation is, as yet, not clear. Two longer-term trials in hypertensive subjects on diurectic treatment, which is considered to lower magnesium status, are conflicting.[7,9]

Several mechanisms to explain a hypotensive effect of magnesium have been proposed. Magnesium may affect uptake and intracellular disposition of calcium ions in the vascular smooth muscle cell by calcium entry antagonizing properties.[1,2] In addition, attenuation of stress-induced noradrenaline release by magnesium supplementation has been suggested.[30] Preliminary reports by Resnick et al have indicated that magnesium administrated intramuscularly lowers diastolic blood pressure singularly in hypertensive subjects with high plasma renin activity.[26,31] We could not confirm this for the effect on diastolic blood pressure and rather the opposite was found for the effect on systolic blood pressure. This casts doubt on the validity of the proposed modifying effect of renin. Considering the conceived calcium entry blocking effect of magnesium, we hypothesized that subjects with a relatively high plasma PTH, which may reflect increased intracellular calcium,[32] would respond better to magnesium supplementation. This was indeed observed for systolic blood pressure, but not for diastolic blood pressure. We have no explanation for this differential response. Plasma PTH level has been shown to fall rapidly in response to magnesium infusion.[33] In the present study, no longterm effect of oral magnesium supplementation on plasma PTH could be detected.

Anti-hypertensive medication, in particular diuretics and beta-blockers, have been suspected of increasing serum cholesterol.[34] Magnesium supplementation has been observed to improve the lipid profile in subjects with ischemic heart disease or lipid abnormalities,[35,36] though a neutral effect on cholesterol was observed in healthy subjects.[37] The latter observation is confirmed by the results of the present study in which no effects on serum total cholesterol and serum HDL-cholesterol were observed. In summary, the findings of this longer-term study suggest that oral supplementation with magnesium aspartate-HCl may be a safe and effective measure for lowering blood pressure in subjects with mild to moderate hypertension, who are not receiving anti-hypertensive treatment with drugs.

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CHAPTER 5

GENEREAL DISCUSSION I

THE PRESENT STUDY AND NEW RESEARCH AREAS

5. General Discussion I

The Present Study and New Research Areas

Introduction

Cardiovascular disease is a major cause of morbidity and mortality in both sexes. At age 40 to 60 the mortality from ischaemic heart disease is about 5 times higher in men compared to women. After this age the relative difference

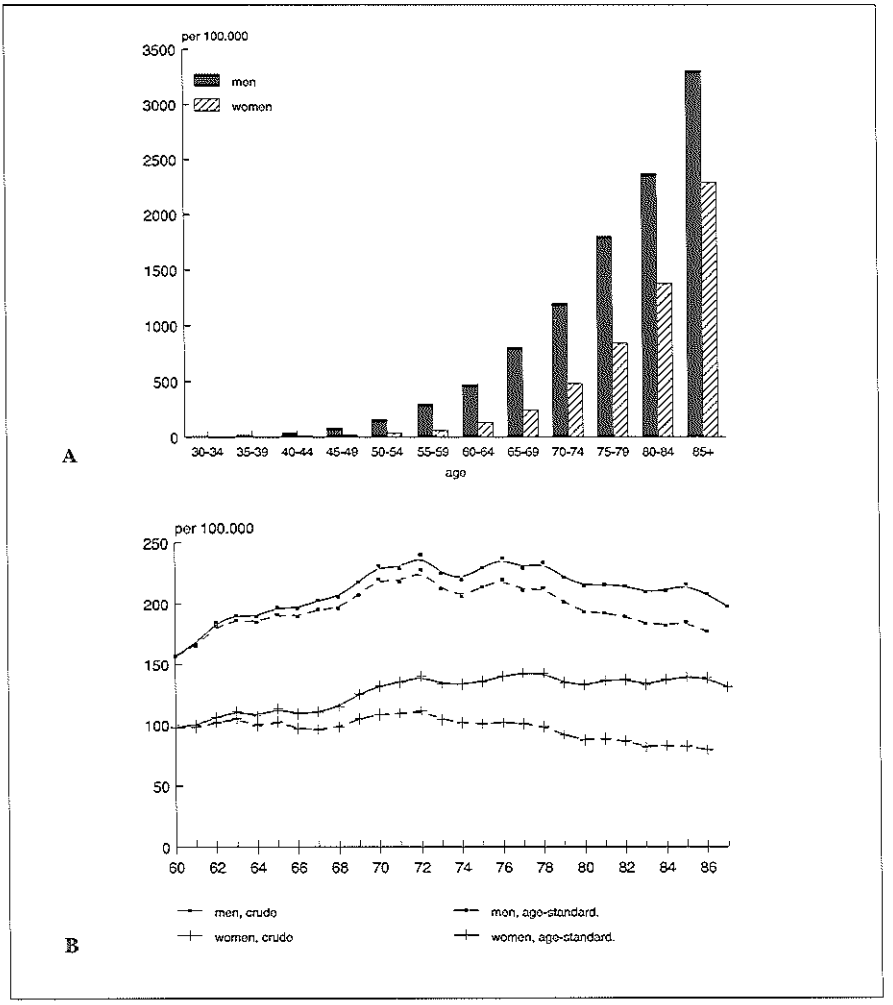

Figure 5.1. Ischaemic heart disease mortality in men and women in the Netherlands: A according to age (1987), B from 1960 to 1987. Source: Stolk, CBS 1987

between the sexes starts to decrease, though the mortality remains lower in women at all ages. The age-standardized mortality rates of ischaemic heart disease in men and women began to decline around 1972. In men, the crude mortality rate also declines. Because the increased longevity in the last decades especially affects the number of older women, the crude mortality rate in women does not decrease (figure 5.1). This makes cardiovascular disease in women a growing public health problem.

This thesis focusses on cardiovascular disease in women. The shortcomings and merits of the presented studies have been discussed in the previous chapters. In this chapter the findings will be placed in the broader context of the levels of research that can be distinguished in the study directed towards primary prevention of cardiovascular disease (figure 5.2). In addition, new areas of research will be delineated.

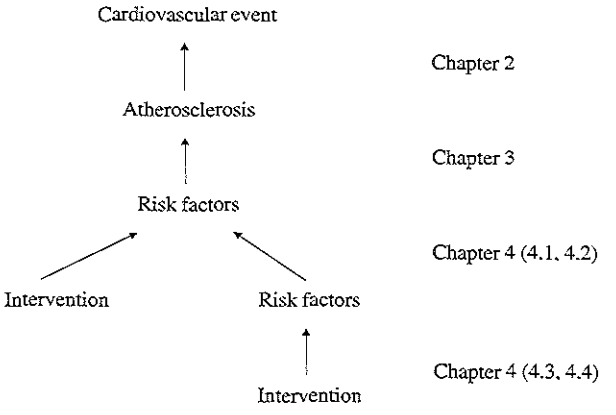

Figure 5.2. Levels of research

Atherosclerosis and cardiovascular disease

Traditionally, epidemiologists have studied cardiovascular disease by examination of the relation between potential risk factors and the presence or occurrence of cardiovascular events. Over the past 40 years, this has resulted in the detection of several important cardiovascular disease risk factors.[1] Yet, the use of the discrete event as study outcome has two disadvantages. The first is that cardiovascular events result from a combination of processes, which makes understanding of the etiology more difficult. The second is that the event reflects a near end-stage of disease, which prohibits the study of risk factor effects at earlier stages of disease. The study of determinants of underlying processes will enhance understanding of the etiology. Atherosclerosis is the major underlying

process. Most information on determinants of atherosclerosis has been derived from autopsy and angiographic studies. The contribution of these studies to the knowledge of the atherosclerotic process has been described in detail.[2,3]

To study atherosclerosis non-invasively in asymptomatic, non-hospitalized subjects it is necessary to rely on vessels other than the coronary and cerebral arteries. Atherosclerosis in these vessels needs to represent a generalized process. This can be ascertained by examination of the correlation between atherosclerosis in different vessel beds at autopsy, or by examination of the association of atherosclerosis in one vessel with the presence of symptomatic atherosclerotic disease in other vessel beds.[4] In the studies presented in this thesis aortic calcifation was used as a measure of atherosclerosis. Aortic calcification has been shown to represent intimal atherosclerosis by comparison with necropsy material.[5] Its association with atherosclerosis in other vessel beds, as observed in autopsy studies* and in a cross-sectional study in the general population, has been described in chapter 2. We prospectively examined the association of aortic calcification with cardiovascular morbidity and mortality. Follow-up of participants of the EPOZ Study aged 45 years and over (1,359 men and 1,598 women) demonstrated that calcification of the abdominal aorta is associated with an increased risk of cardiovascular mortality (chapter 2.1). Subsequently, analysis of longitudinal data of the Framingham Study (2,336 men and 2,873 women) showed that calcification of the thoracic aorta is associated with clinically manifest atherosclerotic disease at several sites (chapter 2.2). These findings support the view that aortic atherosclerosis reflects a generalized process. That the measure suffices for the study of determinants of atherosclerosis is supported by the finding that similar risk factors predispose to atherosclerotic disease at various sites.[6,7] This confirms the view that, in concert with local factors, a general pathogenetic mechanism is present.

Determinants of atherosclerosis in women

Conventional risk factors

Most studies on cardiovascular disease have been performed in men. Elevated blood pressure, elevated serum cholesterol and cigarette smoking have been identified as the major cardiovascular risk factors.[8] It cannot be assumed that the magnitude of the risk associated with these factors and their interactions are the same in men and women. Physiological differences between the sexes may modify the effect of risk factors.[9,10] The changes of blood pressure and serum

* Although the extent of aortic compared to coronary atherosclerosis differs between the sexes (which is in itself interesting, though unexplained, see chapter 2.1), the correlation between aortic and coronary atherosclerosis is similar in men and women.[6]

cholesterol with age are different in women compared to men[1], which results in gender differences in life-time accumulated exposure at any age. The Framingham Study has provided most data on the risk of cardiovascular disease in women.[1,11] Additional information has been provided by a number of prospective follow-up studies[12-20], some of which were confined to women. The studies have generally shown that the relative risks associated with blood pressure and smoking are similar in men and women. In contrast to findings in men[21], present studies suggest that only relatively high levels of serum cholesterol are associated with an increased cardiovascular risk in women, while HDL cholesterol may possibly be more important.[22]

Problems with interpreting the association between exposure to risk factors and cardiovascular disease events have been described in the previous paragraph. Additional limitations in studies among women are the generally low event rates and the lower specificity of some of the endpoints.[23] In this thesis we used data on aortic calcification to study determinants of progression of atherosclerosis in women. The research questions were: (1) Do the recognized risk factors (elevated blood pressure, elevated serum cholesterol and cigarette smoking) predict progression of atherosclerosis in women? (2) Are characteristics unique to women related to progression of atherosclerosis? The first question was examined in a group of 855 women, initially aged 45 to 64 years, who were followed for 9 years.

Moderately elevated and high levels of systolic and diastolic blood pressure were associated with an increased risk of atherosclerotic progression. In addition, a low diastolic pressure was associated with increased progression, which may reflect the presence of a hardened vessel wall. This may be caused in part by atherosclerosis and is likely to show further progress during follow-up. The risk of atherosclerotic progression increased with increasing baseline cholesterol at levels beyond 6.0 mmol/l. This suggests that also moderately elevated levels of cholesterol are atherogenic. During follow-up, cholesterol rose on average by 0.11 mmol/l per year. The contribution of the rise in cholesterol to risk of atherosclerotic progression was lower than expected. Possibly, lifetime accumulated exposure is more important for determination of the atherosclerotic risk. Cigarette smoking was associated with an increased risk of progression in a dose-dependent manner, which did not fully return to the risk of never smokers up to 10 years after quitting. The findings suggest that cigarette smoking, besides acute effects, may also have prolonged effects on the arterial wall. Smoking of 10 cigarettes per day or more doubled the risk of atherosclerotic progression. The magnitude of this effect is comparable with that of a systolic blood pressure level of 160 mmHg or higher versus a level lower than 120 mmHg and with that of a serum cholesterol level of 6.0 mmol/l or higher versus a level lower than 5.0 mmol/l. The risk factor effects were

independent of each other and none of the effects required a critical level of another risk factor. Methodological considerations will be discussed in chapter 6. Clinical and public health implications of the findings will discussed in the next paragraph.

Risk factors unique to women

Of risk factors unique to women endogenous and exogenous estrogen have received most attention (reproductive and menstrual history, pill use and use of postmenopausal hormones).[24] It has long been assumed that the loss of estrogen production with menopause accounts for the marked increase in risk of cardiovascular disease in women, as has been demonstrated in women with surgical menopause.[25] An increased risk of cardiovascular disease with menopause, however, could not be observed in several large prospective follow-up studies, and this has cast doubt on the importance of natural menopause.[24,26] All studies, however, have the inherent difficulty that a change in incidence of cardiovascular disease will not be seen immediately but only 10 to 20 years later, by which time the effects of menopause are hard to distinguish from the effects of ageing. We studied the effects of menopause on atherosclerosis cross-sectionally among 294 premenopausal and 319 postmenopausal women, aged 45 to 55 years, who participated in the EPOZ study (chapter 3.4). A strong association was found between the menopause, either natural or surgically induced, and the presence of aortic atherosclerosis. These findings suggest an increased rate of atherosclerosis in women after the menopause.

Intervention

The ultimate aim of the search for determinants of cardiovascular disease is prevention of the cardiovascular event. The relatively strong and modifiable risk factors, elevated blood pressure, elevated serum cholesterol and cigarette smoking have been considered most suitable for intervention. Attention is growing for the potentially protective effects of postmenopausal estrogen use.

Elevated blood pressure

ANTI-HYPERTENSIVE DRUGS

Anti-hypertensive treatment in mild to moderate hypertension has been proven to be effective for lowering the risk of stroke. The effect on coronary heart disease, however, has been disappointing.[27] This may be because some of these drugs have untoward side effects such as an increase in the concentration of serum lipids.[28] The effect of anti-hypertensive treatment on coronary heart disease in women has been observed to be even lower than in men[29] or

undetectable[30], though care should be taken with the interpretation since the number of events in women were small and the follow-up period may have been too short. Nevertheless, the findings suggest that in women with mild to moderate hypertension, the benefits of drug treatment may not outweigh the potential side-effects. Therefore, non-pharmacological treatment may be preferable. One of the possibilies for non-pharmacological intervention is to promote dietary change.

NUTRITIONAL FACTORS AND HYPERTENSION

Research on the association of diet and hypertension has long focussed on the potentially adverse effect of excess sodium intake. Adverse effects have also been indicated for alcohol consumption and high body weight. Potentially protective effects have been suggested for intake of potassium, calcium, magnesium, fiber and polyunsaturated fatty acids (for discussion of the evidence, see chapter 4.1). Using 4-year follow-up data of the Nurses' Health Study, we prospectively examined the intake of these nutrients, except for sodium, in relation to the incidence of hypertension among 58,218 women, aged 34 to 59 years. Relative weight and alcohol consumption were the strongest predictors of the development of hypertension. Reduction of body weight is universally recommended, though an effect of weight reduction on blood pressure needs further confirmation. Alcohol consumption of 2 glasses per day or more increased the risk of hypertension in a dose-dependent manner. Whether women with moderate alcohol consumption (2 to 3 glasses per day) should be adviced to reduce their consumption is debatable considering the possibility of beneficial effects of moderate alcohol consumption on the occurrence of cardiovascular disease.[31] Women with hypertension, however, who drink 2 glasses of alcohol per day or more will probably benefit from decreasing their alcohol consumption. Dietary calcium and magnesium were the only nutrients inversely related with risk of hypertension. The associations of calcium and magnesium with blood pressure confirm the findings of previous cross-sectional studies (see table 4.3.1). Definite evidence of whether an increase in intake of calcium and magnesium results in blood pressure reduction has to be provided by intervention studies.

CALCIUM AND MAGNESIUM INTERVENTION

A review of the results of reported calcium and magnesium intervention studies has been given in chapter 4.3. Calcium has been studied in a large number of intervention studies. The results are not unanimously supportive of the calcium hypothesis. This may be due to a possible heterogenity in response, as observed in some of the studies. In none of the studies were findings among postmenopausal women considered separately. Few placebo controlled studies

examined the effect of magnesium on blood pressure. Those reported did not support a blood pressure lowering effect of magnesium, but studies were generally small and of short duration. Because of the paucity of data on the effect of magnesium, we performed a randomized controlled magnesium intervention study among 91 women with mild to moderate hypertension. The results suggest that magnesium may be a safe, and effective measure for lowering blood pressure in women with mild to moderate hypertension. We were not able to identify clear modifiers of response, including menopausal status, but numbers in subgroups were small. Future studies should preferably be large enough to examine possible susceptibility of subgroups, defined a priori.

Elevated serum cholesterol

While the need for therapeutic measures in those with very high levels of serum cholesterol is generally agreed upon, the need and methods for lowering moderately elevated levels is controversial. None of the intervention studies on the cardiovascular effects of cholesterol lowering by dietary measures or by drugs included women. Nevertheless, a joint statement of the American Heart Association and the National Heart, Lung, and Blood Institute states that it is justified to consider the same guidelines appropriate for both men and women.[32] The cardiovascular effects of moderately increased cholesterol levels, however, are not yet established for women.[22] Furthermore, as suggested by the findings of our study (chapter 3.2), a cholesterol level that is partially explained by a recent increase, as is the case in postmenopausal women, may not carry the same risk as similar levels observed in men. Therefore, it is not appropriate to base the quidelines for intervention in women on studies performed in men. The public health importance of this issue is underlined by the relatively high percentage of women with an elevated cholesterol level according to the current Dutch Cholesterol Consensus[33], as can be derived from the data presented in chapter 3.2.

Smoking

In the last decades, the percentage of smokers has decreased in men, but not in women.[34] Besides, young women have adopted the more intensive smoking habits of men.[35] Lately, women and the young have become the target of the tabacco industry as 'replacement smokers'.[36] This points to the need of directing special attention to smoking behaviour in these groups. The suggestion of lasting effects of smoking on the blood vessel wall, as observed in our study (chapter 3.3) underlines the need to stop smoking at an early age.

Postmenopausal estrogens

The hypothesis that loss of estrogen production with menopause increases cardiovascular risk in women has led to interest in the potential beneficial effect of postmenopausal estrogen therapy. Large prospective follow-up studies have shown a protective effect of postmenopausal estrogen use on the incidence of coronary heart disease.[37] Intervention studies for confirmation of the finding have not yet been performed. A randomized controlled trial of estrogen therapy has been suggested to evaluate the potential beneficial effects on cardiovascular disease and osteoporosis and the potential adverse effects on endometrium cancer and breast cancer, and to evaluate the effects of addition of progestins.[38]

New research areas

Measurement of atherosclerosis

In the studies presented in chapter 3, progression of atherosclerosis was measured by radiographic detection of calcified plaques, which represents advanced atherosclerosis. Quantification was based on visual examination. Recent developments in ultrasound techniques have made it possible to measure early stages of atherosclerosis in peripheral vessels.[39,40] The accuracy of the measurement also permits reliable detection of small changes in the vessel wall over time.[41] That atherosclerosis in the peripheral arteries reflects a more generalized process is supported by the observation of its association with the presence of atherosclerosis in other vessel beds.[42,43] In time, it may become possible to examine the coronary arteries invasively with ultrasound or non-invasively with magnetic resonance. This would enable the study of earlier stages of coronary disease than is currently possible with angiography.[44] Furthermore, the new techniques will make it possible to specificy of the type of lesion and to study its determinants and predictive value.*

The use of the measurement of atherosclerosis in observational and experimental studies requires increased research activity at a higher level, as illustrated in figure 5.2: in order to estimate the clinical relevance of observed vessel wall changes, the relation between these changes and the occurrence of cardiovascular events needs to be quantified. Concurrently, the presence of atherosclerosis in peripheral vessels may itself be considered as a cardiovascular risk factor, as discussed for aortic atherosclerosis in chapter 2. This upward movement in the determination of the risk factor, however, should not be accompanied by an upward movement of the point of application of primary intervention, which

* In the studies presented in this thesis calcified plaques were viewed as an advanced stage of atherosclerosis, the etiology of which was not considered to be different from that of earlier stages of disease. It is conceivable, however, that advanced lesions or the calcification per se has its own determinants.

would mean increased intervention by pharmacological treatment. When the risk factor is associated with mild or moderately elevated risks of cardiovascular disease, the benefits of pharmacological treatment may not outweigh the harm caused by potential side-effects. The research activity, therefore, should also go downwards: as discussed by Rose, we should search for and intervene on the causes of causes.[45]

Another research area that is also developing rapidly and shows much promise is that of hemostasis. Hemostatic factors trigger the event in vessels already affected by atherosclerosis, but may also be involved in the process of atherosclerosis.[46] Attention for the predictive value of hemostatic factors and its determinants is growing among epidemiologists, supported by the development of reliable biochemical assays.[47] Finally, the expected reduction in risk of cardiovascular disease will probably improve when the effects of intervention on atherosclerosis and thrombosis can be examined. However, when a drug or non-pharmacological measure for a common risk factor is concerned for which relatively large parts of the population might possibly be indicated, intervention studies with the cardiovascular event as outcome, besides other disease endpoints and total mortality, will probably always be nessecary.

Future studies in women

Many questions are still unanswered with respect to the effects of conventional risk factors in women. What are the causes of the rise in blood pressure and serum cholesterol in women after middle-age? What are the determinants and cardiovascular effects of isolated systolic hypertension and why is this condition more prevalent in older women than in older men? Is the association of cholesterol with cardiovascular disease in women a threshold relationship? Does the rise in cholesterol in women after middle-age contribute to the risk of cardiovascular disease? What are the relative importances of HDL cholesterol, triglycerides and other lipids in women? What will happen with the cardiovascular risk associated with smoking in women, now young women have adopted the smoking habits of men? Is part of the cardiovascular risk associated with smoking in women explained by an anti-estrogenic effect, and will body fat protect against these effects in postmenopausal women?

Besides the conventional risk factors other potential determinants of cardiovascular disease have recently received attention. Insuline resistance has been put forward as a potentially important risk factor.[48] Diabetes mellitus is the only risk factor whose relative effect is stronger in women than in men, which may give information about the causes of the female protection for cardiovascular disease.[49] Insuline resistance has been found to cluster with elevated blood pressure and lipid abnormalities.[48] Abdominal obesity and increased levels of serum androgens in women have been suggested to be

associated with the several of these risk factors[50,51], but the biological mechanisms underlying the associations need further investigation. In general, studies in women will benefit from the opportunity of non-invasive measurement of atherosclerosis because accurate risk assessment will no longer be hampered by low rates of cardiovascular events.

The non-invasive measurement of atherosclerosis allows to follow the course of atherosclerosis during specific periods in life. One of the interesting points in time is the menopause, of which the cardiovascular effects are still relatively unknown. As discussed in the previous paragraph, our cross-sectional study suggested an increased rate of progression of atherosclerotic lesions in women after the menopause. The best approach for further study would be to follow women throughout the years of menopause and to examine the changes in cardiovascular risk factors in relation to progression of atherosclerosis.

Finally, as discussed above, the effects of postmenopausal estrogens need further investigation. Examination of the effects of different combinations of estrogen and progestin on progression of atherosclerosis enables selection of the most promising agents to be tested in large trials on cardiovascular events. The ultrasound technique may also allow evaluation of the potential direct effects of estrogen on the vessel wall[52], which have received little attention to date. Estrogen replacement therapy should not be considered as the only means for prevention of the potential effects of estrogen loss. When biological changes occurring at the menopause and their cardiovascular effects can be detected, preventive measures could be directed towards these factors.

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CHAPTER 6

GENERAL DISCUSSION II

METHODOLOGICAL CONSIDERATIONS

6. General Discussion II
Methodological Considerations

Introduction

It has been proposed that progress in the understanding of the epidemiology of diseases depends to a large degree on the incorporation of new leads and methods from other disciplines in epidemiologic research.[1] The enthusiasm shared by epidemiologists for recently developed methods for non-invasive assessment of the process of atherosclerosis supports this view.[2] Large follow-up studies have been started in which atherosclerosis in peripheral vessels rather than its cardiac or cerebral sequelae is a major outcome variable.[3,4] This shift from research into the determinants of the final event to a search for determinants of the underlying process of the disease, is likely to have implications for the design as well as for the analysis of epidemiological studies.

Epidemiology has been defined as the study of the distribution and determinants of disease frequency in human populations.[5] This definition is still applicable in the study of a disease process. The object of research than becomes the change in disease status over time as it relates to determinants and the distribution of changes in populations.[6] Nevertheless, the current methods and practice in epidemiological research are primarily based on the all-or-none outcome of disease. The move from epidemiology of infectious diseases to epidemiology of chronic diseases, though accompanied by increased attention for the latency period, did not change the measure of the outcome as an all-or-none event. The disease process as outcome variable has two characteristics, its time component (status or change) and its measurement scale. Consequences of these features for study design and data analysis are discussed below.

Design considerations

Textbooks on epidemiology distinguish follow-up, case-control and cross-sectional studies. In this, the case-control study can be viewed as an efficient alternative to following up a complete population yielding essentially similar information.[6] In the study of the disease process, which will generally require the follow-up of the whole population, the efficient case-control study is no longer an option. The study of the disease process is not quite analogous to the study of incidence in cohorts. In the latter, the events occur within the follow-up

period. In the study of the process of disease, realizations at the end of the period are examined instead of events within it.[6]

Pre-disease exposure information

One of the major advantages of the follow-up study has been described by Breslow and Day as "the availability of pre-disease information on biological variables".[7] Although the possibility that levels of exposure may be influenced by a preclinical disease state is acknowledged, this possibility is usually not given extensive consideration. The potential difficulties with the interpretation of the order of exposure and disease may be more easily recognized in the study of the disease process: in chronic diseases, like atherosclerosis, a pre-existing state of the disease at baseline is often present. If the pre-existing state of disease is a major determinant of its further development, interpretation of the observed association between baseline exposure and progression of disease is not straightforward. Three different situations are given below.

First, the pre-existing state of disease may be determined by lifetime accumulated exposure. If the baseline level of exposure is associated with lifetime accumulated exposure, an observed association of baseline exposure with subsequent progression of disease may reflect the effect of accumulated exposure rather than the effect of baseline exposure level. For example, the level of atherosclerosis at age 65 may be determined by lifetime cholesterol exposure. In the situation that a) the level of cholesterol at age 65 is associated with lifetime cholesterol exposure and 2) the baseline level of atherosclerosis is an independent determinant of subsequent atherosclerotic progression, an observed association between baseline cholesterol level and progression of atherosclerosis might be spurious. The change in cholesterol may have a weaker association with lifetime exposure than baseline cholesterol level. This may in part explain why the relation of atherosclerotic progression with change in cholesterol was much weaker as compared to the relation with baseline cholesterol level (chapter 3.2).

Second, when the pre-existing state of disease affects the level of the exposure, the observed relation between baseline exposure and progression of disease may not be causal. For example, systolic blood pressure may increase the rate of atherosclerotic progression and atherosclerosis may elevate systolic blood pressure. The association between systolic blood pressure and subsequent progression of atherosclerosis is therefore still difficult to interpret (chapter 3.1). Adjustment for baseline level of disease would address this problem. However, adequate adjustment may not always be possible because of a limited sensitivity of the measurement technique for the detection of early stages of disease. Besides, adjustment for baseline level may introduce spurious results. This is described below with respect to change in the determinant (paragraph 6.4), but

also applies to change in the outcome variable. The problem will be less, however, when the variable measured is relatively stable, which probably holds for atherosclerosis.

A special situation exists when the effects of exposure on disease and vice versa have opposite signs. For example, diastolic blood pressure may increase the rate of atherosclerotic progression, while the presence of atherosclerosis may decrease the diastolic blood pressure. In this situation, it may be possible to disentangle the two effects, as is illustrated by our interpretation of the observed J-shaped relationship between baseline diastolic blood pressure and atherosclerotic progression (chapter 3.1). Because baseline blood pressure level results from both short-term and long-term experiences, the relation between change in diastolic blood pressure and progression of atherosclerosis may give even more information.

In conclusion, a careful interpretation of the data with respect to cause and effect relationships is warranted, even when the baseline level of the exposure is related to subsequent progression of disease.

Status versus change

The advantages of the use of incidence above prevalence of disease as the study outcome have been well described.[6-8] Although there is some analogy of this with the use of status versus change or progression of disease, it cannot be hold that the study of change or progression is to be preferred in all situations above the study of disease status. The latter is preferred in the case of a stable, chronically active determinant presumed to have a cumulative effect.[6] For example, in the described study of smoking and atherosclerosis the interpretation would probably not have been different if we had examined the association between cigarette smoking and baseline atherosclerotic status in stead of the association with 9-year progression of atherosclerosis (chapter 3.3). In fact, the former analysis resulted in similar effect estimates. When the disease affects the determinant, as in the examples of blood pressure given above, the relation of baseline exposure with change or progression is preferred because of its longitudinal character, but difficulties with the interpretion may still be present, as discussed previously.

Data analysis

Measures of association and statistical analysis

The options and properties of the measures of association in the study of event rates are well described, yet little attention has so far been given to the approach when other types of outcome are studied. The disease process can be evaluated using a status or a change variable. Both can be measured on a nominal (e.g.

all-or-none), ordinal or interval scale. Implications for data analysis and measures of association of these different types of outcomes are described below.

THE OUTCOME AS A DICHOTOMOUS VARIABLE

For a dichotomous variable (e.g an all-or-none outcome), the absolute and relative risk are the primary measures of effect in epidemiological studies. Their merits are given by their intelligibility. They quantify the relative of absolute increase in risk per unit change in the determinant. The statistical analysis and interpretation of the model parameters in the study of a disease process, even when dichotomized, may not be similar to that in a study of rare diseases. Implications for the relative risk are given as an example.

The use of the odds-ratio to approximate the relative risk, enables us to use the binomial logistic regression model for multivariate analyses. One condition for the odds-ratio to approximate the relative risk is that the rate of disease is relatively low.[5] This has been the case for most of the chronic diseases studied to date, but for disease processes, broken into two categories (e.g. progression versus no progresson) relatively high rates will be common. In this case the logistic regression analysis still provides a valid estimate of the odds-ratio but will overestimate the corresponding relative risk. An alternative model that directly estimates the relative risk has been described in chapter 3.3, but limitations of this model have been given.

The percentage of subjects with disease in a high risk category can not exceed 100. Therefore, the relative risk becomes dependent on the prevalence or incidence of the disease when the latter are relatively high, as will be common in the study of a disease process. The relative risk estimates will therefore no longer be in the range that we are used to when studying rare chronic disease events. Furthermore, as will become clear in the next paragraph, if the risk factor affects disease progression, it will generally be true that the further down the scale the cut-off is positioned, the stronger the risk estimate will be. Because the choice of the cut-off level may be more dependent of subjective matters and sensitivity of the measurement techniques, than of well described characteristics of the disease process itself, comparison of the size of the risk estimates between studies will become difficult.

THE OUTCOME ON AN ORDINAL SCALE

When the outcome is measured on an ordinal scale, we might want to use ordered outcome categories to increase the use of information present in the data. Analyses of this type have been performed by epidemiologists by forming dichotomies between groups of categories in various combinations.[10] The use of logistic regression when the dependent variable has more than two categories (polytomous logistic regression) has long not been possible for epidemiologists

	Plaques		
	No	Fibrous	Complicated
Non smokers	100	10	10
Smokers	90	10	20

Figure 6.1. The presence of atherosclerotic plaques by smoking status

using standard statistical packages. Some options recently appeared in a new module of BMDP.[11] Interpretation of the model parameters, however, has yet received little formal attention in epidemiological research. One may consider a hypothetical example of smoking and the prevalence of the mild form of atherosclerotic disease, predominantly fibrous plaques, and the severe form of the disease, predominantly complicated plaques (figure 6.1).

At first glance the conclusion would be that smoking has only a weak association with the presence of fibrous plaques. Further insight in the structure of the data shows that this might not be the case. When smoking increases disease progression from fibrous to complicated plaques, an effect on fibrous plaques is masked by the shift of subjects out of this category into the category of complicated plaques. When the severe form of the disease develops via the mild form of disease, the best way to interpret the data is to first combine all disease categories within strata of the exposure and to estimate the ratio of total number of cases and total number of subjects per stratum. When these ratio's differ between exposure groups, the exposure is related to the occurrence of the initial stage of disease. Subsequently, the ratio of cases with severe disease and total number of cases should be calculated. If these ratio's differ between exposure categories, the exposure affects disease progression from the mild to severe form.

In summary, when the outcome is studied on an ordinal scale, the data structure becomes more complex. The data will often be compatible with many underlying processes, the credibility of which may no longer be judged upon by current biological knowledge. Care should therefore be taken with the interpretation of these data.

THE OUTCOME ON A QUANTITATIVE SCALE

When the disease process can be measured on a quantitative scale, linear regression analysis can be used. The measures of association, derived from this model, and their interpretation have been described extensively.[6]

Measurement of the determinant

As discussed above, the study of change requires all subjects to be measured at the beginning and the end of the follow-up period. Besides data on change in the outcome variable, this may provide data on change in the determinant. Analyses of change in the determinant have been reported as early as 1966, when enough data were collected by the Framingham Study to examine changes in the determinant in relation to the occurrence of cardiovascular disease.[14] Subsequently, more studies on change in determinants have been reported.[15] Results of these studies have in general been rather disappointing and only few research papers gave formal attention to its analysis.[16-18]

One of the first problems that has been tried to encounter in the analysis of change is the regression towards the mean effect. The method that has been used to handle this phenomenon is that of residual change. In this, the observed change is corrected for initial level by regressing the follow-up level at the baseline level and the residuals from such a regression model are used as the measure of change.[16] This is comparable to inclusion of the baseline value in the regression model. However, it has been pointed out that this may lead to spurious results, referred to as Lord's paradox. Its principle has been described for the study of determinants of change, but holds in a similar way for the study of change as a determinant of disease: "If, for example, in a study on weight change men and women would have a different initial weight, adjustment for initial level would amount to comparing over-weight women with under-weight men. These are expected to experience a different weight change because they tend to regress to their own population mean, and not to the mean weight of the combined population of men and women. As a consequence, men and women may seem to experience a different change in weight through the adjustment for initial level whereas in reality, the average weight change for men and women is the same".[18] Stratification for baseline level will give spurious results in a similar manner. At present, unadjusted analysis are probably most appropriate in observational studies.[19] Therefore, in the investigations of change in blood pressure and change in cholesterol in relation to progression of atherosclerosis (chapters 3.1 and 3.2), no adjustments for baseline level of the risk factor were made.

Regression to the mean is not the only concern when one studies change. The change in the determinant may be related to the level of the baseline variable by a phenomenon referred to as horse racing, which results in a positive relation between baseline level and change. On the other hand subjects who so far experienced little change may catch up, resulting in a negative relation between baseline level and change. As an example of the latter one may consider the increase in cholesterol in women after middle age. If biology determines that the majority of women will have an increase in cholesterol sooner or later after

middle age (e.g. because they will all experience a loss of estrogen production), those with a low baseline level of cholesterol might be expected to experience a relatively large change in cholesterol during follow-up. At present, there is probably no good way of dealing properly with the correlation between baseline level and change in data analysis.

Conclusions

The study of the disease process in epidemiology requires reconsideration of aspects of study design and data analysis. New statistical models, developed in other disciplines, will probably be introduced in epidemiological research. However, formal attention should be given to the interpretation of the parameters obtained from these models and potential pitfalls. Furthermore, more formal attention should be given to the consequences of the correlation between level and change of the studied variables for data analysis.

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CHAPTER 7

SUMMARY

7. Summary

Cardiovascular disease is generally considered to be a disorder of men. One reason for this is the low incidence of the disease in women at younger age. At older age, however, cardiovascular disease also becomes the most important cause of mortality in women. The main objective of the work presented in this thesis was to study the determinants of cardiovascular risk in women.

Atherosclerosis is the major underlying process of cardiovascular disease. In the studies presented in this thesis, the presence of calcified plaques in the aorta as seen on radiographs was used as a measure of atherosclerosis. Data of two studies were used to examine whether aortic calcification reflects a generalized process. One was the EPOZ study, a population-based study on risk factors for chronic diseases, conducted in 1975-1978 in the Dutch town of Zoetermeer. Lateral X-rays of the lumbar spine were made in all participants, aged 45 years and over (1,359 men and 1,598 women). On these, the presence of calcified plaques in the abdominal aorta was scored. The prevalence of aortic calcification was about 10% in middle-aged subjects, and rose with age to a maximum of 45% in men and 75% in women. The presence of aortic calcification in men under the age of 65 years was associated with an increased risk of cardiovascular mortality during nine years of follow-up. A tendency for an increased risk was observed among older women; the number of deaths in middle-aged women was too small for analysis *(chapter 2.1)*. The relation between the presence of calcified plaques in the thoracic aorta, as detected on chest X-rays, and the development of cardiovascular disease was examined using data of twelve years of follow-up of the Framingham cohort (2,336 men and 2,873 women). The presence of calcified plaques doubled the risk of cardiovascular death in men and women younger than age 65. Similar increases in risk were found for coronary artery disease, stroke and intermittent claudication among middle-aged women. Among middle-aged men these risks were less clear. *(chapter 2.2)*.

The research questions in the study of cardiovascular disease in women were: (1) Do the recognized risk factors (elevated blood pressure, elevated serum cholesterol and cigarette smoking) predict progression of atherosclerosis in women? (2) Are characteristics unique to women related to progression of atherosclerosis? To address the first question, we used data on progression of aortic atherosclerosis of 855 women, initially aged 45 to 64 years. The women had participated in the EPOZ study and were re-examined after nine years. At follow-up, mild and advanced progression of atherosclerosis could be demonstrated in 20 and 17 percent of women, respectively.

Moderately elevated and high levels of systolic and diastolic blood pressure were associated with an increased risk of atherosclerotic progression. This suggests that elevations of both systolic and diastolic blood pressure prior to the development of sustained hypertension may promote the atherosclerotic process. In addition, a low diastolic pressure was associated with increased progression of atherosclerosis, which may reflect the presence of a hardened vessel wall *(chapter 3.1)*.

The risk of atherosclerotic progression increased with increasing baseline cholesterol at levels beyond 6.0 mmol/l. This suggests that also moderately elevated levels of cholesterol are atherogenic in women. During follow-up, cholesterol rose on average by 0.11 mmol/l per year. The rise in cholesterol was associated with only a borderline significant increased risk of mild progression; no association was seen with advanced progression. Whether the rise in cholesterol in women after middle-age contributes to the risk of cardiovascular disease remains to be proven *(chapter 3.2)*.

Cigarette smoking was associated with an increased risk of progression of atherosclerosis in a dose-dependent manner. Among former smokers the risk of progression decreased with increasing duration of stopping but a significant excess risk was still observed after five to ten years since quitting. The findings suggest that cigarette smoking, besides acute effects, may also have prolonged effects on the arterial wall *(chapter 3.3)*. The risks associated with the three risk factors were independent of each other and none of the effects required a critical level of another risk factor.

Of risk factors unique to women, menopause has received much attention, but its cardiovascular effects are still unproven. The association between menopausal state and aortic atherosclerosis was examined cross-sectionally among 294 premenopausal and 319 postmenopausal women, who participated in the EPOZ study. After adjustment for age, women with a natural menopause and women who had had a bilateral oophorectomy had a strongly increased risk of atherosclerosis compared to premenopausal women. These findings suggest an increased risk of atherosclerosis in women after the menopause *(chapter 3.4)*.

One possibility for intervention on risk factors is to promote dietary change. We prospectively examined the relation of various nutritional factors with the incidence of self-reported hypertension among 58,218 US female registered nurses, aged 34 to 59 years *(chapter 4.1)*. During 4 years of follow-up, 3,275 women reported a diagnosis of hypertension. Relative weight and alcohol consumption were the strongest predictors of the development of hypertension. Alcohol consumption of up to 20 g/day (less than 2 glasses) did not increase the risk of hypertension, but beyond this level the risk increased progressively *(chapter 4.2)*. Dietary calcium and magnesium were the only nutrients that were inversely related with risk of hypertension. Whether a change in intake of these

nutrients results in a change in blood pressure needs to be investigated in intervention studies.

A review of the results of reported calcium and magnesium intervention studies has been given in *chapter 4.3*. The results of calcium intervention studies are not unanimously supportive of a blood pressure lowering effect of calcium. This may be due to a possible heterogeneity in response, as observed in some of the studies. Few placebo controlled studies examined the effect of magnesium on blood pressure. Those reported did not support a blood pressure lowering effect of magnesium, but studies were generally small and of short duration. For this reason, the effect of magnesium supplementation (magnesium-aspartate-HCl) on blood pressure was examined in a randomized controlled trial among 91 women with mild to moderate hypertension, who were not receiving anti-hypertensive medication. Subjects received magnesium powders (20 mmol Mg/day) or matched placebo for a period of 6 months. At the end of the study, systolic blood pressure had fallen by 2.7 mmHg (95% confidence interval -1.2 to 6.7) and diastolic blood pressure by 3.4 mmHg (1.3 to 5.6) more in the magnesium group than in the placebo group. The results suggest that magnesium-aspartate-HCl may be a safe and effective measure for lowering blood pressure in women with mild to moderate hypertension. We were not able to clearly identify modifiers of response, but numbers in subgroups were small *(Chapter 4.4)*.

In *chapter 5* the findings of the presented studies are placed in the broader context of research directed towards primary prevention of cardiovascular disease. Subsequently, new research areas are delineated. *Chapter 6* discusses the methodological implications when future research activity shifts from the search for determinants of the final event to the search for determinants of the underlying process of the disease.

Samenvatting

Hart- en vaatziekten worden over het algemeen beschouwd als een ziekte van mannen. Dit komt voornamelijk doordat weinig vrouwen de ziekte ontwikkelen op jonge leeftijd. Op oudere leeftijd, echter, zijn hart- en vaatziekten ook voor vrouwen de belangrijkste doodsoorzaak. Het voornaamste doel van de onderzoeken beschreven in dit proefschrift was het bestuderen van de determinanten van hart- en vaatziekten bij vrouwen.

Het proces dat aan het optreden van hart- en vaatziekten ten grondslag ligt is atherosclerose. In de beschreven onderzoeken werden radiologisch vastgestelde verkalkingen in de aorta gebruikt als maat voor atherosclerose. Of atherosclerose in de aorta samengaat met atherosclerose in overige delen van het vaatstelsel werd nagegaan door het verband te bestuderen met het optreden van hart- en vaatziekten. In een onderzoek naar chronische ziekten dat werd uitgevoerd bij bewoners van Zoetermeer (EPOZ onderzoek) werden zijdelingse röntgenfoto's van de lumbale wervelkolom gemaakt bij alle deelnemers van 45 jaar en ouder (1.359 mannen en 1.598 vrouwen). Op deze foto's werden verkalkingen in de abdominale aorta gescoord. De prevalentie van aorta verkalking was ongeveer 10% op middelbare leeftijd en steeg met het toenemen van de leeftijd tot een maximum van 45% bij mannen en 75% bij vrouwen. Bij mannen onder de 65 jaar met aorta verkalking werd een verhoogde sterfte aan hart- en vaatziekten gevonden gedurende een vervolgperiode van negen jaar. Eenzelfde tendens werd gevonden bij oudere vrouwen; het aantal sterfgevallen onder vrouwen van middelbare leeftijd was te klein voor analyse van de gegevens *(hoofdstuk 2.1)*.

Het verband tussen de aanwezigheid van verkalkingen in de thoracale aorta, gescoord op een voorachterwaartse thoraxfoto, en het optreden van hart- en vaatziekten werd bestudeerd in de Framingham Heart Study (2.336 mannen en 2.873 vrouwen). De aanwezigheid van aorta verkalking bij mannen en vrouwen jonger dan 65 jaar verdubbelde het risico op sterfte aan hart- en vaatziekten tijdens een vervolg periode van 12 jaar. Vergelijkbare verhogingen van het risico werden gevonden voor coronaire hartziekte, beroerte en claudicatio intermittens bij vrouwen van middelbare leeftijd. Bij mannen van middelbare leeftijd waren deze verbanden minder duidelijk *(hoofdstuk 2.2)*.

De vragen die gesteld werden in het onderzoek naar hart- en vaatziekten bij vrouwen waren: (1) zijn de bekende risicofactoren (verhoogde bloeddruk, verhoogd serum cholesterol gehalte en roken) gerelateerd aan progressie van atherosclerose bij vrouwen? (2) zijn karakteristieken die uniek zijn voor

vrouwen gerelateerd aan progressie van atherosclerose? De eerste vraag werd onderzocht bij 855 vrouwen van 45 tot 64 jaar. De vrouwen hadden deelgenomen aan het EPOZ onderzoek in 1975-1978 en namen negen jaar later deel aan een vervolgonderzoek. Tijdens de vervolgmeting werd bij 20% van de vrouwen milde- en bij 17% matig tot ernstige progressie van atherosclerose in de aorta vastgesteld.

Een positief verband werd gevonden tussen matig verhoogde en hoge niveaus van de systolische en diastolische bloeddruk en het risico op progressie van atherosclerose. Deze bevinding suggereert dat verhoging van de systolische en diastolische bloeddruk vóór de ontwikkeling van hypertensie al een effect heeft op de vaatwand. Een lage diastolische bloeddruk was ook geassocieerd met een verhoogd risico op progressie van atherosclerose, wat mogelijk een effect van vaatwand verharding op de diastolische druk reflecteert *(hoofdstuk 3.1)*.

Het risico op progressie van atherosclerose nam toe met het toenemen van het serum cholesterol gehalte vanaf 6.0 mmol/l. Dit betekent dat ook een matig verhoogd niveau van cholesterol atherogeen is bij vrouwen. Gedurende de vervolg periode steeg het cholesterol gehalte met gemiddeld 0.1 mmol/l per jaar. De stijging van het cholesterol gehalte had slechts een zwak verband met progressie van atherosclerose *(hoofdstuk 3.2)*.

Het aantal gerookte sigaretten vertoonde een positief verband met het risico op progressie van atherosclerose. Bij vrouwen die gestopt waren met roken nam het risico op progressie van atherosclerose af naarmate de duur van de gestopte periode langer was, maar een statistisch significant verhoogd risico was na vijf tot tien nog steeds aanwezig. Dit wijst erop dat het roken van sigaretten, naast acute effecten, ook een langdurig effect op de vaatwand kan hebben *(hoofdstuk 3.3)*. De effecten van verhoogde bloeddruk, verhoogd serum cholesterol en roken op progressie van atherosclerose waren onafhankelijk van elkaar.

Dat het optreden van de menopauze het risico op hart- en vaatziekten verhoogt wordt verondersteld maar tot op heden is dit nog niet aangetoond. Het verband tussen menopauzale status en atherosclerose in de aorta werd onderzocht bij 294 premenopauzale en 319 postmenopauzale vrouwen van 45 tot 55 jaar die in 1975-1978 deelnamen aan het EPOZ onderzoek. Vrouwen met een natuurlijke menopauze en vrouwen met een tweezijdige ovariëctomie hadden een verhoogd risico op atherosclerose vergeleken met premenopauzale vrouwen, na correctie voor verschillen in leeftijd. Dit wijst erop dat na de menopauze het proces van atherosclerose wordt versneld *(hoofdstuk 3.4)*.

Eén van de mogelijkheden voor het beïnvloeden van risicofactoren is het bevorderen van veranderingen in de voeding. Het verband tussen verschillende voedingsfactoren en het optreden van hypertensie werd onderzocht bij 58.218 Amerikaanse verpleegsters van 34 tot 59 jaar *(hoofdstuk 4.1)*. Gedurende een vervolgperiode van vier jaar rapporteerden 3.275 vrouwen een diagnose van

hypertensie. Lichaamsgewicht en alcohol consumptie waren het sterkst gerelateerd aan het optreden van hypertensie. Alcohol consumptie tot 20 gram per dag (minder dan 2 glazen) had geen effect, maar bij een hogere consumptie nam het risico op hypertensie progressief toe *(hoofdstuk 4.2)*. Calcium en magnesium waren de enige nutriënten die invers gerelateerd waren aan het risico op hypertensie. Of een verandering in de inneming van deze nutriënten resulteert in een verandering in de bloeddruk dient te worden onderzocht in interventie onderzoek.

Hoofdstuk 4.3 geeft een overzicht van de resultaten van gerapporteerde calcium en magnesium interventie studies. De resultaten van calcium interventie studies zijn niet unaniem ondersteunend voor het bestaan van een bloeddruk verlagend effect van calcium. Mogelijk komt dit doordat slechts een deel van de personen met een hoge bloeddruk gevoelig is voor verhoging van de calcium inneming, zoals in een aantal onderzoeken werd aangetoond. Weinig placebo-gecontroleerde interventie studies onderzochten het effect van magnesium toediening op de bloeddruk. In de meeste van de tot op heden gerapporteerde onderzoeken werd geen effect van magnesium op de bloeddruk gevonden, maar de studies waren over het algemeen klein en hadden een korte interventie periode. Om deze reden werd het effect van magnesium suppletie (magnesium aspartaat-HCl) bestudeerd in een dubbelblind placebo-gecontroleerde trial bij 91 vrouwen met milde tot matige hypertensie die geen bloeddrukverlagende medicijnen gebruikten. De vrouwen kregen gedurende 6 maanden magnesium poeders (20 mmol Mg/dag) of gematchte placebo. Op het einde van de studie was de systolische bloeddruk 2.7 mmHg (95% betrouwbaarheids interval -1.2 tot 6.7) en de diastolische bloeddruk 3.4 mmHg (1.3 tot 5.6) meer gedaald in de magnesium groep dan in de placebo groep. Deze bevinding wijst erop dat magnesium aspartaat-HCl mogelijk een effectief middel is voor het verlagen van de bloeddruk bij vrouwen met milde tot matige hypertensie die geen bloeddrukverlagende medicijnen gebruiken *(hoofdstuk 4.4)*.

In *hoofdstuk 5* worden de bevindingen van de beschreven onderzoeken geplaatst in de bredere context van onderzoek dat zich richt op de primaire preventie van hart- en vaatziekten. Vervolgens worden een aantal nieuwe onderzoeksgebieden aangegeven. *In hoofdstuk 6* worden methodologische problemen besproken die ontstaan wanneer epidemiologisch onderzoek zich richt op het bestuderen van determinanten van het onderliggende ziekte proces in plaats van determinanten van het optreden van de eindpunten van de ziekte.

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