Coronary Calcification and risk of Cardiovascular Disease

An epidemiologic study

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The work presented in this thesis was conducted at the Department of Epidemiology & Biostatistics, Erasmus MC, Rotterdam, at the Department of Radiology of the Daniel den Hoed Clinic, Erasmus MC, Rotterdam, in close collaboration with the Department of Radiology, University Hospital Groningen, Groningen. Financial support for the studies described in this thesis by the Health research and development Council (ZONMw) (grant no. 28-2975) is gratefully acknowledged.

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op hart- en vaatziekten
Een epidemiologisch onderzoek

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Chapter 4.2
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Chapter 5.1

Vliegenthart R, Oudkerk M, Song B, van der Kuip DAM, Hofman A, Witteman JCM. Coronary calcification detected by electron-beam computed tomography: The Rotterdam Coronary Calcification Study. Eur Heart J 2002;23:1596-1603.

Chapter 5.2

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Chapter 5.3

Vliegenthart R, Oudkerk M, Hofman A, Oei HHS, van Dijck W, van Rooij FJA, Witteman JCM. Coronary calcification improves cardiovascular risk prediction in a population of older adults. (submitted)

CHAPTER

one

INTRODUCTION

Already in the eighteenth century, calcification of the coronary artery wall was recognized as being part of the atherosclerotic process.[1] However, only after the recent development of electron-beam tomography (EBT), an ultrafast CT technique, it became possible to accurately quantify the amount of coronary calcification noninvasively. Quantitative measures of coronary calcification, detected by EBT, have been found to be closely related to the amount of atherosclerotic plaque in histopathologic investigations.[2,3] Furthermore, the calcium score derived from EBT is strongly associated with the extent of angiographically detected coronary artery disease.[4,5] Therefore, quantification of coronary calcification using EBT has been proposed as a promising method for noninvasive detection of asymptomatic subjects at high risk of developing coronary heart disease. Studies showing that coronary calcification increases the risk of coronary events have been performed in selected, high-risk populations with small numbers of events.[6-10] There are currently no population-based data on the predictive value of coronary calcification.

The focus of this thesis is to investigate whether coronary calcification predicts cardiovascular disease in the general population. For this purpose, an epidemiologic study was carried out in the population-based Rotterdam Coronary Calcification Study. The Rotterdam Coronary Calcification Study consists of participants from the Rotterdam Study who underwent EBT scanning of the heart. On the scans, the amount of coronary calcification was computed. Scandata were available for 2013 older adults.

In chapter 2, the current knowledge on the pathogenesis, detection and epidemiology of coronary calcification is reviewed. Chapter 3 contains studies on the validation of the scanning and scoring technique. In chapter 4, associations between cardiovascular risk factors and coronary calcification and peripheral atherosclerosis are described. Chapter 5 focuses on the association between coronary calcification and cardiovascular disease. In chapter 6, the main results of the studies described in this thesis are placed in perspective and methodological issues discussed. In addition, this chapter comments on the relevance of the findings and provides suggestions for future research on the topic.

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CHAPTER two

REVIEW OF CORONARY CALCIFICATION

2.1 Pathogenesis of coronary calcification

In the eighteenth century, just after the initial descriptions of coronary sclerosis, pathologists first noted calcium deposits in the coronary arteries.[1,2] Thebesius considered calcified coronary artery lesions to be the most important feature of coronary sclerosis.[1] This was the prevailing view for over 200 years. In 1863 Virchow noticed that the calcification of atherosclerotic lesions in the coronaries was similar to ossification, or bone formation.[3] During the 20th century attention shifted towards cholesterol metabolism and other factors found to play an essential role in atherogenesis. Calcium deposits were regarded as merely a degenerative byproduct of advanced stages of atherosclerosis.[1,4,5] Part of the decreased interest may have been due to the poor resolution of radiographic imaging techniques at the time, with a low sensitivity for detecting calcium. Nevertheless, many researchers recognized that noninvasive imaging of coronary calcification might be useful for the identification of asymptomatic subjects at high risk of acute myocardial infarction or sudden cardiac death. Owing to the development of high-resolution techniques such as fluoroscopy and, more recently, electron-beam tomography (EBT), coronary calcification has come under renewed attention as a way of noninvasively detecting coronary atherosclerosis. Furthermore, fascinating new evidence reveals that coronary calcification is not a passive or degenerative, but an active, organized, regulated, and reversible process with mechanisms resembling bone formation.

Calcification of atherosclerotic lesions

Calcification of the coronary arteries is almost invariably associated with intimal atherosclerosis. The only known exception is Mönckeberg's calcific medial sclerosis,[6,7] which involves mineralization of the media, and occurs particularly among patients with diabetes mellitus.[8] Histological studies show that atherosclerotic calcification starts as early as the second decade of life, very soon after fatty streak formation.[9] In elderly individuals and in more advanced lesions, the frequency and extent of calcified deposits is increased.[9] The predominant mineral within calcified atherosclerotic plaques is hydroxyapatite. This crystalline form of calcium contains 40% calcium by weight,[10] and is identical to the mineral in bone.[11] It has been long known that atherosclerotic calcification and bone share many histologic characteristics[12] such as the formation of bone marrow and active remodeling.[13-15] Ectopic tissues in calcified atherosclerotic arteries appeared to develop as normal bone. It was hypothesized that a recapitulation of embryonic programs might be taking place.[14,15] In addition, electron microscopy revealed matrix vesicles, the initial nucleation sites for hydroxyapatite in bone, in atherosclerotic lesions.[16,17] This evidence supports the theory by which hydroxyapatite develops

primarily in vesicles that pinch off from arterial wall cells, analogous to the process in bone formation. Recently, several bone matrix proteins like collagen type I, matrix gla protein, osteopontin, osteocalcin, and osteonectin have been found in calcified atherosclerotic lesions[18-22] as well as bone morphogenetic protein-2.[23] It is not clear what initiates calcification of atherosclerotic arteries. To study the molecular mechanisms of vascular calcification, many studies have been performed in vitro and in vivo. In cultures of aortic wall cells Boström[23] discovered a subpopulation of arterial wall cells with osteoblastic properties. These cells were called pericyte-like cells[23] or calcifying vascular cells.[24] Evidence by Proudfoot suggests that these cells are a population of smooth muscle cells.[25] A characteristic pattern of growth and differentiation occurred in the cultures. Once the cells reached confluence, they formed multilayered areas, and retracted upon each other forming nodules. The presence of mineral in the nodules has been confirmed by several investigations[26,27] carried out using different methods. In the matrix within the nodules, crystals of hydroxyapatite were deposited, leading to mineralization. Studies have shown that the deposition of the mineralized matrix is associated with stage-specific expression of numerous matrix proteins and markers of osteoblastic cells.[18-23,28-30] Details about the origin of the calcifying cells, cell differentiation and the precise role of the bone matrix proteins have yet to be revealed. Current research focuses on factors that influence and regulate the process of atherosclerotic calcification.

Of particular interest are potential mediators of calcification linking the process to atherosclerosis. Two molecules have been identified that both enhance in vitro mineralization of calcifying vascular cells, and play a role in the atherosclerotic process. The first molecule is transforming growth factor beta, while the second, and most potent inducer of in vitro mineralization detected, is 25-hydroxycholesterol.[24] TGF-β increases the number of mineralized nodules, while 25-hydroxycholesterol increases the rate of mineralized nodule formation and increases the calcium content of the cultures. These findings point to a mechanism that may account for the close relation between atherosclerosis and calcification. Lipids and lipid oxidation products are associated with mineralization in bone and other calcified tissues,[31-33] however in atherosclerosis, this association has not been extensively investigated.

Types of atherosclerotic lesions

Calcium deposits of microscopic size already emerge in atherosclerotic lesions of young people. Atherosclerotic lesions have typical histological and histochemical compositions at different stages of their natural history. Eight different types

TABLE 1
Classification of atherosclerotic lesions according to American Heart Association[35]

Histological composition	Classification
Scattered macrophage foam cells	Type I lesion
Layers of macrophage foam cells, lipid-laden smooth muscle cells – 'fatty streak'	Type II lesion
Accumulation of extracellular lipid droplets in small pools	Type III lesion
Confluent extracellular lipid core – 'atheroma'	Type IV lesion
Fibromuscular tissue layers forming a cap over the lipid core	Type V lesion
Plaque rupture, hematoma, thrombosis in addition to type IV or type V changes – 'complicated lesion'	Type VI lesion
Predominant calcification*	Type VII lesion
Predominant fibrosis*	Type VIII lesion

* Type VII and VIII lesions are considered endstages and may result from regression or change in lipids of type IV-VI.

of lesions have been defined by the American Heart Association's Committee on Vascular Lesions and classified according to the temporal sequence of morphologies[34,35] – see table 1. Type I and type II lesions develop as early as the second decade of life. In these lesion types, no calcium can be detected. Histological evidence of the presence of calcium within atherosclerotic plaques can be found in a type IV lesion, also called 'atheroma'. Calcium granules appear within calcifying vascular cells (or smooth muscle cells) as well as outside cells, scattered among small lipid droplets. Advanced lesions (types III and IV) are also present in young people, however, lesions more advanced than atheroma are hardly found before the fourth decade of life. Erosion of lesions type IV may occur, most frequently leading to mural thrombosis. Type V lesions are characterized by the development of a thin fibrous cap infiltrated by macrophages and lymphocytes. The unstable covering of the necrotic core causes the susceptibility of type V lesions to intraplaque hemorrhage and rupture, which frequently leads to occlusive thrombi. With increasing age, calcium granules grow in size, form lumps and sometimes plates. The lumps and plates tend to be in the periphery of the lipid core, especially in the base. In lesions type VII ('calcific lesion') and type VIII ('fibrocalcific lesion'), the necrotic core has become much smaller and is predominantly replaced by, respectively, calcium and fibrotic tissue. Figure 1 depicts a coronary artery with plaques in different stages.[36] Histological cross-sections of plaques with and without calcification are shown in figure 2. As far, there have been no histological studies investigating what components of the human atherosclerotic lesions regress in the course of clinical intervention studies, for instance during lipid-lowering therapy. An experiment in monkeys on changing atherogenic diets showed that during regression of plaques, the lesion

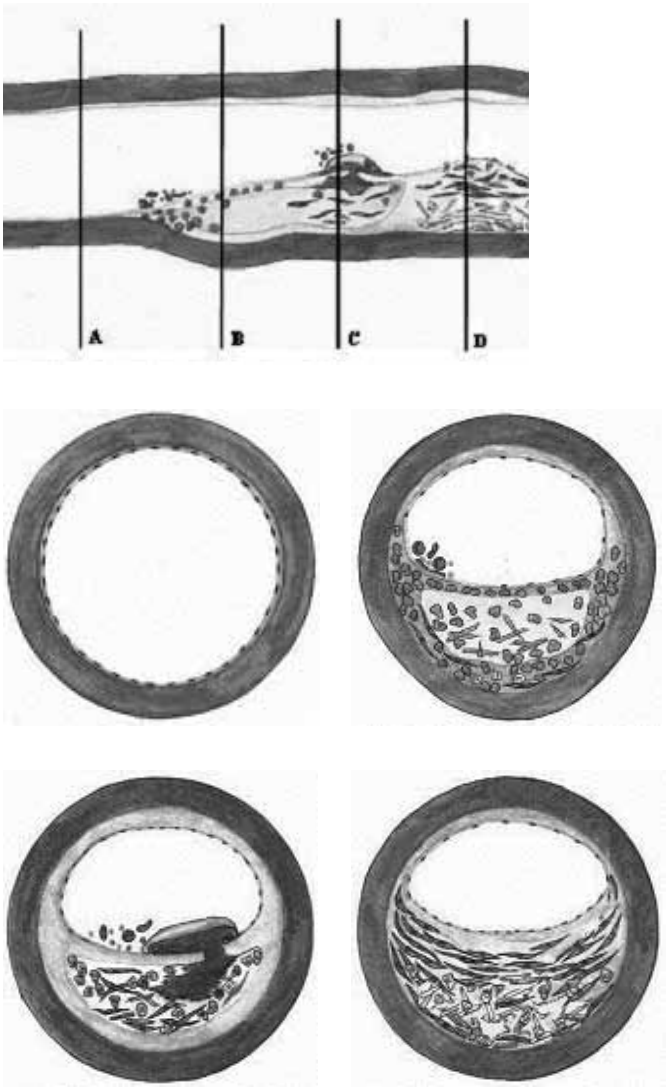

FIGURE 1
Schematic longitudinal and cross-sectional sections of a coronary artery with different stages of plaque. A: normal artery wall, B: vulnerable plaque (AHA type VI), C: ruptured plaque (AHA type VI), D: stable plaque with fibrosis and calcific deposits (AHA type VIII). Modified with permission from Naghavi.[36]

size decreased, but the calcium volume remained the same.[37] Thus, the proportion of calcium in the plaque increased from at most 20% in the baseline lesions to about 50% in the regressed lesions. Whether this is the result only of decreasing lipid content or also of continuing calcium deposition during lesion regression is uncertain.

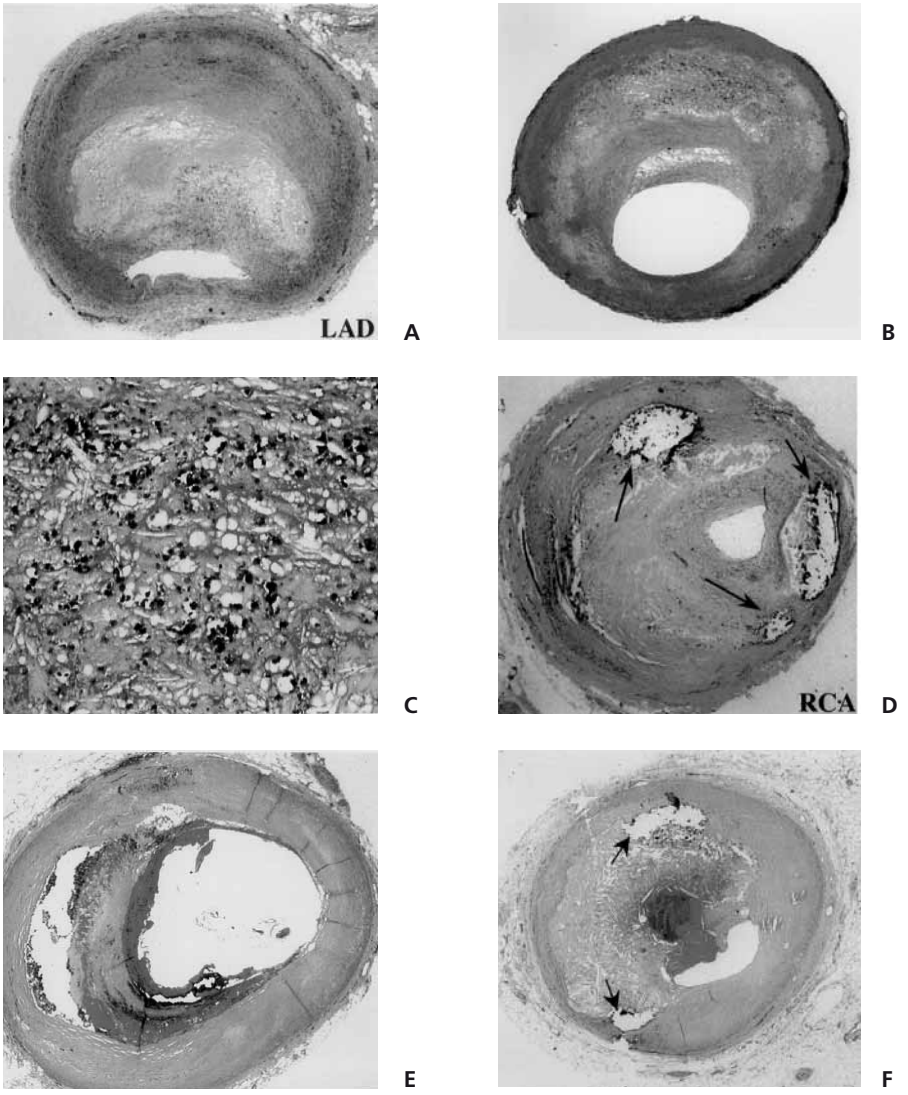

FIGURE 2
Histological cross-sections of coronary arteries. A: plaque without calcification. B: plaque with microcalcification. C: detail of microcalcification. D: plaque with advanced calcification. E: ruptured plaque. F: ruptured plaque with calcification. Printed with permission from Virmani

Amount of calcification and atherosclerotic plaque burden

Observation of calcium in coronary arteries indicates the presence of plaques. Because relatively consistent changes in calcium deposits parallel the natural history of atherosclerotic lesions, it is conceivable that the amount of calcification is associated with the atherosclerotic plaque burden. However, atherosclerotic lesion types in coronary arteries of older adults can be quite heterogeneous, including early and advanced stages. In morphometric analysis of the composition of plaques in coronary events, the overall plaque area and the area of calcification were linearly associated.[38] A study by Rumberger[39] in which the calcium area detected by EBT was compared with the histological plaque area of coronary segments from autopsy hearts confirmed a close relation between coronary calcification and amount of atherosclerosis. The coronary calcium area of hearts as a whole, of individual coronary arteries and of individual coronary segments was highly correlated with the histologically quantified coronary plaque area. The detected amount of coronary calcification was about one fifth of the measured atherosclerotic plaque burden in corresponding segments. It is not surprising that the amount of calcification is so much less than the total amount of plaque: calcium is only one of the many constituents of plaque, and is formed during the natural history of atherosclerotic lesions. During the expansion of atherosclerosis through life, more advanced, calcified lesions exist alongside developing lesions that do not contain detectable calcium yet. Further analyses by Sangiorgi[40] showed that the increase in amount of coronary calcification with advancing age was similar to the increase in coronary atherosclerosis. Although the absence of calcification did not exclude the presence of possibly unstable plaque, there was a low plaque burden on average. Nevertheless, despite significant correlations between calcification area and plaque area, the individual variability in calcification was large.

Calcification and plaque rupture

Most myocardial infarctions are caused by thrombotic occlusion of a coronary artery after plaque rupture. Therefore, it is important to identify the atherosclerotic lesions which are most vulnerable to rupture. The composition of the plaques rather than lumen stenosis is presently regarded as the main determinant of acute coronary events.[41] Evidence on the composition of vulnerable lesions comes predominantly from autopsy studies, and from investigations with intravascular ultrasound (IVUS). The three major determinants of plaque vulnerability are the size and structure of the lipid core, the thickness of the fibrous cap covering the core, and inflammation in and near the cap.[42] If the lipid core of a lesion is large

and soft, the plaque is at higher risk of rupture.[43] Thinning of the fibrous cap and reduction of the collagen content of the cap also increase rupture risk. Part of the last-mentioned increase in risk may be due to break-down of matrix proteins by proteases, released by activated macrophages, and a reduction in synthesis of matrix proteins by smooth muscle cells.[43] If cap thickness is low, circumferential stress at the luminal border of the plaque shows a critical increase.[44] It has been demonstrated that local variations in stress, possibly due to variations in plaque composition, may contribute to rupture of plaques.[45] Furthermore, heavy infiltration of macrophages in the cap and at the shoulder region is associated with plaque rupture. The role of calcification in the pathogenesis of coronary events is unclear. As mentioned before, the amount of calcium is related to the total amount of plaque, including unstable plaque sections. Since the plaque burden is related to myocardial infarction and sudden cardiac death, the quantity of calcification can possibly identify the persons at highest risk of coronary events. Findings from two recent studies support this idea. Schmermund[46] performed a histopathological comparison of coronary arteries of subjects dying from sudden cardiac death (cases) and subjects dying from non-cardiac causes (controls). The plaque area, lipid core size and calcified area were larger in cases than in controls. In an analysis restricted to narrowed coronary sections from cases, ruptured plaques had an increased plaque area, lipid core size, calcified area, and percentage of segments with calcification, compared to stable plaques. It was stated that the surplus of calcium in ruptured plaques could simply reflect the larger plaque burden of ruptured plaques, and not a causal relationship between calcium and rupture. Mascola[47] used EBT to compare infarct-related and non-infarct-related arteries in subjects with myocardial infarction. The amount of calcium, calcified area and number of calcifications were higher for culprit arteries than for non-culprit arteries. Clearly, a relation exists between coronary calcification and severity of coronary disease. However, whether calcification itself is a harmful or protective process, is a matter of debate. One necropsy study found that plaques in severely narrowed vessel segments contained less calcium in subjects with fatal myocardial infarction than in subjects with sudden cardiac death.[38] Doherty and co-workers[48] supposed that calcific deposits may impart stability and tend to reduce vulnerability for plaque rupture. Observations in a helical CT study by Shemesh[49] corroborate this view. In this study myocardial infarction was prone to originate from non- or mildly calcified culprit arteries, while in subjects with stable angina pectoris coronary arteries were generally extensively calcified. Thus, increasing calcification of individual atherosclerotic lesions may decrease the risk of thrombotic obstruction at the lesion site and therefore of a coronary event. Biomechanical results are ambiguous. Calcification of individual plaques were found to make plaques stiffer and induce resistance to fibrous cap rupture,[50]

and extensive calcification may reduce stress in the fibrous cap.[51] However, focal calcification may increase stress at the shoulder regions and in the cap.[52] This leads to the conclusion that coronary calcification may have a different significance in the context of individual plaques and of the total plaque burden. Further research is needed to elucidate the precise role of coronary calcification in plaque formation and stabilization.

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2.2 Detection of coronary calcification

A number of radiological techniques have the potential to detect calcification of the coronary arteries, namely plain chest radiography, fluoroscopy, conventional computed tomography, electron-beam tomography, multi-detector computed tomography, intravascular ultrasound, magnetic resonance imaging, and transthoracic and transesophageal echocardiography. Successively, we will discuss the use of the methods that are most commonly used for visualization of coronary calcification. Coronary calcification has been demonstrated incidentally with plain chest radiography. However, most patients with coronary artery disease have no visible calcifications in the coronary arteries on chest radiographs. The accuracy of chest radiography was 42% compared to fluoroscopy, which is also an insensitive technique (see below).[1]

Fluoroscopy

Already in 1968, coronary calcification seen fluoroscopically was found to be related to the presence of symptomatic heart disease.[2] In the seventies, cardiologists reported the detection of coronary calcification by fluoroscopy as a potential aid in the diagnosis of coronary artery disease (CAD).[3,4] Until then, the only noninvasive method for detecting CAD was exercise testing. There are, however, a number of limitations to exercise testing: it can only detect plaque constricting the lumen severely enough to compromise blood flow, it may cause a coronary event in high-risk subjects, and the possibility to perform the test depends on the exercise-capacity of the patient. In contrast to exercise testing, cardiac fluoroscopy is a rapid, inexpensive and widely available procedure. With different projections, the presence of calcification in the individual coronary arteries can be assessed. A number of studies have been performed that integrated fluoroscopy in the precatheterization evaluation of patients undergoing selective coronary angiography.[3,5-11] After fluoroscopic images were relayed to a TV monitor or recorded on cinefilm, observers rated each coronary artery as to the presence or absence of calcification. Table 1 shows the results of eight studies comparing fluoroscopic detection of coronary calcification with angiographically detected disease (at least 50% luminal narrowing). Because it is difficult to justify an invasive procedure like coronary angiography without suspicion of CAD, these studies were all conducted in patients with symptomatic CAD. Aldrich[3] found that the diagnostic accuracy of fluoroscopy approached that of exercise testing. The accuracy of a positive test result was 86% for fluoroscopy but 69% for exercise testing. In the published studies, the sensitivities for detecting any angiographic disease ranged from 40% to 79%, while the specificities varied from 52% to 93%. The large variability in test parameter values can partly be explained by differences

TABLE 1
Patient studies on the comparison of coronary calcification detected by fluoroscopy with angiographically detected coronary artery disease

Study	Patients (n)	Sensitivity (%)	Specificity (%)
Hamby et al (1974)[5]	500	76	78
Bierner et al (1978)[6]	436	57	92
Aldrich et al (1979)[3]	181	66	52
Margolis et al (1980)[13]	800	40	93
Hung et al (1984) [7]	92	79	83
Detrano et al (1986)[8]	297	66	81
Uretsky et al (1988)[11]	600	76	79
de Korte et al (1995)[9]	778	52	91

in threshold for significant coronary obstruction, differences in disease prevalence in the different populations, and bias caused by evaluation of angiographic or fluoroscopic images without blinding to the result of the other test. Most studies included patients with a history of myocardial infarction, in which the presence of CAD is not a diagnostic issue any more. Only in three studies patients with a previous myocardial infarction were excluded.[7-9] The largest study of these three, by de Korte et al,[9] showed that fluoroscopy discriminated between diseased and non-diseased patients in women with atypical angina and in both men and women with non-specific chest pain. A meta-analytic review on the value of cardiac fluoroscopy reported a weighted mean sensitivity of 59% and a weighted mean specificity of 82%.[12] It can be concluded that the sensitivity of fluoroscopy for detection of CAD is quite low, so many patients with significant luminal narrowing do not have a positive fluoroscopic test result. The low sensitivity is partially due to image quantum noise and to interfering background structures like ribs, spine and great vessels that obscure calcification during the fluoroscopic examination. Notwithstanding the low sensitivity, Margolis et al[13] found that coronary calcification detected by fluoroscopy has prognostic significance: in a population of patients undergoing selective angiography because of suspected CAD, the five-year survival rate was 58% in subjects with calcification while survival was 87% in subjects without calcification. However, the low sensitivity limits the use of fluoroscopy as a screening test for latent CAD. In addition, there are major difficulties in detecting small calcified lesions by fluoroscopy that prohibit its use for mass screening: a high kilovoltage is needed to penetrate subjects with large body habitus, and only trained radiologists can reliably evaluate the presence of coronary calcification.

To improve the sensitivity of conventional fluoroscopy, Detrano et al[10] employed a temporal blurred mask subtraction technique after digitalization of

the fluoroscopic images. With this technique, interfering background structures are subtracted from the image, while moving cardiac structures partly escape from elimination. Furthermore, image averaging over part of the cardiac cycle results in blurring of the subtracted mask and at the same time in enhancement of radiodense objects like calcifications within a less radiodense field. This method was tested in 191 patients referred for coronary angiography. The sensitivity of digital subtraction fluoroscopy for significant luminal narrowing was superior to that of conventional fluoroscopy (92% vs 63%). Despite a decrease in specificity (65% vs 81%), the diagnostic accuracy of digital subtraction fluoroscopy was higher. Reading of coronary arteries on digital subtraction fluoroscopic images, scored as having heavy, mild or no calcification, was found to be highly reproducible.[14] A study of 1461 asymptomatic, high-risk subjects voluntarily undergoing digital subtraction fluoroscopy had some important findings.[15] Firstly, coronary calcification was detectable in the majority of asymptomatic subjects. Secondly, coronary calcification showed the strongest association with age. Since coronary calcification is increasingly common with age, the authors concluded that detection of coronary calcification alone may be inadequate for screening. They proposed that the severity of coronary calcification should be assessed in a quantitative manner, to determine thresholds for different age categories. Two radiological techniques were suggested to quantify coronary calcification: dual energy digital subtraction fluoroscopy[16] and ultrafast computed tomography (see discussion of electron-beam tomography later on).

Dual energy fluoroscopy exploits the energy dependence of tissue attenuation coefficients. Images are obtained by switching a high- and a low-energy beam at a high frequency. After correction for scatter and veiling glare (for the purpose of calcium quantification), weighted logarithmically transformed high-energy images are subtracted from low-energy images. From the resulting dual energy images, the absolute calcium mass can be quantified. Molloi et al[16] reported in 1991 that calcium masses estimated from dual energy images correlated well with true calcium masses in a calcium phantom and in calcified arteries. However, the distribution of calcifications within the coronary arteries has not been determined by this method, and will probably heavily depend on the projection. No in vivo reports have been published using dual energy digital subtraction fluoroscopy to quantitate coronary calcification.

Conventional computed tomography

Because of the high radiation absorption coefficient of calcium, it can be detected by fluoroscopy. CT, however, is far superior in measuring radiation absorption

coefficients of any material or tissue containing calcium, and therefore provides sharply improved soft tissue contrast. Calcification of the coronary arteries is often noted on chest scans performed for noncardiac indications.[17] Rienmüller and Lipton compared CT, fluoroscopy and coronary angiography in 47 patients (mean age 57 years).[18] In vessels with significant CAD, calcification was visible on 62% of the CT scans, but only on 35% of the fluoroscopic images. CT detected calcification in all patients with angiographic coronary stenosis and in all patients with fluoroscopic calcification. Another study of patients referred for coronary angiography evaluated the sensitivity and specificity for significant stenosis in the different coronary arteries.[19] Sensitivity of coronary calcification ranged from 16% for the right coronary artery (RCA) to 78% for the left anterior descending coronary artery (LAD), while the specificities ranged from 78% for LAD to 100% for RCA. The high positive predictive values (between 83% and 100%) suggested that significant CAD is very likely when coronary calcification is present. In a Japanese study 90% of patients with coronary calcification detected by CT had significant angiographic stenosis, while 80% of patients with stenosis showed calcification on the CT scan.[20] On individual artery basis, sensitivity of calcification for stenosis in a coronary artery was 65%, while the specificity was found to be 87%. All three studies recorded calcification in the individual coronary arteries as present or absent. However, more sophisticated determination of the degree of calcification is necessary to predict coronary disease in older subjects, because of the increasing prevalence of coronary calcification with age. Moore et al[21] attempted to design a scoring system to assess the amount of coronary calcification. The length of a calcification at the level of the aortic root (in centimeters), the number of slices with calcification, and the maximum width of calcification (in millimeters) were added. Thus, a score was yielded per vessel. Severe calcification was highly predictive of significant CAD. However, many vessels with significant stenosis did not show calcification. In a substudy of patients undergoing thoracotomy, the presence of coronary calcification increased the risk of cardiac complications. The authors concluded that CT was not sensitive enough to be used for cardiac screening, but recommended to report the presence of calcification on chest CT scans to alert cardiac surgeons. Conventional CT suffers from slow scan times (2 to 4 seconds), resulting in considerable motion artifacts, partial volume effects, breathing misregistration, low sensitivity for small calcified lesions, and inability to quantify calcification accurately.

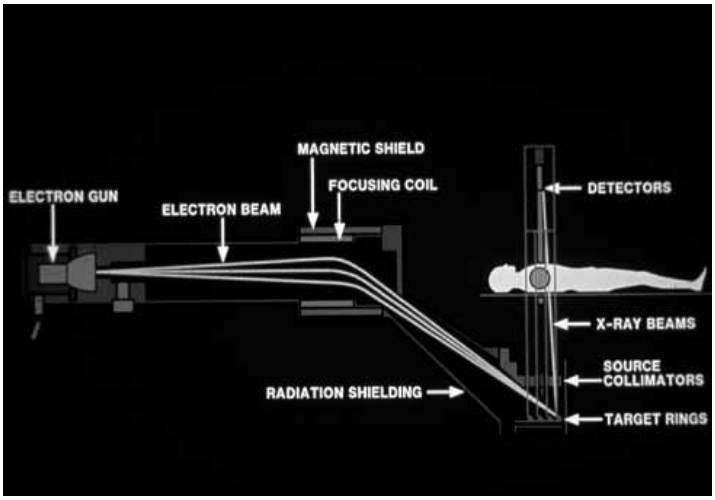


FIGURE 1
Design of the EBT scanner

Electron-beam tomography

Electron-beam tomography (EBT) has resolved many of the problems of conventional CT, due to an acquisition time of 100 ms or less (therefore formerly called 'ultrafast'). This is reached by the implementation of an electron-beam which sweeps along a curved X-ray source beneath the patient table, instead of a mechanically rotating X-ray tube. Figure 1 shows the design of the EBT scanner. The very fast acquisition time freezes cardiac motion, and greatly improves image quality. The scan commonly consists of 20 or 40 adjacent 3-mm images obtained by table incrementation. The images are usually acquired during one or two breath-holds, and are triggered by the electrocardiographic (ECG) signal at the moment of lowest cardiac motion. Previously 80% of the cardiac cycle, near the end of the diastole, was advocated as the ideal acquisition moment, but recent investigations suggest that the lowest velocity of coronary arterial movement is at 48% of the cardiac cycle.[22] Tanenbaum et al[23] were the first to document the detection of coronary calcification by EBT, and studied the correlation with angiographic findings. In 54 patients the angiographic results were compared with the presence of calcific deposits on 50 ms scans with 1.5 mm^2 pixel size. Significant stenosis was present in 43 patients. The sensitivity and specificity of coronary calcification for the presence of significant CAD were 88% and 100%, respectively. Agatston and Janowitz[24] reported the first large study of 584 subjects using EBT for the detection of coronary calcification. Of the subjects, 475 had no history of coronary disease. The scoring protocol consisted of 20 adjacent 3 mm slices with an acquisition

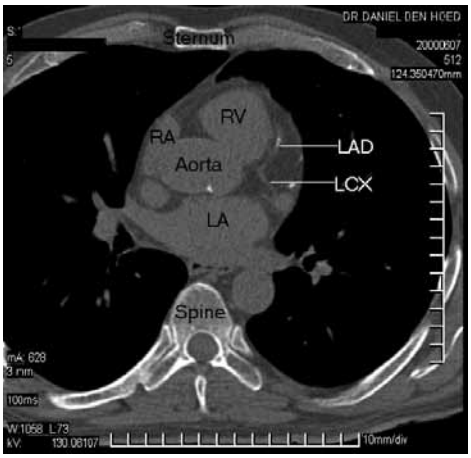


FIGURE 2
Image obtained with EBT. LA: left atrium, LAD: left anterior descending coronary artery, LCV: left circumflex coronary artery, RA: right atrium, RCA: right coronary artery.

time of 100 ms. EBT identified calcification in 90% of subjects, while fluoroscopy detected calcium in only 52% of subjects. Figure 2 shows an image obtained with EBT, with identification of several structures.

Quantification of coronary calcification, detected by EBT

Investigators soon realized the potential of EBT to perform quantitative measurements of coronary calcification. In an attempt to determine the amount of coronary calcification, Agatston and Janowitz devised an arbitrary scoring algorithm.[24] This method has become the standard for the quantification of calcium in the coronary arteries. On each image level, the pixels with a density over 130 Hounsfield Units (HU) are displayed. The value of 130 HU is based on the density 2 standard deviations above the average density of blood in the aorta. The threshold for a calcific lesion is set at 2 (in other studies sometimes 4) pixels. After placing a region of interest around all lesions in a coronary artery, the lesion area and density are determined. The calcium score of the individual calcific lesions is calculated by multiplying the calcium area (in square millimeters) and a factor based on the maximum density of the lesion. This factor ranges from 1 to 4 in the following manner: 1 = 130 to 199 HU, 2 = 200 to 299 HU, 3 = 300 to 399 HU, 4 = at least 400 HU. The total calcium score results from adding up the scores for all individual calcific lesions. The calcium scoring protocol has been incorporated in a number of dedicated software programs. Figure 3 shows the interface of a calcium scoring software program, while examples of different amounts of coronary calcification

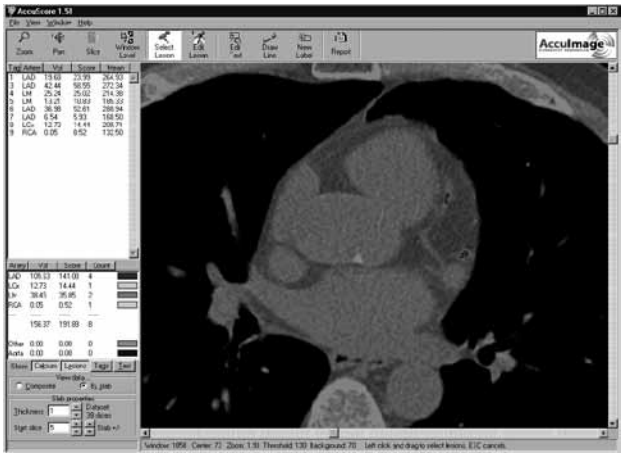
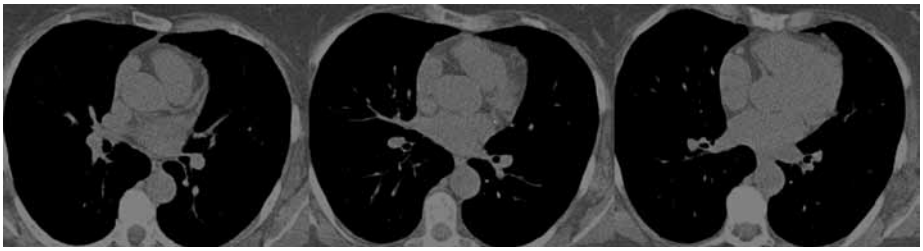
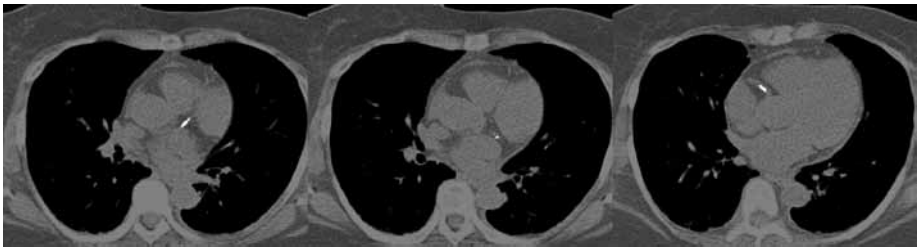


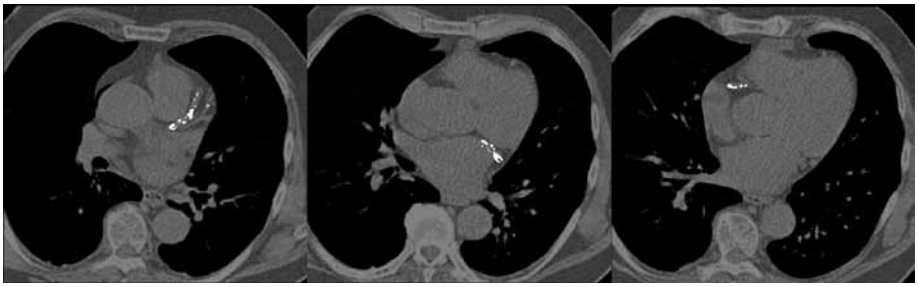
FIGURE 3
Interface of calcium scoring program software



A



B



C

FIGURE 4
Examples of none to mild (A), moderate (B), and extensive (C) coronary calcification, by EBT

TABLE 2
Interscan variability of EBT-derived calcium scores in patient studies

Study	Patients (n)	Variability (%)	
Kajinami et al (1993)[29]	75	34	
Shields et al (1995)[31]	50	38	
Wang et al (1996)[32]			
3-mm slices	72	29	
6-mm slices	77	14	
Callister et al (1999)[33]			
Traditional calcium score	52	19	
Volumetric score		13	
Yoon et al (2000)[34]	1000	39	
Achenbach et al (2001)[35]			
Traditional calcium score	120	20	
Volumetric score		16	
Möhlenkamp et al (2001)[36]			
Traditional calcium score	50	19	(median)
Area score		13	

are visible in figure 4. Agatston et al[24] found an increase in calcium score with age. Sensitivity, specificity and predictive values were calculated for different calcium scores in each decade. The negative predictive value of a calcium score of 0 for age groups 40 to 49, 50 to 59, and 60 to 69 years, was 98%, 94%, and 100%, respectively. Furthermore, the interobserver agreement for 88 scans scored by 2 independent readers was excellent. The authors put forward that the range of calcium scores allows the choice of threshold values tailored to the scan population. Rumberger et al[25] found that the amount of coronary calcification, expressed in the calcium score, was also strongly related to the total amount of plaque. In a study by Mautner et al[26] there was a close correlation between the calcium score and the histomorphometric calcium area in over 4000 segments from heart specimens.

Reliability of calcification measurements is of utmost importance if EBT is to be a screening test for CAD. However, there is debate on the accuracy and reproducibility of calcium scores using EBT. Although the intra- and interobserver variability of calcification measurements are low,[27,28] interscan variability is considerable, ranging from 14% to 37%.[29-36] Reproducibility found in different studies have been summarized in table 2. Different scan protocols have been investigated that could possibly increase the reproducibility of calcium scoring, such as different slice thickness,[32,37] use of a calibration phantom,[38] a different trigger moment in the cardiac cycle,[39] overlapping cross-sections[40] and new scoring methods. In a study by Wang et al,[32] reproducibility increased when scan slices of 6 mm instead of 3 mm were obtained. Mao[39] showed that triggering at 40% of the R-R interval reduced the calcium score variability by

TABLE 3
Different calculation methods for quantification of coronary calcification, detected by EBT

Traditional calcium score[24]:

$$\sum \left[\text{area} \left(\geq 130 \text{ HU} \right) \times \left\{ \begin{array}{l} 1 \text{ if (peak CT number 130-199 HU)} \\ 2 \text{ if (peak CT number 200-299 HU)} \\ 3 \text{ if (peak CT number 300-399 HU)} \\ 4 \text{ if (peak CT number } \geq 400 \text{ HU)} \end{array} \right\} \right]$$

Area score[27]: $\sum \left[\text{area} \left(\geq 130 \text{ HU} \right) \right]$

Lesion score[27]: $\sum \left[n \left(\text{ROI} \{ \geq 130 \text{ HU} \} \right) \right]$

Arterial summation[15]:
$A = \{ \sum [\text{area} (\geq 130 \text{ HU}) \times (\text{mean of each calcified ROI} - \text{mean of ROI without Ca})] \}$
A / slope* × slice thickness = arterial summation

Mass estimation[41]:
$\sum$ ** [proportion Ca × mass of voxel pure Ca]

Proportion Ca can be calculated:

1. CT number = (proportion soft tissue × CT soft tissue) + (proportion Ca × CT Ca)

2. In each voxel, proportion soft tissue + proportion Ca = 1

3. Proportion Ca = $\frac{\text{CT number - CT soft tissue}}{\text{CT Ca - CT soft tissue}}$

Volume score[64]: $\sum$ [area ($\geq$ 130 HU) × slice thickness]
Or: $\sum$ [volume ($\geq$ 130 HU) using isotropic interpolation]

$\sum$ is sum of all slices, except for mass estimation method. Ca is calcium hydroxyapatite. * slope is regression line calculated from known calcium concentrations and the mean CT numbers in each slice of cilindrical inserts in a calcium phantom.
** for all voxels with CT number > 100 HU.

34% as compared to standard triggering at 80%. Furthermore, Achenbach found a decrease in variability from 23% to 9% when overlapping cross-sections were used. Part of the interscan variability can be attributed to the susceptibility of Agatston's scoring method to substantial change in calcium scores with minimal variation in plaque attenuation or area. Other scoring methods have been proposed which seem to be more reproducible than the traditional calcium score according to Agatston.[24] Detrano et al described an arterial summation method[15] and a mass estimation method.[41] Although both the traditional scoring method and the alternative methods reflected the actual mass of calcium, the reproducibility was higher for the alternative scoring methods. Methods summing areas or regions of interest have been proposed by Kaufmann.[27] Callister and co-workers[33] found an improved reproducibility when scoring coronary calcifications with a volumetric method, with a reduction in variability from 19% for the traditional score to 13% for the volume score. An overview of the scoring methods is provided in table 3.

Multi-detector computed tomography

Recent progress in CT has spurred interest in modern mechanical CT scanners for the visualization of coronary calcification, not in the least because of the wide availability of mechanical CT systems. Only after development of subsecond slice acquisition time, continuous rotating CT systems in sequential or spiral mode were considered as a possible imaging modality for detection of coronary calcification. Becker et al[42] compared 50 patients using EBT and conventional 500-ms partial scan CT. The CT scan was acquired during two breath holds without ECG triggering. A high correlation coefficient was found between calcium scores of both modalities, but the variability in calcium scores between the modalities was 42%. Two studies have been performed using a dual-slice spiral CT scanner with 1 second rotation time.[43,44] Scanning was carried out during a single breath hold, without ECG triggering. In the study by Broderick et al[44] different HU thresholds and scoring methods were used. The sensitivity of the presence of coronary calcium for angiographically defined coronary artery disease ranged from 81 to 92%, but the specificity was low (52% to 61%), resulting in an accuracy of 74% to 84%. The test characteristics were comparable to those reported in EBT studies. In both articles spiral CT was suggested to be useful for the noninvasive detection of coronary calcification. Preliminary data from another study have shown that spiral CT derived calcium scores over- and underestimated calcification when compared to EBT derived calcium scores, primarily due to motion artifacts and partial volume effects.[45] By applying retrospective ECG gating, the window with minimal cardiac motion can be selected during each cardiac cycle. In a feasibility study by Woodhouse et al[46] retrospectively gated spiral scan images with 630 ms reconstruction showed less motion artifacts and a higher reproducibility than ungated images. Newer, four-detector CT systems yield an mathematical scan time down to 250 ms per slice with prospective triggering or down to 125 ms with retrospective gating. A couple of studies have been performed comparing multi-detector CT (MDCT) in sequential or spiral mode and EBT.[47-51] Remarks about the MDCT results ranged from disappointing to excellent. Becker et al[48] showed in 100 patients who underwent both EBT and sequential MDCT a high correlation between calcium quantification algorithms applied to images obtained by the two scanning methods, especially in case of volume and mass scoring. Knez et al[52] confirmed in 99 scanned subjects that volume scores yielded for EBT and sequential MDCT correlated very well. The mean variability between the two scanning methods was 17%, comparable to variability in scores obtained by repeated EBT examinations. Recently, Ohnesorge et al[53] used repeated spiral MDCT scanning with down to 125 ms mathematical temporal resolution in 50 patients. The variability of coronary calcium quantification was 23% for the Agatston score, and 18% for the volume

score – again comparable to EBT results. When overlapping increments were used, the variability decreased to 12% and 8%, respectively. The reconstruction of overlapping increments may decrease partial volume effects. A recent study by Schmermund et al[54] found that the percentile scores for over 2000 subjects scanned with sequential MDCT were comparable to percentiles of calcium scores obtained with EBT. Results thus suggest that MDCT may be a valid alternative for calcium scoring. However, due to an effective exposure time of 250 ms, a higher degree of coronary motion artifacts may be present with MDCT. Improvement of the rotational temporal resolution of CT to a slice scan time of at most 100 ms is needed to rule out motion artifacts.[49] The potential value of coronary calcification as a tool in primary prevention becomes even larger because widely available, MDCT scanners can also be applied for the assessment of coronary calcification. However, the technique of MDCT is constantly evolving, which complicates validation. For both MDCT and EBT, standardization in both scanning and scoring protocols are of utmost importance.

Intravascular ultrasound

Intravascular ultrasound (IVUS) is the most sensitive in vivo imaging modality for the characterization of plaque components, including calcium.[55,56] IVUS imaging makes use of a high-frequency ultrasound transducer, and can be performed during coronary angiographic procedures. On IVUS images, calcific deposits appear as bright echoes casting acoustic shadows. Qualitative description of calcifications include location (lesion or reference) and distribution (superficial or deep). Furthermore, the arc of calcium in degrees and the length of calcific deposits can be measured. On sequential images, the arc and length of calcification can be used to calculate the percentage of plaque surface that is calcified.[57] A comparison of IVUS imaging and histology of 54 atherosclerotic lesions showed that IVUS predicted the plaque composition correctly in 96% of cases.[58] All calcified plaque quadrants were identified by IVUS. Friedrich et al[59] examined 50 atherosclerotic arteries by IVUS and histology. Three histological types of calcification were found: dense calcification, microcalcification (size, up to 0.5 mm^2) and a combination of the two. Sensitivity and specificity for densely calcified plaques was 90 and 100%, respectively. However, only 2 of the 12 microcalcifications (17%) were detected by IVUS. In a study comparing IVUS and undecalcified histology of coronary plaques, IVUS identified calcified lesions with a high sensitivity and specificity (89% and 97%, resp.), but underestimated the calcified plaque cross-sectional area by 39%.[60] Two evident factors may contribute to the underestimation of the calcified plaque cross-sectional area. The first is a limited depth of ultrasound

penetration due to the high reflectivity of the acoustic signal by calcium. The second, a nonuniform rotation of the IVUS catheter drive shaft. In a clinical study by Mintz et al[61] 1155 coronary lesions were evaluated by IVUS and coronary angiography. While IVUS detected calcium in 73% of lesions, angiography could only identify calcium in 38%. Compared to IVUS, angiography had a sensitivity of only 48%, and a specificity of 89%. This finding was confirmed by Tuzcu et al[56] the presence of calcium was much higher by IVUS than by angiography. In patients with angiographically visible calcification, the mean arc of calcium on IVUS images was larger (175º vs. 108º). The distribution and amount of calcium within the vessel wall has a major significance in the diagnostic classification of lesion subsets (stable or instable). Furthermore, the identification of calcification patterns is a prognostic indicator for the outcome after coronary interventions, and can influence the choice of treatment. The disadvantages of IVUS are the invasive nature of the imaging modality and the limited portion of the coronary tree that is visualized. Thus, IVUS has no importance in screening for subclinical CAD.

Magnetic resonance imaging

Magnetic resonance imaging (MRI) receives increasing attention for the characterization of atherosclerotic plaque. Noninvasive MRI has been shown to have the potential to examine the fibrous and lipid components of coronary plaque. However, the ability of MRI to detect calcification is limited. Calcium structures do not respond to the incoming RF signal. The most common finding of calcification on T1- and T2-weighted spin-echo images is reduced signal intensity, primarily as a result of a low mobile proton density.[62] However, another study found extremely variable spin-echo findings for calcifications.[63] In contrast, gradient-echo images showed a marked (but nonspecific) decrease in intensity in case of calcification, due to T2 shortening.[63] Interestingly, concentrations of calcium particulate of up to 30% in weight reduce T1 relaxation times, resulting in increased signal intensity.[64] Although an important role of MRI in the detection of coronary calcification is not expected, MRI is a very promising technique to detect vulnerable plaques and evaluate plaque components.

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Epidemiology of coronary calcification 2.3

The prevalence of coronary calcification

The amount of coronary calcification, detected by electron-beam tomography (EBT) depends on sex and age. Studies among self-referred, asymptomatic subjects have shown that men generally have higher calcium scores than women and that calcium scores increase with age.[1-5] Table 1 shows sex- and age-stratified calcium scores of the largest study, which comprised 35246 self-referred subjects. More than 50 percent of the men already had detectable coronary calcification at the age of 40. Median calcium scores increased from 0.5 in men below 40 years to 473 in men over 74 years. Calcium scores in women were comparable to calcium scores in men who were 15 year younger. Until the age of 54 the median calcium score in women was 0. Median calcium scores in women increased to 75 in women over 74 years. Although the amount of coronary calcification increases with age, age itself is not a risk factor for coronary calcification. Rather, age is a cumulative measure of exposure to cardiovascular risk factors.

Cardiovascular risk factors and coronary calcification

It is commonly accepted that cardiovascular risk factors like obesity, hypertension, hypercholesterolemia, smoking and diabetes increase the risk of coronary heart disease. These risk factors are therefore called traditional risk factors. More recently, studies have identified new markers for cardiovascular disease like C-

TABLE 1
EBT calcium score percentiles for 25251 men and 9995 women within age strata. Reprinted from Hoff, et al,[80] with permission from Excerpta Medica Inc.

Calcium scores	Age (years)								
	<40	40-44	45-49	50-54	55-59	60-64	65-69	70-74	>74
Men (n)	3504	4238	4940	4825	3472	2288	1209	540	235
25th percentile	0	0	0	1	4	13	32	64	166
50th percentile	1	1	3	15	48	113	180	310	473
75th percentile	3	9	36	103	215	410	566	892	1071
90th percentile	14	59	154	332	554	994	1299	1774	1982
Women (n)	641	1024	1634	2184	1835	1334	731	438	174
25th percentile	0	0	0	0	0	0	1	3	9
50th percentile	0	0	0	0	1	3	24	52	75
75th percentile	1	1	2	5	23	57	145	210	241
90th percentile	3	4	22	55	121	193	410	631	709

reactive protein, fibrinogen and homocysteine. In the following paragraphs, we will discuss the effect of traditional risk factors and newer risk factors on coronary calcification.

Subjects with obesity have a relative risk of 2 to 2.5 for coronary heart disease as compared to subjects without obesity.[6] Population-based studies in young and generally middle-aged subjects have shown that measures of obesity are strongly associated with coronary calcification. Studies in these populations found that body mass index, waist to hip ratio, abdominal height and intra-abdominal fat were associated with coronary calcification.[7-9] In contrast, in older adults body mass index was no risk factor for coronary calcification.[10] Whether the lack of an association between obesity and coronary calcification in elderly is caused by selection due to survival, by frailty due to underlying disease (e.g. cancer) or by another underlying mechanism is unclear.

Blood pressure is positively and linearly related to cardiovascular disease.[11,12] A net reduction of 5-6 mm Hg in diastolic blood pressure is associated with a 38% reduction in stroke risk and a 16% reduction in coronary heart disease risk.[12] Furthermore, hypertension is an important risk factor for atherosclerosis at extracoronary sites.[13,14] Population-based studies in young and generally middle-aged subjects have shown that systolic and diastolic blood pressure are important risk factors for coronary calcification.[7-9,15] Adults with coronary calcification had a higher systolic blood pressure (123 mm Hg vs 117 mm Hg, $p<0.01$) and a higher diastolic blood pressure (82 mm Hg vs 77 mm Hg, $p<0.001$) than adults without any coronary calcification. Adults above the 90th percentile of systolic blood pressure were 6.4 times more likely to have coronary calcification. The relative risk for a diastolic blood pressure above the 90th percentile was 4.2 for men and 3.2 for women.[9] Analogous to the attenuation of the predictive value of hypertension for coronary heart disease in the very elderly, no association of hypertension and coronary calcification was found in subjects with a mean age of 80 years.[10]

Compared to subjects who have cholesterol levels below 5.0 mmol/l, the risk of coronary heart disease is 3-fold greater among subjects who have cholesterol levels between 6.5 mmol/l and 7.9 mmol/l and 5-fold greater among subjects who have cholesterol levels >8.0 mmol/l.[16] Population-based studies with an age range of 20 to 70 years have shown that total cholesterol, triglycerides and cholesterol/HDL cholesterol ratio are positively associated with coronary calcification while HDL cholesterol is inversely associated with coronary calcification in both men and women.[7-9] However, in the very elderly an association of cholesterol and coronary calcification is lacking.[10]

Pathologic studies in the 1970s already showed that smoking increases the amount of coronary and aortic atherosclerosis.[17] The development of EBT offered the opportunity to study the association of smoking and coronary atherosclerosis

TABLE 2
Distribution of coronary calcium scores among males: patients with diabetes versus those without by age category*. Reprinted from Mielke et al,[82] with permission from Excerpta Medica Inc.

Age	N	Mean	Median	SD
Without diabetes				
0-39	219	9.9	0	55
40-49	626	46	0	144
50-59	906	160	15	392
60-69	629	332	126	562
>69	211	635	330	799
With diabetes				
0-39	3	0	0	0
40-49	21	168	1	591
50-59	39	415	164	589
60-69	48	690	320	738
>69	13	787	569	775

*P=0.000 via the Mann-Whitney, for those with versus those without diabetes.

in vivo. In a population-based study among 740 adults between 20 and 59 years of age, Maher showed that subjects with a history of smoking had higher calcium scores than subjects who never smoked. In a multivariate model a history of smoking was only in men associated with coronary calcification.[7] Even in elderly aged 80 years the number of packyears smoked is strongly associated with coronary calcification.[10] In a self-referred high-risk population (72% of the subjects had ≥ 1 and 42% had ≥ 2 cardiovascular risk factors) a history of smoking was associated with a higher prevalence of detectable coronary calcification.[4] On the other hand, studies using intravascular ultrasound to detect coronary calcification in patients who underwent coronary angiography found a similar or even lower amount of calcification in smokers than in non-smokers.[18-20] This apparent contradiction is likely due to selection bias. Therefore, the results of the latter studies cannot be extrapolated to the general population.

Studies on diabetes and coronary calcification consistently showed that diabetes and markers of insulin resistance increase the amount of coronary artery calcification.[8,21-23] Table 2 shows calcium scores for men with diabetes and for men without diabetes in different age-categories. Men with diabetes have higher calcium scores than men without diabetes. Similarly, women with diabetes have higher calcium scores than women without diabetes (table 3).[22] A study in 139 diabetes patients (mean age 58) showed that subjects with diabetes had a mean calcium score of 344 while the control group, which was matched for age, sex and

TABLE 3
Distribution of coronary calcium scores among females: patients with diabetes versus those without by age category*. Reprinted from Mielke et al,[82] with permission from Excerpta Medica Inc.

Age	N	Mean	Median	SD
Without diabetes				
0-39	86	3.7	0	17
40-49	319	13	0	67
50-59	572	38	0	132
60-69	436	119	8	305
>69	174	197	65	313
With diabetes				
0-39	3	1.3	2	1.15
40-49	20	8.3	0	22
50-59	26	56	1	107
60-69	31	221	28	341
>69	7	300	193	314

*P=0.003 via Mann-Whitney, for those with versus those without diabetes.

cardiovascular risk factors, had a mean calcium score of 242. Moreover, this study showed that a calcium score of at least 400 was present in 26% of the diabetes patients and only in 7% of the subjects without diabetes.[21] On the other hand, a population-based study in elderly (mean age 80 years) showed no association between diabetes mellitus and coronary calcification.[10] This is considered to be due to the older age of the subjects. In conclusion it can be stated that diabetes mellitus is strongly associated with coronary calcification.

Population-based follow-up studies have shown that moderate alcohol consumption diminishes the risk of coronary heart disease.[24-28] Although it has been postulated that alcohol increases HDL cholesterol levels, the mechanism by which alcohol intake exerts this effect is not well understood. So far, only one study investigated whether alcohol consumption was associated with coronary calcification. In 1196 high-risk subjects no association was found between alcohol consumption and coronary calcification.[24]

C-reactive protein is a sensitive marker of inflammation that increases the risk of coronary heart disease in healthy subjects,[29-31] in patients with stable and unstable angina pectoris,[32-34] and in high-risk patients.[35] In addition, C-reactive protein has been related both cross-sectionally and prospectively to peripheral arterial disease.[36,37] However, C-reactive protein is not associated with the amount of coronary calcification in most studies.[10,38-40] As a possible explanation for the lack of the association, it has been suggested that hsCRP, in addition to being a

marker of atherosclerotic burden, may reflect an underlying propensity to plaque instability whereas coronary calcification may be a marker for mature and hence stable atherosclerotic plaque.[38]

Increased plasma fibrinogen concentration is an independent risk factor for cardiovascular disease.[41,42] There are several mechanisms by which fibrinogen may increase the risk of cardiovascular disease. Fibrinogen is the main coagulation protein in plasma, is an important determinant of blood viscosity and can act as a cofactor for platelet aggregation.[41,43] Fibrinogen may also contribute to cardiovascular disease by other direct effects: it is a component of atherosclerotic plaques and stimulates smooth muscle cell migration and proliferation.[43] Furthermore, the correlation with C-reactive protein suggests that fibrinogen reflects the inflammatory activity of progressing atherosclerosis.[44] Studies on the association of fibrinogen and coronary calcification have found conflicting results. A population-based study in 114 men and 114 women found that subjects who were selected on the basis of their high calcium score had higher fibrinogen than the control group.[40] In addition, a study in hypercholesterolemic patients found that fibrinogen was positively associated with coronary calcification.[45] However, other studies were not able to confirm these findings.[10,39] In conclusion, studies have suggested that fibrinogen may play a role in the process of atherosclerosis. Results from larger population-based studies have to be awaited before conclusions can be drawn on the association of fibrinogen and coronary calcification.

Although elevated serum homocysteine levels have been shown to correlate with coronary heart disease risk in cross-sectional studies, results from prospective studies are conflicting.[46] A recent meta-analysis of 57 studies showed that homocysteine is only weakly related to coronary heart disease and somewhat stronger related to cerebrovascular disease.[47] Experimental studies have shown that hyperhomocysteinemia is atherogenic, at least at early stages and in the presence of another potent risk factor,[48,49] while a recent clinical trial has shown that homocysteine lowering negatively affects intimal hyperplasia and reduces restenosis after coronary angioplasty.[50] However, epidemiologic studies found no effect of serum homocysteine levels on coronary calcification.[9,51,52] Whether this is due to lack of power or due to the measurement of calcification, which occurs late in the atherosclerosis process, remains to be established.

Racial differences in coronary calcification

There is still uncertainty regarding differences between black and white subjects in the prevalence, progression, and risk of coronary artery disease (CAD). Pathological studies have found more extensive fatty streaks in the coronary

arteries of blacks than of whites[53-55] but similar amount of raised lesions,[54] which are likely to contain calcium. In accordance with pathological studies a population-based study in young adults showed no racial difference in the presence of coronary calcification.[56] On the other hand population-based studies in elderly found that calcium scores were lower in black than in white subjects.[57,58] It has been suggested that this difference at older ages is due to a higher survival rate in white subjects as compared to black subjects with similar coronary calcium scores. Another explanation could be racial differences in the process of calcifying plaques.[57]

Coronary calcification and measures of extracoronary atherosclerosis

Pathology studies in the 1960s already revealed that atherosclerosis is a generalized process that is not limited to the coronary arteries but is present in the entire arterial system. Measures of extracoronary atherosclerosis have been found to predict the risk of coronary heart disease.[59-62] Recently, the development of EBT offered the opportunity to study the association of extracoronary atherosclerosis and coronary atherosclerosis in the living. Since then several studies have examined to what extent atherosclerosis in the extracoronary arteries reflects coronary atherosclerosis. Carotid atherosclerosis, as measured by intima media thickness and the number of plaques, is strongly associated with the amount of coronary calcification.[63-65] Similar associations with coronary atherosclerosis are present for aortic atherosclerosis[63,65,66] and peripheral atherosclerosis.[63,65]

Coronary calcification: a prognostic factor for manifest atherosclerotic disease

The most important cause of morbidity and mortality in individuals with cardiovascular risk factors is coronary atherosclerosis. Atherosclerotic plaques in a coronary artery can rupture or erode, which can lead to thrombosis and partial or complete occlusion of the culprit artery. If occlusion of the coronary artery lasts for some time, ischemia and subsequently infarction of the cardiac tissue, which is normally nourished by the culprit artery, can occur – causing a coronary event. Most subjects at risk do not develop symptoms of CAD until the occurrence of acute myocardial infarction or sudden cardiac death. The ability to predict and prevent the majority of coronary events is limited. Research has shown that asymptomatic individuals at risk benefit from agressive risk factor

modification or further testing.[67] However, risk factor assessment to identify high-risk subgroups is neither highly sensitive nor highly specific:[67] many individuals, considered to be at high risk of a coronary event will not experience myocardial infarction or sudden cardiac death, while many other subjects, considered to be low-risk, will suffer a coronary event. Thus, there is a clear need for new strategies for the primary prevention of clinical manifestations of CAD. In recent years, an alternative approach to risk stratification has been proposed: noninvasive evaluation of coronary calcification by EBT. This strategy is based on the close histopathological correlation between coronary calcium deposits and the total amount of coronary atherosclerosis.[68]

To draw conclusions about the possible clinical use of the calcium score, prospective results from large follow-up studies in unselected populations are of utmost importance.[69] So far four studies have been published on the prognostic value of coronary calcification for coronary events in asymptomatic subjects – see the overview provided in table 4. In a study by Arad et al[70] 39 coronary events were observed in 1172 asymptomatic subjects (mean age 53 years) during an average follow-up of 3.6 years. The population consisted of subjects who were either referred by physicians or self-referred in response to advertisements. The 39 events included 3 sudden cardiac deaths, 15 (nonfatal) myocardial infarctions, and 21 revascularizations. The mean calcium score among subjects with events was much higher than the mean calcium score among subjects without events (764 ± 935 vs 135 ± 432). A calcium score above 160 was associated with an odds ratio of all coronary events of 15.8 (95% CI, 7.4-33.9), while the odds ratio of only myocardial infarction and coronary death was 22.2 (95% CI, 6.4-77.4). Wong et al[71] observed in a study among 926 self-referred and GP-referred subjects a relative risk of cardiovascular events of 8.8 in the highest calcium score quartile compared to subjects without coronary calcification. However, of the 28 cardiovascular events that occurred during a mean of 3.3 years after scanning,

TABLE 4

Relative risks of coronary calcification for coronary heart disease in prospective EBT studies

Study	Subjects (n)	Mean age (y)	Follow-up (mo)	Events (n)	Cut-off calcium score	Relative risk
Arad et al (2000)[70]	1173	53	43	39	160	22.2
Wong et al (2000)[71]	926	54	40	28	5	4.5
Raggi et al (2000)[72]	632	52	37	27	median	21.5
Detrano et al (1999)[73]	1196	66	41	46	44	2.3
Park et al (2002)[74]	967	66	77	50	142	4.9/6.1*

* Relative risk was 4.9 for subjects with low C-reactive protein level, and 6.1 for subjects with a high C-reactive protein level.

23 were revascularizations. Referral bias could have influenced the results since revascularizations may partially have taken place on the basis of a high calcium score. Raggi et al[72] reported a relative risk of 21.5 (95% CI, 2.8-162.4) for myocardial infarction or cardiac death in the fourth quartile of the calcium score when compared to the lowest quartile. The subjects in the study population were referred for scanning because of the presence of cardiovascular risk factors. The investigation was based on calcium scores of 632 subjects (mean age 52 years) and 27 coronary events in 32 months. In the South Bay Heart Watch Study, 1196 high-risk asymptomatic subjects (mean age 66 years) were followed up during 41 months.[73] During the follow-up time, 29 myocardial infarctions and 17 coronary deaths occurred. The ability of EBT to distinguish subjects at high risk of coronary events from subjects at low risk was not better than that of risk factor assessment. In nondiabetic subjects from the same study, coronary calcification contributed independent information to the six-year risk prediction based on traditional risk factors and C-reactive protein.[74] The clinical value of coronary calcification needs to be confirmed in general populations of varying ages and background.

Atherosclerosis is thought to be a generalized process. Manifestations of atherosclerosis not only occur in the coronary arteries but also at other sites of the vascular system, such as the arteries in the leg (manifest as claudication intermittens), and the carotid or cerebral arteries (transient ischemic attack or stroke). Since calcification of the coronary arteries is also a marker of the amount of atherosclerosis elsewhere in the vessels, detection of the amount of coronary calcification could be related to manifestations of atherosclerosis distant from the heart. Vliegenthart et al recently showed an association between the amount of coronary calcification and the presence of stroke in the Rotterdam Coronary Calcification Study.[75] People whose calcium score was high (above 500) were three times more likely to have experienced stroke, compared to those with low calcium scores (0 to 100). Those with an intermediate score (101 to 500) were twice as likely to have had a stroke in comparison to the reference category. The results support the view that similar processes underlie coronary heart disease and stroke. However, cross-sectional studies can suffer from bias. Results from prospective studies are needed to determine if people with high levels of coronary calcification are at an increased risk of stroke.

EBT could provide a non-invasive method to identify asymptomatic people at high risk of coronary events as well as those with an increased risk of cerebrovascular events. If it is possible to assess a person's combined risk of coronary heart disease and stroke by identifying the amount of coronary calcification, risk factor modification could be more rigorously applied in those with the highest combined risk. A subsequent question is, whether the adding the calcium score to risk factor assessment in asymptomatic populations increases

the predictive power of cardiovascular disease. Prospective investigations in population-based studies should focus on the questions to what extent coronary calcification increases the risk of cardiovascular disease and whether coronary calcification adds incremental value to risk factor assessment for the prediction of cardiovascular disease.

Progression and regression of coronary calcification

A method to document the atherosclerotic process in the general population is invaluable for the assessment of interventions or lifestyle modifications aimed at reducing cardiovascular risk. Accurate measurements of coronary calcification can be obtained by EBT, with reasonable variability (down to about 10% with the new protocols). Since the amount of coronary calcium has been shown to be closely related to the total amount of coronary atherosclerosis,[68] the assessment of coronary calcification may facilitate the study of the progression of coronary atherosclerosis. A number of studies have been conducted in which changes in coronary calcification were evaluated over time (table 5). Annual progression rates in the different studies ranged from 20% to 52%, depending on the population. Neither age nor sex was found to be a significant predictor of progression.[76,77] Whether cardiovascular risk factors increase the rate of calcium progression is a

TABLE 5
Annual progression of coronary calcification in generally asymptomatic subjects, in observational EBT studies

Study	Subjects (n)	Follow-up (months)	Progression without therapy, %	Progression with statins, %
Yoon et al (2002)[77]	217	25	38	
Maher et al (1999)[84]	82	42	24	
Pohle et al (2001)[85]	104	15	27	
Janowitz et al (1991)[86]	20	13		
Asymptomatic			20	
CAD			48	
Callister et al (1998)[87]	27	12	52	
Callister et al (1998)[81]	149* (105 treated)	14	52**	5**
Budoff et al (2000)[76]	131* (60 treated)	26	39	15
Achenbach et al (2002)[88]	66	14/12†	25	9

* study among hypercholesterolemic patients. ** progression rate for whole follow-up period. † 14 months without treatment, then 12 months with treatment.

matter of debate. Hypertension and diabetes were found to be independent factors for progression of coronary atherosclerosis in a recent study by Yoon and others.[77] In contrast, Budoff et al[76] found in a smaller population that no cardiovascular risk factor affected the rate of progression. The most important factor for the prediction of progression seems to be the initial calcium score.

The progression of coronary atherosclerosis may not only be evaluable by EBT, multi-detector CT has also been proposed for this purpose. So far, one study, by Shemesh et al,[78] examined the rate of calcium progression using dual-slice spiral CT in 246 hypertensive subjects, who underwent a second scan after three years. Of the patients with a baseline calcium score of 0 28% had calcium on the second scan, while of those with calcium on the first scan, 70% showed progression. Although the absolute progression was highest in subjects with a high baseline calcium score, the relative progression was highest in those with initially little coronary calcification. Furthermore, the relative rate of calcium progression was found to be higher in hypertensive patients who had had a coronary event compared to those without an event, although the initial calcium score was on average higher in those with an event than in asymptomatic subjects.[79]

To accurately evaluate progression of coronary calcification over time, it is necessary to take into account the variability of calcium scores, which can occur in dual scan runs. Therefore, Bielak et al[80] designed a regression method to evaluate the change in calcium scores over time, while accounting for disagreement in the quantity of calcium between dual scan runs. The regression method was then tested on 81 participants who were scanned after a mean interval of 3.5 years. No significant change in calcium quantity was present in 73% of participants, while 26% had a large increase. One percent of the participants had a significant decrease in calcium score.

Prospective trials to assess the effectiveness of an intervention on cardiovascular event rate require large numbers of subjects to be followed for many years, at high cost. As a means to obtain effect estimates for all participants in a clinical trial, and after a shorter period, the use of surrogate end-points was introduced. Surrogate end-points have the advantage that much smaller numbers of subjects can be studied for a shorter period of time, with sufficient statistical power. Before a marker can be used as a surrogate end-point, the validity of the marker should be established, i.e. whether the marker can be used to assess the risk of cardiovascular events. A number of potential end-points have been investigated, that are indeed able to predict cardiovascular events. Furthermore, most surrogate end-points, like lumen diameter by coronary angiography and carotid intima-media thickness, are continuous variables, which provide a much more precise measure of the effect of the studied intervention. In addition to the mentioned benefits, surrogate markers provide information on the natural history

of and factors associated with progression. Coronary calcification by EBT, which is non-invasive and a direct measure of coronary atherosclerosis, may be very worthwhile as a surrogate end-point. The use of coronary calcification as end-point in clinical studies is gaining interest.

Until now, a couple of observational studies have published results on the amount of coronary calcification as end-point. Callister et al[81] investigated the effect of the statin HMG-CoA reductase inhibitor on the progression of coronary calcium in 149 patients. Coronary calcification progressed by 52% on average in the untreated patients (44 patients). Among the treated patients, the calcium volume score increased 25% in those whose mean LDL cholesterol level was above 120 mg/dL (40 patients), while there was a reduction in calcium score of 7% in those whose final LDL cholesterol level was below 120 mg/dL (65 patients). Thus, the extent of plaque progression, stabilization or regression was strongly related to the effectiveness of the cholesterol-lowering treatment. Another study on the effect of statins showed an increase in calcium scores of 15% in treated hypercholesterolemic patients, while the calcium progression in untreated patients amounted to 39%. A recent prospective study,[88] in which subjects eligible for cholesterol-lowering treatment were first followed for one year, and then treated for a year, with repeated EBT evaluation, showed that the progression of coronary calcium with treatment, was 8.8%, but 25% without treatment. The natural history of coronary calcification is also the focus of a clinical trial on the effect of aggressive versus moderate lipid-lowering treatment in postmenopausal women with hypercholesterolemia, called BELLES (beyond endorsed lipid lowering with EBT scanning). In this study, the percent change in calcium volume scores will be assessed after a year of randomized treatment. In conclusion, the assessment of coronary calcification shows great promise to serially monitor the effectiveness of medical therapy to prevent cardiovascular disease.

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CHAPTER

three

VALIDATION OF THE DETECTION OF CORONARY CALCIFICATION

3.1 Effect of slice thickness in electron-beam tomography scanning on calcium scoring

ABSTRACT

Purpose: To compare the accuracy of 3-mm and 1.5-mm slice thickness EBT scan protocols for calcium quantification and the prevalence of coronary calcifications at 3 mm and 1.5 mm.

Materials and Methods: EBT images were acquired with non-overlapping 1.5-mm and 3-mm slice protocols. Scans were obtained from an antropomorphic thorax phantom with calcium cylinders of different sizes and densities, and from 1302 study participants. A calcified lesion was defined as a minimum of two pixels (area, 0.52 mm^2) with an attenuation of minimally 130 HU. Quantification of the calcified lesions was performed using a volumetric method with isotropic interpolation. Participants were classified in categories based on cut-off levels for volume score quartiles for the 1.5mm scan.

Results: In the phantom, deviation of calculated volumes from the true cylinder volumes as well as measurement variation was generally higher for the 3-mm protocol than for the 1.5-mm protocol. In the participants, the median volume score was 100 (interquartile range, 11-409) for the 3-mm protocol, and 144 (35-513) for the 1.5-mm protocol. The agreement between classification of volume scores for the 1.5-mm and 3-mm scans was good (kappa, 0.62; $P < .001$). However, compared to the quartile classification for the 1.5-mm scan, 28% of all participants would be classified into a different category using the 3-mm protocol.

Conclusion: EBT scans with 3-mm slice thickness yield less accurate calcified volume estimates than 1.5-mm scans in a phantom. Use of thinner slice EBT protocols considerably affects the classification of individuals based on the amount of coronary calcification depicted.

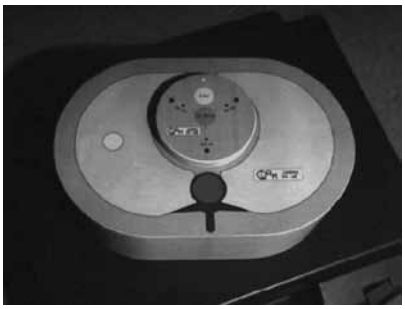
Introduction

Coronary calcification detected by electron-beam tomography (EBT) is a non-invasive tool for the identification of asymptomatic subjects at high risk of coronary heart disease. Several studies have shown that the amount of coronary calcification, expressed in a calcium score, is related to the risk of myocardial infarction and sudden cardiac death.[1-4] Furthermore, quantification of coronary calcification by EBT may enable study of progression and regression of coronary atherosclerosis.[5,6] Especially for the evaluation of change in the amount of coronary calcification over time, a high reproducibility and accuracy of calcium quantification are obligatory. Discussion has arisen whether measurement of coronary calcification by EBT meets these requirements. Firstly, the repeatability of traditional calcium scores on sequential EBT scans has been found to be poor.[7-9] Compared to the widely-used calcium scoring method according to Agatston, a volumetric measurement of coronary calcification yields more reproducible scores.[9,10] Secondly, the commonly used imaging protocol for coronary calcification may cause variability in calcium quantification. Partial volume effects considerably influence the results obtained with the standard 3-mm protocol, leading to loss of sensitivity for detection of small, relatively low-attenuation calcifications, which are also of clinical significance. It has been suggested that thinner slice protocols may substantially improve the accuracy of calcium scoring by decreasing partial volume effects.[9,11-13] The purpose of our study was to compare the accuracy of 3-mm and 1.5-mm slice thickness EBT scan protocols for calcium quantification in a phantom and the prevalence of coronary calcifications at 3 mm and 1.5 mm in a study population.

Materials and methods

Cardio CT Phantom

A stationary cardio CT phantom (QRM, Möhrendorf, Germany) was imaged. The phantom was antropomorphic, and resembled a thorax with artificial lungs and a spine insert, surrounded by soft tissue equivalent material. At the position of the heart, a hole was present in which an cylindrical insert fitted. The insert contained nine cylinders, consisting of calcium hydroxyapatite with densities of 200, 400 and 800 mg per cm^3. There were three cylinders of each density, with diameters of 1, 3 and 5 mm. The length of the cylinders was equal to their diameter. Volumes of the calcium cylinders were thus 0.8, 21.2, and 98.2 mm^3, respectively. The phantom is shown in figure 1.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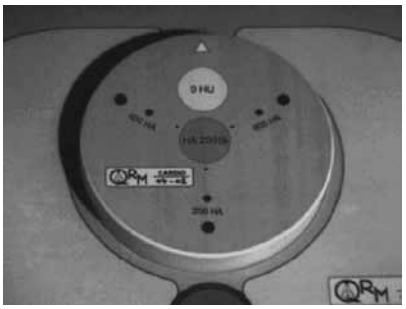
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FIGURE 1
Design of cardio CT phantom (A), detailed view of insert with calcium cylinders (B)

Study Population

The participants were a subgroup of a large population-based study, invited for EBT scanning. From 1997 to 2000, 3-mm EBT scanning was performed in the entire study population, consisting of volunteers from 60 through 85 years of age. A subgroup of 1302 participants also underwent scanning with a slice thickness of 1.5 mm. The mean age ± standard deviation (SD) of the participants was 71.2 ± 5.7 years, 48% were men. The Medical Ethics Committee approved the study, and all participants gave informed consent. To conform with the protocol outlines as approved by the Medical Ethics Committee, participants were not informed about their calcium score, since the clinical value of the detection of coronary calcification has not been defined

Scan Protocol

Imaging was performed with a C-150 Imatron scanner (Imatron, South San Francisco, California). Every day that the scanner was used, we calibrated the scanner using a water phantom. EBT scanning was performed with 100 ms acquisition time, 130 kV and 630 mA. Two scan protocols with different slice thickness were used: 3-mm collimation with 3-mm table increment, and 1.5-mm collimation with 1.5-mm table increment. The cardio CT phantom was positioned with the spine centered on the table, and the rear edge of the phantom in the middle of the gantry. We positioned the phantom isocentrically with the horizontal and vertical laser lights. The whole phantom was scanned, and the scan protocols were repeated four times. The participants underwent two consecutive studies within a few minutes of each other. Before the participants were scanned, they exercised adequate breath-holding. Images were acquired at 80% of the cardiac cycle, using electrocardiogram (ECG) triggering. Both scans were performed

during a single breath-hold. Images were obtained from the level of the root of the aorta through the entire heart. The first scan consisted of 38 3-mm slices, while the second scan consisted of 60 1.5-mm slices. Total radiation exposure associated with the two scans for the participants was approximately 2.5 mSv. The radiation exposure of the dual scanning was considered low, since this is no more than the normal annual background radiation (2-5 mSv). For both the phantom and participants, scan data were reconstructed with a 260-mm field of view by using a 512x512 matrix and a sharp reconstruction filter. The pixel area was 0.26 mm^2, and the voxel volume was 0.39 mm^3 for the 1.5-mm protocol, and 0.77 mm^3 for the 3-mm protocol.

Calcium Scoring Method

Quantification of calcifications was performed with AccuImage software (AccuImage Diagnostics Corporation, South San Francisco, California). A calcification was defined as a minimum of two adjacent pixels (area = 0.52m^2) with an attenuation over 130 Hounsfield Units. Reading of the scans was performed by two readers (RV, BS), who both have 3 years of experience in calcium scoring. The scan readers were blinded to the clinical data of the participants. The scans obtained from the phantom were read by one reader (RV). The scans from the participants were read by either of the two readers. The reader who performed the scoring of the 3-mm scan, also read the corresponding 1.5-mm scan, in a random order. On the scans of the phantom, a region of interest was placed around each calcium insertion visible. On the scans of the participants, a region of interest was placed around each high-attenuation lesion in the epicardial coronary arteries. The peak attenuation in Hounsfield Units and the area in mm^2 of the individual calcifications were calculated. For this study, the calcifications were quantified by a volumetric method with isotropic interpolation, as described by Callister et al.[10] We computed these volume scores, because of the reported improved reproducibility for volumetric calcium scoring, and because calculation of volumes enabled us to compare results for the phantom directly with the true volumes. For the participants, the scores for individual calcifications were summated, resulting in a volume score for the entire epicardial coronary system.

Statistical Analysis

For the phantom measurements, mean volume scores and standard deviations were computed. We calculated variation coefficients, defined as the standard

deviation divided by the mean, to assess the measurement variation. To compare the mean volume scores for the two slice protocols a paired t-test was applied.

The prevalence of calcifications on the two scans of the participants was compared by the McNemar test. Because of the skewed distribution of volume scores in the study population, medians and interquartile ranges were computed. Variability of the volume scores between the two scan protocols was calculated as follows: (volume score 1.5-mm scan – volume score 3-mm scan)/ 0.5x (volume score 1.5-mm scan + volume score 3-mm scan). Volume scores were logarithmically transformed by calculating natural log (volume score + 1) to reduce skewness. A paired t-test was applied to compare the mean log volume scores. The systematic error (D) and the limit of agreement between the measurements (D ± 1.96 SD) were determined according to the method described by Bland and Altman.[14] Because the magnitude of the differences depended on the mean of the volume scores, we used the aforementioned logarithmic transformation of the measurements. The limit of agreement was calculated to establish a range of values within which 95% of the differences between the measurements of the two slice protocols will fall.

Furthermore, to exclude possible breathing artifacts which could have occurred at the end of the 1.5-mm scan, we scored corresponding slices in a subset of participants (n=100), consisting of the first 40 1.5-mm slices and the first 20 3-mm slices starting from the aortic root. Medians, ranges, variability and log volume scores were computed for these corresponding scanned sections, and the t-test was used to compare mean log volume scores.

Quartiles of the volume score for the 1.5-mm scan were defined in categories of age and sex. The cut-off levels for volume score quartiles for the 1.5-mm scan were also applied to categorize the volume scores for the 3mm scan. An intraclass correlation coefficient (kappa) was calculated as a measure of correlation between categories. SPSS 10.0 for Windows (SPSS Inc., Chicago, Illinois) was used for data analysis. A statistically significant difference was assumed when the P value was less than .05.

Results

Phantom

The calcium cylinders with a diameter of 3 and 5 mm were identified on the 1.5- and 3-mm scans from all four scan sessions. The cylinder with a diameter of 1 mm and a density of 800 mg/cm^3 was also scored as a calcification on all scans. However, the cylinder with a diameter of 1 mm and a density of 400 mg/cm^3

TABLE 1
Mean volume scores for calcium cylinders by slicing protocol

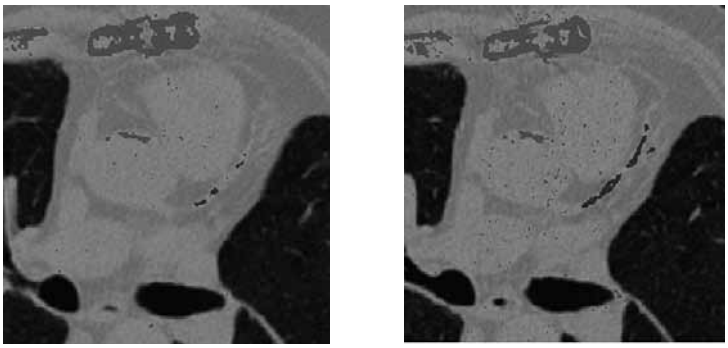
Calcium cylinder (diameter, density)	1.5-mm scan Mean ± SD (VC)	3.0-mm scan Mean ± SD (VC)	p*
1 mm			
200 mg/cm³	---	---	---
400 mg/cm³	1.1**	---	---
800 mg/cm³	4.1 ± 0.6 (14.2)	2.6 ± 0.5 (20.7)	0.03
3 mm			
200 mg/cm³	26.2 ± 2.5 (9.5)	25.1 ± 1.4 (5.6)	0.45
400 mg/cm³	44.0 ± 1.2 (2.7)	48.5 ± 5.0 (10.3)	0.19
800 mg/cm³	61.7 ± 2.3 (3.7)	73.1 ± 6.0 (8.2)	0.04
5 mm			
200 mg/cm³	115.0 ± 6.7 (5.8)	113.6 ± 9.6 (8.5)	0.78
400 mg/cm³	158.7 ± 5.5 (3.5)	185.5 ± 5.9 (3.2)	<0.01
800 mg/cm³	203.2 ± 3.6 (1.8)	260.2 ± 23.6 (9.1)	0.02

Values are mean volume scores ± standard deviation (SD) and the variation coefficient (VC) in % in parentheses.
* Paired t-test to compare volume scores for the two slicing protocols.
** Volume score of the one scan on which the calcium cylinder was quantifiable.

was only identified once, on a 1.5-mm scan, while the same size cylinder with a density of 200 mg/cm³ was not detected on any scan. Table 1 describes the mean volume score ± SD and variation coefficient for the cylinders. The measurement variation ranged from 1.8% to 14.2% for the scores of the thinner slice protocol, and from 3.2% to 20.7% for the scores of the standard protocol. For the majority of the calcium cylinders (71%), the measurement variation was lower in case of the 1.5-mm scans in comparison to the 3-mm scans. The volume scores obtained for both slice protocols deviated from the true volumes. Comparison of the volume scores for the two protocols showed significant differences for the 1-mm cylinder, for the 3-mm cylinder with the highest density, and for the 5-mm cylinders with density of 400 and 800 mg/cm³. In case of significant differences in volume scores, the 1.5-mm collimation resulted in volume scores that were closer to the true volumes than the volume scores obtained for the 3-mm collimation, except for the volume of the 1-mm cylinder, which showed a larger deviation in case of the 1.5-mm protocol.

Study population

On the 1.5-mm scan, calcium was detectable in 99% of the participants, while the 3-mm protocol detected calcium in 90% of the participants. On the basis of the



A B

FIGURE 2
Example of discrepancy between 3.0-mm slicing (A) and 1.5-mm slicing (B): 74-year old man

McNemar test, the prevalence of calcification was significantly higher with 1.5-mm collimation than with 3-mm collimation. Of the participants with a positive 1.5-mm scan, but without calcification on the 3-mm scan (n = 113), 92% had a volume score up to 20, while the maximum score was 149.9. When participants with a positive volume score up to 20 on the 1.5-mm scan were selected (n = 233), 45% had no detectable coronary calcification on the 3-mm scan. In no instance did participants without coronary calcification on the 1.5-mm scan have a positive score on the 3-mm scan. An example of discrepancy between the standard and the thinner slice protocol is shown in figure 2. Extensive calcification was visible on the 1.5-mm scan, but only a small part of the calcification was detected by the 3-mm protocol. The difference in volume scores for this participant was 474.2.

The distribution of volume scores in the study population was highly skewed. Median scores (interquartile range) were 144.0 (34.6-513.0) for the thinner slice protocol, and 100.3 (11.1-409.3) for the standard protocol. The median difference (interquartile range) between the 1.5-mm protocol and the 3-mm protocol was 25.6 (3.6 - 87.9). The variability in volume scores was 59%. The mean logarithmically transformed volume scores differed significantly for the 1.5-mm and 3-mm scan (4.8 ± 1.9 vs 4.2 ± 2.3[$p < 0.001$]). There was a systematic error between the volume score obtained for the thinner slice protocol and that for the standard protocol (D = 179%, 95% confidence interval 59% - 680%) (figure 3).

In a subset of 100 participants, the difference between volume scores for corresponding slices (40 1.5-mm slices and 20 3-mm slices) was examined, as well as the variability. The median difference between the volume scores for the selected scan sections was equal to the median difference for the whole scans (30.2), while the variability for the selections was comparable to that obtained for the entire scans (55% vs 52%).

TABLE 2
Cross-tabulation of the classification according to volume score quartiles, for the two slicing protocols

		Volume score quartiles 1.5-mm				Total
		1	2	3	4	
Volume score quartiles 3-mm*	1	311	147	9	1	468 (36%)
	2	10	170	110	3	293 (23%)
	3	1	12	196	63	272 (21%)
	4			14	255	269 (21%)
Total		322 (25%)	329 (25%)	329 (25%)	322 (25%)	1302 (100%)

* Cut-offs for quartiles based on volume score distribution for 1.5mm scans.

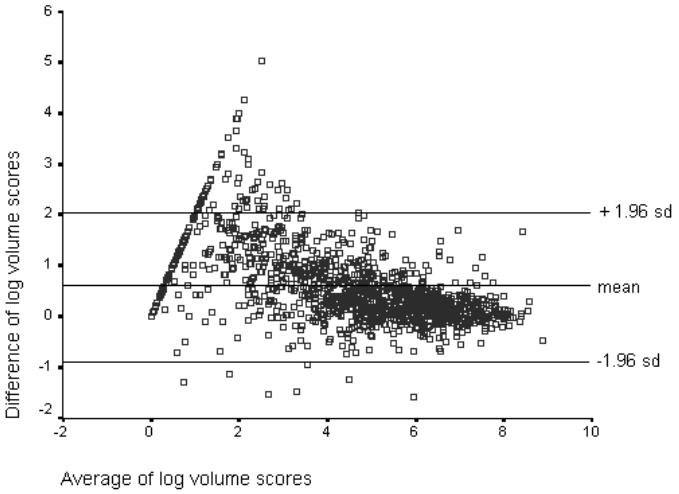


FIGURE 3
Bland-Altman plot comparing mean logarithmically transformed volume scores of 1.5-mm and 3-mm scans with the differences between the two scans (log volume score 1.5-mm – log volume score 3-mm)

Table 2 shows the classification of age- and sex-adjusted volume score categories for the two slice protocols. The intraclass correlation coefficient was .62 ($p<0.001$), indicating good agreement of classification between the 1.5-mm and 3-mm scan. However, compared to the quartile classification for the 1.5-mm scan, 28% (n = 370) of all participants would be classified into a different category, of which 90% into a lower category, in case of the standard, 3-mm protocol.

Discussion

In this study we compared a standard 3-mm EBT scan protocol for the detection of coronary calcification to a thinner, 1.5-mm protocol. The phantom results show that the detection of calcifications was more accurate using the 1.5-mm collimation concerning measurement estimates and measurement variation. In the population study, there was a significant difference in volume scores obtained for both scans. The agreement between classification of volume scores for the 1.5-mm and 3-mm scans of the participants was good. However, compared to volume score quartiles for the 1.5-mm protocol, 28% of all participants would be classified into a different category using the standard 3-mm protocol.

The reproducibility of calcium scoring on sequential EBT scans has been found to be poor.[7-9] One of the suggested improvements concerned the scoring algorithm. The drawbacks of the quantification of calcium according to Agatston et al[15] are its dependence on the peak voxel attenuation of a calcified lesion, which varies between scans, and an arbitrary scaling factor based on the peak attenuation. Callister et al[10] found a reduced variability when using a volumetric scoring method with isotropic interpolation in comparison to the standard scoring method (median variability, 9% vs 15%). A study by Yoon et al[9] confirmed improved reproducibility in case of the volumetric scoring algorithm, as well as for the calculation of calcium mass, based on voxel volume and attenuation. In accordance with these studies, we quantified calcifications using a volumetric scoring method.

To increase the accuracy of calcium quantification, not only different scoring algorithms were suggested, but also alterations in the commonly used scan protocol.[9,10,13] Wang et al[8] found that the variability in 6-mm scans was significantly lower than the variability in the standard 3-mm scans. However, in a study by Callister et al[11] the 6-mm protocol was shown to be significantly less sensitive for the detection of particularly small calcified lesions, in comparison to the 3-mm protocol. Several studies have suggested that use of slice thickness smaller than 3 mm may be necessary to decrease partial volume effects and prevent the loss of diagnostic and prognostic information, especially on small calcifications.[9,11-13] This is the first population study investigating the accuracy of a 1.5-mm slice protocol for EBT. Our results confirm the inferior detection of calcifications by the standard 3-mm protocol, compared to the thinner slice thickness. Calcified lesions were detected in significantly more participants when using 1.5-mm collimation, and almost half of the small calcifications detectable on 1.5-mm scans were missed using 3-mm collimation.

The volume scores calculated for the calcium cylinders in the phantom overestimated the true calcified volumes, with in general significantly higher

estimates for the 3-mm scan than for the 1.5-mm scan. The main cause for the overestimation of the calcifications in the stationary phantom is partial volume effect. The partial volume effect has a larger impact in case of the 3-mm collimation, since the volume averaging is greater in this scan protocol. Voxels containing part of a calcification, yet with a high density, contribute as calcified volumes if the average density of the entire voxel is over 130 HU. This effect was increasingly visible with higher densities: the volume overestimation increased, up to a factor 3.5. In the calcium cylinders with the lowest density, the volume estimates for the 3-mm scan were on average lower than for the 1.5-mm scan, indicating a partial volume effect neglecting partially calcified voxels with lower calcium density. In the population the mean volume score for the standard protocol was significantly lower than for the thinner slice protocol. This can probably be explained by the fact that many calcium deposits in atherosclerotic plaques have a density comparable to that of the lowest density calcium cylinder in the phantom. In the population, partial volume effects may therefore have led to underestimation, or even completely discarding, of small or relatively low density calcifications. Using a 1.5-mm collimation halves the size of the voxel, reduces the partial volume effect, and improves the accuracy. This was indeed shown by the improved volume estimates in the phantom, and the lower measurement variation for the thinner slice protocol. Also in the participants, the detection of calcified lesions was better on the 1.5-mm scan than on the 3-mm scan, as shown by the increased prevalence of calcifications and the significantly higher mean volume score. The partial volume effect will possibly continue to diminish with slice thicknesses smaller than 1.5 mm.

Some issues concerning the scanning and calcium scoring methodology have to be addressed. First, the use of 1.5-mm collimations implies the acquisition of more slices, and thus, the necessity of longer breath-holding. Before scanning the participants were trained to hold their breath for the duration of the scan. Especially in the distal part of the 1.5-mm scan, breathing artifacts may have occurred, leading to blurred images. We hypothesized that the occurrence of breathing artifacts in the distal scan section would not considerably affect the quantification of calcified lesions. Large or high-density calcifications in the distal parts of the coronary arteries are rare. To explore the possible influence of breathing artifacts in 1.5-mm scanning, we selected 20 3-mm slices and 40 1.5-slices in a corresponding region starting at the aortic root on scans from 100 subjects. The selection of these numbers of slices was made because of the following considerations. The standard 3-mm scan, consisting of almost 40 slices, requires a breath-hold time which can be maintained by most individuals. In addition, by selecting the first 6 cm of the axial scan, the largest part of the coronary arteries was evaluable. Scanning of the first 6 cm of the heart has previously been applied by Wang et al.[8] When the

selected scan section was evaluated, the mean difference between the volume scores obtained for the two slice thicknesses as well as the variability was very comparable to the results for the entire scans. Therefore, it is very unlikely that possible breathing artifacts on distal 1.5-mm slices have caused the difference in volume scores between both slice thicknesses. Secondly, in the study population, images were acquired at 80% of the cardiac cycle. A recent study[16] has reported that ECG triggering tailored to the heart rate of the individual patient, results in an improved reproducibility and less motion artifacts as compared to triggering at 80%. However, individual ECG triggering optimization will be a difficult procedure for screening purposes. Therefore, we think that a standardized ECG triggering protocol has to be established in the future.

EBT scanning of the heart is a sensitive non-invasive method to detect calcified lesions in coronary arteries. The quantification of coronary calcification may not only provide a means to categorize asymptomatic individuals according to their risk of coronary heart disease,[1-4] but may also enable evaluation of progression or regression of atherosclerosis.[5,6] Accurate measurement of coronary calcification, especially for the latter purpose, is obligatory. Using cut-off values of volume scores based on age- and sex-adjusted quartiles for the 1.5-mm scan, a considerable percentage of individuals was classified into a different category in case of the 3-mm scan. Further studies are necessary to investigate the reproducibility of the 1.5-mm protocol.

In conclusion, this study shows that EBT scans with a slice thickness of 3 mm yield less accurate calcified volume estimates than 1.5-mm scans, neglecting especially small calcifications. The use of thinner slice protocols considerably affects the classification of individuals based on the amount of coronary calcification.

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Effect of scoring parameter settings on calcium scoring 3.2

ABSTRACT

Introduction: Current multi-detector CT and electron-beam tomography (EBT) technology enable the evaluation of coronary calcification. Multiple software packages are available to quantify calcification using several scoring algorithms. All software packages allow adjustment of a number of scoring parameters. We investigated the effect of scoring parameters on the calcium score outcome.

Materials and Methods: Three parameters (4- or 8-connected, lesion size threshold, and interpolation) can be set in calcium scoring software, each theoretically having influence on the scoring outcome. This is shown using simplified examples. To evaluate the effect in real data we used AccuImage software to perform calcium scoring on randomly chosen EBT scans from 10 participants in an ongoing study. Both Agatston scores and volume scores were calculated.

Results: The decrease in connectivity from 8- to 4-connected had an overall negative effect on Agatston and volume scores; the effect on the volume score was larger (mean variability Agatston –0.3% and volume –0.6%). Decreasing the threshold from 4 to 2 pixels increased the calcium scores because smaller lesions were also selected as calcified plaques (mean variability Agatston 2.9% and volume 5.0%). Finally, the use of interpolation had a large negative effect on the volume score (mean variability -27.6%) and no effect on the Agatston score.

Conclusion: Parameter settings in software for quantification for coronary calcification affect the calcium score outcome. Therefore, parameter settings for calcium scoring should be standardized.

Introduction

Current multi-detector CT and electron-beam tomography (EBT) technology enable the evaluation of coronary calcification. Multiple software packages are available to quantify calcification using either Agatston or volume scoring algorithm. The software packages allow for quantification of coronary calcification with poor to good interscan reproducibility,[1] depending on acquisition technique and scoring method. Quantitative volumetric scoring has been shown to have better inter-scan reproducibility than Agatston scoring.[2-4] Multi-detector CT has been reported to have better inter-scan reproducibility than EBT. Main factors for the variability between scans are partial volume errors, inconsistent breathhold, and inconsistent ECG triggering or gating (especially for arrhythmia).[4,5] A potentionally important, frequently ignored factor affecting reproducibility is that all software packages allow adjustment of a number of scoring parameters and have different standard settings. Differences in scoring parameters could affect the outcome both in Agatston scoring and volume scoring, and thus may possibly result in differences in classification and subsequent strategies.

Materials and Methods

Theoretical background

Three parameters that can be specified by the user have been evaluated. Firstly, two dimensional connectivity can be set on 4- or 8-connected.[6] Secondly, lesion size threshold can be specified with frequently used cutoff points at 2, 3, or 4 pixels. Third, interpolation can be defined as either on or off. These parameters each have their own theoretical influence on the score outcome.

4- or 8-connected

Considering a pixel P, at row i and column j of an image, we can define its four horizontal and vertical neighbors.
(i-1, j), (i+1, j), (i, j-1), (i, j+1)
In addition four diagonal neighbors exist
(i-1,j-1), (i+1,j-1), (i+1,j+1), (i-1,j+1)
Two pixels are 4-adjacent if they are horizontal or vertical neighbors. Pixels that are 4-adjacent are called 4-neighbors. Two 4-adjacent pixels are connected if they have the same pixel value, in which case they are 4-connected (figure 1). Two

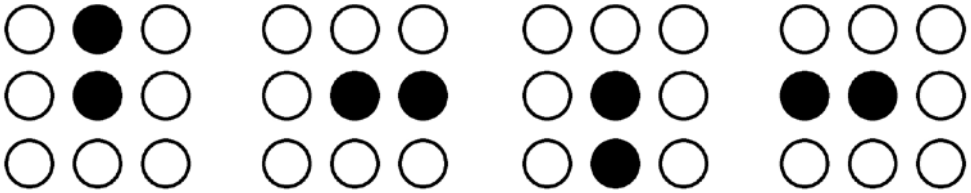


FIGURE 1.
Possible combinations of 4-connected pixels in a binary image

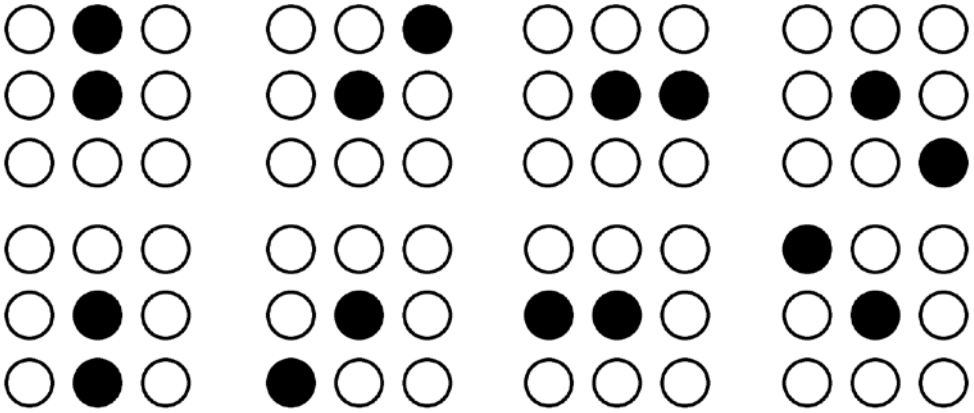


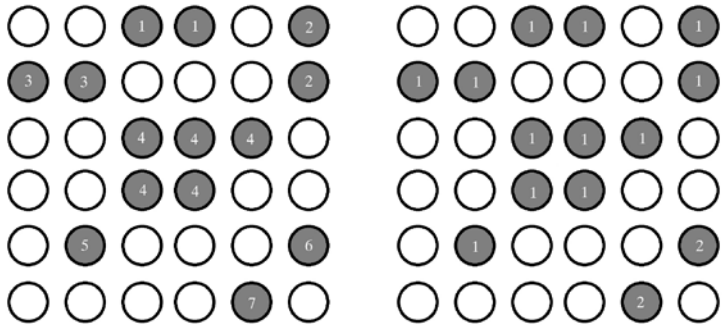
FIGURE 2.
Possible combinations of 8-connected pixels in a binary image

pixels are 8-adjacent if they are 4-adjacent or if they are diagonal neighbors. As before, pixels are 8-connected if they are 8-adjacent and have the same pixel value (figure 2).

Using the concept of connectivity we can define connected regions. Two pixels P and Q are 8-connected if a path of 8-adjacent pixels (including P and Q) having the same value can be traced between them. A similar definition applies to 4-connected pixels. In a connected region all the pixels are connected to each other, in either the 8-connected or 4-connected sense. Locating the connected regions is useful, since they often correspond to objects or groups of objects in the image such as calcified regions. After locating a connected region its size can be determined. An image can contain any number of connected regions not only depending on the image itself, but also on the connectivity (figure 3).

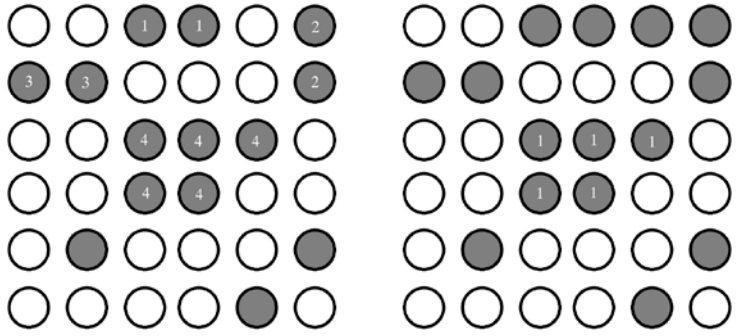
Lesion size threshold

After the connected regions are determined, the lesion size threshold is used to classify the connected regions into either calcified plaques or noise. The lesion



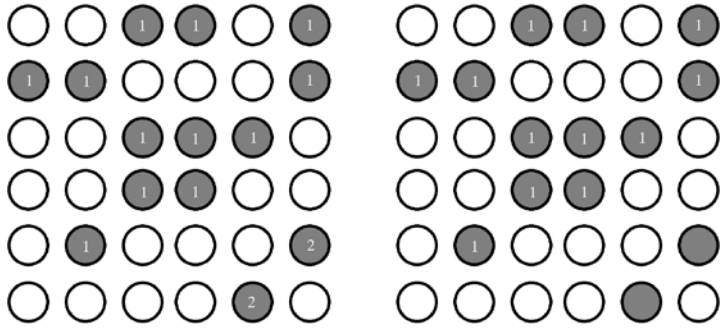
A B

FIGURE 3.
The displayed pattern results in 7 connected regions using 4-connectivity (A) and 2 connected regions when using 8-connectivity (B)



A B

FIGURE 4.
Using a threshold of 2 pixels, 4 regions are 4-connected (A); using a threshold of 4 pixels, 1 region is 4-connected (B)



A B

FIGURE 5.
Using a threshold of 2 pixels, 2 regions are 8-connected (A); using a threshold of 4 pixels, 1 region is 8-connected (B)

size threshold is set at a certain value t. All connected regions with size < t are classified as noise, where typical values for t are 2, 3, and 4 pixels. Obviously, the selection of the threshold influences the number of lesions detected, and thus the resulting calcium score outcome (figures 4 and 5).

Interpolation

Inter-slice interpolation is the retrospective calculation of in-between slices using post-processing software to increase the quality of rendered images. The calculation is based on the information contained in the surrounding (measured) slices. The main reason for the use of inter-slice interpolation is to obtain a smoother transition between slices, and to obtain more isotropic voxels with a similar resolution in all three axes. In case of volume measurement of calcifications, interpolation can be used to obtain a more reliable measure of the calcified plaque burden. Because of the calculation of in-between slices, the intra- and inter-observer variability decreases.

TABLE 1.
Settings for the eight different measurements

Measurement nr:	1	2	3	4	5	6	7	8
4-connectivity	X	X	X	X				
8-connectivity					X	X	X	X
Threshold 2 pixels	X	X			X	X		
Threshold 4 pixels			X	X			X	X
Interpolation	X		X		X		X	
No interpolation		X		X		X		X

TABLE 2.
Classification of plaque burden and implications based on calcium score using Agatston scoring method

Calcium score	Plaque burden	Probability of significant CAD	Implications for CV risk
0	No identifiable plaques	Very low	Very low
1-10	Minimal identifiable plaques	Very unlikely	Low
11-100	Mild atherosclerotic plaque	Mild or minimal coronary stenosis likely	Moderate
101-400	Moderate atherosclerotic plaque	CAD highly likely	Moderately high
>400	Extensive atherosclerotic plaque burden	High likelihood of significant coronary stenosis	High

Scan data analysis

EBT scans were used, from 10 randomly chosen participants in an ongoing population-based study. Acquisition was performed within one breath hold on a C-150 EBT scanner (Imatron, San Francisco, CA). With a 100ms exposure per slice, 38 slices were acquired with a 3 mm slice thickness and a field of view of 260 mm. ECG triggering was applied at 80% of the cardiac cycle. To evaluate the effect in scan data we used Accuscore calcium scoring software (AccuImage, San Francisco, CA) with 8 different settings (table 1) of the scoring parameters. Agatston scores and volume scores were computed. The severity of calcification was classified by Rumberger et al[7] (table 2) to determine the likelihood of significant coronary artery disease (CAD). The variability in calcium scores for each change in scoring parameters was calculated by dividing the difference between the two resulting calcium scores, by a half times the sum of the two calcium scores, and multiplying the result with 100%.

Results

To show the severity of calcification in our subject group the mean Agatston scores over the eight measurements were calculated (figure 6). No subjects had a calcium score of 0 or of 1 to 10. Four subjects had a calcium score of 11 to 100, three had a calcium score of 101 to 400, and another three a calcium score above 400. One subject (subject 7) had a very high calcium score. Because the selection of calcified plaques had to be performed manually for each of the eight measurements intra-observer variation was present in this subject. This could be demonstrated by the fact that certain measurements that are supposed to be identical provided different results. An example of this is that turning on the interpolation, which only affects the volumetric measurement, had a negative effect on the Agatston score and the number of lesions, while these effects should be zero just like for the other subjects. Therefore, all results are shown both with and without this subject.

The decrease in connectivity from 8- to 4-connected had an overall negative effect on both Agatston and volume score. The effect on the volume score was larger than on the Agatston score (table 3, figures 7-9). Decreasing the threshold from 4 to 2 pixels had an overall positive effect ranging from 0.4% to 8.8% for the Agatston score, 0% to 20.1% for the volume score, and 12.5% to 100.0% for the number of lesions (figures 10-12). Finally, the use of interpolation had a large negative effect on the volume score (figure 13) ranging from -2.4% to -55.2%. Table 3 shows the range in variability for the separate adjustments, averaged for the four combinations of changes, as well as the mean variability over the scans.

TABLE 3.
Mean variability and range of the variability of the three parameter changes for both Agatston and volume score (bottom 3 measurements excluding patient 7)

Score type	Variability 8 to 4 connectivity		Variability threshold 4 to 2		Variability interpolation off to on	
	Mean	Range	Mean	Range	Mean	Range
Agatston	-0.34	-4.25 - 3.21	2.89	-1.22 - 8.41	---	---
Volume	-0.60	-9.95 - 4.10	4.95	-0.86 - 18.19	-27.64	-55.18 - -1.73
Lesion number	-2.88	-23.53 - 10.53	35.74	10.53 - 66.67	-0.44	-5.06 - 0.00
Agatston	-0.63	-4.25 - 3.21	5.50	0.04 - 18.19	---	---
Volume	-0.99	-9.95 - 0.00	3.24	0.37 - 8.41	-30.46	-55.18 - -2.37
Lesion number	-3.61	-23.53 - 10.53	38.19	11.76 - 66.67	0.00	0.00 - 0.00

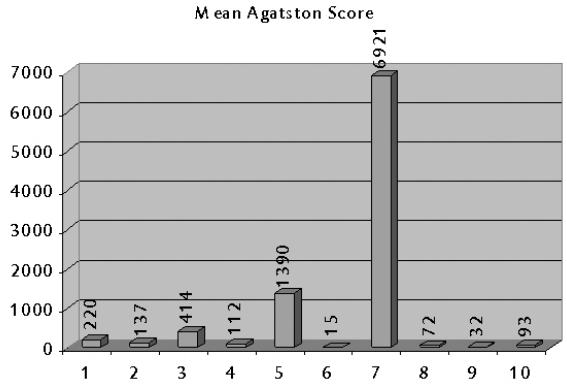


FIGURE 6.
Mean Agatston scores of the 10 subjects

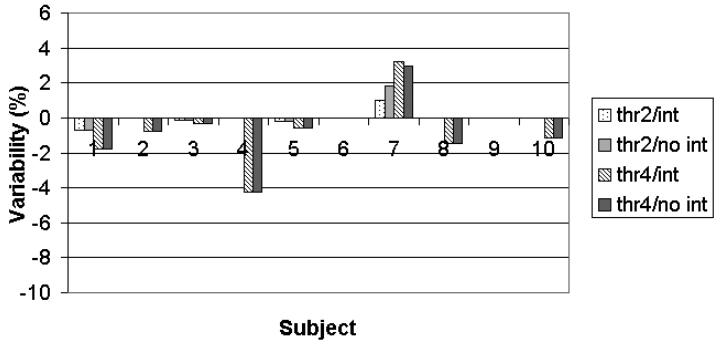


FIGURE 7.
Variability of the Agatston score with change in connectivity from 8 to 4

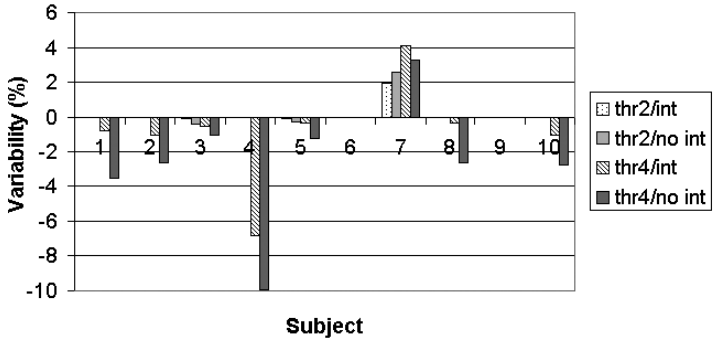


FIGURE 8.
Variability of the volume score with change in connectivity from 8 to 4

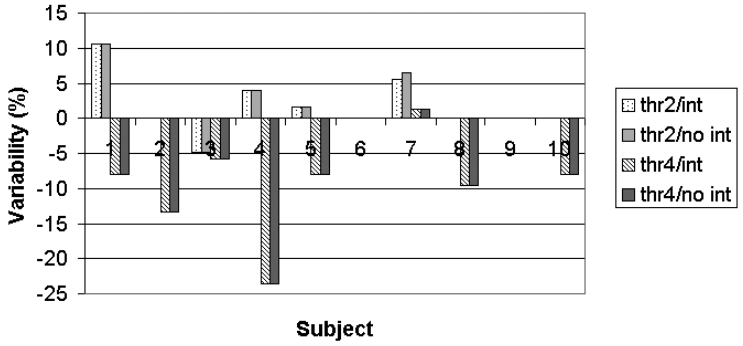


FIGURE 9.
Variability in the number of lesions with change in connectivity from 8 to 4

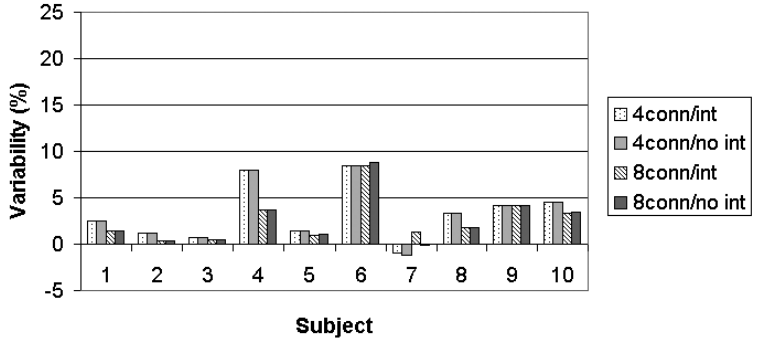


FIGURE 10.
Variability of the Agatston score with change in threshold from 4 to 2 pixels

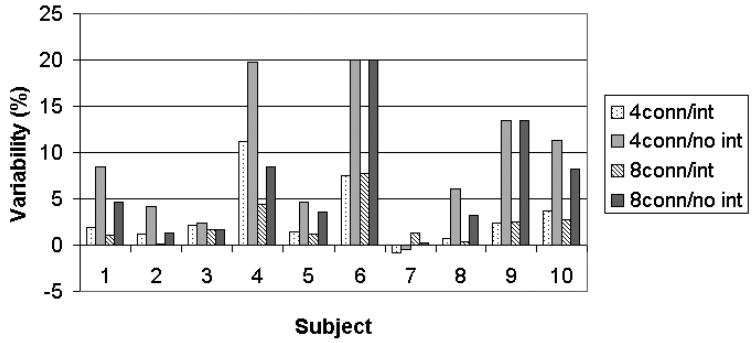


FIGURE 11.
Variability of the volume score with change in threshold from 4 to 2 pixels

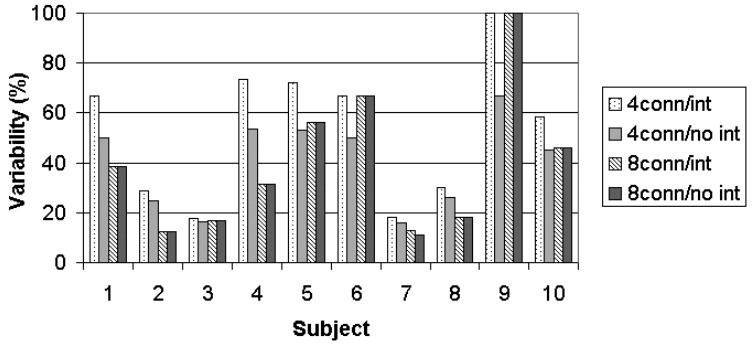


FIGURE 12.
Variability of the number of lesions with threshold from 4 to 2

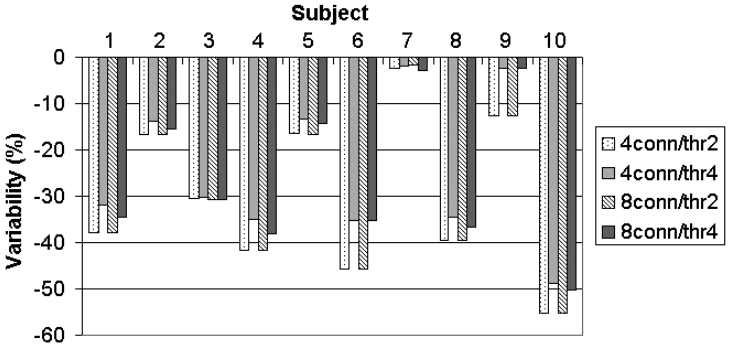


FIGURE 13.
Variability of the volume score when turning on interpolation

Discussion

In this study, not only the theoretical effects of different parameter settings in calcium scoring software were shown, but also the in vivo effects. We found that the scoring parameter settings influenced the calcium scoring outcomes. Decreasing the definition of pixel connectivity resulted in a decrease in calcium score. This effect is logical because of the elimination of all corner-connected pixels, and all small lesions with 8-connectivity from the measurement. Because of the weighting factors used in Agatston scoring, the small lesions which were eliminated only had little impact because they were usually lesions with lower HU values. Furthermore, removal of boundary pixels of larger lesions was less important in Agatston scoring than in volume scoring because of the weighting factor used in the algorithm according to Agatston.

Decreasing the threshold from 4 to 2 pixels had an overall positive effect because smaller lesions were also selected as calcified plaques. Again the effect in the Agatston score was smaller because small lesions were usually of lower density, and thus, had a smaller influence on the weighted Agatston score than on the volume score.

Finally, the use of interpolation had a large negative effect on the volume score. A possible explanation is that interpolation smoothes out the edges which are overestimated without interpolation, and thus provides a lower (and more accurate) volumetric calcium score. Overview of the number of lesions clearly showed that no change was found, except in subject 7. Overall, volume score showed a larger variation with changing parameter settings than the Agatston score.

One of the limitations of our study is the small number of subjects. In this small study, no changes in classification were found caused by the change of the parameter settings. A larger study with more subjects should evaluate the possible effect on classification. Furthermore, our software did not support the use of newer scoring techniques, such as the mass score. The mass score is an absolute mass quantification that uses different scan parameters and a calibration phantom in each scan. However, it is likely that these newer techniques also suffer from dependence on certain parameter definitions.

It has been demonstrated that calcium scoring results obtained with different calcium scoring software may differ significantly.[8,9] The differences in calcium scores were mainly attributed to differences in the selection of the lesions (ranging from automatic to manual). The conclusion was that the software used for calcium scoring should be acknowledged. The possibility of different score parameter settings as a cause for these different results was not discussed.

According to previously published results, calcified plaque scoring using a volumetric method shows a higher accuracy and better reproducibility than when using the Agatston score.[2,3] However, changing parameter settings had a higher effect on volume scoring than on Agatston scoring in our study. This is in accordance with results from Yamamoto et al[9] indicating that for different software systems, differences in volume scores were larger than for Agatston scores.

Repeated assessment of coronary calcification has been proposed as a promising method to evaluate progression or regression of atherosclerosis[10] for example during lipid lowering treatment. Budoff et al showed a 15% per year increase in Agatston score in patients with treatment and a 39% per year increase in patients without treatment.[11] In 66 subjects with treatment the volume score was found to increase by a median of 9% per year, and without treatment by 25% per year.[12] In our study difference in scoring parameters resulted in a mean variability up to 6% for the Agatston score and up to 30% for the volume score. Because the change in calcium scores on repeated EBT scans may be in the same range as the variability between software systems or parameter settings, inferences from repeated measurements should be made with caution. Our findings emphasize the need for further improvement in the reproducibility of calcium scoring.

In conclusion, software parameter settings affect the calcium scoring outcome. Since software packages often have different standard parameter settings, calcium scores for the same individual may differ in different institutes. This could be particularly important in case of repeated measurements in different institutes. The variability in calcium scores due to different parameter settings underlines the importance of standardization of the technique to assess and quantify coronary calcification.

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CHAPTER four

DETERMINANTS OF CORONARY CALCIFICATION AND ATHEROSCLEROSIS

Cardiovascular risk factors and coronary calcification

4.1

ABSTRACT

The present study was designed to examine the associations between classical cardiovascular risk factors and coronary calcification assessed by electron-beam tomography (EBT) in older subjects. The Rotterdam Coronary Calcification Study is embedded in the Rotterdam Study, a population-based study in subjects aged 55 years and over. Participants of the Rotterdam Coronary Calcification Study underwent an EBT scan between 1997 and 2000. Coronary calcification was quantified according to the Agatston score. Body mass index, systolic blood pressure, diastolic blood pressure, total cholesterol, HDL-cholesterol, diabetes and smoking were assessed 7 years before scanning (1990-1993) and concurrently to scanning (1997-2000). We used the first 2013 participants for the present analyses. Calcium scores were 5 times higher in men than in women. Calcium scores increased with a factor 1.5 in men and with a factor 1.9 in women for every 5 years increase in age. While all risk factors assessed 7 years before scanning were strongly associated with the amount of coronary calcification, associations for blood pressure and cholesterol attenuated or even disappeared when measured concurrently to scanning. Calcium scores in subjects with 3 or more risk factors were 5 times higher than in subjects without risk factors. This population-based study in older subjects shows that risk factors are associated with coronary calcification. Associations with risk factors were stronger when measured at earlier age.

Introduction

Classical cardiovascular risk factors like obesity, hypertension, hypercholesterolemia, diabetes and smoking are associated with atherosclerosis at different sites,[1-7] and with an increased risk of coronary heart disease.[8-12] Several population-based studies have investigated the association between these classical risk factors and coronary calcification. In asymptomatic adults, classical risk factors were strongly associated with the amount of coronary calcification.[13-20] However, only one study has been performed in older subjects (mean age 80 years).[21] In the latter study, only smoking and triglycerides were associated with coronary calcification.

We investigated the associations between classical cardiovascular risk factors and coronary calcification in a population of older subjects. Classical risk factors were measured on average 7 years before electron-beam tomography (EBT) scanning and at the time of EBT scanning.

Materials and Methods

Study population

The Rotterdam Coronary Calcification Study was designed to study determinants and consequences of coronary calcification, detected by EBT. The study is embedded in the Rotterdam Study. The Rotterdam Study is a population-based study, which started with a baseline visit between 1990 to 1993. All inhabitants of a suburb of Rotterdam, aged 55 years and over, were invited (response 78 percent). The rationale and design of the Rotterdam Study have been described elsewhere.[22] Follow-up visits took place in 1993 to 1994 and 1997 to 1999. From 1997 onward, participants through 85 years of age were invited to participate in the Rotterdam Coronary Calcification Study and to undergo an EBT scan. Subjects in nursing homes did not visit the research center and thus were not invited for the study. Of the 3371 eligibles, scans were obtained for 2063 subjects (response: 61 percent). Due to several causes, i.e. metal clips from cardiac surgery, severe artifacts and registration errors (ECG, acquisition), image acquisition data could not be reconstructed or analyzed in 50 subjects. Thus, scores were available for 2013 participants. The median duration between risk factor assessment and EBT scanning was 7 years (1990-1993) and 50 days (1997-2000). The Medical Ethics Committee of Erasmus Medical Center Rotterdam approved the study, and all participants gave informed consent.

Coronary calcification

We assessed coronary calcification in the epicardial coronary arteries detected on EBT scans. Imaging was performed with a C-150 Imatron scanner (Imatron, South San Francisco, California, U.S.A.). Before the subjects were scanned, they exercised breath holding. From the level of the root of the aorta through the heart, 38 images were obtained with 100 ms scan time and 3 mm slice thickness. We acquired images at 80% of the cardiac cycle, using electrocardiogram triggering, during a single breath-hold. The scanner was calibrated on a daily basis using a water phantom. Quantification of coronary calcification was performed with AccuImage software (AccuImage Diagnostics Corporation, South San Francisco, California, U.S.A.) displaying all pixels with a density of over 130 Hounsfield Units. Trained scan readers were blinded to the clinical data of the participants. A calcification was defined as a minimum of two adjacent pixels (area = 0.52 mm^2) with a density over 130 Hounsfield Units. We placed a region of interest around each high-density lesion in the epicardial coronary arteries. The peak density in Hounsfield Units and the area in mm^2 of the individual coronary calcifications were calculated. A calcium score was obtained by multiplying each area of interest with a factor indicating peak density within the individual area, as proposed by Agatston et al.[23] We added the scores for individual calcifications, resulting in a calcium score for the entire epicardial coronary system.

Cardiovascular risk factors

The Rotterdam Coronary Calcification Study is embedded in the ongoing Rotterdam Study. Therefore, information was available on risk factors assessed 7 years before EBT scanning (1990-1993) and concurrent with scanning (1997-2000). Apart from blood sampling methods, protocols for the interview and clinical examination were identical at both examinations. Information on smoking and medication was obtained during the home interview of the Rotterdam Study and the number of packyears of smoking was computed. Clinical measures were obtained during a visit at the Rotterdam Study research center. Height and weight were measured and body mass index (BMI) was calculated (weight (kg)/height $(m)^2$). Blood pressure was measured at the right brachial artery using a random-zero sphygmomanometer with the participant in sitting position. The mean of two consecutive measurements was used in the analyses.

Blood samples obtained in 1990-1993 were non-fasting while blood samples obtained in 1997-2000 were fasting. Laboratory techniques were identical for both periods: serum total cholesterol was determined by an enzymatic procedure,

high-density lipoprotein (HDL) was measured similarly after precipitation of the non-HDL fraction[24] and glucose was determined enzymatically by the hexokinase method. Diabetes was defined as the use of anti-diabetic medication and/or non-fasting glucose levels ≥ 11.1 mmol/l (1990-1993) and/or fasting glucose levels ≥ 7.0 mmol/l (1997-2000). Impaired glucose tolerance was defined as no use of antidiabetic medication and/or non-fasting glucose levels of 7.8-11.0 mmol/l (1990-1993) and/or fasting glucose levels of 6.1-7.0 mmol/l (1997-2000).

Statistical analysis

The distribution of the calcium score is highly skewed and therefore, log (total calcium score + 1) was used for linear regression analysis. Age-adjusted regression coefficients were computed using cardiovascular risk factors as independent variables and the log calcium score as dependent variable. For age and sex the exponential increase in calcium score was computed by taking the exponent of the regression coefficient. Except for the analysis of sex and the log calcium score, all analyses were performed in men and women separately.

Analysis of covariance was used to compute age-adjusted geometric mean calcium scores for categories of risk factors. For this, we categorized BMI, systolic blood pressure, diastolic blood pressure, total cholesterol, HDL-cholesterol into quartiles and divided the number of packyears smoked into four categories (0, 1-10, 11-20, ≥ 21).

We computed a risk score by adding the number of risk factors. We defined risk factors as follows: (1) BMI ≥ 30 kg/m^2, (2) systolic blood pressure ≥ 160 mm Hg and/or diastolic blood pressure ≥ 100 mm Hg, (3) total cholesterol ≥ 6.2 mmol/l, (4) HDL-cholesterol < 0.9 mmol/l, (5) diabetes (6) ≥ 1 packyear of smoking. SPSS 10.0 for Windows (SPSS, Inc., Chicago, Illinois) was used for data analysis.

Results

Table 1 shows the baseline characteristics of the study population. The mean age at the time of EBT scanning was 71 years. Calcium scores in men were approximately 5 times higher than in women. Age was positively associated with coronary calcification (table 2): in men calcium scores increased with a factor 1.53 for every 5 years increase in age. In women calcium scores increased with a factor 1.87.

Table 3 shows that BMI, systolic blood pressure, diastolic blood pressure, total cholesterol, diabetes and smoking measured 7 years before scanning were

TABLE 1

Characteristics of the study population at the time of EBT scanning

Variable	Men (n = 933)	Women (n = 1080)
Age (years)	71.2 ± 5.6	71.3 ± 5.8
Body mass index (kg/m^2)	26.5 ± 3.2	27.4 ± 4.4
Systolic blood pressure (mm Hg)	144 ± 21	142 ± 21
Diastolic blood pressure (mm Hg)	77 ± 11	75 ± 11
Total cholesterol (mmol/l)	5.6 ± 0.9	6.0 ± 0.9
HDL-cholesterol (mmol/l)	1.2 ± 0.3	1.5 ± 0.4
Serum glucose (mmol/l)	6.1 ± 1.7	5.8 ± 1.3
Smokers (%)		
Current	18	15
Past	72	39
History of MI (%)	18	6
Calcium score *	312 (62-969)	56 (5-261)
Log calcium score	5.3 ± 2.1	3.7 ± 2.3

Categorical variables are expressed as percentage. Values of continuous variables are expressed as mean ± standard deviation.
* Value of the calcium score is expressed as median (interquartile range) because of its skewed distribution.
MI = myocardial infarction.

TABLE 2

Exponential increase in calcium score for male sex and 5 year older subjects

	Exponential increase (95% CI)			
	Men		**Women**	
Male sex	4.95	(4.14-5.99)	---	
Age (per 5 years)	1.53	(1.36-1.72)	1.87	(1.67-2.10)

TABLE 3

Age-adjusted regression coefficients for risk factors measured 7 years before EBT scanning, describing the increase in log calcium score per unit increase of the cardiovascular risk factors

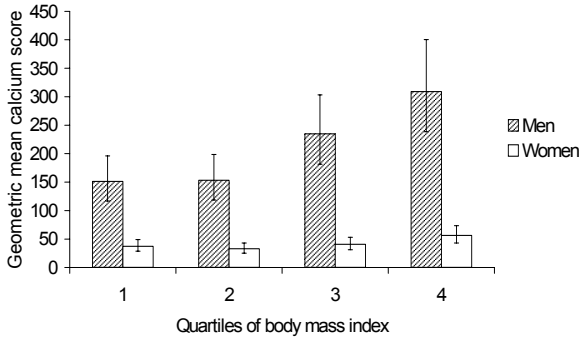
	Age-adjusted Beta coefficients (95% CI)			
Variable	**Men**		**Women**	
Body mass index (kg/m^2)	0.10	(0.06-0.14)	0.05	(0.02-0.09)
Systolic blood pressure (mm Hg)	0.017	(0.009-0.025)	0.012	(0.004-0.020)
Diastolic blood pressure (mm Hg)	0.015	(0.003-0.027)	0.011	(-0.003-0.025)
Total cholesterol (mmol/l)	0.26	(0.15-0.38)	0.31	(0.21-0.41)
HDL-cholesterol (mmol/l)	-0.35	(-0.73-0.04)	-0.49	(-0.84- -0.14)
Diabetes	0.63	(0.19-1.11)	0.66	(0.00-1.31)
Smoking (packyears)	0.007	(0.001-0.013)	0.018	(0.010-0.025)

TABLE 4
Age-adjusted regression coefficients for risk factors measured concurrently to EBT scanning, describing the increase in log calcium score per unit increase of the cardiovascular risk factors

	Age-adjusted Beta coefficients (95% CI)			
Variable	**Men**		**Women**	
Body mass index (kg/m^2)	0.08	(0.04-0.12)	0.04	(0.01-0.07)
Systolic blood pressure (mm Hg)	0.008	(0.002-0.014)	0.005	(-0.001-0.011)
Diastolic blood pressure (mm Hg)	0.0002	(-0.012-0.012)	0.0002	(-0.014-0.014)
Total cholesterol (mmol/l)	-0.08	(-0.23-0.06)	0.04	(-0.11-0.20)
HDL-cholesterol (mmol/l)	-0.16	(-0.57-0.25)	-0.58	(-0.93- -0.22)
Diabetes	0.59	(0.11-1.07)	0.63	(-0.03-1.26)
Smoking (packyears)	0.009	(0.003-0.012)	0.017	(0.009-0.025)

positively associated with the calcium score while HDL-cholesterol was inversely associated with the calcium score. Table 4 shows regression coefficients when risk factors were measured at the time of EBT scanning. The strength of the association did not change for BMI when measured concurrently with coronary calcification. The associations of blood pressure and the calcium score attenuated when measured concurrently. Systolic blood pressure was weakly associated with the calcium score and this association did not reach statistical significance in women ($p = 0.16$), while diastolic blood pressure was not associated with the calcium score. After exclusion of subjects with blood pressure lowering medication the association of systolic blood pressure and the calcium score was statistically significant in both men and women while no association was present for diastolic blood pressure (data not shown). The association of total cholesterol and the calcium score was absent when we measured cholesterol concurrently to coronary calcification. After exclusion of subjects with lipid lowering medication, total cholesterol was strongly associated in women while no association was present in men (data not shown). HDL-cholesterol measured at the time of scanning was only in women associated with the calcium score; in men no association was present even after exclusion of subjects with lipid lowering medication (data not shown). Associations for diabetes were slightly stronger and associations for smoking were similar when measured at the time of scanning.

Figure 1 shows geometric mean calcium scores for categories of risk factors measured 7 years before scanning. Calcium scores increased with increasing BMI, systolic blood pressure, diastolic blood pressure and level of total cholesterol while calcium scores decreased with increasing level of HDL-cholesterol. Compared to subjects with normal glucose tolerance, calcium scores were elevated in subjects with diabetes but not in subjects with an impaired glucose tolerance. The number


Geometric mean calcium score
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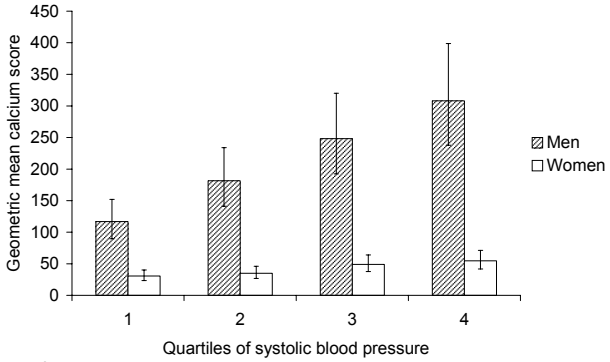
400
350
300
250
200
150
100
50
0
1
2
3
4
Quartiles of body mass index
Men
Women


Geometric mean calcium score
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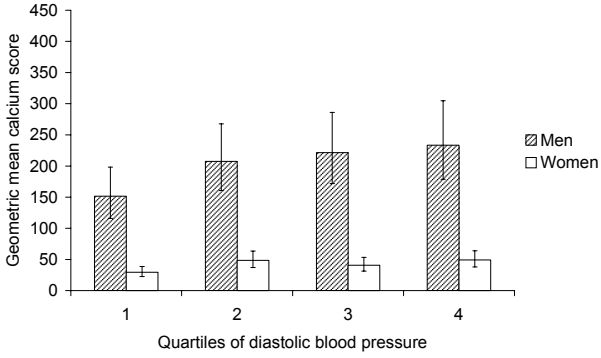
Quartiles of systolic blood pressure
Men
Women


Geometric mean calcium score
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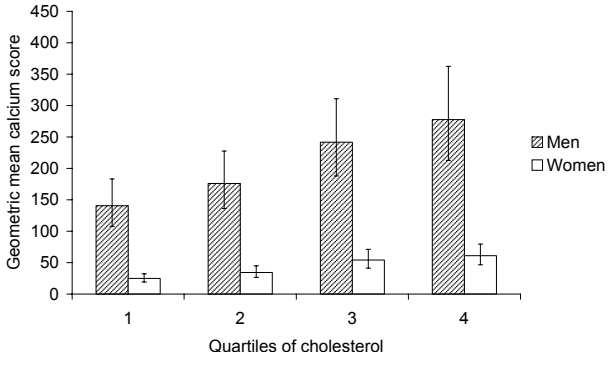
250
200
150
100
50
0
1
2
3
4
Quartiles of diastolic blood pressure
Men
Women


Geometric mean calcium score
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300
250
200
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100
50
0
1
2
3
4
Quartiles of cholesterol
Men
Women

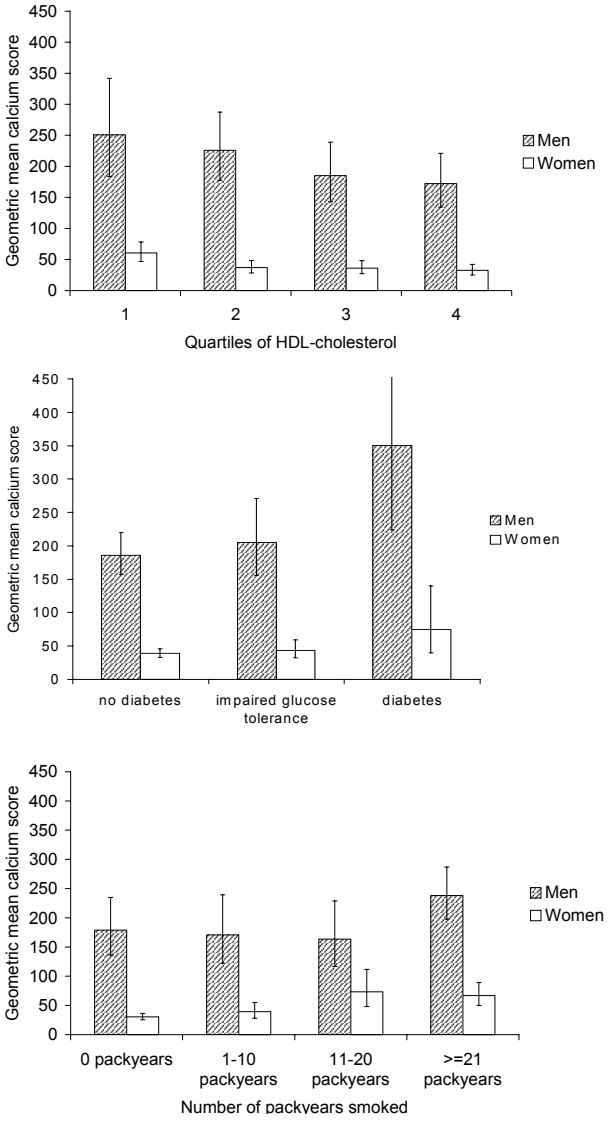

FIGURE 1
Age-adjusted geometric mean calcium scores for categories of cardiovascular risk factors assessed 7 years before EBT scanning

of packyears smoked showed a positive association with coronary calcification. Associations were strongest for BMI, systolic blood pressure, total cholesterol, diabetes (men) and smoking (women). Figure 2 shows a gradual increase in the calcium score with increasing number of risk factors. Calcium scores in subjects with three or more cardiovascular risk factors were five times higher than those in subjects without cardiovascular risk factors. Women with at least three risk factors had calcium scores comparable to men without risk factors.

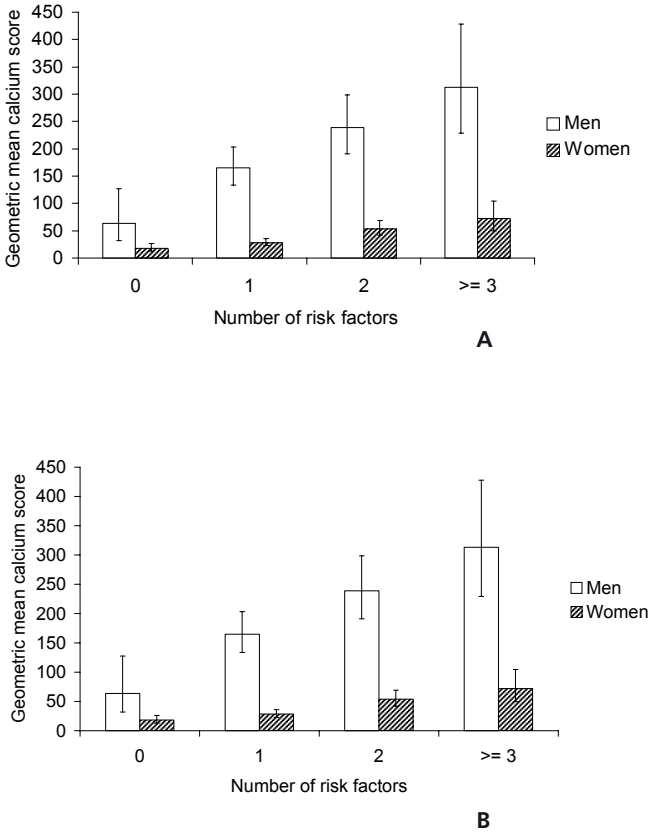

FIGURE 2.
Age-adjusted geometric mean calcium scores for the number of cardiovascular risk factors assessed 7 years before and concurrently to EBT scanning

Discussion

The present study shows that age and male sex are the most important risk factors for coronary calcification. Cardiovascular risk factors measured 7 years before EBT scanning were strongly associated with the amount of coronary calcification. Associations for blood pressure and cholesterol attenuated or even disappeared when measured concurrently to EBT scanning. Calcium scores increased gradually with increasing number of cardiovascular risk factors.

EBT scans were obtained in 2063 subjects. Subjects undergoing an EBT scanning had approximately the same levels of cardiovascular risk factors as the non-responders. There were slight differences between responders and non-responders in age (70.6 vs 72.4 years), sex (46% vs 38% male), BMI (27.0 vs 6.7 kg/m^2) and ever smoking (90% vs 86% for men, 53% vs 49% for women). It is unlikely that these differences have affected our results.

Age and male sex are important risk factors for coronary heart disease.[25-29] The present study shows that calcium scores in 5-year older men were 1.53 times higher. Calcium scores in 5-year older women were 1.87 times higher. While calcium scores in men were five times higher than calcium scores in women, men have calcium scores comparable to 10 to 15 year older women. These findings are in accordance with previous studies in referral populations.[30-32]

The present study shows that cardiovascular risk factors measured 7 years before EBT scanning were strongly associated with the amount of coronary calcification while associations were weaker for blood pressure and cholesterol when measured at the time of EBT scanning. Several causes should be considered. Firstly, the observation of weaker associations for blood pressure and cholesterol in 7 year older subjects is in line with the observation that the predictive value of cardiovascular risk factors attenuates with increasing age.[33-35] Recently, a study in older adults with a mean age of 80 years found no association of BMI, hypertension, total cholesterol, HDL-cholesterol, diabetes with coronary calcification. Only the number of packyears smoked and triglycerides were associated with coronary calcification in men and women.[21] Conversely, studies in young and middle-aged adults found that BMI, systolic blood pressure, diastolic blood pressure, total cholesterol, diabetes and smoking were positively associated with the amount of coronary calcification while an inverse association with HDL-cholesterol was observed.[13-19] Secondly, at the time of EBT scanning more subjects were treated with blood pressure lowering medication (39% vs 24%) and with cholesterol lowering medication (14% vs 6%) than 7 years before EBT scanning. Misclassification of risk factors due to treatment will lead to an underestimation of the strength of the associations. The stronger associations for systolic blood

pressure and cholesterol (women) after exclusion of subjects with medication use support this hypothesis.

Body mass index is strongly associated with the amount of coronary calcification assessed by EBT. However, image noise is correlated with BMI in EBT scans and may confound coronary calcium readings in obese individuals.[36] Therefore, we may have overestimated the association of obesity and coronary atherosclerosis.

In the present study, a gradual increase in coronary calcification, up to fivefold, was seen from subjects without cardiovascular risk factors to subjects with three or more cardiovascular risk factors. Similarly, a study in middle-aged adults found a fourfold increase in calcium score from subjects without cardiovascular risk factors to subjects with five cardiovascular risk factors.[15] While calcium scores in women were only one fifth of calcium scores in men, women with three or more cardiovascular risk factors had calcium scores comparable to men without cardiovascular risk factors.

In conclusion, age and male sex are the most important risk factors for coronary calcification. While cardiovascular risk factors assessed 7 years before EBT scanning are strongly associated with coronary calcification, associations of blood pressure and cholesterol with the calcium score attenuated when risk factors were measured concurrently to EBT scanning. Calcium scores in subjects with three or more cardiovascular risk factors are five times higher than in subjects without cardiovascular risk factors in both men and women. Women with three or more cardiovascular risk factors have calcium scores comparable to men without risk factors.

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Alcohol consumption and coronary calcification

4.2

ABSTRACT

Background: A U- or J-shaped association exists between alcohol consumption and coronary heart disease. One of the proposed mechanisms is through atherogenesis. There are no data on the association between alcohol consumption and coronary atherosclerosis in asymptomatic subjects. Coronary calcification, a noninvasive measure of the amount of coronary atherosclerosis, provides the opportunity to study this association.

Methods: This cross-sectional study was performed in the population-based Rotterdam Coronary Calcification Study. Data on alcohol consumption were available for 1795 participants without coronary heart disease. Mean age of the participants was 71 years (standard deviation, 5.7 years). Coronary calcification was detected on electron-beam tomography scans, and quantified in a calcium score according to Agatston's method. A calcium score above 400 was considered a high calcium score.

Findings: In this population of asymptomatic older adults, 15.8% consumed no alcohol, 46.5% consumed up to 1 glass per day, 16.9% 1 to 2 glasses per day, and 20.9% more than 2 glasses per day. A U-shaped association was found between alcohol consumption and coronary calcification. Compared to non-drinkers, the odds ratio of a high calcium score for daily consumption up to 1 drink was 0.60 (95% confidence interval, 0.44-0.82), for 1 to 2 drinks 0.51 (0.35-0.76), and for more than 2 drinks 0.90 (0.62-1.29). The association remained after multivariate adjustment.

Interpretation: Alcohol consumption up to 2 drinks per day was inversely associated with a high amount of coronary calcification. Subjects who consumed 1 to 2 drinks of alcohol per day had a 50% lower risk of a high calcium score compared to non-drinkers.

Introduction

A U- or J-shaped association exists between alcohol consumption and coronary morbidity and mortality, with light-to-moderate drinkers facing a lower risk than abstainers or heavy drinkers.[1-7] The underlying mechanism of the reduced risk associated with moderate levels of alcohol is not well understood. One of the potential mechanisms is the effect of alcohol on atherogenesis.[7,8] Previously, the association between alcohol and coronary atherosclerosis has been investigated by assessing the severity of angiographically determined coronary artery disease.[9-11] However, studies involving invasive coronary angiography as a measure of coronary atherosclerosis can only be conducted in symptomatic subjects suspected for coronary artery disease. Whether alcohol consumption is associated with coronary atherosclerosis in asymptomatic subjects is unknown.

The development of electron-beam tomography (EBT) has enabled non-invasive measurement of the amount of coronary calcification, which is part of the process of atherosclerosis. Coronary calcification, assessed by EBT, has been found to be closely related to the atherosclerotic plaque burden,[12] and can therefore be used as a measure of coronary atherosclerosis. In addition, the risk of coronary heart disease increases with the amount of coronary calcification.[13-16] How alcohol consumption affects coronary atherosclerosis, as reflected by the amount of coronary calcification, has not been established. One study in which the amount of coronary calcification was compared between drinkers and non-drinkers, found no difference.[17] However, in this study, information on alcohol consumption was limited to a dichotomous variable (daily consumption of at least one drink versus abstainers).

The Rotterdam Coronary Calcification Study is a prospective population-based study in older adults with detailed data on alcohol consumption. In 1795 subjects free of coronary heart disease at baseline, we studied the consumption of different levels and types of alcohol in relation to coronary calcification.

Methods

Study population

The Rotterdam Coronary Calcification Study was designed to study determinants and consequences of coronary calcification, detected by EBT. The study was embedded in the Rotterdam Study, a prospective, population-based study among 7,983 subjects aged 55 years and older, which started in 1990. The rationale and design of the Rotterdam Study have been described elsewhere.[18] From 1997 onward,

participants through 85 years of age were invited to participate in the Rotterdam Coronary Calcification Study and to undergo an EBT scan. Subjects in nursing homes did not visit the research center and thus were not invited for the study. Of the 3370 eligibles, scans were obtained for 2063 subjects (61%). Due to several causes, e.g. metal clips from cardiac surgery, severe artifacts, and registration errors (electrocardiography, acquisition), image acquisition data could not be reconstructed or analysed in 50 subjects, and therefore data were available for 2013 participants. All other information was obtained from the examinations of the Rotterdam Study. The median duration between the examination at the Rotterdam Study center and EBT scanning was 50 days. The Medical Ethics Committee of Erasmus University Rotterdam approved the study, and all participants gave informed consent.

Alcohol intake

Alcohol consumption was assessed as part of the interview at the study center. The dietary interviews were performed using a computer programme that simultaneously checked the data. Participants reported the number of alcoholic beverages they consumed on a weekly basis, in each of four categories: beer, wine, liquor, and moderately strong alcohol types. The latter category contained predominantly fortified wines, namely sherry and port. Non-drinkers were considered abstainers. Non-drinkers were asked whether they had been alcohol consumers in the past. By adding the number of drinks of specific alcoholic beverages consumed per week, the total daily consumption of alcohol in drinks per day was calculated. Since most of the moderately strong alcoholic drinks were wine types, this category was combined with the wine category in the analyses. The alcohol consumption was divided into daily consumption of 0 drinks (non-drinking), up to 1 drink, 1 to 2 drinks, and more than 2 drinks.

Cardiovascular risk factors and medical history

Blood pressure was measured at the right brachial artery using a random-zero sphygmomanometer with the participant in sitting position. The mean value of two consecutive measurements was used in the analyses. After an overnight of fasting, blood samples were obtained at the research center. Serum total cholesterol was determined by an enzymatic procedure. High-density lipoprotein (HDL) was measured similarly after precipitation of the non-HDL fraction.[19] Diabetes mellitus was considered present with current use of antidiabetic medication, or when

fasting glucose levels exceeded 7.0 mmol/l.[20] Height and weight were measured, and the body mass index (BMI) was calculated (weight (kg)/height $(m)^2$). Smoking status was assessed, and subjects were categorized as current, past or never smokers. We used information about the highest attained level of education as an indicator of social economic status. This variable was categorized as low (primary education), intermediate (secondary general or vocational education), and higher (higher vocational education or university).

Information on myocardial infarction, coronary artery bypass graft surgery (CABG) and percutaneous transluminal coronary angiography (PTCA) in the subjects' history before the start of the Rotterdam Study were obtained by direct questioning, while subjects were continuously monitored for the occurrence of coronary events after the start of the Rotterdam Study. History of coronary heart disease was considered positive when myocardial infarction, CABG or PTCA was reported, and confirmed by physicians' records. Subjects in whom coronary heart disease was present at the time of scanning were excluded from the analysis.

Coronary calcification

We assessed coronary calcifications in the epicardial coronary arteries detected on EBT scans. Imaging was performed with a C-150 Imatron scanner (GE Imatron). Before the subjects were scanned, they exercised adequate breath-holding. From the level of the root of the aorta through the heart, 38 images were obtained with 100 ms scan time and 3 mm slice thickness. We acquired images at 80% of the cardiac cycle, using electrocardiogram triggering, during a single breath-hold. Quantification of coronary calcifications was performed with AccuImage software (AccuImage Diagnostics Corporation) displaying all pixels with a density of over 130 Hounsfield units. A calcification was defined as a minimum of two adjacent pixels (area = 0.65 mm^2) with a density over 130 Hounsfield Units. Calcium scores were calculated according to Agatston's method.[21] The trained scan readers were blinded to the clinical data of the participants. To conform with the protocol outlines as approved by the Medical Ethics Committee, participants were not informed about the calcium score.

Statistical analysis

Levels of potential confounders were compared between categories of alcohol consumption, using a general linear model. In this model, age and sex were included as covariates. A calcium score of 400 was the cutpoint for a high calcium

score, in concordance with the calcium score categorization by Rumberger.[22] Logistic regression analysis adjusted for age and sex was used to calculate odds ratios of a high calcium score in categories of alcohol consumption, using non-drinkers as the reference category. The logistic regression analysis was repeated with additional adjustment for cardiovascular risk factors (body mass index, diabetes mellitus, smoking, educational level). The two logistic models were also conducted after exclusion of past-drinkers. Furthermore, the two logistic models were performed for each alcoholic beverage type separately, with additional adjustment for total alcohol consumption. All measures of association are presented with 95% confidence intervals (CI). SPSS 11.0 for Windows (SPSS, Inc., Chicago, Illinois) was used for data analysis.

Results

Characteristics of the study population

Table 1 shows the characteristics of the study population. The study population consisted of somewhat more women than men (57.5% vs 42.5%). Mean age of the population was 70.6 years (standard deviation, 5.6 years). In this population of asymptomatic older adults, 15.8% consumed no alcohol, 46.5% consumed up to 1 glass per day, 16.9% 1 to 2 glasses per day, and 20.9% more than 2 glasses per day. Only 10.9% of the population consumed more than 3 alcoholic drinks per day. The percentage of men increased with increase in alcohol consumption. Furthermore, increasing levels of alcohol consumption were associated with statistically significant differences in body mass index, presence of diabetes mellitus, smoking status and educational level. Of the subjects who did not drink alcohol at the time of scanning, 38.2% (n = 108) had consumed alcohol in the past, but had quit drinking. Of those who had stopped drinking, 4.0% reported daily alcohol consumption of more than 3 drinks in the past. The distribution of consumption of different alcoholic beverages is shown in figure 1. While beer and liquor were consumed by only a minority of the population (29.3% and 35.4%, respectively), almost three-quarters drank wine types (73.3%). Of the population, 18.9% consumed at least 1 glass of wine types per day. Corresponding percentages for beer and liquor were 5.0% and 10.9%, respectively. The distribution of the calcium score was highly skewed, with a median of 97 and interquartile range of 10 to 430. A high calcium score was present in 25.9% of the study population.

TABLE 1
Baseline characteristics of 1795 subjects without coronary heart disease by alcohol consumption category

	Alcohol consumption (drinks/day)			
	0 (n = 283)	Up to 1 (n = 834)	1 – 2 (n = 303)	More than 2 (n = 375)
Men (%)	26.1	33.1	53.8	66.4*
Age (y)	71.3 (5.5)	70.7 (5.6)	70.9 (5.6)	69.6 (5.4)
Systolic BP (mm Hg)	145 (21)	144 (36)	146 (53)	148 (66)
Diastolic BP (mm Hg)	75 (12)	77 (34)	80 (54)	81 (68)
Serum total cholesterol (mmol/l)	7.9 (13.6)	7.8 (13.3)	6.5 (7.7)	7.2 (10.8)
Serum HDL cholesterol (mmol/l)	6.9 (22.8)	4.7 (17.8)	4.0 (15.8)	4.6 (17.4)
Body mass index (kg/m2)	27.7 (4.9)	27.1 (4.0)	26.6 (3.5)	26.8 (3.5)*
Diabetes mellitus (%)	16.0	12.5	8.6	11.1*
Smoking (%)				
Current	14.1	14.0	14.9	24.5
Former	42.4	46.9	65.7	60.8
Never	43.5	39.1	19.5	14.7*
Education level (%)				
Low	38.5	28.8	23.8	18.4
Intermediate	54.4	57.9	61.7	61.3
Higher	7.1	13.3	14.5	20.3*
Calcium score†	121 (9-500)	76 (7-339)	90 (11-346)	164 (23-640)

BP indicates blood pressure, chol indicates cholesterol.

Values are means (SD) or percentages.

* $p < 0.05$ in chisquare test (for percentages) or in ANOVA (for means).

† Value of the calcium score is expressed as median (inter-quartile range) because of its skewed distribution.

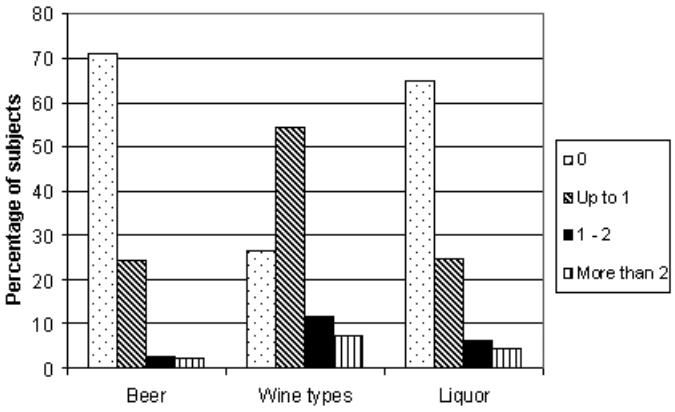

FIGURE 1
Consumption of alcoholic beverages in 1795 subjects without coronary heart disease

TABLE 2
Risk of a high calcium score according to level of alcohol consumption among 1795 subjects without coronary heart disease

Daily alcohol consumption	Cases/ Controls	Age- and sex-adjusted OR (95% CI)	Multivariate OR † (95% CI)
Non-drinker	85/198	1.00 (reference)	1.00 (reference)
Up to 1 drink	181/653	0.60** (0.44-0.82)	0.57** (0.41-0.81)
1 – 2 drinks	70/233	0.51** (0.35-0.76)	0.47** (0.31-0.72)
More than 2 drinks	129/246	0.90 (0.62-1.29)	0.83 (0.56-1.23)

* $p < 0.05$; ** $p < 0.01$.
† Multivariate analysis: additionally adjusted for body mass index, diabetes mellitus, smoking, educational level.

Alcohol and coronary calcification

Table 2 presents odds ratios of a high calcium score for levels of alcohol consumption after adjustment for age and sex, and after multivariate adjustment. The association between alcohol consumption and coronary calcification appeared to be U-shaped. The risk of a high calcium score was reduced by 10% to 49% for alcohol drinkers compared to non-drinkers. The inverse association was statistically significant for daily consumption up to 2 drinks of alcohol. Multivariate adjustment showed similar results (see table 2). Exclusion of subjects who had been drinkers, but who had stopped, resulted in similar risk estimates: age- and sex-adjusted odds ratios were 0.70 (95% CI, 0.47-1.03) for consumption up to 1 drink per day, 0.60 (0.38-0.95) for 1 to 2 drinks per day, and 1.04 (0.68-1.61) for more than 2 drinks per day, compared to non-drinkers.

The odds of a high calcium score associated with the use of separate alcoholic beverage types was computed, adjusted for age, sex, and total alcohol consumption, and in a multivariate model additionally adjusted for body mass index, diabetes mellitus, smoking, and educational level (table 3). Daily consumption up to 1 drink of all alcoholic beverage types was inversely associated with a high calcium score. The strongest inverse association, an odds ratio of 0.50 (0.32-0.77), was found for a daily consumption of 1 to 2 glasses of wine types. Associations remained after multivariate adjustment.

Discussion

This is the first study that has assessed the effect of alcohol consumption on coronary atherosclerosis in a general population of asymptomatic subjects. In 1795 asymptomatic subjects, a strong inverse association was found between daily

TABLE 3
Risk of a high calcium score according to level of consumption of alcoholic beverage types among 1795 subjects without coronary heart disease

Daily alcohol consumption	Cases/ controls	OR model I†	(95% CI)	OR model II‡	(95% CI)
Beer					
Non-drinker	302/967	1.00	(reference)	1.00	(reference)
Up to 1 drink	129/307	0.76	(0.57-1.01)	0.80	(0.59-1.08)
1 – 2 drinks	19/30	1.11	(0.58-2.11)	1.07	(0.54-2.10)
More than 2 drinks	15/26	0.96	(0.45-2.04)	0.93	(0.43-2.03)
Wine types					
Non-drinker	159/321	1.00	(reference)	1.00	(reference)
Up to 1 drink	229/746	0.71**	(0.55-0.91)	0.68**	(0.51-0.89)
1 – 2 drinks	38/169	0.50**	(0.32-0.77)	0.54**	(0.34-0.85)
More than 2 drinks	39/94	0.90	(0.55-1.49)	0.93	(0.55-1.59)
Liquor					
Non-drinker	265/894	1.00	(reference)	1.00	(reference)
Up to 1 drink	116/325	0.68*	(0.51-0.92)	0.65**	(0.48-0.89)
1 – 2 drinks	53/60	1.59*	(1.02-2.50)	1.49	(0.93-2.40)
More than 2 drinks	31/51	0.93	(0.53-1.63)	0.87	(0.48-1.58)

* $p < 0.05$; ** $p < 0.01$.
† Model I: adjusted for age, sex, and total alcohol consumption.
‡ Model II: additionally adjusted for body mass index, diabetes mellitus, smoking, educational level.

alcohol consumption up to 2 drinks and coronary atherosclerosis, as reflected by coronary calcification. The largest risk reduction of a high calcium score, 50%, was found in subjects consuming 1 to 2 drinks per day. The inverse associations remained after adjustment for body mass index, diabetes mellitus, smoking, and educational level.

Some methodological issues concerning this study need further attention. First, we used coronary calcification, a measure of subclinical coronary atherosclerosis,[12] as the outcome. Because most subjects with coronary calcification are asymptomatic, changes in drinking habits as a result of clinical symptoms were not likely to affect the associations. Secondly, it is known that with increasing age, most drinkers reduce the level of alcohol consumption.[23] Our study population consisted of asymptomatic older adults, of which less than a quarter drank more than 2 drinks per day. Current levels of alcohol consumption in these subjects may not reflect the possibly higher level of consumption during earlier decades. This will have led to an underestimation of the strength of the association between alcohol drinking and coronary calcification. Because of the limited range of alcohol consumption, it was not possible to examine the relation between heavy drinking

and coronary calcification. Thirdly, non-drinkers may not be the most appropriate reference category, since this category consists of lifelong teetotallers and past-drinkers.[24] Abstainers could have an adverse risk profile, while past-drinkers may have stopped drinking due to ill health, particularly ischemic heart disease. Thus, use of non-drinkers as reference category has been suggested to exaggerate the apparent benefits of light-to-moderate alcohol consumption.[24] However, bias in the category of non-drinkers did probably not play a considerable role in our study. In our population, 15.8% did not drink alcohol at the time of scanning, including 38.2% past-drinkers. The majority of teetotallers (73.0%) were women, in whom abstaining from alcohol is an accepted and common phenomenon in society. Of the past-drinkers, only four percent had been moderate or heavy drinkers in the past, while subjects with a history of coronary heart disease were excluded from our study. Fourthly, the analyses were based on self-reported drinking habits. Alcohol consumption may have been underreported, especially in heavy drinkers.[25,26] Underreporting of the level of consumption would tend to weaken the associations found. Finally, drinking pattern may play influence the association between alcohol intake and the risk of coronary heart disease.[27,28] However, we did not ascertain regularity in alcohol drinking.

It has been long known that alcohol drinking affects the occurrence of coronary heart disease.[1-7] One of the potential mechanisms is the effect of alcohol on coronary atherosclerosis.[7] A number of studies has shown that light-to-moderate alcohol consumption is inversely associated with extracoronary measures of atherosclerosis, such as peripheral arterial disease,[29,30] and carotid plaques.[8,31,32] However, there are no population-based data on the effect of alcohol consumption on atherosclerosis in the coronary arteries. This is the first population-based study in asymptomatic subjects that has assessed the association between alcohol consumption and coronary atherosclerosis, as reflected by coronary calcification. Only one other study has investigated the association between alcohol drinking and coronary calcification.[17] In 1196 high-risk subjects, the amount of coronary calcification did not differ between drinkers and non-drinkers. The association between amount of alcohol consumption and coronary calcification was not studied. Previously, the association between alcohol and coronary atherosclerosis has been investigated by assessing the severity of angiographically determined coronary artery disease.[9-11] Drinkers had a lower risk of severe coronary stenosis compared to non-drinkers, but no dose-response effect was found. Since these studies involved invasive coronary angiography only symptomatic subjects suspected for coronary artery disease were studied. Symptoms of coronary artery disease may have caused a change in the level of alcohol consumption, leading to spurious associations. In addition, coronary calcification has been found to have a

closer association with the extent of coronary atherosclerotic plaque burden than the level of stenosis.[33]

Several mechanisms have been proposed through which alcohol affects atherogenesis. Part of the protective effect is mediated by elevation of serum HDL cholesterol.[34,35] Apart from its effect on blood lipids, alcohol consumption influences haemostasis,[36] and levels of adhesion molecules,[37] and enhances insulin resistance.[38,39] Whether specific alcoholic beverages are equivalent in the ability to protect for coronary heart disease is still a matter of debate. Some studies have suggested that wine consumption exerts a more protective effect than beer or liquor.[40,41] The hypothesis that wine may contain additional beneficial substances is supported by a number of clinical and experimental studies.[42-47] A recent meta-analysis on beer and wine consumption in relation to vascular risk concluded that both beer and wine drinkers faced a lower risk of cardiovascular disease, but an inverse dose-response effect was only found for light-to-moderate consumption of wine.[48] Thus, the authors warned to interpret the finding for consumption of beer with caution. However, others have questioned the presence of components in wine that offer additional cardiovascular benefit, pointing to possible differences in risk factors, diet, or drinking pattern associated with the consumption of wine.[43,49,50] Evidence exists that wine drinkers are more likely to consume alcohol during a meal, and generally have a healthier lifestyle. In our study, daily consumption of up to 1 glass of beer, wine and liquor showed a similar reduction in risk of a high calcium score, while daily consumption of 1 to 2 glasses seemed to reduce the risk of a high calcium score strongest for wine types. However, this finding should be interpreted cautiously: the numbers of subjects with higher consumption of beer were very small, and the apparent stronger protective effect of wine consumption may be confounded by life style and drinking pattern. Larger studies with a larger range in levels of consumption of the different alcoholic beverage types are needed to confirm our findings.

In conclusion, this is the first population-based study in asymptomatic subjects on the effect of alcohol consumption on coronary atherosclerosis, as reflected by coronary calcification. The association between alcohol consumption and coronary calcification appeared to be U-shaped. Alcohol consumption up to 2 drinks per day was inversely associated with a high amount of coronary calcification. Subjects who consumed 1 to 2 drinks of alcohol per day had a 50% lower risk of a high calcium score compared to non-drinkers.

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Alcohol consumption and peripheral arterial disease

4.3

ABSTRACT

Moderate alcohol consumption is associated with a reduced risk of cardiovascular disease. Data on alcohol consumption and atherosclerosis are scarce. To determine the association between alcohol consumption and risk of peripheral arterial disease, a cross-sectional study (1990-1993) was carried out in the population-based Rotterdam Study among men and women aged 55 years and over. Data on alcohol consumption and peripheral arterial disease, as measured by the ankle to brachial blood pressure index, were available for 3975 participants without symptomatic cardiovascular disease. Male drinkers consumed beer, wine and liquor, while female drinkers predominantly drank wine and fortified wine types. An inverse relation between moderate alcohol consumption and peripheral arterial disease was found in women, but not in men. Because of residual confounding by smoking, analyses were repeated in non-smokers. In non-smoking men, odds ratios were 0.86 (95% confidence interval, 0.46-1.63) for daily alcohol consumption up to 10 grams, 0.75 (0.37-1.55) for 11 to 20 grams, and 0.68 (0.35-1.34) for more than 20 grams, compared to non-drinking. In non-smoking women, corresponding odds ratios were 0.65 (0.48-0.87), 0.66 (0.42-1.05) and 0.41 (0.21-0.77), respectively. In conclusion, an inverse association between alcohol consumption and PAD was found in non-smoking men and women.

Introduction

Alcohol drinking affects the occurrence of ischemic heart disease. The association of alcohol with coronary morbidity and mortality is U- or J-shaped.[1-5] The underlying mechanism of the reduced risk associated with moderate levels of alcohol is not known. One of the potential mechanisms is the effect of alcohol on atherosclerosis.[3] The presence of peripheral arterial disease (PAD), which is largely asymptomatic, is an indicator of a long-term atherogenic process in the peripheral blood vessels. PAD is considered present below a certain cut-off point of the ankle to brachial blood pressure index (ABI),[6] and is associated with atherosclerotic diseases in other vessel beds[7] and with cardiovascular morbidity and mortality.[8-12] Only few studies have investigated the relation between alcohol consumption and PAD. Among 1592 participants of the Edinburgh Artery Study, a positive linear association of alcohol consumption and ABI was found in men, but not in women.[13] The protective effect was attributable to wine drinking in particular, but was no longer significant after additional adjustment for social class. A recent study in 4549 American Indian men and women showed a significant inverse association of alcohol consumption with PAD.[14] However, the level of alcohol intake and the type of beverages consumed were not taken into account. The prospective Physicians' Health Study demonstrated an inverse association of moderate alcohol use with symptomatic PAD.[15] Though, the range of alcohol intake in the study population, was small, and this population comprised relatively healthy men. In the Framingham Heart Study an inverse association was found between moderate alcohol consumption and the occurrence of intermittent claudication.[16] However, only a small proportion of subjects with peripheral arterial disease is symptomatic. Furthermore, alcohol might have influenced the clinical symptoms of PAD.[17]

The population-based Rotterdam Study of 7983 people aged 55 years and over provides the opportunity to investigate in detail the association of alcohol consumption with PAD. In 3975 subjects free of cardiovascular disease at baseline, we studied the consumption of different levels and types of alcohol in relation to PAD, taking into account important confounders.

Materials and Methods

The Rotterdam Study

The Rotterdam Study is a prospective study designed to investigate the occurrence and determinants of chronic and disabling cardiovascular, neurogeriatric, locomotor, and ophthalmologic diseases in an ageing population. The rationale

and design of the study have been described previously.[18] The cohort includes 7983 men and women aged 55 and over (78% of the eligible population), living in a suburb of Rotterdam, the Netherlands. Of these, 879 subjects lived in nursing homes. From August 1990 until June 1993, baseline data were collected during a home interview by a trained research assistant and at two visits to the study center for clinical examination and assessment of diet.

Assessment of alcohol intake and diet

Alcohol consumption was assessed as part of a dietary interview. A trained dietician interviewed the participants at the study center, using a validated, semiquantitative food frequency questionnaire.[19] The interview was based on a checklist on which the subjects had indicated the foods and beverages consumed more than once a month during the preceding year. The dietary interviews were performed using a computer programme that simultaneously checked the data. Participants reported the number of alcoholic beverages they consumed on a weekly basis, in each of four categories: beer, wine, liquor, and moderately strong alcohol types. The latter category contained predominantly fortified wines, namely sherry and port. Non-drinkers were considered abstainers. The subjects were asked whether the level of alcohol use had changed during the last five years and if so, whether the amount had increased or decreased. For each of the different beverages, the number of drinks was multiplied by the average amount of ethanol in one drink of the alcoholic beverage. A drink was defined as 200 ml of beer containing 8.0 g of ethanol, 100 ml of wine containing 10.0 g of ethanol, 50 ml of liquor containing 14.0 g of ethanol, or 75 ml of moderately strong alcohol types containing 10.5 g of ethanol. By adding the amounts of ethanol in the four groups, the total amount of alcohol in grams/day was calculated. A validation study comparing the nutrient intake derived from the food frequency questionnaire and a 15-day food record showed a correlation coefficient of 0.89 for the intake of alcohol.[19] Since most of the moderately strong alcoholic drinks were wine types, this category was combined with the wine category in the analyses. The alcohol consumption was divided into non-drinking, use of $\leq$ 10 grams, $>$ 10 - $\leq$ 20 grams, and $>$ 20 grams/day.

Study interview

Smoking status was assessed, and subjects were categorized as current, past or never smokers. In ever smokers, the average number of cigarettes smoked

was asked, as well as the number of smoking years. From this information, the number of cigarette pack-years was computed. We used information about the highest attained level of education as an indicator of social economic status. This variable was categorized as low (primary education), intermediate (secondary general or vocational education), and higher (higher vocational education or university). Intermittent claudication was diagnosed according to the criteria of the World Health Organization by means of the Rose questionnaire.[20] Information on myocardial infarction and stroke in the subjects' history was obtained by direct questioning, and considered positive when confirmed by physicians' records. Data on previous coronary artery bypass graft surgery (CABG) or percutaneous transluminal coronary angiography (PTCA) were collected during the interview.

Clinical examination

Clinical examinations were performed during a visit at the research center. Height and weight were measured with participants wearing light clothes and without shoes. Body mass index (BMI) was calculated as weight(kg)/height(m)^2. Serum total cholesterol was determined by an enzymatic procedure. High density lipoprotein (HDL) was measured similarly after precipitation of the non-HDL fraction.[21] Diabetes mellitus was considered present with current use of antidiabetes medication, or when non-fasting random or post-load glucose levels exceeded 11.0 mmol/l.[22,23]

Blood pressure was measured at the right brachial artery using a random-zero sphygmomanometer with the participant in sitting position. The mean of two consecutive measurements was used in the analysis. Hypertension was defined as a systolic blood pressure of 160 mm Hg or higher or a diastolic blood pressure of 95 mm Hg or higher, or current use of antihypertensive drugs for the indication of hypertension. An 8-MHz continuous wave Doppler probe (Huntleigh 500 D, Huntleigh Technology) and a random-zero sphygmomanometer were used to measure the systolic blood pressure level of the posterior tibial artery at both legs.[6] The blood pressure was measured once for each leg, with the participant in supine position. The ratio of the ankle systolic blood pressure to the brachial systolic blood pressure, the ABI, was calculated for each leg. PAD was considered present when the ABI was < 0.9 in at least one leg.[24]

Population for analysis

Non-institutionalized participants who visited the study center were eligible for a dietary interview (n = 6521). Of these, diet could not be assessed in 271 subjects of the pilot phase, and in 122 subjects suspected of dementia. Furthermore, a random group of 481 participants was not interviewed because of logistic reasons. Of the dietary reports, 212 were considered unreliable by the dietician, and were excluded. Thus, dietary data were available for 5435 subjects. Of these 5435, data on ABI was missing for 535 participants. Additionally, subjects with an ABI of more than 1.5 (n = 272) were excluded because this index usually results from arterial rigidity preventing compression of the ankle artery. Subjects with complete data on diet and ABI (n = 4900) differed from excluded subjects (n = 1621) only in mean age and in diabetes status. The mean age was higher in those excluded due to exclusion of subjects suspected for dementia, while the percentage of diabetes mellitus was higher in subjects excluded because of missing data, probably due to non-assessable arteries on both sides. To avoid a spurious association due to change in alcohol consumption resulting from symptomatic cardiovascular disease, subjects with a history of myocardial infarction, stroke, coronary artery bypass graft surgery or percutaneous transluminal coronary angiography were excluded from the analysis (n = 653). Ultimately, 3975 subjects were included in the present analysis.

Data analysis

Levels of potential confounders were compared between categories of alcohol consumption, using a general linear model. In this model, age and sex were included as covariates. Risk estimates were obtained for men and women separately. The relation between alcohol consumption and PAD was assessed using logistic regression analysis adjusting for age. The risk of PAD for each category of alcohol consumption, was compared to the risk among non-drinkers. The associations were expressed as odds ratios (OR) with 95% confidence intervals (CI). A second model additionally adjusted for possible confounding factors, namely cigarette pack-years, body mass index and diabetes mellitus. A next model also adjusted for education as a measure of social economic status. The first two logistic models were also conducted after exclusion of subjects who had reduced their alcohol consumption in the last five years. Because of potential residual confounding by smoking, we stratified according to smoking status and repeated the logistic regression analysis, adjusting for age and cigarette pack-years. To study the association of drinking (yes/no) of specific types of alcohol,

the two aforementioned logistic regression models were used, but simultaneously taking into account the amount of consumption of other types of alcohol. The data were analysed using SPSS 7.5 for Windows.

Results

General characteristics of the study population are presented in table 1. Of the participants, 62.5 percent was female. The percentage of drinkers among men (88.5) was higher than among women (74.7). Furthermore, the average daily amount of alcohol consumed by drinkers was much higher for men than

TABLE 1
Baseline characteristics of 3975 men and women aged 55 years and over without cardiovascular disease: The Rotterdam Study, 1990 – 1993

Characteristic*	Men (n = 1489)	Women (n = 2486)
Age (y)	66.5 (7.2)	67.4 (7.8)
Systolic blood pressure (mmHg)	139 (22)	139 (22)
Diastolic blood pressure (mmHg)	75 (12)	73 (11)
Serum total cholesterol (mmol/l)	6.3 (1.2)	6.8 (1.2)
Serum high-density lipoprotein cholesterol (mmol/l)	1.23 (0.3)	1.46 (0.4)
Body mass index (kg/m2)	25.7 (2.8)	26.6 (4.0)
Diabetes mellitus (%)	8.1	8.8
Smoking (%)		
Current	30.1	19.2
Former	61.9	28.6
Never	9.0	52.2
Daily alcohol consumption (%)		
Non-drinker	11.5	25.3
≤ 10 grams	37.3	51.6
> 10 - ≤ 20 grams	19.1	12.7
> 20 grams	32.1	10.4
Ankle/brachial index	1.11 (0.2)	1.07 (0.2)
Peripheral arterial disease (%)	13.4	14.4
Intermittent claudication (%)	1.4	0.9
Education level (%)		
Low	20.7	40.4
Intermediate	58.2	52.8
Higher	21.1	6.8

* Values are means (SD) or percentages. SD is standard deviation.

TABLE 2
Consumption of alcoholic beverages in 3975 men and women withoutcardiovascular disease: The Rotterdam Study, 1990 - 1993

Beverage type	Men (n = 1489)		Women (n = 2486)	
Beer				
drinkers (%)	46.6		5.2	
drinks/day (SD)*	1.19	(1.7)	0.48	(0.7)
Liquor				
drinkers (%)	40.1		17.5	
drinks/day (SD)	1.20	(1.3)	0.76	(1.0)
Wine and fortified wine				
drinkers (%)	41.4		66.4	
drinks/day (SD)	0.74	(0.9)	0.61	(0.8)

* Average number of drinks per day in drinkers. SD is standard deviation.

for women (16.4 grams (SD 18.8) and 6.2 grams (SD 10.2) respectively). The prevalence of PAD was slightly lower in men (13.4%) than in women (14.4%). Of those with PAD only few subjects reported intermittent claudication as assessed by the Rose questionnaire (7.5% of male cases, 3.9% of female cases). In a general linear model adjusted for sex, the mean level of alcohol intake decreased with increase in age category (p for trend < 0.001). After adjustment for age and sex, the level of alcohol intake was associated with cigarette pack-years (p < 0.001), diabetes mellitus (p = 0.12), and attained level of education (p = 0.02) (data not shown). The level of HDL cholesterol was significantly higher in male and female drinkers compared to non-drinkers. In addition, women who consumed alcohol were on average older, more often diabetic, more likely smokers, and higher educated compared to non-drinking women.

Table 2 shows the distribution of consumption of various alcoholic beverages. Among men, beer, liquor and wines were consumed by almost equal percentages (46.6%, 40.1% and 41.4%, respectively). The average daily amount consumed by drinkers of the specific beverages was higher for beer and liquor (1.2 drinks), than for wine and fortified wine (0.74 drinks). The percentage of women drinking wine and fortified wine was much higher (66.4%) than the percentages of women drinking beer or liquor (5.2% and 17.5%, respectively). Wine-, beer- or liquor-drinking women drank less than men drinking the same alcoholic beverages.

Table 3 presents odds ratios of PAD for varying levels of alcohol consumption adjusted for age, and in multivariate analyses additionally adjusted for cigarette pack-years, body mass index, and diabetes mellitus. In men, there was no inverse

TABLE 3
Risk of peripheral arterial disease according to level of alcohol consumption among 3975 men and women without cardiovascular disease: The Rotterdam Study, 1990 - 1993

Daily alcohol consumption	Cases/ controls	Age-adjusted OR (95 % CI)		Multivariate OR † (95 % CI)	
Men (n=1489)					
Non-drinker	26/145	1.00	(reference)	1.00	(reference)
≤ 10 grams	71/485	0.84	(0.51, 1.38)	0.97	(0.58, 1.63)
> 10 - ≤ 20 grams	37/247	0.91	(0.52, 1.58)	1.02	(0.57, 1.80)
> 20 grams	65/413	0.97	(0.59, 1.61)	0.97	(0.57, 1.65)
Women (n=2486)					
Non-drinker	122/507	1.00	(reference)	1.00	(reference)
≤ 10 grams	163/1119	0.66**	(0.51, 0.91)	0.70*	(0.53, 0.91)
> 10 - ≤ 20 grams	39/277	0.69	(0.46, 1.02)	0.66*	(0.43, 1.00)
> 20 grams	34/225	0.78	(0.51, 1.19)	0.64	(0.41, 1.01)

* $p < 0.05$; ** $p < 0.01$.
† Multivariate analysis: additionally adjusted for cigarette pack-years, body mass index, diabetes mellitus.
OR is odds ratio; (95% CI) denotes 95 percent confidence interval.

association between alcohol consumption and the risk of PAD. In the age-adjusted model, a slightly protective, but statistically non-significant association with PAD was observed for men consuming up to 10 grams of alcohol daily compared to non-drinkers (OR = 0.84; 95% CI, 0.51-1.38). However, this association disappeared in multivariate analysis. In women, a risk reduction of 22% to 36% was observed for alcohol drinkers, compared to non-drinkers. This reduced risk among women was significant for daily consumption up to 10 grams of alcohol, and in multivariate analysis for up to 20 grams of alcohol daily. Additional adjustment for social economic status did not change the results for either sex (data not shown). There was no significant interaction between alcohol consumption and sex (data not shown). Exclusion of subjects who had reduced their alcohol consumption in the last five years (190 subjects, 4.0% of the women and 6.1% of the men) did not affect the associations (data not shown).

Stratified analysis according to smoking status revealed different odds ratios of PAD by category of alcohol consumption in smokers and non-smokers (table 4). In logistic regression analysis adjusted for age and cigarette pack-years, odds ratios of PAD in increasing categories of alcohol consumption were lower among past and never smokers than among current smokers. In past and never smokers, an inverse association was found between alcohol consumption and PAD. In non-smoking men, odds ratios were 0.86 (0.46-1.63) for daily alcohol consumption up to 10 grams, 0.75 (0.37-1.55) for 11 to 20 grams, and 0.68 (0.35-1.34) for more than 20 grams, compared to non-drinking (p for trend = 0.21). In non-smoking women,

TABLE 4
Risk of peripheral arterial disease by level of alcohol consumption according to smoking status among 3975 men and women without cardiovascular disease: The Rotterdam Study, 1990 - 1993

Daily alcohol consumption	Current smokers			Past and never smokers		
	Cases/ Controls	OR (95% CI) *		Cases/ Controls	OR (95% CI) *	
Men (n=1489)						
Non-drinker	10/42	1.00	(reference)	16/103	1.00	(reference)
≤10 grams	26/125	0.85	(0.38, 2.00)	45/360	0.85	(0.46, 1.63)
>10 - ≤20 grams	17/65	1.19	(0.49, 2.93)	20/182	0.75	(0.36, 1.55)
>20 grams	37/126	1.31	(0.59, 2.91)	28/287	0.68	(0.35, 1.34)
Women (n=2486)						
Non-drinker	22/77	1.00	(reference)	100/430	1.00	(reference)
≤10 grams	33/176	0.75	(0.41, 1.39)	130/943	0.65**	(0.48, 0.87)
>10 - ≤20 grams	11/62	0.70	(0.32, 1.59)	28/215	0.66	(0.42, 1.05)
>20 grams	22/74	1.05	(0.53, 2.08)	12/151	0.41**	(0.21, 0.77)

* Adjusted for age and cigarette pack-years.

** $p < 0.01$.

OR is odds ratio; (95% CI) denotes 95 percent confidence interval.

corresponding odds ratios were 0.65 (0.48-0.87), 0.66 (0.42-1.05) and 0.41 (0.21-0.77), respectively (p for trend < 0.001). The lowest odds ratio was found in never smoking women with a daily alcohol consumption of more than 20 grams (OR = 0.32; 0.11-0.91). In smoking subjects there was no inverse association between alcohol consumption and PAD.

The risk of PAD associated with the use of the separate alcoholic beverages was computed, adjusted for age and use of other alcoholic beverages, and in a second model also adjusted for cigarette pack-years, body mass index and diabetes mellitus. The association was stronger for the consumption of wine and fortified wine than for the consumption of beer or liquor. In women, the risk of PAD associated with the use of wine types was statistically significant (OR = 0.72; 0.57-0.91, first model, and OR = 0.74; 0.58-0.95, second model). In men, odds ratios for wine were 0.81 (0.59-1.13) and 0.88 (0.63-1.24), respectively. The estimates of the risk of PAD associated with beer and liquor consumption for both men and women ranged from 0.86 to 1.14, and were not statistically significant. In logistic regression analysis of non-smokers, no consistent association between an alcoholic beverage and PAD was found, except for wine and fortified wine in women (OR = 0.67; 0.51-0.88). There was no significant inverse association between any type of alcoholic beverage and PAD in current smokers (odds ratios ranging from 0.92 to 1.17).

Discussion

In the large population-based Rotterdam Study, we found an inverse association of alcohol consumption with PAD in women. The association was present already at low levels of alcohol use, and still present for levels of moderate alcohol consumption. In men, there was virtually no association of alcohol intake with PAD. Among non-smoking subjects an inverse association was found between alcohol consumption and PAD in both men and women. The largest risk reduction, 59 percent, was found in women consuming over 20 grams per day. The strengths of the present study include a large and well-described cohort of elderly men and women, classification of consumption of alcohol and the types of beverages based on a validated food checklist, and a relatively large number of mostly asymptomatic PAD cases (558 subjects).

In the present study, drinking habits were self-reported. This might have caused underreporting of alcohol use, especially among heavy drinkers. This is more likely to have occurred for men than for women, since more men were heavy drinkers. Imprecision in the reporting of alcohol consumption would tend to weaken the associations found. Shaper et al. has argued that non-drinkers might not be suitable for use as reference group in examining the effects of alcohol on PAD.[4] The group of non-drinkers could be less healthy than expected, due to inclusion of former heavy drinkers and people who have stopped drinking because of ill-health, particularly ischemic heart disease. Furthermore, it is probable that lifelong non-drinkers have reasons for being abstainers that introduce other biases, and have an adverse risk profile. This is most likely for men, for whom abstaining from alcohol is rather uncommon in society, at least in the age-category of our cohort. However, additional exclusion of subjects who had reduced their alcohol consumption in the last five years did not change our results. Furthermore, participants with prevalent symptomatic cardiovascular disease were excluded from the analysis. In addition, drinking pattern may influence the risk of cardiovascular diseases.[25] In our study, there was no information about the regularity of alcohol consumption.

Using PAD as an indicator of atherosclerosis has the advantage that most subjects with PAD are asymptomatic.[6] Thus, spurious associations between alcohol and PAD, resulting from symptoms that cause a change in alcohol consumption, are not likely to occur. Possible misclassification of PAD cases was probably non-differential, and would only weaken the real associations. In the Rotterdam Study, subjects did not report their physical activity, one of the confounders of the relation between alcohol consumption and PAD. Since measurement of ABI was performed only on non-institutionalized subjects visiting the research center and participants with dementia were not interviewed about their diet, our subjects were relatively healthy and mobile. They may not be a representative sample

of the whole elderly population without prevalent symptomatic cardiovascular disease.

Only a few other studies have investigated the association of alcohol consumption and the presence of peripheral atherosclerosis. Jepson et al. found in the Edinburgh Artery Study a higher ABI (thus less PAD) in men with high alcohol consumption, but no association in women.[13] ABI was associated with wine consumption, but not beer or liquor. After additional adjustment for social class the positive associations disappeared, possibly indicating confounding by social class. The findings for women in this study were considered the result of the relatively low consumption of alcohol in this group (median consumption of one drink per week). In the prospective Physicians' Health Study, the relative risk of symptomatic PAD associated with moderate alcohol use was 0.74 in multivariate analysis.[15] This cohort comprised subjects who were on average 10 years younger and had a higher average educational level than the men in our study. Furthermore, the overall alcohol consumption was very low in comparison with our study and other studies: 97% of the men reported use of less than two drinks a day. The consumption of different types of alcoholic beverages was not taken into account in this study. The Framingham Heart Study found the lowest risk of intermittent claudication at levels of 13-24 grams of alcohol per day in men (hazard ratio = 0.67; 95% CI, 0.42-0.99), and 7-12 grams in women (hazard ratio = 0.44; 95% CI, 0.23-0.80), compared to non-drinkers.[16] Especially beer and wine were negatively associated with the occurrence of intermittent claudication. The study population, although younger than our population, had a similar distribution of alcohol consumption as ours. In this study and the Physicians' Health study, only subjects with onset of intermittent claudication or peripheral arterial surgery were considered PAD cases. Alcohol may influence the clinical symptoms of PAD by preferentially dilating diseased arteries.[16] In a study among American Indians, a study population with fewer cases of PAD than ours, a statistically significant inverse association between alcohol drinking and peripheral arterial disease was found in multiple logistic regression analysis. However, there was no information on the types and amount of alcohol consumed.[14]

We found an inverse association between alcohol consumption and PAD for women, but not for men. Due to the small range of alcohol intake among women, with only 10% consuming over 20 grams of alcohol daily, it was not possible to examine the risk of PAD in heavy drinking women. Although the range of alcohol consumption among men was wider, the odds ratio of PAD was not significantly different from one for any of the alcohol consumption categories compared to non-drinking. Part of the gender difference may be explained by a strong confounding effect of smoking. In non-smoking subjects, an inverse association between alcohol consumption and PAD was present in both men and women.

The inverse association was strongest in never smoking women. Unfortunately, we were not able to study the association between alcohol consumption and PAD in never smoking men due to small numbers. In our population, only eight percent of men had never smoked. The percentage of never smoking men were higher in the Edinburgh Artery Study (25%) and in the Physicians' Health Study (50%). The effect of smoking may have been less distorting in these studies. Furthermore, the discrepancy concerning the observed associations for men and women can possibly partly be explained by differences in the distribution of the beverage types consumed. In our study population women consumed mainly wine and fortified wine types, and we found the association to be strongest for these alcoholic beverages. The inverse association of wine consumption and PAD is in concordance with results from the Edinburgh Artery Study.[13] Contrary to their results, the associations we found remained after additional adjusting for social class. Although our data in men are not incompatible with an effect of wine, the association was weak and not significant. Furthermore, due to small numbers of cases among beer drinkers and liquor drinkers in women, possible inverse associations between these beverages and PAD cannot be excluded.

An extensive review on alcohol and risk of coronary heart disease concluded that there is strong evidence that beer, liquor, and wine are all three associated a lower risk of coronary heart disease.[26] The review focused primarily on acute events of coronary atherosclerotic disease, in which clotting and fibrinolysis are also thought to play a major role. We studied the long-term process of atherogenesis, in which the effect of alcoholic beverages on atherosclerosis is more important. Alcohol itself affects haemostasis,[27] and affects atherosclerosis through its effect on lipid profile.[28] Possibly additional effects on atherosclerosis may be mediated by substances only present in wines. Wine and fortified wines contain phenolic substances, which have been shown to have antioxidant effects on low-density lipoproteins, thus decelerating atherogenesis.[29,30]

In summary, in this large population-based study moderate alcohol consumption was inversely associated with peripheral arterial disease in women but not in men. Residual confounding by smoking may have influenced the results. Among non-smokers an inverse association was found between alcohol consumption and PAD in both men and women.

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CHAPTER five

CORONARY CALCIFICATION AND RISK OF CARDIOVASCULAR DISEASE

5.1 Coronary calcification and the presence of myocardial infarction

ABSTRACT

Aims: Available data are insufficient to determine the relation between coronary calcification and coronary events in the general population. We cross-sectionally examined the association between coronary calcification and myocardial infarction in the prospective Rotterdam Coronary Calcification Study.

Methods and Results: From 1997 onwards, subjects were invited for electron-beam tomography scanning to detect coronary calcification. The study was embedded in the population-based Rotterdam Study. Calcifications were quantified in a calcium score according to Agatston's method. Calcium scores were available for 2013 participants with a mean age of 71 years (standard deviation, 5.7 years). A history of myocardial infarction prior to scanning was present in 229 subjects. Compared to subjects in the lowest calcium score category (0-100), the age-adjusted odds ratio for myocardial infarction in subjects in the highest calcium score category (above 2000) was 7.7 (95% confidence interval, 4.1-14.5) for men, and 6.7 (95% confidence interval, 2.4-19.1) for women. Additional adjustment for cardiovascular risk factors only slightly altered the estimates. The association was observed across all age subgroups, i.e. also in subjects of 70 years and older.

Conclusion: A strong and graded association was found between coronary calcification and myocardial infarction. The association remained at high ages.

Introduction

Electron-beam tomography (EBT) is a highly sensitive technique to detect coronary calcification. Quantitative measures of coronary calcification are closely related to the amount of atherosclerotic plaque in histopathologic investigations.[1,2] Furthermore, the calcium score derived from EBT is strongly associated with the extent of angiographically detected coronary artery disease.[3,4] Consequently, quantification of coronary calcification using EBT has been proposed as a promising method for non-invasive detection of asymptomatic subjects at high risk of developing coronary heart disease. Until now four prospective studies have been published addressing the association between the amount of coronary calcification, detected by EBT, and risk of coronary events.[5-8] The studies enrolled either self-referred subjects or subjects referred for scanning by the general practitioner because of cardiovascular risk factors. Furthermore, the studies were all performed on less than fifty coronary events. In three of these studies, the majority of the end points were revascularizations, possibly partially performed on the basis of the scan results. Since large prospective studies in unselected populations using EBT for quantification of coronary calcification have started rather recently, unbiased results have to be awaited. Meanwhile, results of a cross-sectional study in a large, unselected population will provide an estimate of the strength of the association between coronary calcification and coronary heart disease. So far no study has examined the relation between coronary calcification and presence of myocardial infarction in a general population.

We cross-sectionally examined whether coronary calcification detected by EBT is associated with myocardial infarction in 2013 elderly men and women participating in the Rotterdam Coronary Calcification Study.

Methods

Study population

The Rotterdam Coronary Calcification Study was designed to study determinants and consequences of coronary calcification, detected by EBT. The study was embedded in the Rotterdam Study. The Rotterdam Study is a population-based study that started in 1990 to 1993. All inhabitants of a suburb of Rotterdam, aged 55 years and over, were invited (response 78%). The rationale and design of the Rotterdam Study have been described elsewhere.[9] Follow-up visits took place in 1993 to 1994 and 1997 to 1999. From 1999 onwards the study population is extended with a second cohort comprising inhabitants who reached the age of 55 years after

the baseline examination in 1990 to 1993 and subjects aged 55 years and over who migrated into the research area. Baseline and follow-up visit examinations included non-invasive measurements of atherosclerosis. Measurement protocols for the first and second cohort were identical.

From 1997 onwards, participants not older than 85 years of age who completed the third phase of the first cohort or the baseline examination of the second cohort of the Rotterdam Study, were invited to participate in the Rotterdam Coronary Calcification Study and undergo an EBT scan. Subjects in nursing homes did not visit the research center and thus were not invited for the Rotterdam Coronary Calcification Study. We restricted the present analysis to participants recruited from the first cohort, who were scanned from 1997 to 2000. Of the 3371 eligibles, scans were obtained for 2063 subjects (response: 61%). Due to several causes i.e. metal clips from cardiac surgery, severe artifacts, and registration errors (electrocardiogram (ECG), acquisition), image acquisition data could not be reconstructed or analysed in 50 subjects. Therefore, scores were available for 2013 participants. All other measurements were obtained from the examinations of the Rotterdam Study. The median duration between the examination at the Rotterdam Study center and EBT scanning was 50 days. The Medical Ethics Committee of Erasmus University Rotterdam approved the study, and all participants gave informed consent.

Coronary calcification

We assessed coronary calcifications in the epicardial coronary arteries detected on EBT scans. Imaging was performed with a C-150 Imatron scanner (Imatron, South San Francisco, California). Before the subjects were scanned, they exercised adequate breath-holding. From the level of the root of the aorta through the heart, 38 images were obtained with 100 ms scan time and 3 mm slice thickness. We acquired images at 80% of the cardiac cycle, using ECG triggering, during a single breath-hold. Every day that the scanner was used, we calibrated the scanner using a water phantom. Scanning took about 15 minutes per subject, while up to 10 minutes of time were involved in quantification of the coronary calcifications. Quantification of coronary calcifications was performed with AccuImage software (AccuImage Diagnostics Corporation, South San Francisco, California) displaying all pixels with a density of over 130 Hounsfield units. The trained scan readers were blinded to the clinical data of the participants. A calcification was defined as a minimum of two adjacent pixels (area = 0.65 mm^2) with a density over 130 Hounsfield Units. We placed a region of interest around each high-density lesion in the epicardial coronary arteries, ensuring that the complete calcified lesion

was incorporated in the region of interest. The peak density in Hounsfield Units and the area in mm^2 of the individual coronary calcifications were calculated. A calcium score was obtained by multiplying each area of interest with a factor indicating peak density within the individual area, as proposed by Agatston et al.[10] We added the scores for individual calcifications, resulting in a calcium score for the entire epicardial coronary system. Interobserver reliability for calcium scoring has been found to be excellent, with correlation coefficients of calcium scores obtained by different observers greater than 0.99, and only small differences in absolute calcium scores.[11] Conform the protocol outlines as approved by the Medical Ethics Committee, participants were not informed about their calcium score.

Diagnosis of myocardial infarction

Myocardial infarction was classified as present if the myocardial infarction had occurred before the baseline examination from 1990 to 1993 or after baseline but prior to EBT (1997 to 2000). Myocardial infarction at baseline was based on medical records from general practitioner or cardiologist and/or on ECG evidence collected on instigation of participants'report. Infarctions detected by the modular ECG analysis system without a history of symptoms were verified by an experienced cardiologist,[12,13] and classified as present or absent. General practitioners in the research area of the Rotterdam Study (85% of the cohort) reported myocardial infarctions occurring after the baseline examination. For 15% of the cohort, of which the general practitioners had practices outside the research area, information was obtained through checking the participant's file and by interviewing the general practitioner annually. When an event was reported, additional information was collected by interviewing the general practitioner and scrutinising information from hospital discharge records and letters of medical specialists. Two research physicians independently coded the possible cardiac events, according to the International Classification of Diseases, 10^{th} version.[14] A cardiologist reviewed the coded events and performed the definitive coding. The research physicians and cardiologist were not aware of the calcium score of the participants who had had an EBT scan. Comparison with the national morbidity registry of hospitals showed that 98% of all myocardial infarctions occurring after the baseline examination were detected by the above mentioned data collection procedures. Myocardial infarction was classified as present when a hospital discharge diagnosis of myocardial infarction was present, or, in case a patient was not hospitalized, when signs and symptoms, ECG recordings and cardiac enzyme data were diagnostic of myocardial infarction.[15]

Cardiovascular risk factors

Information on smoking was obtained during the home interview of the Rotterdam Study. We categorised subjects as current, past or never smokers. Furthermore, history of myocardial infarction occurring before the 65th year of age in first degree family members was ascertained by self-report. Anthropometric measures were obtained during a visit at the research center. Height and weight were measured, and the body mass index was calculated (weight (kg)/height $(m)^2$). Blood pressure was measured at the right brachial artery using a random-zero sphygmomanometer with the participant in sitting position. The mean value of two consecutive measurements was used in the analyses. After an overnight of fasting, blood samples were obtained at the research center. Serum total cholesterol was determined by an enzymatic procedure. High-density lipoprotein (HDL) was measured similarly after precipitation of the non-HDL fraction.[16] Diabetes mellitus was considered present with current use of antidiabetic medication, or when fasting glucose levels exceeded 7.0 mmol/l.[17]

Statistical analysis

The distribution of calcium scores was skewed, and therefore, medians and ranges were reported. Linear regression analysis was applied to compare the age-adjusted continuous baseline characteristics of the Rotterdam Coronary Calcification Study participants and the non-responders. To compare smoking status and gender between the study population and the group of non-responders, the chi-square test was used. Five absolute calcium score categories were defined, based on cut-points that we chose before examining the association with presence of myocardial infarction: 0-100, 101-500, 501-1000, 1001-2000, and above 2000. Age-adjusted odds ratios for presence of myocardial infarction were calculated per calcium score category using logistic regression analysis, for men and women separately. Calcium score category 0-100 was used as reference. Analyses for calcium score categories were repeated with additional adjustment for cardiovascular risk factors (smoking, body mass index, blood pressure, total cholesterol, HDL cholesterol, diabetes mellitus). Furthermore, we performed age-adjusted and multivariate logistic regression analyses after dividing the calcium score distribution into sex-specific quartiles, using the lowest quartile as reference. Age-adjusted odds ratios for presence of myocardial infarction were also calculated for absolute calcium score categories after dividing the subjects into two age strata, younger than 70 years and 70 years and older. Because of small number of cases, we considered calcium score above 1000 as the highest category in the age subgroup analysis.

All measures of association are presented with 95% confidence intervals (95% CIs). A P value less than 0.05 was considered statistically significant. SPSS 9.0 for Windows (SPSS, Inc., Chicago, Illinois) was used for data analysis.

Results

Characteristics of study population

Table 1 shows characteristics of the Rotterdam Coronary Calcification Study population. The study population consisted for 46% of men. The mean age of the study participants was 71 years (standard deviation (SD), 5.7 years). The median calcium score differed for men and women: 312 (interquartile range 62-970) and 55 (interquartile range 5-261), respectively. The mean of the logarithmically transformed calcium scores (SD) was 5.3 (2.1) for men and 3.7 (2.3) for women. Information about history of myocardial infarction was available for 1936 scanned subjects. In 18% (166 cases) of men and 6% (63 cases) of women who underwent EBT myocardial infarction was reported prior to scanning. Among the cases, 46% of men and 62% of women had suffered a myocardial infarction during the last ten years. The characteristics of the study population were compared with the non-responders. The study population did not differ from the non-responders with regard to systolic and diastolic blood pressure, total and HDL cholesterol level,

TABLE 1
Characteristics of the Rotterdam Coronary Calcification Study population

Characteristic*	Men (n = 932)	Women (n = 1081)
Age (years)	71.2 ± 5.6	71.3 ± 5.8
Systolic blood pressure(mm Hg)	144 ± 21	142 ± 21
Diastolic blood pressure (mm Hg)	77 ± 11	75 ± 11
Total cholesterol (mmol/L)	5.6 ± 0.9	6.0 ± 0.9
HDL cholesterol (mmol/L)	1.2 ± 0.3	1.5 ± 0.4
Body mass index (kg/m^2)	26.5 ± 3.2	27.4 ± 4.4
Smokers (%)		
Current	18	15
Past	72	39
Diabetes mellitus (%)	14	12
History of myocardial infarction (%)	18	6
Family history of myocardial infarction (%)	19	20
Calcium score †	312 (62–970)	55 (5–261)
Log calcium score	5.3 ± 2.1	3.7 ± 2.3

* Categorical variables are expressed as percentage. Values of continuous variables are expressed as mean (standard deviation).
† Value of the calcium score is expressed as median (inter-quartile range) because of its skewed distribution.

body mass index, diabetes mellitus and history of myocardial infarction. However, in comparison to the non-responders, the scanned population was significantly younger (mean age difference 1.8 years), consisted of relatively more men (46% vs 38%), and was more likely to have a history of smoking (70% vs 63%).

Coronary calcification and myocardial infarction

Compared to subjects without a history of myocardial infarction, the calcium score distribution of the cases of myocardial infarction was shifted to the right (Figure 1). Of subjects without a previous myocardial infarction, 50% had a calcium score of 0-100, and 11% had a calcium score above 1000. In contrast, only 19% of subjects with a history of myocardial infarction had a calcium score of 0-100, while 41% had a calcium score above 1000.

In men, age-adjusted odds ratios for previous myocardial infarction ranged from 1.9 (95% CI, 1.0-3.4) in the calcium score category 101-500, to 7.7 (95% CI, 4.1-14.5) in those with a calcium score above 2000, when compared to subjects in the reference category (table 2). The corresponding age-adjusted odds ratios

TABLE 2
Risk of myocardial infarction in calcium score categories for men and women

	n	Events	Model 1 * Odds Ratio (95% CI)	Model 2 † Odds Ratio (95% CI)
Men				
Calcium score:				
0–100	296	19	1.0 (reference)	1.0 (reference)
101–500	267	33	1.9 (1.0–3.4)	1.9 (1.0–3.7)
501–1000	145	33	3.8 (2.1–7.0)	3.4 (1.7–6.6)
1001–2000	128	46	7.6 (4.2–13.8)	9.0 (4.7–17.4)
>2000	96	35	7.7 (4.1–14.5)	7.9 (3.9–15.7)
Women				
Calcium score:				
0–100	631	25	1.0 (reference)	1.0 (reference)
101–500	266	14	1.3 (0.7–2.6)	1.4 (0.7–2.8)
501–1000	109	12	3.0 (1.4–6.2)	3.2 (1.4–7.3)
1001–2000	48	6	3.5 (1.3–9.3)	4.5 (1.6–12.7)
>2000	27	6	6.7 (2.4–19.1)	5.8 (1.9–17.6)

† Model 1: adjusted for age.
‡ Model 2: adjusted for age, smoking, blood pressure, body mass index, total cholesterol, HDL cholesterol, and diabetes mellitus.
The number of subjects in model 2 is somewhat lower than for model 1, due to missing values for cardiovascular risk factors.
CI = confidence interval.

in women were 1.3 (95% CI, 0.7-2.6) and 6.7 (95% CI, 2.4-19.1). Additional adjustment for cardiovascular risk factors only slightly changed the results (model 2 in table 2). In men, 49% of the myocardial infarctions occurred in the calcium score category above 1000, while in women this percentage was 19.

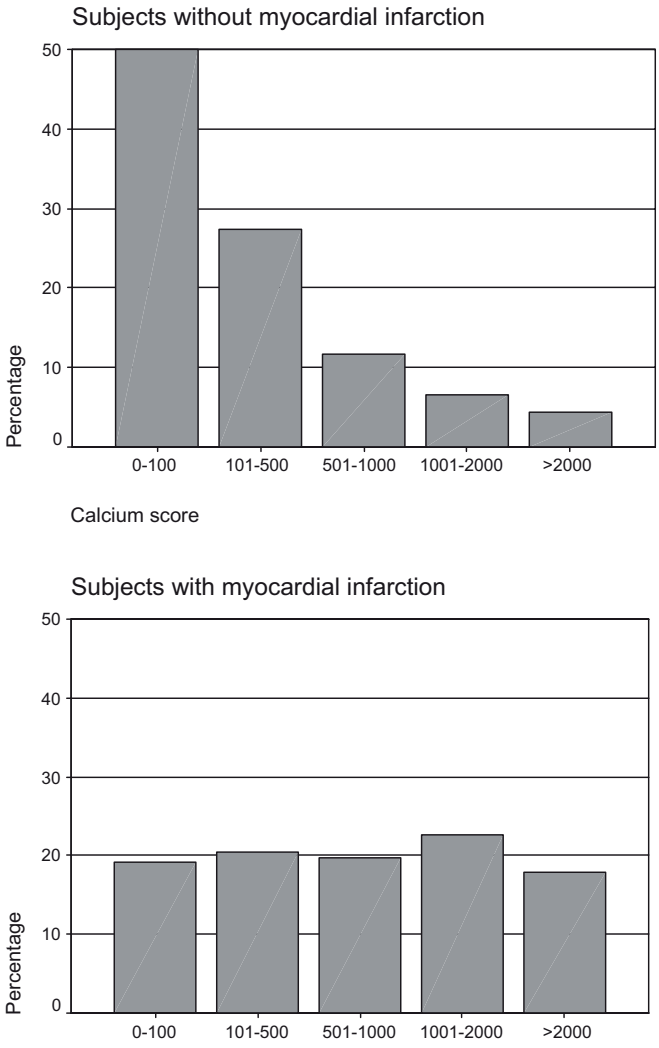

FIGURE 1
Distribution of calcium scores by myocardial infarction status

TABLE 3
Risk of myocardial infarction in sex-specific calcium score quartiles for men and women

	n	Events	Model 1 * Odds Ratio (95% CI)	Model 2 † Odds Ratio (95% CI)
Men				
Q1 (0–62)	223	11	1.0 (reference)	1.0 (reference)
Q2 (63–311)	230	25	2.2 (1.0–4.6)	2.5 (1.1–5.6)
Q3 (312–969)	227	46	4.4 (2.2–8.8)	4.3 (2.0–9.4)
Q4 (>969)	224	84	10.3 (5.3–20.2)	12.3 (5.8–25.8)
Women				
Q1 (0–4)	262	11	1.0 (reference)	1.0 (reference)
Q2 (5–55)	261	11	0.9 (0.4–2.2)	0.9 (0.3–2.2)
Q3 (56–261)	263	13	1.1 (0.5–2.4)	1.1 (0.5–2.6)
Q4 (>261)	247	28	2.5 (1.2–5.3)	2.5 (1.1–5.5)

† Model 1: adjusted for age.
‡ Model 2: adjusted for age, smoking, blood pressure, body mass index, total cholesterol, HDL cholesterol, and diabetes mellitus.
The number of subjects in model 2 is somewhat lower than for model 1, due to missing values for cardiovascular risk factors.
CI = confidence interval.

TABLE 4
Age-adjusted odds ratio of myocardial infarction in calcium score categories, for men and women, in two age strata

	Men			Women		
	n	Events	Odds Ratio (95% CI)	n	Events	Odds Ratio (95% CI)
< 70 years						
Calcium score:						
0–100	176	9	1.0 (reference)	338	7	1.0 (reference)
101–500	111	8	1.4 (0.5–3.7)	108	3	1.3 (0.3–5.1)
501–1000	58	12	4.8 (1.9–12.2)	36	3	4.3 (1.0–17.8)
>1000	84	24	7.1 (3.1–16.5)	15	3	10.0 (2.3–44.2)
≥ 70 years						
Calcium score:						
0–100	120	10	1.0 (reference)	293	18	1.0 (reference)
101–500	156	25	2.0 (0.9–4.5)	158	11	1.3 (0.6–2.8)
501–1000	87	21	3.4 (1.5–7.6)	73	9	2.4 (1.0–5.5)
>1000	140	57	7.7 (3.7–16.1)	60	9	3.8 (1.6–9.3)

CI = confidence interval. Associations were observed between coronary calcification and myocardial infarction in subjects younger than 70 years as well as in subjects of 70 years and older (table 4). Although estimates varied in strata of age among both men and women, probably due to small numbers, in all subgroups a graded association was present.

Table 3 shows odds ratios obtained when the calcium score distribution was divided into quartiles for men and women separately. Compared to men in the lowest calcium score quartile, the odds ratios of myocardial infarction for those in the second, third and fourth quartile were significantly higher than one. In men, increasing calcium score quartiles were associated with a graded increase in odds of having experienced a myocardial infarction. In women, compared to those in the lowest calcium score quartile, only women in the highest calcium score quartile were more likely to have experienced a myocardial infarction (age-adjusted OR; 95% CI, 2.5; 1.5-5.3).

Associations were observed between coronary calcification and myocardial infarction in subjects younger than 70 years as well as in subjects of 70 years and older (table 4). Although estimates varied in strata of age among both men and women, probably due to small numbers, in all subgroups a graded association was present.

Discussion

The amount of coronary calcification showed a strong and graded association with the presence of myocardial infarction in a general population. Men and women with a calcium score above 2000 were seven to eight times more likely to have experienced a myocardial infarction compared to subjects with calcium score up to 100. Associations were present in subjects younger than 70 years as well as in subjects of 70 years and older. This is the first large study on the association between coronary calcification and coronary events in an elderly population.

Comparison of all characteristics of subjects who participated in the Rotterdam Coronary Calcification Study and the non-responders demonstrated a relevant difference in the percentage of men, in the percentage of smokers, and in the mean age, but otherwise small, non-significant differences. However, subjects with severe disability may not have agreed to undergo EBT scanning. If reasons for non-participation are related to the amount of coronary calcification, this may have limited the range of calcium scores in this study. The threshold for detection of coronary calcifications in this study was two consecutive pixels. Various minimal numbers of pixels have been used in different studies for distinguishing true foci of calcium from noise. Some studies have used higher thresholds to reduce the contribution of noise. Nevertheless, when we compared calcium scoring with a threshold of two pixels and scoring with a threshold of four pixels in a subgroup of subjects, we found a very high correlation coefficient ($r = 0.99$) between calcium scores.

There are some drawbacks due to the cross-sectional study design. Since only survivors of a myocardial infarction were included, it is uncertain whether the same risk estimates would have been found for fatal events. The time lag between the occurrence of myocardial infarction and scanning may have resulted in classification of subjects into a different calcium score category than the classification would have been if coronary calcification had been measured at the time of the coronary event. When we limited the logistic regression analysis to events in the past ten years, the odds ratios for myocardial infarction were comparable to the odds ratio for all events: up to 6.6 (data not shown). Occurrence of myocardial infarction in the past may have led to changes in life-style and medication use to reduce cardiovascular risk. This could have diminished the difference in amount of coronary calcification between subjects with and without myocardial infarction, and therefore the risk estimates may have been underestimated.

Controversy exists on the relation between atherosclerosis and calcium deposits. Coronary calcification is closely related to the amount of atherosclerotic plaque in the coronary artery tree.[1] Doherty and co-workers have suggested that coronary calcification is an active process that stabilises vulnerable plaques and protects against rupture.[18] Thus, increasing calcification of individual atherosclerotic lesions may decrease the risk of obstruction at the lesion site and therefore of a coronary event. This view is supported by the finding that myocardial infarction was prone to originate from non- or mildly calcified culprit arteries, while in subjects with stable angina pectoris coronary arteries were generally extensively calcified.[19] However, a recent study comparing culprit and non-culprit arteries in subjects with myocardial infarction showed that culprit arteries were more calcified than non-culprit arteries.[20] If plaque calcification protects against plaque rupture and if, therefore, subjects with intermediate calcification are more prone to have myocardial infarctions than subjects with severe calcification, more cases among the subjects with intermediate calcification will have died from a myocardial infarction before entering the study. Studying the association between amount of calcification and myocardial infarction in a cross-sectional design may have transformed the true shape of the association. It is not clear whether the progression rate of calcification is equal for culprit vessels and non-culprit vessels. However, Schmermund et al.[21] reported that the relative progression of overall coronary calcification resulted largely from uniformly relative progression of calcification at the typical predilection sites, suggesting a coronary systemic process.

There are only few published prospective studies addressing the association between coronary calcification detected by EBT and the risk of coronary events. Arad et al.[8] found relative risks of myocardial infarction or coronary death of 22.3 (95% CI, 5.1-97.4) and 22.2 (95% CI, 6.4-77.4) for thresholds of calcium scores of

80 and 160, respectively, among 1173 self-referred asymptomatic subjects (mean baseline age 53 years) with 18 myocardial infarctions and coronary deaths during a follow-up of 3.6 years. In the South Bay Heart Watch Study, 29 myocardial infarctions and 17 coronary deaths occurred in 1196 high-risk asymptomatic subjects (mean age 66 years) during 41 months.[5] The ability of EBT to distinguish subjects at high risk of coronary events from subjects at low risk was not better than that of risk factor assessment. Raggi et al.[6] reported a relative risk of 21.5 (95% CI, 2.8-162.4) for myocardial infarction or cardiovascular death in the fourth quartile of the calcium score when compared to the lowest quartile. This study in a population referred for scanning because of the presence of cardiovascular risk factors was based on calcium scores of 632 subjects (mean age 52 years) with 27 events in 32 months. Wong et al.[7] observed in a recent study among 926 self-referred or GP-referred subjects a relative risk of cardiovascular events of 8.8 in the highest calcium score quartile compared to subjects without coronary calcification. However, of the 28 cardiovascular events that occurred during a mean of 3.3 years after scanning, 23 were revascularizations, which partially may have taken place on the basis of a high calcium score. A cross-sectional study in 1218 selected high-risk subjects observed a higher mean calcium score in subjects with than in subjects without a self-reported history of coronary heart disease.[22]

It is well-known that the prevalence and amount of coronary calcification rises with increasing age, and that the distribution of coronary calcification is lower in women than in men at all ages.[18] Raggi et al.[6] found in a relatively young population with 50% women that age- and sex-adjusted calcium score percentiles were appropriate to identify high-risk subjects independent of the absolute calcium score. Our study consisted of elderly subjects. Only 2% of women had a calcium score above 2000. When we analysed the association with myocardial infarction in quartiles of the calcium score, the odds ratio for women remained 1 up to the third quartile compared to the lowest quartile, while in men gradually increasing odds ratios were seen (see table 3). Although extreme absolute calcium scores do not seem to be appropriate for selection of women at high risk, risk elevation in this group was only seen in the upper part of the calcium score distribution.

Risk factor assessment and noninvasive measurement of atherosclerosis in the population aim at selecting subjects at high risk of coronary events. Cardiovascular risk factors have been found to increase the risk of cardiovascular disease approximately 1.5 to 5 times.[23,24] Studies investigating the relation of carotid intima-media thickness and the ankle to brachial blood pressure index with coronary events, have found relative risks ranging between 2 and 4.[24-30] No studies on risk factors or atherosclerosis and coronary events have reported relative risks of seven to eight, as observed with coronary calcification detected by EBT in our cross-sectional study.

In conclusion, we observed a strong and graded association between the amount of coronary calcification and the presence of myocardial infarction in both men and women, which remained at high ages. This is the first study on the association between coronary calcification detected by EBT and coronary heart disease in an unselected older population. Although prospective data need to confirm our findings, this population-based cross-sectional study suggests that EBT is a powerful tool for the selection of subjects at high-risk of coronary events.

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Coronary calcification and the presence of stroke

5.2

ABSTRACT

Background and Purpose: Coronary calcification as detected by electron-beam tomography (EBT) measures the atherosclerotic plaque burden, and has been reported to predict coronary events. Since atherosclerosis is a generalized process, coronary calcification may also be associated with manifest atherosclerotic disease at other sites of the vascular tree. We examined whether coronary calcification as detected by EBT is related to the presence of stroke.

Methods: From 1997 onwards, subjects were invited to participate in the prospective Rotterdam Coronary Calcification Study and undergo EBT to detect coronary calcification. The study was embedded in the population-based Rotterdam Study. Calcifications were quantified in a calcium score according to Agatston's method. Calcium scores were available for 2013 subjects (mean age [SD], 71 [5.7] years). Fifty subjects had experienced stroke prior to scanning.

Results: Subjects were 2 times more likely to have experienced stroke when their calcium score was between 101 and 500 (odds ratio [OR], 2.1; 95% confidence interval [CI], 0.9-4.7), and 3 times more likely when their calcium score was above 500 (OR, 3.3; 95% CI, 1.5-7.2), compared to subjects in the lowest calcium score category (0 to 100). Additional adjustment for cardiovascular risk factors did not materially alter the risk estimates.

Conclusions: In this population-based study, a markedly graded association was found between coronary calcification and stroke. The results suggest that coronary calcification as detected by EBT may be useful to identify subjects at high risk of stroke.

Introduction

Several studies have shown that non-invasive measures of atherosclerosis predict cerebrovascular events.[1-6] Coronary calcification as detected by electron-beam tomography (EBT) is closely related to the amount of coronary atherosclerotic plaque,[7] and has been reported to predict coronary events.[8-12] There is a close relation between calcification of the coronary arteries and the extracoronary plaque burden.[13,14] Therefore, coronary calcification may also be associated with manifest atherosclerotic disease at other sites of the vascular tree. No data are available on the association between coronary calcification and cerebrovascular events. We studied the association of coronary calcification as detected by EBT and the presence of stroke in 2013 elderly men and women who participated in the population-based Rotterdam Coronary Calcification Study.

Materials and Methods

Study population

The Rotterdam Coronary Calcification Study was designed to study determinants and consequences of coronary calcification, detected by EBT. The study was embedded in the Rotterdam Study. The Rotterdam Study is a population-based study that started in 1990 to 1993. All inhabitants of a suburb of Rotterdam, aged 55 years and over, were invited (response 78%). The rationale and design of the Rotterdam Study have been described elsewhere.[15] Follow-up visits took place in 1993 to 1994 and 1997 to 1999. From 1999 onwards, the study population is extended with a second cohort comprising inhabitants who reached the age of 55 years after the baseline examination from 1990 to 1993, and subjects aged 55 years and over who migrated into the research area. Baseline and follow-up visit examinations included non-invasive measurements of atherosclerosis. Measurement protocols for the first and second cohort were identical.

From 1997 onwards, participants through 85 years of age who completed the third phase of the first cohort or the baseline examination of the second cohort of the Rotterdam Study were invited to participate in the Rotterdam Coronary Calcification Study and undergo an EBT scan. Subjects in nursing homes did not visit the research center and thus were not invited for the Rotterdam Coronary Calcification Study. We restricted the present analysis to participants from the first cohort, who were scanned from 1997 to 2000. Of the 3371 eligibles recruited from the first cohort, scans were obtained for 2063 subjects (response: 61%). Due to several causes i.e. metal clips from cardiac surgery, severe artefacts,

and registration errors (ECG, acquisition), image acquisition data could not be reconstructed or analysed in 50 subjects. Consequently, scores were available for 2013 participants. All other measurements were obtained from the examinations of the Rotterdam Study. The median duration between the examination at the Rotterdam Study center and EBT scanning was 50 days. The Medical Ethics Committee of Erasmus University Rotterdam approved the study, and all participants gave informed consent.

Measurement of coronary calcifications

We assessed coronary calcifications in the epicardial coronary arteries detected on EBT scans. Imaging was performed with a C-150 Imatron scanner (Imatron, South San Francisco, California). Before the subjects were scanned, they exercised adequate breath-holding. From the level of the root of the aorta through the heart, 38 images were obtained with 100 ms scan time and 3 mm slice thickness. We acquired images at 80% of the cardiac cycle, using electrocardiogram triggering, during a single breath-hold. Every day that the scanner was used, we calibrated the scanner using a water phantom. Quantification of coronary calcifications was performed with AccuImage software (AccuImage Diagnostics Corporation, South San Francisco, California) displaying all pixels with a density of over 130 Hounsfield units. The trained scan readers were blinded to the clinical data of the participants. A calcification was defined as a minimum of two adjacent pixels (area = 0.65 mm^2) with a density over 130 Hounsfield Units. We placed a region of interest around each high-density lesion in the epicardial coronary arteries. The peak density in Hounsfield Units and the area in mm^2 of the individual coronary calcifications were calculated. A calcium score was obtained by multiplying each area of interest with a factor indicating peak density within the individual area, as proposed by Agatston et al.[16] We summated the scores for individual calcifications, which resulted in a calcium score for the entire epicardial coronary system. Conform the protocol outlines as approved by the Medical Ethics Committee, participants were not informed about their calcium score.

Diagnosis of stroke

A stroke was categorized as present if stroke had occurred before the baseline examination from 1990 to 1993 or after baseline but prior to EBT scanning (1997 to 2000). Of subjects that reported a history of stroke at the baseline examination, the general practitioner (GP) was asked for supplementary medical information,

including a detailed history, information on neuro-imaging, and copies of hospital discharge records. After the baseline examination, GPs in the research area, covering 85% of the cohort, reported the possible stroke events to the research center. For 15% of the cohort, of which the GPs had practices outside the research area, information was obtained annually by checking the participant's GP file and by interviewing the GP. When an event was reported, additional information including the date of the possible stroke was obtained by interviewing the GP and scrutinising information from hospital discharge records and/or neuro-imaging in case of admission or referral. Medical information was checked and evaluated by an experienced neurologist from the Erasmus Medical Center Rotterdam. Events were coded according to the International Classification of Diseases, 10th version.[17] The neurologist classified the events as definite, probable, possible, and no stroke. A stroke was classified by the neurologist as definite when the event led to a hospitalization, and the hospital discharge record indicated a diagnosis of a new stroke, based on clinical signs and symptoms and/or on neuro-imaging during hospital stay. When, in case of no hospitalization, signs and symptoms associated with the event obtained from the GP records and interview were highly suggestive of a stroke according to the neurologist, the stroke was classified as probable. Definite and probable events were used in the analyses.

Measurement of cardiovascular risk factors

Information on smoking was obtained during the home interview of the Rotterdam Study. We categorised subjects as current, past or never smokers. Anthropometric measures were obtained during the visit at the Rotterdam research center. Blood pressure was measured at the right brachial artery using a random-zero sphygmomanometer with the participant in sitting position. The mean value of two consecutive measurements was used in the analyses. Hypertension was defined as a systolic blood pressure of 140 mm Hg or higher or a diastolic blood pressure of 90 mm Hg or higher. After an overnight of fasting, blood samples were obtained at the research center. Serum total cholesterol was determined by an enzymatic procedure. High-density lipoprotein (HDL) was measured similarly after precipitation of the non-HDL fraction.[18] Diabetes mellitus was considered present with current use of antidiabetic medication, or when fasting glucose levels exceeded 7.0 mmol/l.[19]

Statistical analysis

The distribution of calcium scores was skewed, and therefore, medians and ranges were reported. Linear regression analysis was applied to compare the age-adjusted continuous baseline characteristics of the Rotterdam Coronary Calcification Study and the non-responders. To compare sex, smoking status, and the percentage of subjects with hypertension, diabetes mellitus, stroke and myocardial infarction between the study population and the group of non-responders, the chi-square test was used. Three absolute calcium score categories were defined, based on cut-points that were chosen before examining the association with the presence of stroke: 0 to 100, 101 to 500, and above 500. Age- and sex-adjusted odds ratios (OR) with 95% confidence interval (CI) for the presence of stroke were calculated per calcium score category using logistic regression analysis. Calcium score category 0-100 was used as reference. Analyses for calcium score categories were repeated with additional adjustment for cardiovascular risk factors (smoking, total cholesterol, HDL cholesterol, hypertension, diabetes mellitus). Age-adjusted ORs (95% CI) were also calculated for men and women separately. SPSS 9.0 for Windows (SPSS Inc., Chicago, Illinois) was used for data analysis.

Results

Table 1 describes the characteristics of the 2013 participants of the Rotterdam Coronary Calcification Study. The mean age (standard deviation [SD]) of the study population was 71 years (5.7 years). Comparison of characteristics of the study participants and the non-responders demonstrated no significant differences with regard to total and HDL cholesterol levels, hypertension, diabetes mellitus, and history of myocardial infarction. However, the scanned population was significantly younger (mean age difference 1.7 years), consisted of relatively more men (46% vs 38%), was more likely to have a history of smoking (70% vs 63%), had a slightly higher body mass index (27.0 vs 26.7). Furthermore, compared to the non-responders, a slightly lower percentage of study participants had a history of stroke (2.5% vs 3.6%). The median calcium score was 135 (interquartile range 13-578). The median calcium score was higher for men than for women: 312 (interquartile range 62-970) and 55 (interquartile range 5-261), respectively. In 34 men (3.6%) and 16 women (1.5%) stroke was recorded before EBT scanning. The mean interval (SD) between the stroke and scanning was 8.8 years (7.8).

TABLE 1
Characteristics of the Rotterdam Coronary Calcification Study population and the non-responders

Variable	Study population (n = 2013)	Non-responders (n = 1308)
Age (years)	70.8 ± 5.5	72.5 ± 6.5*
Male (%)	46.3	37.8*
Smokers (%)		
Current	16.2	16.4
Past	54.2	46.5*
Body mass index	27.0 ± 3.9	26.7 ± 4.1*
Total cholesterol (mmol/l)	5.8 ± 1.0	5.9 ± 1.0
HDL cholesterol (mmol/l)	1.4 ± 0.4	1.4 ± 0.4
Hypertension (%)	54.4	54.5
Diabetes mellitus (%)	13.2	13.2
History of stroke (%)	2.5	3.6*
History of myocardial infarction (%)	11.8	11.6
Calcium score	135 (13 – 578)	
Log calcium score	4.4 ± 2.4	

Values are unadjusted proportions or means ± standard deviation, except for the calcium score, which is expressed as median (inter-quartile range) because of its skewed distribution.
* p <0.05 compared with non-responders, adjusted for differences in age and sex (except for the variable age: sex-adjusted).

TABLE 2
Risk of stroke in calcium score categories

	n	events	Model 1*		Model 2†	
			OR	95% CI	OR	95% CI
All						
Calcium score:						
0 – 100	927	10	1.0	…	1.0	…
101 – 500	533	14	2.1	0.9 – 4.7	1.5	0.6 – 3.8
> 500	553	26	3.3	1.5 – 7.2	3.0	1.3 – 6.8
Test for trend			p = 0.001		p = 0.007	

OR is odds ratio, CI is confidence interval, n is number of subjects.
* Model 1: adjusted for age and sex.
† Model 2: adjusted for age, sex, smoking, total cholesterol, HDL cholesterol, hypertension and diabetes mellitus. The number of subjects in model 2 is somewhat lower than for model 1 (n = 1795), due to missing values for cardiovascular risk factors.

TABLE 3
Risk of stroke in calcium score categories for men and women, adjusted for age

	n	events	OR	95% CI
Men				
Calcium score:				
0 – 100	296	3	1.0	…
101 – 500	267	10	3.5	0.9 – 12.8
> 500	369	21	5.2	1.5 – 17.8
Test for trend			p = 0.005	
Women				
Calcium score:				
0 – 100	630	7	1.0	…
101 – 500	266	4	1.3	0.4 – 4.6
> 500	184	5	2.4	0.7 – 7.8
Test for trend			p = 0.16	

OR is odds ratio, CI is confidence interval, n is number of subjects.

In logistic regression analysis, a graded association was found between the amount of coronary calcification and the presence of stroke (table 2). The age-adjusted odds ratios of stroke was 2.1 (95% CI, 0.9-4.7) in the calcium score category 101-500, and 3.3 (95% CI, 1.5-7.2) in subjects with a calcium score above 500, when compared to subjects in the reference category (calcium score of 0-100). Additional adjustment for cardiovascular risk factors did not substantially alter the results (model 2).

Table 3 shows risk estimates for men and women separately. In men, age-adjusted odds ratios for the presence of stroke increased from 3.5 (95% CI, 0.9-12.8) in the calcium score category 101-500, to 5.2 (95% CI, 1.5-17.8) in those with a calcium score above 500, when compared to subjects in the reference category (calcium score of 0-100). The corresponding age-adjusted odds ratios in women were 1.3 (95% CI, 0.4-4.6) and 2.4 (95% CI, 0.7-7.8), respectively. In men, 62% of the strokes occurred in the calcium score category above 500, while in women this percentage was 31.

Discussion

The amount of coronary calcification showed a graded association with the presence of stroke in a general population of elderly subjects. Subjects in the highest calcium score category (above 500) were three times more likely to have experienced a stroke compared to subjects in the reference calcium score category (up to 100). The odds ratio of stroke was 5.2 for men with a calcium score above

500 in comparison to men with a calcium score up to 100. In women with a calcium score above 500, stroke had occurred 2.4 times more often than in women in the reference calcium score category, although the estimates are less certain for women due to small numbers.

Scans were obtained from 2063 subjects recruited from the first cohort of the Rotterdam Study. Comparison of all characteristics of the participants of the Rotterdam Coronary Calcification Study with the non-responders only showed a relevant difference in the mean age, the percentage of men, and the percentage of subjects with a history of smoking. Subjects with severe disability, possible resulting from stroke, may not have shown up for EBT scanning. If reasons for non-participation are related to the amount of coronary calcification, this may have limited the range of calcium scores in this study, since the highest calcium scores are to be expected in the subjects with disabling cardiovascular disease. Thus, we were not able to assess the possibly stronger association between coronary calcification and stroke in subjects with severe cardiovascular disease. Secondly, because of small numbers of cases, the confidence intervals of the sex-specific odds ratios are wide. This should induce caution when drawing conclusions on the strength of the association in men and women. To obtain more reliable risk estimates on the association between coronary calcification and stroke by gender, more cases are needed. Furthermore, the threshold used in this study for detection of coronary calcifications was two consecutive pixels. Some studies have used higher thresholds to reduce the contribution of noise. However, in a subgroup of subjects, we found a very high correlation coefficient ($r = 0.99$) between calcium scores, obtained using a threshold of two pixels and a threshold of four pixels. Thirdly, due to the fact that the association of coronary calcification with stroke was evaluated in a cross-sectional study design, only survivors of a cerebrovascular event were included. It is uncertain whether the same risk estimates would have been found for fatal events. Moreover, survivors of stroke are more likely to have had less severe types of stroke like lacunar infarctions, possibly limiting the range of stroke severity. Since there was an interval between the occurrence of stroke and scanning of 8.8 years on average, subjects may have been classified into a different calcium score category than the classification would have been if coronary calcification had been measured at the time of the cerebrovascular event. Furthermore, the cerebrovascular event in the past may have initiated medication use to reduce cardiovascular risk. This could have diminished the difference in coronary calcium load between subjects with and without stroke, resulting in underestimation of the odds ratios. After adjustment for cardiovascular determinants, risk estimates changed only slightly. This lack of change may in part be due to modification of risk factors in subjects after the cerebrovascular event, leading to misclassification of risk factors.

Non-invasive measures of atherosclerosis have been shown to predict cerebrovascular events. Studies investigating the relation of carotid intima-media thickness with stroke, have presented relative risks ranging between 3.4 and 8.5 for highest versus lowest quintile or category of intima-media thickness.[1] Low ankle brachial pressure index also indicates an increased risk of stroke, but relative risks of stroke associated with this measure were lower.[4] Since we investigated the association between coronary calcification and stroke in a cross-sectional design, the risk estimates cannot be compared directly with the results from these prospective studies. However, in our study coronary calcification was strongly related to the presence of stroke. Therefore, coronary calcium detection by EBT may not only be useful to identify subjects at high risk of coronary heart disease, but additionally those at high risk of stroke.

In conclusion, we observed a markedly graded association between the amount of coronary calcification and stroke in an elderly population. This is the first study on the association between coronary calcification as detected by EBT and stroke. Although prospective data need to confirm our findings, this population-based cross-sectional study suggests that the amount of coronary calcification identifies subjects at high risk of cerebrovascular events.

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5.3 Coronary calcification and incident coronary heart disease, cardiovascular disease and mortality

ABSTRACT

Background: Coronary calcification detected by electron-beam tomography (EBT) may improve the assessment of cardiovascular risk. There are currently no population-based data on the association of coronary calcification with cardiovascular disease. We investigated the predictive value of coronary calcification for coronary heart disease, cardiovascular disease, and total mortality in a general population of older adults.

Methods: From 1997 to 2000, EBT scanning for assessment of coronary calcification was performed in subjects of the population-based Rotterdam Study. Coronary calcium scores were available for 2013 participants with a mean age of 71 years.

Results: During a mean follow-up period of 3.3 years, 116 cardiovascular events occurred, including 73 coronary events. Only one coronary event occurred in the lowest calcium score quartile, while two-thirds occurred in the highest quartile. The risk of coronary events gradually increased with increasing calcium score. Subjects with a calcium score above 1000 had an eight times increased risk of coronary heart disease compared to those with a calcium score of 0 to 100 (age- and sex adjusted relative risk 8.0 (95% confidence interval 3.6-18.2)). The corresponding relative risk in asymptomatic subjects was 8.2 (3.3-20.4). The relative risks only slightly changed after adjustment for cardiovascular risk factors (6.8, 2.9-16.1 and 8.3, 3.3-21.2, respectively).

Conclusions: Coronary calcification is a strong and independent predictor of coronary heart disease. The amount of coronary calcification may not only identify subjects at high risk of coronary heart disease, but also those in which the risk of coronary heart disease is negligible.

Introduction

Coronary calcification, assessed by electron-beam tomography (EBT) may be a useful tool for the identification of subjects at high risk of coronary heart disease. The technique is based on a close correlation between coronary calcification and atherosclerotic plaque burden.[1] Several studies have shown that the amount of coronary calcification is associated with the risk of coronary heart disease.[2-6] However, there are currently no population-based data on the association of coronary calcification with risk of cardiovascular disease. Previous studies were performed in selected, high-risk populations, and the majority included small numbers of events. Furthermore, whether assessment of coronary calcification improves risk prediction when cardiovascular risk factors are known is uncertain. Most studies used self-reported information on risk factors and results of the additive predictive value of coronary calcification are inconsistent.

The Rotterdam Coronary Calcification Study is a prospective population-based study among 2013 older adults. We studied the predictive value of coronary calcification for coronary heart disease, cardiovascular disease, and total mortality.

Methods

Study population

The Rotterdam Coronary Calcification Study was designed to study determinants and consequences of coronary calcification, detected by EBT. The study was embedded in the Rotterdam Study, a prospective, population-based study among 7983 subjects aged 55 years and older, which started in 1990. The rationale and design of the Rotterdam Study have been described elsewhere.[2] From 1997 onward, participants through 85 years of age were invited to participate in the Rotterdam Coronary Calcification Study and to undergo an EBT scan. Subjects in nursing homes did not visit the research center and thus were not invited for the study. Of the 3370 eligibles, scans were obtained for 2063 subjects (61%). Due to several causes, e.g. metal clips from cardiac surgery, severe artifacts, and registration errors (electrocardiography, acquisition), image acquisition data could not be reconstructed or analysed in 50 subjects, and therefore data were available for 2013 participants. All other information was obtained from the examinations of the Rotterdam Study. The examinations included an extensive interview on life-style, medical history and medication use, physical examination, blood pressure measurement, 12-lead electrocardiography, and laboratory measurements. Details of risk factor assessment methodology have been published previously.[3,4] The

median duration between the examination at the Rotterdam Study center and EBT scanning was 50 days. The Medical Ethics Committee of Erasmus University Rotterdam approved the study, and all participants gave informed consent.

Coronary calcification

We assessed coronary calcifications in the epicardial coronary arteries detected on EBT scans. Imaging was performed with a C-150 Imatron scanner (Imatron). Before the subjects were scanned, they exercised adequate breath-holding. From the level of the root of the aorta through the heart, 38 images were obtained with 100 ms scan time and 3 mm slice thickness. We acquired images at 80% of the cardiac cycle, using electrocardiogram triggering, during a single breath-hold. Quantification of coronary calcifications was performed with AccuImage software (AccuImage Diagnostics Corporation) displaying all pixels with a density of over 130 Hounsfield units. A calcification was defined as a minimum of two adjacent pixels (area = 0.65 mm^2) with a density over 130 Hounsfield Units. Calcium scores were calculated according to Agatston's method.[5] The trained scan readers were blinded to the clinical data of the participants. To conform with the protocol outlines as approved by the Medical Ethics Committee, participants were not informed about the calcium score.

Clinical outcomes

Two participants were lost to follow-up, in which cases the last date of contact was used as census date. Information concerning the vital status of the participants was obtained from the municipal health service of Rotterdam. Subjects in the Rotterdam Study were continuously monitored for the occurrence of cardiovascular events through automated linkage with the files from general practitioners in the research area of the Rotterdam Study (85% of the cohort). For 15% of the cohort of which the general practitioners had practices outside the research area, information was obtained through checking the participant's file and by interviewing the general practitioner regularly. When myocardial infarction, coronary artery bypass grafting (CABG), percutaneous transluminal coronary angioplasty (PTCA), stroke or death was reported, the research assistants collected additional information from medical records of the general practitioner and in addition, obtained information from hospital discharge records or nursing home records, including letters from medical specialists. Two research physicians independently coded the possible cardiovascular events, according to the International Classification of Diseases, 10^{th} version.[6] In case the research physicians disagreed on the diagnosis of a coronary

event or stroke, a cardiologist or neurologist, respectively, reviewed the coded event and performed the definitive coding. The research physicians, cardiologist and neurologist were not aware of the calcium score outcome. In the analyses, we used the following outcome measures: coronary heart disease (incident myocardial infarcion, CABG, PTCA and coronary heart disease mortality), cardiovascular disease (incident myocardial infarction, CABG, PTCA, stroke and cardiovascular mortality), coronary heart disease mortality, cardiovascular mortality, and total mortality.

Statistical analysis

The calcium score was divided into four absolute categories: 0 to 100, 101 to 500, 501 to 1000, and above 1000. Age- and sex-adjusted Cox regression analysis was conducted to compute event-free survival curves for the absolute calcium score categories. Hazard ratios of events in increasing absolute calcium score categories were computed as estimates of relative risk. Subjects with a calcium score of 0 to 100 were used as the reference group. We also computed relative risks in a multivariate model, containing the following cardiovascular risk factors in addition to age and sex: body mass index, systolic blood pressure, diastolic blood pressure, total cholesterol, HDL cholesterol, smoking, diabetes mellitus, history of coronary heart disease (for outcomes: coronary heart disease and coronary heart disease mortality) or cardiovascular disease (for the other outcomes), and family history of myocardial infarction. Cox analyses were repeated in asymptomatic subjects. Subjects with a history of coronary heart disease were excluded in the analyses with coronary heart disease or coronary heart disease mortality as outcome, while subjects with a history of cardiovascular disease were excluded for the other outcomes. Furthermore, age- and sex-adjusted relative risks were computed for dichotomized cardiovascular risk factors.

In the asymptomatic population, two additional analyses were conducted. First, the Framingham risk model as derived by Anderson et al.[7] was applied to calculate 10-year risk probabilities. The risk probabilities were divided into quartiles. Cox regression analysis adjusted for age and sex was conducted in categories based on calcium score (categories: 0-100, 101-500, >500) and the Framingham risk function (below or above 75th percentile). For this analysis, three categories of calcium scores were used to increase statistical power. Secondly, we computed probabilities of coronary heart disease and cardiovascular disease for each subject as predicted by the multivariate model containing only age, sex and cardiovascular risk factors, and by the multivariate model that also included the calcium score. We applied the probability values as thresholds to categorize the results as positive or negative. True- and false-positive rates were determined for each threshold, and used to construct receiver operating characteristic (ROC) curves. Differences in

the predicted values were estimated by comparing the areas under the ROC curve, taking correlation between the areas into account.[8]

All measures of association are presented with 95% confidence intervals (CI). SPSS 11.0 for Windows (SPSS, Inc., Chicago, Illinois) was used for data analysis. Of the population, 8% missed information on one cardiovascular risk factor, while 3% missed information on two or more risk factors. Before multivariate Cox regression analyses were performed, missing risk factor values were imputed using the multivariate imputation by chained equations (MICE) approach in S-Plus 2000 (MathSoft, Inc., Cambridge, Massachussetts).

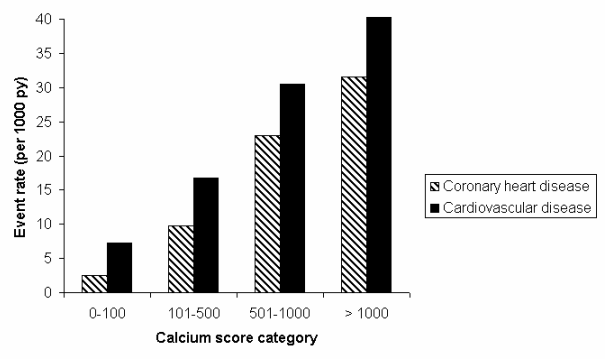
Results

Baseline characteristics of the study population are shown in table 1. The distribution of the calcium score was highly skewed, with a median of 134 and a range of 0 to 12611. The mean follow-up duration was 3.3 years (standard deviation, 0.8 years; maximum, 4.9 years). Of the 2013 participants, 140 had died during the follow-up period. Hundred and sixteen subjects suffered a cardiovascular event, including

TABLE 1
Baseline characteristics of the study population (n=2013)

Variable	Mean or percentage*
Age, y	71.3 ± 5.7
Male	46.3
Body mass index, kg/m^2	27.0 ± 3.9
Systolic blood pressure, mm Hg	143 ± 21
Diastolic blood pressure, mm Hg	76 ± 11
Total cholesterol, mmol/L	5.8 ± 1.0
HDL cholesterol, mmol/L	1.4 ± 0.4
Smokers	
Current	16.2
Past	54.2
Diabetes mellitus	13.2
History of myocardial infarction	11.4
History of stroke	2.5
History of CABG/PTCA	6.0
Family history of myocardial infarction	19.6
Calcium score†	134 (13-578)

* Categorical variables are expressed as percentage. Values of continuous variables are expressed as mean (standard deviation).
† Median (interquartile range) reported because of skewed distribution of the calcium score.



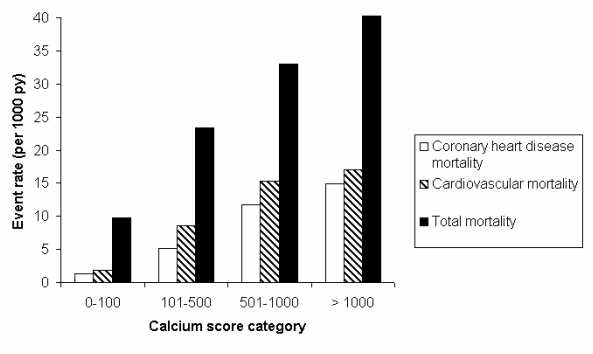


FIGURE 1
Event rates according to calcium score category

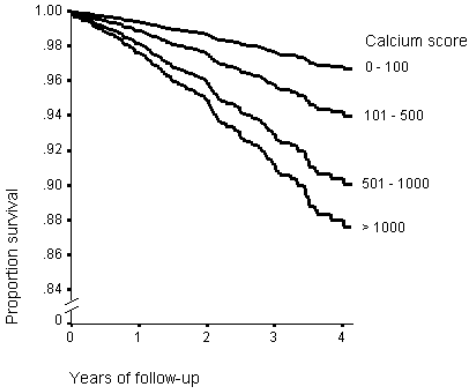


FIGURE 2
Cardiovascular event-free survival curves according to calcium score category

TABLE 2
Relative risks of events according to calcium score category in all subjects

	Total/events (n)	Relative risk (95% confidence interval)	
		Age- and sex-adjusted	Multivariate-adjusted
CHD*			
Calcium score:			
0-100	927/8	1.0 (reference)	1.0 (reference)
101-500	533/17	3.1 (1.3-7.2)	2.9 (1.2-6.8)
501-1000	254/19	6.8 (2.9-15.7)	6.2 (2.6-14.6)
>1000	299/29	8.0 (3.6-18.2)	6.8 (2.9-16.1)
CVD*			
Calcium score:			
0-100	927/23	1.0 (reference)	1.0 (reference)
101-500	533/29	1.8 (1.1-3.2)	1.7 (1.0-3.0)
501-1000	254/25	3.1 (1.7-5.6)	2.8 (1.6-5.1)
>1000	299/39	3.9 (2.3-6.8)	3.4 (1.9-6.1)
CHD mortality			
Calcium score:			
0-100	927/4	1.0 (reference)	1.0 (reference)
101-500	533/9	2.8 (0.9-9.2)	2.6 (0.8-8.6)
501-1000	254/10	5.6 (1.7-18.5)	5.8 (1.8-19.1)
>1000	299/14	6.1 (1.9-19.3)	5.7 (1.7-18.8)
CVD mortality			
Calcium score:			
0-100	927/6	1.0 (reference)	1.0 (reference)
101-500	533/15	3.2 (1.2-8.4)	3.0 (1.1-7.9)
501-1000	254/13	5.1 (1.9-13.8)	5.0 (1.8-13.6)
>1000	299/16	5.0 (1.9-13.2)	4.4 (1.6-12.1)
Total mortality			
Calcium score:			
0-100	927/31	1.0 (reference)	1.0 (reference)
101-500	533/41	1.7 (1.0-2.7)	1.7 (1.1-2.8)
501-1000	254/28	2.3 (1.3-3.8)	2.2 (1.3-3.8)
>1000	299/40	2.6 (1.6-4.2)	2.7 (1.6-4.5)

* CHD is coronary heart disease, CVD is cardiovascular disease

27 myocardial infarctions, 21 revascularizations, 43 strokes, and 50 cardiovascular deaths. The rates of events in the calcium score categories are shown in figure 1.

Figure 2 shows the association between the calcium score categories at the start of follow-up and survival free of new cardiovascular events, adjusted for differences in age and sex. The event-free survival decreased with increase of the calcium score, with a cumulative incidence at four years of 3% for a calcium score up to 100, and of 12% for a calcium score above 1000. Of the 73 coronary events, 64% occurred in the highest calcium score quartile, while 86% occurred in the upper two quartiles. Only one coronary event (1%) occurred in the lowest

TABLE 3
Relative risks of events according to calcium score category in asymptomatic subjects

	Total/events (n)	Relative risk (95% confidence interval)	
		Age- and sex-adjusted	Multivariate-adjusted
CHD*			
Calcium score:			
0-100	905/7	1.0 (reference)	1.0 (reference)
101-500	492/16	3.4 (1.4-8.3)	3.2 (1.3-8.0)
501-1000	202/10	4.8 (1.8-12.8)	5.1 (1.9-13.8)
>1000	196/17	8.2 (3.3-20.4)	8.3 (3.3-21.2)
CVD*			
Calcium score:			
0-100	893/21	1.0 (reference)	1.0 (reference)
101-500	477/26	2.0 (1.1-3.6)	1.8 (1.0-3.3)
501-1000	191/14	2.6 (1.3-5.2)	2.4 (1.2-4.8)
>1000	185/22	4.2 (2.2-7.9)	3.7 (2.0-7.1)
CHD mortality			
Calcium score:			
0-100	905/4	1.0 (reference)	1.0 (reference)
101-500	492/9	2.9 (0.9-9.7)	2.7 (0.8-8.9)
501-1000	202/8	5.4 (1.6-18.5)	5.5 (1.6-19.1)
>1000	196/8	5.4 (1.6-18.7)	5.0 (1.4-17.4)
CVD mortality			
Calcium score:			
0-100	893/6	1.0 (reference)	1.0 (reference)
101-500	477/14	3.4 (1.3-8.9)	3.1 (1.2-8.2)
501-1000	191/9	4.9 (1.7-14.1)	4.7 (1.6-13.5)
>1000	185/7	3.8 (1.2-11.7)	3.3 (1.1-10.2)
Total mortality			
Calcium score:			
0-100	893/27	1.0 (reference)	1.0 (reference)
101-500	477/37	2.0 (1.2-3.3)	2.1 (1.2-3.4)
501-1000	191/22	2.8 (1.6-5.1)	2.8 (1.6-5.0)
>1000	185/23	3.0 (1.7-5.3)	3.0 (1.7-5.4)

* CHD is coronary heart disease, CVD is cardiovascular disease.

quartile. Using quartiles based on age- and sex-adjusted calcium score percentiles resulted in 51% of events in the highest quartile, and 6% in the lowest quartile.

Table 2 shows the relative risks of events for categories of coronary calcification. There was an increasing risk of events with increasing calcium score (test for trend, $p<0.01$ for all outcomes). Compared to a calcium score of 0 to 100, relative risks of events in subjects with a calcium score above 1000 ranged from 2.6 for total mortality and 3.6 for cardiovascular disease to 8.0 for coronary heart disease. Additional adjustment for cardiovascular risk factors resulted in generally similar risk estimates for all outcomes (2.5, 3.7, and 8.1, respectively). When also a history

TABLE 4
Relative risks of coronary heart disease and cardiovascular disease for cardiovascular risk factors

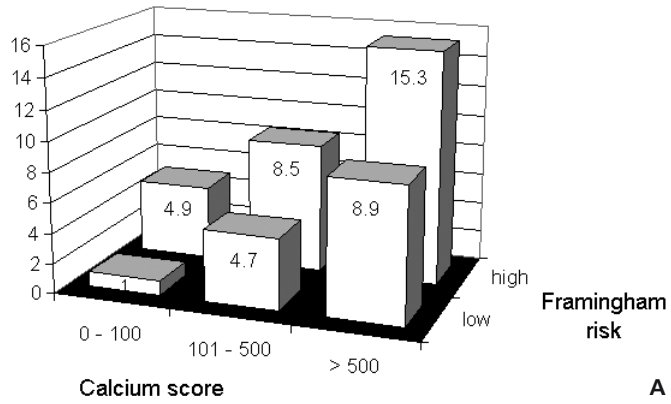
Variable	Relative risk (95% confidence interval)	
	Coronary heart disease	Cardiovascular disease
Overweight	1.0 (0.6-1.5)	1.0 (0.7-1.5)
Hypertension	2.4 (1.2-4.9)	2.4 (1.4-4.3)
Hypercholesterolemia	1.2 (0.7-2.0)	0.9 (0.6-1.4)
Current smoking	1.3 (0.7-2.3)	1.5 (0.9-2.3)
Diabetes mellitus	2.5 (1.5-4.1)	2.3 (1.5-3.5)
History of disease*	2.6 (1.5-4.3)	1.9 (1.2-2.9)
Family history of MI†	0.8 (0.5-1.6)	0.9 (0.5-1.4)

* History of coronary heart disease in case of coronary heart disease as outcome, history of cardiovascular disease in case of cardiovascular disease.
† MI indicates myocardial infarction.

of coronary heart disease was added as variable, the risk estimate for coronary heart disease decreased to 6.8. Eleven percent of the population had a history of coronary heart disease, and 13% had a history of cardiovascular disease. Relative risks among asymptomatic subjects were in general similar to the ones found for the whole population (table 3). However, due to the relatively small number of events in the analyses of coronary heart disease mortality and cardiovascular mortality, the relative risks found for those outcomes had somewhat wider confidence intervals. Additional adjustment for cardiovascular risk factors did not materially change the risk estimates. When the Cox regression analyses were restricted to subjects over 70 years of age, similar relative risks of coronary and cardiovascular events were found. For example, among asymptomatic subjects over 70 years, the relative risks of coronary heart disease for increasing calcium score categories compared to the reference category were 3.4 (95% CI, 1.1-18.8), 5.9 (95% CI, 1.8-19.5), and 8.2 (95% CI, 2.6-26.0), respectively (data not shown).

To compare the strength of the association between coronary calcification and events with the associations of cardiovascular risk factors, age- and sex-adjusted relative risks of coronary heart disease and cardiovascular disease were computed for dichotomized risk factors (Table 4). The strength of the associations between cardiovascular risk factors and events ranged from no association to relative risks of 2 to 2.5 for hypertension, diabetes mellitus and a history of disease.

Figure 3 presents age- and sex-adjusted relative risks of coronary heart disease and cardiovascular disease by categories based on the calcium score and the Framingham risk function. Compared to the reference category (subjects with a calcium score of 0 to 100 and a Framingham risk score below the 75th percentile), there was an increasing risk with increase in calcium score and Framingham



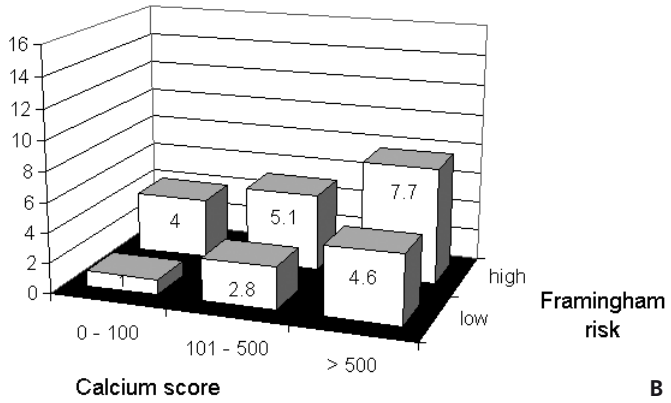


FIGURE 3
Relative risks of coronary heart disease (A) and cardiovascular disease (B) by low and high Framingham risk score and categories of the calcium score, in asymptomatic subjects

risk score ($p<0.05$ for all hazard ratios). Relative risks ranged from 4.7 to 15.3 for coronary heart disease, and from 2.8 to 7.7 for cardiovascular disease.

The area under the ROC curve (AUC), which indicates the power to discriminate subjects who will have an event from those who will not, was 0.748 (standard error, 0.035) for the multivariate model based on age, sex, and cardiovascular risk factors. When the amount of coronary calcification was added to the multivariate model, the discriminatory power significantly improved (AUC, 0.771; standard error, 0.035; difference in AUC, 0.023; p for change = 0.03).

Discussion

The results from this population-based study on coronary calcification among older adults show that coronary calcification is a strong and independent predictor of coronary heart disease. Coronary calcification adds independent predictive information to risk prediction based on cardiovascular risk factors. The amount of coronary calcification not only identifies subjects at high risk of coronary heart disease, but also subjects in which the risk of coronary heart disease is negligible.

Before interpreting the results, some methodological issues need to be discussed. This is the first population-based study on the prognostic value of coronary calcification. Of the invited population, 61% participated in the study. Characteristics of the study population were similar to those of the non-responders, except that the study population included more men and more smokers. If reasons for non-participation were related to the amount of coronary calcification, this could have resulted in a slightly restricted range of calcium scores in this study. Additional strengths of the current study include the higher numbers of events in comparison to the majority of previous studies, standardized assessment of risk factor levels, and subjects' unawareness of the calcium scoring result.

Four prospective studies have shown that the amount of coronary calcification increases the risk of coronary heart disease.[9-13] The relative risks showed a large variation with wide confidence intervals, ranging from 2.3 for a calcium score above the median to 22.3 for a calcium score above 80. Previous studies were conducted in selected, high-risk populations. In three prospective studies,[10-12] subjects were self-referred for EBT scanning or referred by primary care physicians because of the presence of risk factors. The South Bay Heart Watch study, in which two prospective investigations were performed,[9,13] consisted of subjects who were invited to participate in the study if they had at least two risk factors. In all published studies, participants were notified of their calcium score. Awareness of a high calcium score may have motivated a positive change in health behaviour.[14] Severe coronary calcification may have led to diagnostic evaluation and subsequent revascularization, which was included as cardiovascular outcome in two of the prospective studies.[11,12] Therefore, these studies are likely to have yielded biased results on the association between coronary calcification and the risk of cardiovascular disease. Furthermore, whether measurement of coronary calcification improves the identification of high-risk subjects, in addition to cardiovascular risk factors, is uncertain. Three studies with self-reported data on risk factors showed that coronary calcification was a strong predictor of coronary events, independent of cardiovascular risk factors.[10-12] However, the predictive value of coronary calcification may have been overestimated due to misclassification of self-reported risk factors. In the only prospective study

in which levels of cardiovascular risk factors were assessed, three-year risk prediction did not significantly improve when coronary calcification was added.[9] In nondiabetic subjects from the same study, coronary calcification contributed independent information to the six-year risk prediction based on traditional risk factors and C-reactive protein.[13]

The predictive power of cardiovascular risk factors decreases with age, partly due to selective survival and the influence of co-morbidity on risk factor levels.[15-17] Calcification of the coronary arteries can be seen as a cumulative measure of life-time exposure to cardiovascular risk factors, and may therefore improve risk stratification at older age. In our study among older adults, coronary calcification was a strong predictor of coronary heart disease, but also of cardiovascular disease and total mortality. The relative risks for coronary calcification are an order of magnitude higher than those found for cardiovascular risk factors. Only one of the 73 coronary events occurred in the lowest coronary calcium quartile. The calcium score in this quartile ranged from 0 to 13, indicating very low levels of atherosclerosis in this part of the population. Coronary calcium may thus not only identify subjects at high risk of coronary heart disease, but also identify a substantial proportion of the population at very low risk. Therefore, with EBT, the population can possibly be stratified into subjects in which no preventive regimes are necessary, independent of risk factor values, and subjects in which aggressive risk factor modification is appropriate.

In summary, this population-based study among older adults shows that coronary calcification is a strong and independent predictor of coronary heart disease. Relative risks for coronary calcification were an order of magnitude higher than those for cardiovascular risk factors. The amount of coronary calcification may not only identify subjects at high risk of coronary heart disease, but also those in which the risk of coronary heart disease is negligible.

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CHAPTER

six

General Discussion

The aim of the research described in this thesis is to assess the predictive value of coronary calcification, as detected by electron-beam tomography (EBT), for cardiovascular disease in the general population. The merits and shortcomings of the described studies have been discussed in the previous chapters. This chapter will provide a more general discussion of the main findings, and will consider methodological issues. Furthermore, this chapter will comment on the clinical relevance of the findings, and give suggestions for further research on coronary calcification.

Background

Most individuals at risk of coronary heart disease do not experience symptoms until the occurrence of acute myocardial infarction or sudden cardiac death. The ability to predict and prevent, however, the majority of coronary events is limited. Risk factor assessment to identify high-risk subjects is neither highly sensitive nor highly specific.[1] Therefore, new strategies are needed for the identification of individuals at high risk of coronary events. In recent years, noninvasive evaluation of coronary calcification by EBT has been proposed as a method to improve risk stratification. EBT scanning has been shown to accurately detect coronary calcification. The amount of coronary calcification is expressed as a calcium score. The assessment of coronary calcification is based on the close histopathological correlation between calcium deposits in the coronary arteries and the total amount of coronary atherosclerosis.[2-5] To draw conclusions about the possible clinical use of the calcium score in the population, prospective studies in unbiased, general populations are obligatory. There are currently no population-based data on the predictive value of coronary calcification for cardiovascular disease.

The Rotterdam Coronary Calcification Study is a population-based cohort study which started in 1997. The study was embedded in the Rotterdam Study, an ongoing population-based cohort study among 7983 subjects of 55 years and older, who were living in Ommoord, a suburb in Rotterdam, The Netherlands. The baseline examination of the Rotterdam Study took place from 1990 to 1993. At the third examination (1997 to 1999), participants were invited to undergo EBT scanning of the heart for assessment of coronary calcification. All non-institutionalised subjects of 85 years and younger were invited. Of the 3371 eligible subjects, 61% (n = 2063) agreed to participate in the Rotterdam Coronary Calcification Study, and were scanned. The Rotterdam Coronary Calcification Study consists of 2013 subjects with complete scandata. The studies described in this thesis were mainly performed in this group of elderly subjects. For the validation study (chapter 3.1), a cardiac phantom was additionally used, while one other study (chapter 4.3) was carried out in the Rotterdam Study.

Main Findings

Assessment of coronary calcification by EBT

EBT emerged in the eighties as a new imaging method for evaluation of the heart. The potential of EBT scanning to perform quantitative measurements of coronary calcification was realized soon after. Agatston and Janowitz devised an arbitrary calcium scoring algorithm,[6] which subsequently became the standard for the quantification of calcium in the coronary arteries. Reliability of measurements is of utmost importance if EBT is to be used as a tool to quantify coronary calcification in large groups of individuals, and evaluate calcium build-up over time. A measurement can suffer from two types of errors: a systematic deviation from the true value or variability in the measurement. When the estimated value is close to or equal to the true value, the measurement is called 'accurate'. When repeated measurement of the same value yields comparable results, the measurement is called 'reproducible'. Some debate exists on the accuracy and reproducibility of calcium scores obtained by EBT scanning.[7-14] These factors may improve by lowering the slice thickness,[8, 15] by implementing a scoring method based on volumetric measurements,[9,15] and by ECG-triggering at 40% instead of at 80% of the cardiac cycle.[16] To validate the technique of EBT scanning and calcium scoring, we performed two studies which are described in this thesis (chapter 3). First, we compared the standard 3-mm slice protocol with a thinner, 1.5-mm slice protocol. On a 3-mm scan, small, low density calcifications may be missed, because of partial volume effect, leading to a lower sensitivity. We hypothesized that a thinner slice thickness, because of a decrease in partial volume effect, would lead to more accurate estimates of the calcified volume. A cardiac phantom with calcium inserts of specified volumes was scanned with two different slice thicknesses: the standard 3-mm protocol, and a thinner, 1.5-mm protocol. The volume scores (estimates of the calcified volume) were generally closer to the true volumes for the 1.5-mm scan, compared to the 3-mm scan, while the measurement variation was in general lower. Differences in volume scores were also found in 1302 participants which underwent both 1.5-mm and 3-mm scanning. When examining categories based on volume score quartiles for the 1.5-mm protocol, 28% of the subjects were classified into a different category in case of the 3-mm protocol. Especially in repeated assessment of coronary calcification to measure progression, a thinner slice protocol may have importance. However, the use of 1.5-mm slices also has some drawbacks: since more slices are obtained than in 3-mm scanning, a longer breath-hold is necessary. In general, this posed no problems in our study population of older adults. Furthermore, the radiation exposure is higher for the 1.5-mm protocol, about 1.6 times the exposure of a 3-

mm scan. While the exposure of a 3-mm scan is 0.8 mSv for EBT,[17] the exposure of a 1.5-mm scan is approximately 1.5 mSv. However, compared to the natural background radiation, which is approximately 3 mSv, the radiation dose of a 3-mm EBT scan, and the increase in radiation dose when applying the 1.5-mm protocol are relatively low. Before final conclusions on the recommendability of 1.5-mm scanning for assessment of coronary calcification can be drawn, more research needs to be conducted: not only is the reproducibility of the 1.5-mm protocol unknown, but, most importantly, the level of accuracy in calcium scoring needed for accurate cardiovascular risk stratification of subjects in clinical practice has not been determined.

The results on reproducibility and accuracy of coronary calcium scoring with different techniques have led to the recognition that standardization is needed. Not only concerning the scan protocol but also concerning the scoring algorithm. We investigated how different parameter settings in calcium scoring software affect the resulting scores in scans from ten participants. When the settings for connectivity of pixels, lesion size threshold or slice interpolation were changed, the calcium estimates showed significant differences up to 6% for the calcium score, and up to 30% for the volume score. Especially the use of interpolation had a considerable effect on the volume scores. Since available software systems have different standard settings, calcium scores obtained from different scan institutes can show differences just due to the software used. This finding underlines the importance of standardization of the technique to assess and quantify coronary calcification.

In the epidemiological studies described in this thesis, we used calcium scores based on the standard 3-mm protocol, and the standard scoring method according to Agatston.[6] Although we have shown that using the thinner, 1.5-mm protocol instead of the standard 3-mm protocol caused a difference in our categorization in 28% of the study participants, it is not certain whether future therapeutic strategies based on the calcium scoring outcome will be affected by a thinner slice protocol. Implementation of this thinner slice protocol is at this moment premature and requires further research. Furthermore, the calcium scoring method according to Agatston was used because most of the knowledge concerning EBT detected coronary calcification relates to this scoring algorithm. The possible differences in acquisition and evaluation parameters result in large differences in calcium scoring outcomes between scan centers, and between patient studies. Most large participant studies on EBT lack validation of scanning and scoring technique. Since acquisition parameters do not allow alterations after a study has been performed, it is not possible to re-evaluate scans with different acquisition parameters. However, post-processing parameters in calcium scoring do allow alterations and re-evaluation. The results from our study call for standardization of settings in

calcium scoring software, which can then be applied in available studies to re-evaluate scans and improve comparability of calcium scoring results.

Determinants of coronary calcification and atherosclerosis

The development of EBT has enabled non-invasive measurement of the amount of coronary calcification, a measure of largely asymptomatic coronary plaque burden. Numerous studies have shown that classical cardiovascular risk factors are strongly associated with coronary calcification.[18-25] However, these studies did not address the associations between risk factors and coronary calcification in older adults. In the only study performed in elderly (mean age 80 years), only smoking and triglycerides were related to the amount of coronary calcification. We studied the association between cardiovascular risk factors measured at the time of scanning, and at a mean of 7 years prior to scanning. The strongest risk factors for coronary calcification were male sex and age. While strong associations were found between all risk factors measured 7 years before scanning and coronary calcification, the associations with blood pressure and cholesterol attenuated when measured concurrently. It is known that in older adults, the predictive value of cardiovascular risk factors for coronary heart disease decreases.[26-28] Causes for the fall in predictive value of cardiovascular risk factors include selective survival of subjects relatively resistant to cardiovascular risk factors, the fact that current risk factor levels in elderly do not reflect the life-time risk factor exposure, and influence of co-morbidity on risk factor levels.[29-33] Our finding of a stronger association of coronary calcification with risk factor levels at years before EBT scanning than the levels measured concurrently with EBT scanning supports these ideas.

A U- or J-shaped association exists between alcohol consumption and coronary heart disease, with light-to-moderate drinkers being at a lower risk than either abstainers or heavy drinkers.[34-40] One of the proposed mechanisms is through atherogenesis.[40,41] There are no data on the association between levels of alcohol consumption and coronary atherosclerosis in asymptomatic subjects. We cross-sectionally studied the association between alcohol consumption and coronary calcification in 1795 asymptomatic subjects. Since coronary calcification indicates largely asymptomatic coronary atherosclerosis, spurious associations due to clinical symptoms which cause a change in alcohol consumption are not likely to occur. A clear U-shaped association was found between alcohol consumption and coronary calcification. Alcohol consumption up to 2 drinks per day protected against coronary calcification. Subjects who consumed 1 to 2 drinks of alcohol per day had a 50% lower risk of a high calcium score compared to non-drinkers.

In the past, extracoronary measures of atherosclerosis have been investigated in relation to alcohol consumption. One of these measures is peripheral arterial disease (PAD), present below a certain cut-off point of the ankle-brachial blood pressure index. In the Physicians' Health Study and the Framingham Heart Study, inverse associations were found between alcohol consumption and the occurrence of symptomatic PAD or intermittent claudication.[37,42] However, alcohol may have influenced the clinical symptoms of PAD by preferentially dilating diseased arteries.[43] The association between alcohol consumption and PAD was cross-sectionally studied in 3975 asymptomatic subjects from the Rotterdam Study. In our study, only 7.5% of the men and 3.9% of the women with PAD had symptoms of intermittent claudication. An inverse association was found between alcohol consumption and PAD in women, with risk reductions of 22% to 36% for alcohol drinkers, compared to non-drinkers. In men, there was no inverse association between alcohol consumption and the risk of PAD. Similar findings were visible in analyses in which the levels of cardiovascular risk factors were taken into account. The lack of association in men was partly explained by residual confounding due to smoking. Smokers not only are more likely to drink alcohol, and in higher amounts, but smokers are also at a higher risk of atherosclerotic disease. It is possible that the protective effect of alcohol on atherosclerosis is counteracted by smoking. Because of the strong association between alcohol consumption and smoking, it was not possible to assess levels of alcohol consumption in relation to PAD in smokers. Since most men were current or past smokers, the effect of smoking could not be completely adjusted for in the analyses. When the analysis was restricted to nonsmokers, daily alcohol consumption showed an inverse association with PAD in both men and women, with the largest risk reduction, 60%, in women consuming more than 2 drinks per day.

Studies on alcohol consumption encounter some methodological difficulties. First of all, alcohol consumption which is self-reported, as in our study, is likely to result in underreporting, especially in heavy drinkers. If alcohol consumption up to moderate intake has a protective effect on atherosclerosis, and if heavy drinkers reported moderate alcohol use, the resulting effect of moderate alcohol consumption may have appeared less protective. However, if heavy drinkers reported no alcohol consumption, the protective effect of light and moderate alcohol consumption may have appeared stronger. Thus, underreporting may have lead to biased risk estimates. Furthermore, in elderly, the current levels of alcohol consumption may not reflect the mostly higher consumption during earlier decades. But also, in cross-sectional studies, clinical symptoms of the outcome may have affected the level of alcohol consumption. This was less likely a problem in our studies, because we used largely asymptomatic outcome measures. In addition, the selection of the reference category is difficult: the often

used reference category of non-drinkers consists of abstainers and past-drinkers. Abstainers possibly have reasons for being abstainers that introduce other biases, and may have an adverse risk profile.[44] This is most likely for men, in which abstaining from alcohol was rather uncommon in society. Past-drinkers may have an increased risk of cardiovascular disease because ill-health, especially ischemic heart disease, may well be the cause for stopping drinking. These issues may have affected our results, in different directions and sizes. However, the latter issue has not had a large effect on our results: after exclusion of drinkers who had reduced or stopped alcohol consumption, we found similar risk estimates. In conclusion, although our study design and statistical methods were aimed at reducing distortion of the association between alcohol consumption and atherosclerosis, certain bias in the results can not be completely excluded.

Whether a specific alcoholic beverage exerts a more protective effect than others is a matter of vivid debate. Some experimental and clinical evidence suggests that wine contains substances that decelerate atherosclerosis in addition to the effect of alcohol. Daily consumption of up to 1 glass of beer, wine and liquor showed a similar reduction in risk of a high calcium score, while daily consumption of 1 to 2 glasses showed the strongest risk reduction for wine types. Furthermore, in women only consumption of wine types was associated with a decreased risk of PAD was significantly reduced. However, these findings should be interpreted with caution: the numbers of subjects with higher consumption of beer and liquor, and of wine types in men, were very small, and the apparent stronger protective effect of wine consumption may be confounded by life style and drinking pattern. Larger studies with a larger range in levels of consumption of the different alcoholic beverage types are needed to confirm our findings.

Coronary calcification and cardiovascular disease

Although coronary calcification has been shown to be closely related to the amount of coronary plaque burden and the extent of angiographically defined coronary artery disease,[2-5] controversy exists on the question whether coronary calcification is a useful tool for the identification of subjects at high-risk of cardiovascular disease. To draw conclusions on the possible value of coronary calcification in primary prevention, the predictive value in the general population should be known, as well as the power to predict events independent of cardiovascular risk factors. Four prospective studies have shown that the amount of coronary calcification increases the risk of coronary heart disease.[45-49] The relative risks showed a large variation with wide confidence intervals, ranging from 2.3 for a calcium score above the median to 22.3 for a calcium score above 80. Previous studies were conducted

in selected, high-risk populations. In three prospective studies,[46-48] subjects were self-referred for EBT scanning or referred by primary care physicians because of the presence of risk factors. The South Bay Heart Watch study, in which two prospective investigations were performed,[45,49] consisted of subjects who were invited to participate in the study if they had at least two risk factors. Since participation in the previous studies was affected by the exposure level, the results on the predictive value from these studies can not be generalized to the general population. A further complication in the published studies is that participants were notified of their calcium score. Awareness of a high calcium score may have motivated a positive change in health behaviour.[50] Severe coronary calcification may have led to diagnostic evaluation and subsequent revascularization, which was included as cardiovascular outcome in two of the prospective studies.[47,48]

In contrast, our study was performed in a general population of older adults, who were not referred or selected on the basis of risk factors. Furthermore, characteristics from the responders did not differ much from the non-responders. In addition, the participants were not aware of the calcium score. Therefore, possible modifications in lifestyle on the basis of a high calcium score did not occur. In the cross-sectional analyses, a strong and graded association was found between the amount of coronary calcification and the presence of myocardial infarction and stroke. In subjects with a calcium score above 2000, a myocardial infarction was 6.7 (women) to 7.7 (men) times more likely to be present than in those with a calcium score of 0 to 100. Because of a smaller number of stroke cases in the population, a calcium score above 500 was used as the highest calcium score category in this analysis. Those with a calcium score above 500 were 3.3 times more likely to have a history of stroke, compared to the same reference category. The risk estimates hardly changed when levels of risk factors were taken into account. Although the cross-sectional results suggest a strong association between coronary calcification and cardiovascular disease, there are some drawbacks in this study design, which are discussed as part of the Methodological Considerations.

We also used a longitudinal study design to investigate the association between coronary calcification and incident events. The amount of coronary calcification was strongly associated with the risk of coronary heart disease, cardiovascular disease and mortality. The relative risks found for coronary calcification were much higher than those found for cardiovascular risk factors,[51,52] and for extracoronary measures of atherosclerosis.[52-57] Adjustment for cardiovascular risk factors hardly changed the risk estimates. It is well-known that the predictive power of cardiovascular risk factors decreases with age, partly due to selective survival and the influence of co-morbidity on risk factor levels.[29,31,33] Calcification of the coronary arteries can be seen as a cumulative measure of life-time exposure to cardiovascular risk factors, and may therefore improve risk stratification at

older age. Only one of the 73 coronary events occurred in the lowest coronary calcium quartile. The calcium score in this quartile ranged from 0 to 13, indicating a very low level of atherosclerosis in this part of the population. Coronary calcium may thus not only identify subjects at high risk of coronary heart disease, but may also identify a substantial proportion of the population at very low risk. Therefore, the amount of coronary calcification by EBT can possibly be used to stratify the population into subjects in which no preventive regimes are necessary, independent of risk factor values, and into subjects in which aggressive risk factor modification is appropriate.

Methodological considerations

Study design

Most of the studies described in this thesis have a cross-sectional design. In a cross-sectional study, the exposure and outcome variables are measured at the same point in time. This study design is adequate when the association between two variables is studied without making an inference on etiology. However, a cross-sectional study design yields biased results, when the outcome has affected the exposure or when the exposure changes over time. In our studies on the association between alcohol consumption and atherosclerosis, this issue is not of great importance: both coronary calcification and peripheral arterial disease are measures of subclinical atherosclerotic disease. Therefore, subjects were not aware of possible atherosclerotic disease. This was different in the cross-sectional studies on coronary calcification and cardiovascular events. The time lag between the occurrence of cardiovascular events and scanning may have resulted in classification of subjects into a different calcium score category than the classification would have been if coronary calcification had been measured before the event. Secondly, occurrence of a cardiovascular event in the past may have led to changes in lifestyle and medication use to reduce cardiovascular risk. This could have diminished the difference in coronary calcium load between subjects with and without an event, resulting in underestimation of the odds ratios. After adjustment for cardiovascular determinants, risk estimates changed only slightly. This lack of change may in part be due to modification of risk factors in subjects after the cardiovascular event, leading to misclassification of risk factors. Thirdly, only survivors of myocardial infarction and stroke were included. It is uncertain whether the same risk estimates would have been found for fatal events.

Finally, whether the rate of calcification remains the same after a coronary event is not certain. However, a clinical study showed that the progression of

calcification did not accelerate when significant coronary artery disease was present.[58]

To draw conclusions on etiology, a longitudinal study design is preferable. In this type of study design determinants are measured before the occurrence of events, which enables to follow the course from a determinant to disease. In addition, longitudinal studies are considered to suffer les from bias. We investigated the predictive value of coronary calcification in a longitudinal study. The follow-up from EBT scanning up to now is 3.3 years on average, during which 116 subjects had a cardiovascular event, including 73 coronary events. Even in this relatively short follow-up time, we found very strong results on the predictive value of coronary calcification. However, longer follow-up time and more events are necessary to study the predictive value of coronary calcification in detail.

Bias

The possibility of selection bias in our study needs some consideration. Selection bias occurs when participation in a study is related to the exposure, and, independent of the exposure, to the outcome. Selection bias can lead to either under- or overestimation of the association. The studies in this thesis were performed in the Rotterdam Coronary Calcification Study, for which participants were derived from the population-based Rotterdam Study. The Rotterdam Study is a study among 7983 subjects aged 55 years and over, living in a suburb of Rotterdam (response rate 78%). The baseline examination took place from 1990 to 1993, while the third examination was from 1997 to 1999. Not all subjects who were still alive in 1997 returned for the third examination, which was possibly related to health status. Subjects up to 85 years of age who completed the third examination were eligible for participation in the Rotterdam Coronary Calcification Study, and were invited to undergo EBT scanning for detection of coronary calcification. Of the 3371 eligible subjects, 2063 (61%) participated. The eligible subjects who did not undergo EBT scanning (non-responders) may differ from responders concerning risk factor levels, health status, and attitude towards health. We compared the characteristics of the responders to the non-responders: the scanned population was significantly younger (1.8 years on average), consisted of relatively more men (46% vs 38%) and was more likely to have a history of smoking (70% vs 63%). The other characteristics, including a history of myocardial infarction, did not differ. The range of calcium scores in our study population may be somewhat limited because subjects with disabling cardiovascular disease did not participate. Selection bias will probably not have had a considerable effect on our results.

Information bias occurs when the exposure or outcome is misclassified, either non-differential or differential. Non-differential misclassification in the ascertainment of the outcome or the exposure generally results in weakening of the associations. Differential misclassification occurs in longitudinal studies when the assessment of the outcome in the exposed differs from the assessment in the non-exposed. Neither kind of misclassification has played a large role in our study. The amount of coronary calcification, detected on EBT, (exposure) is an accurate measurement. Furthermore, reported events (outcome) were ascertained using medical records, and coded by two independent physicians. Biased ascertainment of the amount of coronary calcification (in the cross-sectional studies) and the events (in both cross-sectional and prospective studies) was prevented by blind assessment.

When an association, found between the exposure and the outcome, can be attributed to a third, extraneous factor, confounding is present. The factor that causes the apparent association is called a confounder. The studies in this thesis on the association between coronary calcification and cardiovascular disease are studies on predictive value, in which confounding is not an issue. In the prospective study we also used a model with adjustment for cardiovascular risk factors, in order to investigate the predictive value of coronary calcification independent from the known risk factors.

Subclinical measures of atherosclerosis

Cardiovascular disease has traditionally been studied by examining the association between potential risk factors and the presence or occurrence of clinically manifest atherosclerotic events. However, a long follow-up is required before the number of outcomes is sufficient to study associations in detail. Measures of subclinical atherosclerotic disease as outcome have several advantages over discrete cardiovascular outcomes, which can enhance the study of cardiovascular risk and prevention. First of all, subclinical measures of atherosclerosis enable study of early stages of atherosclerotic disease, and not only the final stage of this process (a clinical event). Secondly, the continuous nature of most subclinical measures of atherosclerosis substantially increases the power to detect associations. Thirdly, because subclinical disease measures are asymptomatic and previously unknown to the participants, it is unlikely to have an effect on lifestyle, that can alter associations between risk factors and disease. This is particularly advantageous in cross-sectional studies. Finally, subclinical measures of atherosclerotic disease enable the study of the progression of atherosclerosis by repeated measurement. In conclusion, the assessment of coronary calcification

is a very promising method to use in the detection and monitoring of coronary atherosclerosis.

Categorization of calcium scores

In studies on the predictive value of coronary calcification, different categorizations have been used to define relative risks. Generally speaking, two methods can be distinguished: categorizations based on plain calcium scores, and categorizations based on age- and sex-adjusted calcium score percentiles. The plain calcium scores have also been addressed as absolute calcium scores, however, this assumes that the absolute amount of coronary calcium is measured, while we are in fact dealing with an arbitrary scoring method. The choice of categorization reflects the idea on the significance of coronary calcification. If coronary calcification (reflecting coronary atherosclerosis) is regarded merely as a disease, categories based on plain calcium scores will be appropriate: the more coronary calcification, the more disease. If coronary calcification can be regarded as part of the aging process, then categories should be determined depending on age (and sex), in which subjects with an abnormal amount of coronary calcification for their age group can be identified. In fact, no clear choice is made here in the literature. Coronary event prediction in asymptomatic individuals has so far mainly been based on categories of the plain calcium score.[45,47-49] In these prospective studies, a graded increase in risk was found for increasing calcium score categories. One prospective study[46] hypothesized that categories based on age- and sex-adjusted calcium score percentiles could be more appropriate than plain categories, since previous studies showed that coronary events occur at all levels of coronary calcification.[59-61] The calcium score quartiles were based on calcium scores from self-referred subjects and subjects referred to a CT screening center. The results seemed to confirm the hypothesis: risk stratification on the basis of age- and sex-adjusted quartiles was superior to that based on plain calcium score categories. However, a direct comparison of the prognostic value of plain and relative calcium score categories found comparable results.[62] In both our cross-sectional and prospective study on coronary calcification and myocardial infarction, a strong and graded association was found for increasing plain calcium score categories. In the cross-sectional study, numbers of myocardial infarctions permitted analysis by sex. Relative risks in plain calcium score categories were comparable for men and women. When the cross-sectional analyses were performed in sex-specific quartiles, a graded association, similar to that shown for plain categories, was found in men only – in women, there was only an increased risk in the upper quartile. Whether sex- and

age-specific quartiles improve risk stratification compared to plain calcium score categories remains to be resolved in prospective studies.

Generalizability of results

Our study was performed in a general population of older men and women, aged 60 to 85 years. In this age category, coronary calcification strongly predicted coronary heart disease and cardiovascular disease, with relative risks surpassing those found for cardiovascular risk factors and extracoronary measures of atherosclerosis. The strength of the association remained after adjusting for levels of cardiovascular risk factors – showing that the calcium score is an independent predictor of events. As a population ages, selective survival occurs of subjects who are probably less vulnerable to risk factors, since those who were vulnerable have probably died. Calcification of the coronary arteries can be seen as a cumulative measure of life-time exposure to cardiovascular risk factors. The predictive power of cardiovascular risk factors decreases with age, partly due to selective survival and the influence of co-morbidity on risk factor levels.[29,31,33] Therefore, coronary calcification may improve cardiovascular risk stratification in particularly in older adults.

Whether the predictive value of coronary calcification is similar to that in general populations with younger age remains to be investigated. In younger populations, cardiovascular risk factors are strongly associated with coronary heart disease, and coronary risk prediction models based on the risk factors have been shown to have a good predictive value.[51] It remains to be resolved whether coronary calcification predicts coronary events in younger populations, and whether the calcium score adds information to cardiovascular risk prediction based on risk factors in young and middle-aged individuals.

Clinical implications

In this thesis, results are presented from the first population-based study on the predictive value of coronary calcification. In this population of older adults, the amount of coronary calcification was strongly associated with the risk of coronary heart disease, cardiovascular disease and mortality. Only one of the 73 coronary events occurred in the lowest coronary calcium quartile. The calcium score in this quartile ranged from 0 to 13, indicating a very low level of atherosclerosis in this part of the population. Coronary calcium may thus not only identify subjects at high risk of coronary events, but may also identify a substantial proportion of the

population at very low risk. Our study provides strong support for the predictive power of coronary calcification. The results suggest that the assessment of coronary calcification has a potential role in primary prevention of coronary heart disease in older adults, complementing cardiovascular risk factors. The amount of coronary calcification by EBT may possibly be used to stratify the population into subjects in which no preventive regimes are necessary, independent of risk factor values, and into subjects in which aggressive risk factor modification is appropriate.

Measurements of subclinical atherosclerosis in the carotid arteries, including intima-media thickness and the presence of plaques, have also been proposed as a promising method to assist in cardiovascular risk prediction. Studies investigating the relation of carotid intima-media thickness and carotid plaques with coronary events and stroke, have found multivariate-adjusted relative risks ranging from 2 to 3. Two studies on intima-media thickness have been performed in elderly.[52,54] Compared to the lowest two quintiles of intime-media thickness, odds ratios of 2.8 for stroke, and 1.4 for myocardial infarction were found for the upper quintile.[54] In the other study, comparing the upper quintile to the lowest quintile of intima-media thickness resulted in a relative risk of 3.2 for all cardiovascular events.[52] No studies on atherosclerosis and cardiovascular events have reported relative risks of four to eight, persisting in multivariate analysis, as observed with coronary calcification in our prospective study. In conclusion, coronary calcification is very promising for improvement of cardiovascular risk prediction, more than measures of carotid atherosclerosis.

Future research

Our prospective study on coronary calcification had a mean follow-up of 3.3 years, during which 73 coronary and 116 cardiovascular occurred. To study the predictive value of coronary calcification in subgroups, a longer follow-up period with more clinical events is obligatory. In addition, future studies should investigate whether coronary calcification is associated with stroke in a prospective design. Furthermore, to answer the question whether coronary calcification also predicts cardiovascular disease in younger populations, new studies should be performed in general populations at younger ages. Population-based studies with sufficient numbers of events and a longer follow-up should answer whether coronary calcification can be used to identify subgroups in the population at high risk of coronary heart disease independently of cardiovascular risk factors, and in addition, if assessment of coronary calcification can be implemented in primary prevention in a cost-effective manner.

Until recently, EBT was the only technique to accurately detect coronary calcification. However, EBT scanners are not widely available, which limits possible use in primary prevention. Newest generation multi-detector CT (MDCT) scanners may also have the ability to detect and quantify coronary calcification. A recently published study among 2030 subjects scanned by MDCT suggests that calcium scores obtained by MDCT are comparable to those obtained by EBT.[63] The potential value of coronary calcification as a tool in primary prevention becomes even larger when more widely available, MDCT scanners can also be applied for the assessment of coronary calcification. However, the technique of MDCT is constantly evolving, which complicates validation. Furthermore, MDCT-based calcium scoring has not been validated using the amount of coronary calcification in specimens from pathology, but has been designed to resemble EBT-based calcium scoring outcomes. For both methods, standardization in both scanning and scoring protocols are of utmost importance.

A number of studies have shown that coronary calcification can be used to assess progression of subclinical atherosclerosis over time.[64-67] Repeated measurement of coronary calcification in general populations may provide valuable information on the progression of atherosclerosis, and the influence cardiovascular risk factors on progression. However, further improvements in the reproducibility of calcium scoring are needed to make an inference on possible progression of atherosclerotic disease.

Since atherosclerosis is a generalized process, calcification is likely to occur in atherosclerotic plaques throughout the arterial vessels. Calcification of atherosclerotic plaques is a process which already starts at a young age. The first vessel bed in which atherosclerotic plaques appear, is the aorta. The application of EBT for detection of calcium in the aorta is therefore very promising, and deserves further study, especially in younger populations. Aortic calcification by X-ray has been shown to predict cardiovascular disease, and has been shown to be closely related to the amount of coronary calcification.[68-70] Therefore, aortic calcification at young ages may predict coronary calcification later in life. Assessment of aortic calcification by EBT may provide a more sensitive and accurate method to detect aortic calcification. Using repeated measurement, that would provide a method to trace the development of atherosclerosis, as reflected by calcification, from the earliest stages. In addition, since carotid atherosclerosis increases the risk of stroke,[52,54] future studies should investigate whether assessment of calcium in the carotid arteries provides a sensitive tool to identify subjects at high risk of stroke.

Detecting calcified areas in the coronary arteries does not equate to identifying the most probable site of plaque rupture, but rather coronary calcification indicates the presence and the extent of atherosclerosis – stable and vulnerable.[2,5] In theory, a method to detect and measure vulnerable plaque holds even greater promise

for cardiovascular risk prediction. A new development is imaging of plaques with magnetic resonance imaging (MRI). Cardiovascular MRI has the potential to measure components of vulnerable plaque, including fibrous cap thickness, necrotic core size, and lipid pool size[71,72] – components which experimentally have been shown to be related to plaque rupture. Results from a number of studies, including patient studies, have shown that noninvasive assessment of carotid plaques by MRI is very accurate.[73,74] Furthermore, the characteristics of plaques, as detected by MRI, may have clinical impact: in a cross-sectional study, rupture of the fibrous cap of carotid plaques, assessed by MRI, was strongly related to stroke.[75] Although prospective studies are needed to confirm this finding, assessment of carotid plaques by MRI may have some role in the prevention of stroke. Accurate characterization of coronary plaques can not be accomplished with the current external MRI techniques, due to cardiac and respiratory motion. Therefore, a clinical role for coronary MRI in asymptomatic individuals is not to be expected in the near future.

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CHAPTER

seven

SUMMARY/SAMENVATTING

Summary 7.2

The recent development of electron-beam tomography (EBT) enables the noninvasive assessment of coronary calcification. The amount of coronary calcification, as detected by EBT, has a close relation with the amount of coronary atherosclerosis. Therefore, coronary calcification is a promising method for noninvasive detection of asymptomatic subjects at high risk of developing coronary heart disease. There are currently no population-based data on the predictive value of coronary calcification. The aim of this thesis is to study whether coronary calcification predicts coronary heart disease and cardiovascular disease in the general population. For this purpose, an epidemiologic study was carried out in the population-based Rotterdam Coronary Calcification Study, consisting of 2013 older adults.

Chapter 2 reviews the current knowledge on the pathogenesis, detection and epidemiology of coronary calcification. Recent studies have found that calcification of atherosclerotic plaques is an active, organized process. The precise role of calcification in the pathogenesis of atherosclerosis is not clear (chapter 2.1). For individual atherosclerotic plaques, calcification may impart stability and reduce the risk of plaque rupture. Overall, the more extensive the coronary calcification is, the more atherosclerosis is present in the coronary arteries. Therefore, coronary calcification can be used as a reflection of the extent of coronary atherosclerosis. A number of radiological techniques has the ability to detect coronary calcification (chapter 2.2). For accurate noninvasive detection, a very fast CT scan technique is obligatory. EBT enables detection and quantification of coronary calcification due to very rapid acquisition times: instead of a mechanically rotating X-ray tube, an electron-beam is implemented that sweeps along the X-ray source. The newest generation of mechanical CT scanners, multi-detector CT scanners, is also being considered for the assessment of coronary calcification. Most studies on determinants and consequences of coronary calcification so far have used EBT for the measurement of coronary calcification (chapter 2.3). The amount of coronary calcification increases with age, and is higher for men than for women. Coronary calcification can be viewed as a cumulative measure of life-time exposure to cardiovascular risk factors. Obesity, hypertension, hypercholesterolemia, smoking, and diabetes mellitus are associated with more extensive coronary calcification at different ages, although most associations were not present in elderly. Measurements of atherosclerosis at other sites of the vascular tree are related to coronary calcification. Studies in selected, high-risk populations with small numbers of coronary events have shown that the amount of coronary calcification increases the risk of coronary heart disease. Measurement of progression or regression of coronary calcification by EBT may be valuable for the evaluation of interventions or lifestyle modifications aimed at reducing cardiovascular risk.

Chapter 3 contains studies on the validation of the scanning and scoring technique. Chapter 3.1 describes a validation study in which the effect of different EBT slice thickness on calcium scoring was assessed. Scanning with a standard 3-mm slice protocol was compared with a thinner, 1.5 mm slice protocol. For this purpose, a cardiac phantom with calcium inserts was scanned. Furthermore, scans of 3-mm and 1.5-mm slice thickness were available from a subgroup of study participants. In this study, the amount of coronary calcification was expressed in a volume score, which takes into account the slice thickness. In the phantom, the calculated volume scores for 1.5-mm scanning were generally closer to the true volumes than the volume scores for 3-mm scanning, and the measurement variation lower. In the participants, the volume scores for both protocols showed a good agreement, but more than a quarter of all participants was classified in a different calcium score category using the 1.5-mm protocol than in case of the 3-mm protocol. The 1.5-mm slice protocol yielded more accurate calcium scores – however, it is not certain whether this level of accuracy is needed for accurate cardiovascular risk stratification. In chapter 3.2 the effect of parameter settings in calcium scoring software on the resulting calcium scores is described. Differences in scoring parameter settings may contribute to variability in calcium scores between scanners and repeated scans. The effect of changes in pixel connectivity, lesion size threshold, and interpolation between slices, was demonstrated by theoretical examples, and evaluated in a number of EBT scans from study participants. Changes in scoring parameter settings influenced the resulting calcium scores. It is not only necessary that the applied calcium scoring software is acknowledged in reports, but more importantly, scoring parameter settings should be standardized.

In chapter 4, associations between cardiovascular risk factors and coronary calcification and atherosclerosis are addressed. The association of cardiovascular risk factors, measured on average 7 years prior to EBT scanning, and measured at the time of EBT scanning, with coronary calcification, is described in chapter 4.1. Body mass index, blood pressure, cholesterol levels, diabetes and smoking measured 7 years before scanning were positively associated with coronary calcification, while HDL-cholesterol showed an inverse association. The most important risk factors for coronary calcification were age and male sex. Calcium scores gradually increased with increasing number of cardiovascular risk factors. The associations for blood pressure and cholesterol decreased in strength or even disappeared when the risk factors were measured concurrently with scanning.

Moderate alcohol consumption has been found to reduce the risk of coronary heart disease. One of the possible mechanisms is through the effect of alcohol on atherosclerosis. Two cross-sectional studies in this thesis investigate the association of alcohol consumption with coronary calcification and with peripheral

arterial disease. The study of alcohol consumption and coronary calcification was performed in 1795 asymptomatic subjects (chapter 4.2). Compared to non-drinkers, daily alcohol consumption up to 2 drinks was inversely associated with a high amount of coronary calcification (a calcium score above 400). The largest risk reduction, 50%, was found in subjects consuming 1 to 2 drinks per day. The inverse associations remained after adjustment for body mass index, diabetes, smoking, and educational level. The association between alcohol consumption and peripheral arterial disease was assessed in participants from the Rotterdam Study (chapter 4.3). Data on drinking habits and peripheral arterial disease, as determined by the ankle-brachial blood pressure index, were available for 3975 asymptomatic subjects. Male drinkers consumed beer, wine, and liquor, while female drinkers consumed predominantly wine and fortified wine types. Alcohol consumption was inversely associated with peripheral arterial disease in women, but not in men. When the analyses were repeated in non-smoking subjects, moderate alcohol consumption reduced the risk of peripheral arterial disease in men and women.

Chapter 5 focuses on the association between coronary calcification and cardiovascular disease. Chapter 5.1 describes whether coronary calcification is related to the presence of myocardial infarction. At the time of scanning, 229 subjects had a history of myocardial infarction. The amount of coronary calcification showed a strong and graded association with the presence of myocardial infarction. Compared to subjects with a calcium score of 0 to 100, men and women with a calcium score above 2000 were seven to eight times more likely to have a history of myocardial infarction. Associations were found for subjects younger than 70 years as well as in subjects of 70 years and older.

Since atherosclerosis is a generalized process, coronary calcification may also be associated with manifest atherosclerotic disease at other sites of the vascular tree, such as cerebrovascular events. Chapter 5.2 addresses the association between coronary calcification and the presence of stroke. Fifty subjects had experienced a stroke prior to EBT scanning. A markedly graded association was found between coronary calcification and stroke. Compared to subjects with a calcium score of 0 to 100, subjects with a calcium score of 101 to 500 were 2 times more likely to have a history of stroke, and those with a calcium score above 500 3 times more likely. Additional adjustment for cardiovascular risk factors yielded similar risk estimates.

Studies in selected, high-risk populations with small numbers of events have shown that coronary calcification increases the risk of coronary events. The predictive value of coronary calcification in our general population of older adults is described in chapter 5.3. During a mean follow-up of 3.3 years, 116 cardiovascular events occurred, including 73 coronary events. Only one coronary

event occurred in the lowest calcium score quartile, while two-thirds occurred in the highest quartile. The risk of coronary events gradually increased with increasing calcium score. Subjects with a calcium score above 1000 had an eight times increased risk of coronary heart disease compared to those with a calcium score of 0 to 100 (95% confidence interval, 3.6-18.2). The corresponding relative risk in asymptomatic subjects was 8.2 (3.3-20.4). The relative risks only slightly decreased after adjustment for cardiovascular risk factors. The amount of coronary calcification significantly improved risk prediction based on cardiovascular risk factors. Coronary calcification was found to be a strong predictor of coronary heart disease, cardiovascular disease, and mortality. The amount of coronary calcification not only identifies subjects at high risk of coronary heart disease, but also those in which the risk of coronary heart disease is negligible

In chapter 6, the main results of the studies described in this thesis are discussed, the implications of the findings are mentioned and suggestions for future research are provided. Our study provides strong support for the predictive power of coronary calcification. The results suggest that the assessment of coronary calcification has a potential role in primary prevention of coronary heart disease in older adults, in addition to cardiovascular risk factors. Population-based studies with sufficient numbers of events and a longer follow-up should answer whether coronary calcification can be used to identify subgroups in the population at high risk of coronary heart disease independently of cardiovascular risk factors, and in addition, if assessment of coronary calcification can be implemented in primary prevention in a cost-effective manner.

Samenvatting 7.2

Het optreden van hart- en vaatziekten verloopt voor een belangrijk deel via de ontwikkeling van preklinische aandoeningen, zoals atherosclerose. Meting van atherosclerose in een asymptomatische populatie kan selectie van mensen met een hoog risico op hart- en vaatziekten wellicht verbeteren. Een nieuwe radiologische scantechniek, electronenbundel tomografie (EBT), maakt het mogelijk om verkalkingen in de kransslagaders te kwantificeren op een noninvasieve wijze. De mate van verkalking in de kransslagaders hangt nauw samen met de hoeveelheid atherosclerose. Daarom is de meting van kransslagaderverkalking veelbelovend voor noninvasieve opsporing van asymptomatische mensen met een hoog risico op coronaire hartziekte. In dit promotie-onderzoek is nagegaan wat de voorspellende waarde van verkalking in de kransslagaders is voor klinisch manifeste hart- en vaatziekten in de algemene bevolking. Voor dit doel is een epidemiologisch onderzoek uitgevoerd in de Rotterdam Coronary Calcification Study, een populatie studie die bestaat uit 2013 ouderen.

Hoofdstuk 2 geeft een overzicht van de huidige kennis omtrent het ontstaan, de detectie, en het voorkomen van verkalking in de kransslagaders. Recente studies hebben gevonden dat verkalking van atherosclerotische plaques een actief, georganiseerd proces is. De precieze rol van verkalking in de ontwikkeling van atherosclerose is niet geheel opgehelderd (hoofdstuk 2.1). Verkalking van individuele atherosclerotische plaques in de kransslagaders leidt wellicht tot verbetering van de stabiliteit en verlaging van het risico van plaque ruptuur. Voor de kransslagaders tezamen geldt: hoe meer verkalking gedetecteerd wordt, hoe meer atherosclerose aanwezig is. Daarom is verkalking een maat voor de atherosclerotische plaque vorming in de kransslagaders. Een aantal radiologische technieken kan kransslagaderverkalking detecteren (hoofdstuk 2.2). Voor accurate noninvasieve detectie van verkalking is een zeer snelle CT scantechniek nodig, aangezien de kransslagaders zeer snel bewegen, synchroon aan de beweging van het hart. De EBT techniek maakt het mogelijk verkalking in de kransslagaders te detecteren en kwantificeren door een zeer snelle scantijd: in plaats van een mechanisch roterende röntgenbron, wordt gebruik gemaakt van een elektronenbundel die langs de röntgenbron zwiept. Ook de nieuwste generaties mechanische CT scanners, multi-detector CT scanners genaamd, bieden de mogelijkheid om kransslagaderverkalking te meten. Tot nu toe hebben de meeste studies over determinanten en gevolgen van verkalking in de kransslagaders de meting verricht met EBT (hoofdstuk 2.3). De hoeveelheid verkalking in de kransslagaders neemt toe met de leeftijd, en is hoger voor mannen dan voor vrouwen. Kransslagaderverkalking is een marker voor de som van cardiovasculaire risicofactoren tot het moment van meting. Vetzucht, een verhoogde bloeddruk, een verhoogd cholesterol gehalte, roken en suikerziekte laten een associatie zien met de mate van verkalking in de kransslagaders. Echter deze verbanden verminderen

of verdwijnen in ouderen. Metingen van atherosclerosis op verschillende plaatsen van het vaatstelsel zijn gerelateerd aan verkalking in de kransslagaders. Studies in geselecteerde, hoog-risico populaties met kleine aantallen ziektegevallen hebben getoond dat een toenemende hoeveelheid kransslagaderverkalking het risico van coronaire hartziekte verhoogt. Meting van progressie van kransslagaderverkalking door middel van EBT kan waardevol zijn voor de evaluatie van medische interventies of veranderingen in de manier van leven die bedoeld zijn om het cardiovasculair risico omlaag te brengen.

Hoofdstuk 3 bevat studies op het gebied van de validatie van de techniek voor het scannen en scoren van verkalking in de kransslagaders. Hoofdstuk 3.1 beschrijft een validatie studie waarin het effect van verschillende EBT coupedikten op de kalkscores werd bepaald. Scanning met een standaard 3-mm coupedikte werd vergeleken met een dunnere, 1.5-mm coupedikte. Voor dit doel werd een hartfantoom gescand waarin cylinders met verschillende kalkconcentraties waren geplaatst. Daarnaast waren scans met een coupedikte van 3 mm en 1.5 mm beschikbaar voor een subgroep van de studiedeelnemers. In deze studie werd de hoeveelheid kransslagaderverkalking uitgedrukt in een volumescore, die rekening houdt met de coupedikte. In het fantoom waren de berekende volumescores voor de 1.5-mm coupedikte in het algemeen dichter bij de werkelijke kalkvolumes dan voor de 3-mm coupedikte, en de meetvariatie kleiner. In de deelnemers toonden de volumescores voor de twee coupedikten een goede overeenkomst. Echter, meer dan een kwart van de deelnemers werd in een andere categorie ingedeeld wanneer de 1.5-mm coupedikte werd toegepast, vergeleken met de 3-mm coupedikte. De 1.5-mm coupedikte leverde nauwkeuriger kalkscores op, maar het is niet zeker, of deze mate van nauwkeurigheid nodig is voor accurate cardiovasculaire risicostratificatie. Hoofdstuk 3.2 toont het effect van parameter settings in kalkscore software op de resulterende kalkscores. De hypothese is dat verschillen in score parameter settings bijdragen aan variabiliteit in kalkscores tussen scanners en bij herhaald scannen. Het effect van het veranderen van parameter settings werd gedemonstreerd aan de hand van theoretische voorbeelden, en daarnaast geëvalueerd in een aantal EBT scans van studiedeelnemers. Verschillen in score parameter settings beïnvloedden de resulterende kalkscores. Het is niet alleen noodzakelijk dat toegepaste kalkscore software wordt vermeld in rapporten, maar nog belangrijker, dat standaardisatie plaatsvindt van score parameter settings.

In hoofdstuk 4 worden associaties tussen cardiovasculaire risicofactoren en verkalking in de kransslagaders en perifere atherosclerose gerapporteerd. Het verband tussen cardiovasculaire risicofactoren, gemeten ten tijde van EBT scannen en gemiddeld 7 jaar ervoor, en verkalking in de kransslagaders werd onderzocht in hoofdstuk 4.1. Oplopende Quetelet index, bloeddruk, en cholesterol waarde, benevens suikerziekte en roken, allen gemeten 7 jaar voor

scannen, waren geassocieerd met een ernstiger mate van kransslagaderverkalking, terwijl een toename in HDL-cholesterol waarde een inverse relatie toonde met kransslagaderverkalking. De belangrijkste risicofactoren voor verkalking in de kransslagaders waren leeftijd en mannelijk geslacht. Kalkscores namen gradueel toe met het aantal aanwezige cardiovasculaire risicofactoren. In tegenstelling, het verband van bloeddruk en cholesterol waarde, gemeten ten tijde van EBT scannen, met verkalking in de kransslagaders was minder sterk of zelfs afwezig.

Het is gebleken dat matige alcohol consumptie het risico van coronaire hartziekte reduceert. Een van de mogelijke mechanismen is door het effect van alcohol op atherosclerose. In twee cross-sectionele studies in dit proefschrift werd de relatie tussen alcohol consumptie en verkalking in de kransslagaders en perifeer vaatlijden onderzocht. De studie aangaande alcohol consumptie en verkalking in de kransslagaders werd uitgevoerd in 1795 asymptomatische deelnemers (hoofdstuk 4.2). Vergeleken met niet-drinken, reduceerde een dagelijkse alcohol consumptie tot 2 glazen het risico op een hoge kalkscore (een kalkscore boven 400). De grootste risico reductie, 50%, werd gevonden in deelnemers die 1 tot 2 glazen alcohol per dag dronken. Het inverse verband bleef aanwezig na correctie voor Quetelet index, suikerziekte, roken, en opleidingsniveau (als maat voor socioeconomische status). De associatie tussen alcohol consumptie en perifeer vaatlijden werd bepaald in deelnemers van het ERGO onderzoek (hoofdstuk 4.3). Gegevens over drinkgewoonten en perifeer vaatlijden, bepaald door middel van de enkel-arm bloeddruk index, waren beschikbaar voor 3975 asymptomatische deelnemers. Mannelijke drinkers consumeerden bier, wijn en sterke drank, terwijl vrouwelijke drinkers met name wijn en matig sterke drank consumeerden. Toename in het alcoholgebruik was geassocieerd met een afname van het voorkomen van perifeer vaatlijden in vrouwen, maar niet in mannen. Toen de analyses werden uitgevoerd in niet-rokende deelnemers, liet matig alcoholgebruik een inverse verband zien met het voorkomen van perifeer vaatlijden in mannen en vrouwen.

Hoofdstuk 5 richt zich op het verband tussen verkalking in de kransslagaders en klinisch manifeste hart- en vaatziekten. Hoofdstuk 5.1 beschrijft de relatie tussen verkalking in de kransslagaders en de aanwezigheid van een hartinfarct. Ten tijde van het scannen hadden 229 deelnemers een hartinfarct in de voorgeschiedenis. De hoeveelheid kransslagaderverkalking toonde een sterk en gradueel verband met de aanwezigheid van een hartinfarct. Vergeleken met deelnemers met een kalkscore van 0 tot 100, was de waarschijnlijkheid dat mannen en vrouwen met een kalkscore boven 2000 een hartinfarct hadden doorgemaakt 7 tot 8 keer zo groot. Associaties werden gevonden voor deelnemers jonger dan 70 jaar en voor deelnemers van 70 jaar en ouder.

Aangezien atherosclerose een gegeneraliseerd proces is, kan verkalking in de kransslagaders mogelijk ook geassocieerd zijn met manifeste atherosclerotische ziekte op andere plaatsen van het arterieel systeem, zoals een beroerte. In hoofdstuk 5.2 wordt de associatie tussen verkalking in de kransslagaders en de aanwezigheid van een beroerte besproken. Vijftig deelnemers hadden een beroerte gehad voor de EBT scan. In deze cross-sectionele studie werd een gradueel verband gevonden tussen kransslagaderverkalking en de aanwezigheid van een beroerte. In vergelijking tot deelnemers met een kalkscore van 0 tot 100, was de waarschijnlijkheid van de aanwezigheid van een beroerte 2 keer zo groot in deelnemers met een kalkscore van 101 tot 500, en 3 keer zo groot in deelnemers met een kalkscore boven de 500. Vergelijkbare bevindingen werden gevonden na correctie voor cardiovasculaire risicofactoren.

Onze studie is de eerste die de voorspellende waarde van kransslagaderverkalking in de algemene bevolking heeft onderzocht (hoofdstuk 5.3). Tijdens een gemiddelde follow-up periode van 3.3 jaar, traden 116 gevallen van hart- en vaatziekten op, waaronder 73 gevallen van coronaire hartziekte. Slechts één geval van coronaire hartziekte trad op in het laagste kalkscore kwartiele, terwijl tweederde van de ziektegevallen optrad in het hoogste kwartiele. Het risico op coronaire hartziekte nam gradueel toe met de mate van verkalking in de kransslagaders. Deelnemers met een kalkscore boven 1000 hadden een 8 keer verhoogd risico op coronaire hartziekte in vergelijking tot deelnemers met een kalkscore van 0 tot 100. Een vergelijkbaar relatief risico werd gevonden toen deze analyse in asymptomatische deelnemers werd uitgevoerd. De relatief risico's werden slechts iets lager na correctie voor cardiovasculaire risicofactoren. Risicopredictie gebaseerd op cardiovasculaire risicofactoren verbeterde significant door toevoeging van de hoeveelheid kransslagaderverkalking. Onze bevindingen wijzen uit dat verkalking in de kransslagaders een sterke voorspeller is van coronaire hartziekte, cardiovasculaire ziekte, en overlijden. De hoeveelheid kransslagaderverkalking identificeert niet alleen individuen met een hoog risico op coronaire hartziekte, maar ook individuen in wie het risico van coronaire hartziekte te verwaarlozen is.

In hoofdstuk 6 worden de bevindingen in het licht van andere studies besproken. Daarnaast worden de implicaties van de bevindingen uitgewerkt, en suggesties voor toekomstig onderzoek gegeven. Dit onderzoek is de eerste studie naar de voorspellende waarde van kransslagaderverkalking in een algemene bevolking. Vier studies met kleine aantallen ziektegevallen hebben getoond dat verkalking in de kransslagaders een verhoogd risico op coronaire hartziekte aanduidt. Echter deze studies werden uitgevoerd in geselecteerde, hoog-risico populaties van mensen die ofwel zich zelf aanmeldden voor een scan, of doorverwezen werden door de huisarts vanwege de aanwezigheid van cardiovasculaire risicofactoren. Daarnaast werd in deze studies de kalkscore meegedeeld aan de deelnemers,

wat ongetwijfeld van invloed is geweest op de manier van leven. Onze studie toont voor de eerste keer in een algemene bevolking van ouderen dat verkalking in de kransslagaders een zeer sterke voorspeller is voor het optreden van coronaire hartziekte. Relatief risico's gevonden voor kransslagaderverkalking zijn veel hoger dan die voor cardiovasculaire risicofactoren. Toevoeging van de meting van verkalking in de kransslagaders verbetert risicoschatting op basis van cardiovasculaire risicofactoren. De hoeveelheid kransslagaderverkalking identificeert niet alleen individuen met een hoog risico van coronaire hartziekte, maar ook individuen in wie het risico van coronaire hartziekte te verwaarlozen is. Daarom is de meting van verkalking in de kransslagaders een veelbelovende methode voor toepassing in de primaire preventie van hart- en vaatziekten in de algemene bevolking.

Dankwoord

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Het idee voor de Rotterdam Coronary Calcification Study komt van promotor Professor Oudkerk. Beste Professor Oudkerk, ik ben u zeer erkentelijk voor de tijd die u besteed hebt aan mijn begeleiding. Daarnaast heb ik van uw radiologische kennis, uw heldere kijk op het onderzoek en op het bredere perspectief, geleerd. U hebt al veel vooruitstrevend onderzoek geïnitieerd. Hieraan hoop ik ook de komende jaren mijn steentje bij te dragen. Ik voel me vereerd uw eerste promovendus te mogen zijn.

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gegevens – Frank, hartelijk bedankt voor de moeite! Deirdre heeft een belangrijke bijdrage geleverd als coördinator van het ERGO onderzoek en als codeur van HVZ gevallen. Ook de andere codeurs dank ik voor hun tijd.

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Na het vertrek van professor Oudkerk naar Groningen, is het radiologische deel van het onderzoek aldaar voortgezet. Aangezien naast professor Oudkerk ook de Rotterdamse collega's Fons, Peter, Paul, en Jan Cees naar het Groningse verhuisden, waren er heel wat bekende gezichten op de nieuwe afdeling in een onbekende stad. Met z'n vieren op een kleine werkkamer leer je elkaar goed kennen. Daarnaast konden we alle beslommeringen rond de aanschaf van een huis in het Noorden delen… Jan Cees ben ik dankbaar voor zijn gastvrijheid – fantastisch dat er, toen ik nog geen eigen woonruimte had, altijd een slaapplaats voor mij beschikbaar was bij mijn bezoeken aan Groningen! (en nog op steenworp afstand van het AZG ook)

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Tijdens het promotie-onderzoek was het prettig om contact te hebben met andere onderzoekers – dit zorgde o.a. voor interessante discussies en gezelligheid. Ten eerste op de afdeling Epidemiologie&Biostatistiek: door de tijd heen maak je heel wat promovendi mee, wat de afdeling behoorlijk dynamisch maakt. Het was leuk om deel uit te maken van de hart-vaat groep. Ik dank mijn kamergenoten, Marianne, Irene, Joost, Liesbeth, Hok-Hay, Annette en Toos, voor de goede sfeer op de kamer. De laatste jaren van het onderzoek heb ik meer tijd doorgebracht in Groningen dan in Rotterdam. Het was goed toeven op de kamer met regelmatig wisselende agnio´s, Allard, Roy, Henriëtte en Haider - alhoewel soms wel wat erg rumoerig! Joost, Dennis, Tineke en Stella zorgden de afgelopen tijd voor welkome vrolijke onderbrekingen in het werk. Ik wil jullie hartelijk bedanken voor de vele gezellige en goede gesprekken en de belangstelling voor mijn onderzoek. Het is zeer plezierig dat jullie als collega´s dichtbij in het Triadegebouw verblijven. Op de vele m.n. radiologisch gerichte congressen heb ik aangename en interessante dagen gehad, met collega´s en andere onderzoekers. It has been a pleasure to meet many researchers in cardiovascular and radiologic research these past years. In particular, I would like to acknowledge Yadon Arad, Thomas Flohr, David Hill, Udo Hoffmann, David King, Stefan Möhlenkamp, Bernd Ohnesorge, Axel Schmermund and Stefan Ulzheimer. I have enjoyed our talks on various research-related topics and hope the fruitful discussions will continue in coming years. A special thanks goes out to Stefan Ulzheimer and his co-worker who kindly agreed to visit us at the Daniel den Hoed clinic for measurements with their own designed moving phantom - that was great!

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About the Author

Rozemarijn Vliegenthart was born in Rotterdam, The Netherlands, on March 28, 1976. In 1994 she graduated from secondary education (Gymnasium) at the Gereformeerde Scholengemeenschap Rotterdam. After that she spent a year at a liberal arts college, Dordt College, in Sioux Center, USA, which resulted in an Associate of Arts degree.

In 1995, she commenced her medical study at the Erasmus University in Rotterdam. During medical school, she did a clinical elective at the department of Surgery, George Provincial Hospital, South Africa. In 1996, at the end of the second year of the medical study, she was selected for a Master of Science program in Clinical Epidemiology at the Netherlands Institute for Health Sciences in Rotterdam. As part of this program, she attended courses at the Epidemiology Research Institute in Boston, USA. In September 1999, she obtained the Doctoral degree in Medicine as well as the Master of Science degree in Clinical Epidemiology.

In October 1999, she started the work presented in this thesis. The work was performed at the department of Epidemiology&Biostatistics, Erasmus MC, Rotterdam (head: Prof.dr. A.Hofman), and the department of Radiology, Daniel den Hoed Clinic, Erasmus MC, Rotterdam (former head: Prof.dr. M.Oudkerk). From October 2000, part of the work was continued in Groningen, after Professor Oudkerk was appointed head of the department of Radiology at the University Hospital Groningen. In October 2001, she received a Young Investigator Award from the North American Society for Cardiac Imaging and the American Heart Association Committee on Cardiovascular Radiology.

In April 2003, she will start her internships at the University Hospital Groningen. During this time, she will remain involved in cardiovascular research.