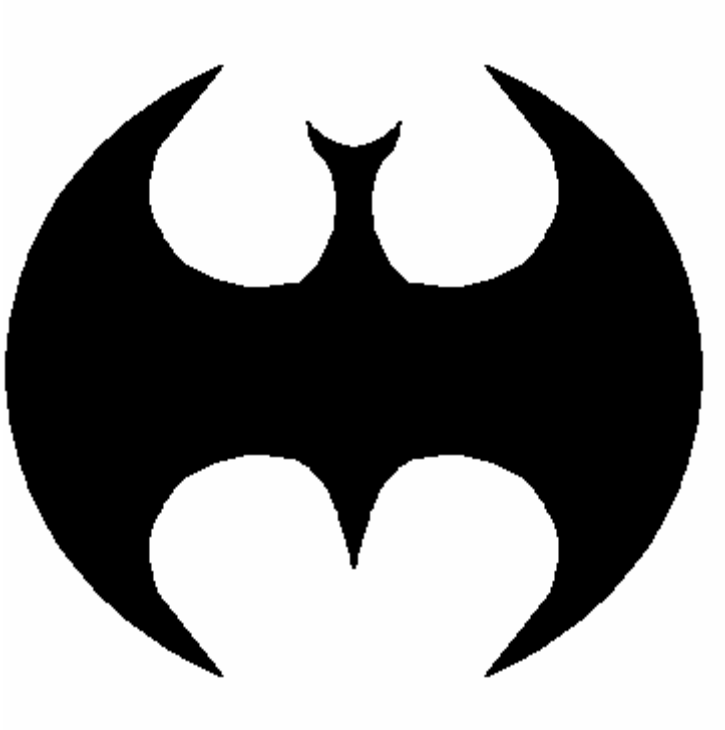
STUDIES ON ARTERIAL ENDOTHELIAL FUNCTION AND INTIMA-MEDIA THICKNESS USING ULTRASOUND TECHNIQUE

Morteza Rohani



Stockholm 2008

DEPARTMENT OF MEDICINE, HUDDINGE
KAROLINSKA INSTITUTET, STOCKHOLM, SWEDEN

STUDIES ON ARTERIAL ENDOTHELIAL FUNCTION AND INTIMA-MEDIA THICKNESS USING ULTRASOUND TECHNIQUE

Morteza Rohani

Stockholm 2008

Front cover by my wife Naghmeh Khamoosh: The origin of ultrasound
All previously published papers were reprinted with permission from the publisher.
Published and printed by US-AB Universitetsservice
Address: Nanna Svartz väg 4, 171 77 Solna

ISBN 978-91-7409-009-3

Imagination is more important than knowledge.
For knowledge is limited to all we now know
and understand, while imagination
embraces the entire world,
and all there ever will
be to know and
understand.

Albert Einstein (1879-1955)

List of original papers

I. Rohani M, Agewall S

Oral snuff impairs endothelial function in healthy snuff users

Journal of Internal Medicine 2004; 255: 379–383

II. Rohani M, Jogestrand T, Ekberg M, Linden J, Källner G, Jussila R, Agewall S

Interrelation between the extent of atherosclerosis in the thoracic aorta, carotid intima-media thickness and the extent of coronary artery disease

Atherosclerosis 2005; 179:311–316

III. Rohani M, Jogestrand T, Källner G, Jussila R, Agewall S

Morphological changes rather than flow-mediated dilatation in the brachial artery are better indicators of the extent and severity of coronary artery disease

Journal of Hypertension 2005; 23:1397–1402

IV. Hafström I, Rohani M, Denberg S, Wörnert M, Jogestrand T, Frostegård J

Effects of low-dose prednisolone on endothelial function, atherosclerosis, and traditional risk factors for atherosclerosis in patients with rheumatoid arthritis — A randomized study

J Rheumatol 2007; 34:1810–6

Abstract

Background: The development of ultrasonographic arterial imaging, high-resolution B-mode ultrasonography, has created a technical basis for a new diagnostic approach to atherosclerotic disease by offering the possibility of accurate and reproducible quantification of the structural and functional changes in the arterial wall. Flow-mediated dilation (FMD) of the brachial artery and carotid intima-media thickness (IMT) have been shown to be good surrogate markers of atherosclerosis. Atherosclerotic lesions detected in the thoracic aorta by transesophageal echocardiography (TEE) are markers of diffuse atherosclerotic disease, often associated with coronary, carotid and peripheral vascular disease.

Aims: The aims of this thesis were to study

- whether intake of oral moist snuff causes acute endothelial dysfunction in the brachial artery of long-term snuff users.
- to assess the strength of any relation between walls changes in the thoracic aorta and carotid arteries and the angiographic severity and extent of coronary artery lesions in patients with angiographically verified CAD.
- to examine the relationship between morphological and functional parameters of the brachial and carotid arteries and the angiographic extent and severity of coronary artery stenosis in patients with severe CAD.
- to determine the influence of low-dose prednisolone on atherosclerosis, endothelial function, and risk factors for atherosclerosis in patients with early rheumatoid arthritis (RA).

Methods: The acute effect of 1 g oral snuff on endothelial function in 20 healthy, long-term snuff users was measured by FMD. The effect on heart rate and blood pressure was also measured.
The strength of any relation between the angiographic severity and extent of coronary artery lesions on the one hand and ultrasonographically measured walls changes in the thoracic aorta (n=37), carotid arteries and brachial artery and endothelial function in the brachial artery on the other hand was investigated by TEE, carotid and brachial B-mode scanning and FMD in 58 patients who had undergone coronary angiography.
In a randomized study 67 patients with early, active rheumatoid arthritis (RA) were randomized to either 7.5 mg prednisolone daily (n=34) or no prednisolone (n=33). The effects of the low-dose treatment with prednisolone on the brachial endothelial function and the carotid wall thickness were studied by FMD and B-mode ultrasonography, respectively, after a mean of 5 years treatment. The relation between risk factors for atherosclerosis and the above mentioned ultrasonographic parameters was investigated.

Results: FMD values declined significantly after the administration of oral moist snuff. Heart rate, systolic and diastolic blood pressure increased significantly after snuff administration. All parameters remained unchanged after placebo.

A significant correlation was seen between the extent of coronary artery stenosis and aortic plaques score. Mean carotid IMT was also significantly correlated with coronary artery stenosis extent score. Moreover, a significant correlation was seen between the aortic plaque score and the mean carotid IMT.

A significant correlation was seen between the extent of coronary artery stenosis defined as the coronary angiographic score and both the mean brachial artery IMT and intima–media area (IMa). There was no significant correlation between FMD and the extent of coronary artery stenosis. A significant correlation was seen between the mean carotid artery IMT and the mean brachial artery IMT. However, there was no significant correlation between FMD and the mean carotid artery IMT or IMa.

Carotid IMT and IMa, prevalence of carotid atherosclerotic plaques and FMD in brachial artery did not differ between RA patients treated with and those not treated with prednisolone. Carotid IMT and IMa, but not brachial FMD, were significantly associated with traditional risk factors for atherosclerosis in patients with RA.

Conclusions:

- Intake of oral moist snuff significantly impaired endothelial function, measured as FMD of the brachial artery, in long-term snuffers. As endothelial dysfunction predicts cardiovascular morbidity, use of oral snuff should be discouraged.
- Morphological parameters of the carotid arteries and the thoracic aorta were related to severity and extent of coronary artery stenosis in patients with severe CAD. B-mode ultrasonography of the carotid arteries and transesophageal echocardiography of the aorta therefore have a potential for diagnostic evaluation of patients with suspected CAD.
- Morphological but not functional parameters of the brachial artery were associated with the extent of coronary artery stenosis and atherosclerotic wall changes in the carotid arteries in patients with severe CAD. These findings indicate a potential of B-mode ultrasonography of morphological parameters in the brachial artery in the diagnostic evaluation of patients with suspected CAD.
- Low-dose prednisolone did not influence endothelial function or subclinical atherosclerosis in patients with RA. The study verifies the relation between risk factors for atherosclerosis and carotid IMT and IMa.

Keywords: Ultrasound, atherosclerosis, flow mediated dilatation, endothelial function, carotid arteries, thoracic aorta, brachial artery, coronary artery disease, nicotine, snuff, prednisolone, rheumatoid arthritis

Abbreviations

BARFOT	Better Anti-Rheumatic FarmacO-Therapy
BH4	Tetrahydrobiopterin
BMI	Body mass index
CAD	Coronary artery disease
CCA	Common carotid artery
CRP	C-reactive protein
CVD	Cardiovascular disease
DAS	Disease Activity Score
DBP	Diastolic blood pressure
DMARD	Disease modifying antirheumatic drug
ESR	Erythrocyte sedimentation rate
FMD	Flow-mediated dilatation
GC	Glucocorticoid
HAQ	Health Assessment Questionnaire
HDL	High density lipoprotein
HR	Heart rate
IMT	Intima-media thickness
IMa	Calculated intima-media area
bIMT	Brachial artery intima-media thickness
cIMT	Common carotid artery intima-media thickness
LDL	Low density lipoprotein
LP	Lipoprotein
NO	Nitric oxide
NOS	Nitric oxide synthase
NSAID	Nonsteroidal anti-inflammatory drugs
RA	Rheumatoid arthritis
SBP	Systolic blood pressure
SLE	Systemic lupus erythematosus
TEE	Transesophageal echocardiography

Contents

Introduction

The high-resolution B-mode ultrasound imaging has been widely used in the last two decades for non-invasively detection of endothelial dysfunction, as a functional parameter of endothelium, and early atherosclerosis wall changes in different arterial walls as morphological changes. These two variables have different pathophysiological background even though it has been shown a good correlation between each variable with conventional risk factors for coronary artery disease (CAD) and ability to predict the occurrence of cardiovascular events. The correlation between these two methods in different studies and in different populations has been a controversial since each method can be influenced by different factors as age independent of the presence or absence of CAD.

Endothelial function and flow mediated dilatation (FMD)

The vascular endothelium plays a critical role in the homeostatic and physiologic functions of the vasculature. Endothelium keeps a dynamic balance in vascular tone by releasing vasoactive mediators as nitric oxide (NO). NO plays an important role in maintenance of basal vascular tone, and exerts a potent dilator effect on vascular smooth muscle. NO also supports a number of additional antiatherogenic functions through inhibition of platelet aggregation, leukocyte adhesion, monocyte chemotaxis, and smooth muscle cell proliferation. However, endothelial dysfunction, especially reduction in the bioavailability of endothelium derived nitric oxide is a key early event in atherogenesis, appearing long before the formation of structural atherosclerotic changes [1, 2]. Endothelial dysfunction is associated with a growing number of risk factors for cardiovascular disease, including smoking, diabetes mellitus, hypertension, hyperlipidemia, hyperhomocystinemia, menopause, and advanced age [1, 3,

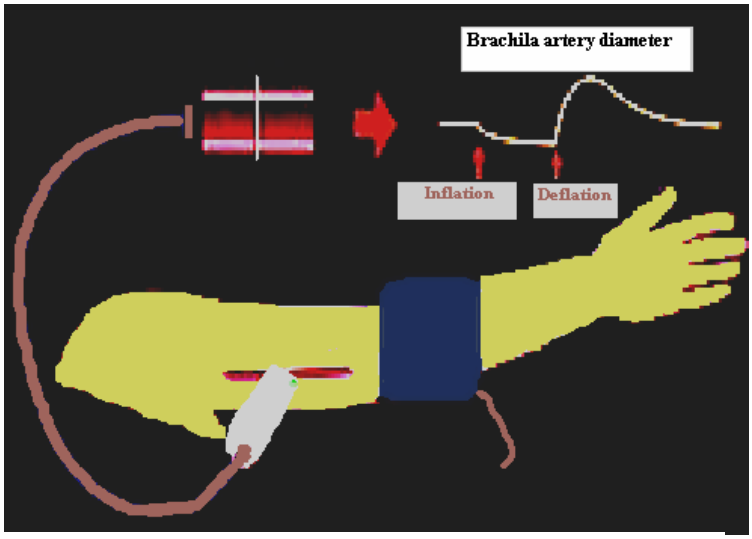


Fig. 1 Measurement of FMD in the brachial artery

4]. A non-invasive ultrasound-based method to assess the endothelial function was developed in 1992 [1] (Fig. 1). This method is based on the flow-mediated dilatation (FMD) in the brachial artery and provides valuable insights into early atherogenesis. An impaired FMD of the brachial artery has been demonstrated in asymptomatic individuals with established cardiovascular risk factors such as smoking, hypercholesterolemia and diabetes [1, 3]. FMD correlates with an invasive testing of coronary endothelial function, as well as to the severity and extent of coronary atherosclerosis [5, 6]. Flow-mediated dilation is also a surrogate variable that may predict cardiovascular events [7, 8].

Arterial wall thickness and B-mode ultrasonography

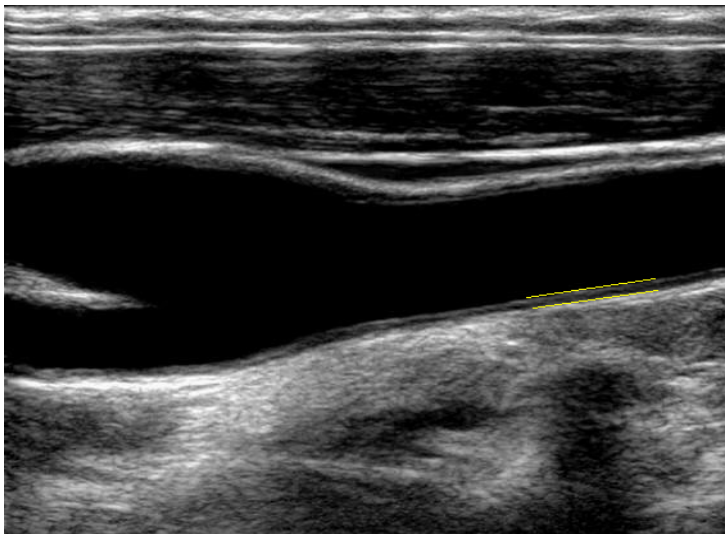


Fig. 2 Measurement of IMT in common carotid artery

Atherosclerosis evolves from the vascular wall. The initial build-up starts in the intima-layer of the arterial wall. To track these early phases of disease requires a technique that can reliably detect and follow the extent of the intima thickening non-invasively over time. B-mode ultrasonography can not be used to measure the intima thickness but allows the measurement of the combined thickness of the intima and media-layers, the so called intima-media-complex (Fig. 2). The ultrasonographic method can be used for evaluation of lumen diameter, intima-media thickness (IMT) and presence and extent of plaques in superficial arteries such as the carotid arteries. The quantitative assessment of the intima-media thickness using ultrasound is safe, validated and inexpensive. Several articles during the past 20–30 years have shown the value of B-mode ultrasonography to detect and monitor atherosclerosis. An increased intima-media thickness has been shown to be associated with unfavourable levels of established cardiovascular risk factors [9], prevalent cardiovascular disease [10] and atherosclerosis elsewhere in the arterial system [11]. Several investigators have observed that the severity of carotid artery disease as measured by ultrasound correlates with CAD as

defined by angiography [12 - 14]. Furthermore, several large prospective studies have evaluated the usefulness of ultrasonographic measurement of IMT and have demonstrated its role in predicting future cardiovascular events. IMT is commonly considered to be an early index of atherosclerosis and in longitudinal studies was identified as a risk factor for myocardial infarction and stroke [15].

Thoracic aorta wall changes and transesophageal echocardiography

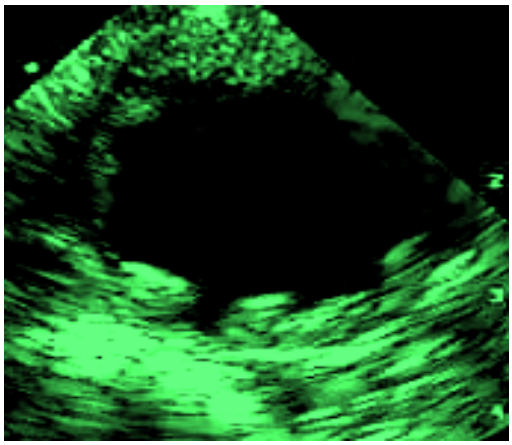


Fig. 3 TEE of thoracic aorta

Transesophageal echocardiography (TEE) is a semi-invasive procedure with a proven utility in identifying aortic atherosclerosis as a source of embolism and in evaluating other aortic pathologies (Fig 3). It can accurately detect aortic atheromatous plaques and assess their size and specific characteristics. Atherosclerotic lesions in the aorta detected by TEE are markers of diffuse atherosclerotic disease, often associated with coronary, carotid and peripheral vascular disease [16, 17]. Moreover, qualitative evaluation of thoracic aortic atherosclerosis by TEE has been related to coronary artery disease (CAD), with a good negative predictive value for the disease [18]. Autopsy studies have also shown that atherosclerotic disease of the thoracic aorta has a direct correlation with the degree of CAD [19]. In addition, a strong association between the presence and severity of aortic plaque imaged by TEE and the presence and extent of CAD has been identified [20 - 23]. Patients with complex plaque, defined as a protuberant plaque of $\geq$ 4 mm in thickness or any plaque with a mobile component detected on routine TEE, are at high risk for cardiac death and coronary event [24].

Oral snuff

The use of oral moist snuff (ground and moistened dark tobacco, buffered to a pH of about 8.5 with sodium carbonate) is a widespread habit in Sweden (Fig. 4). During the last two decades snuff sales have steadily increased and in 2001, 198 million cans were sold. Today, 10.4% of the population between 15 and 75 years of age in Sweden are snuff users. This

increase has occurred in conjunction with a decrease in the consumption of cigarettes. In Sweden, which is the largest market in northern Europe, there are almost a million snuff consumers; about half of them are former smokers. Cardiovascular health hazards and endothelial damage associated with cigarette smoking are well known [25, 26] but conflicting results have been obtained regarding the role of smokeless tobacco as an important cardiovascular risk factor [27–29]. Previous data show that snuff use, in contrast to tobacco smoking, is not associated with an increase of the intima-media thickness (IMT) in the carotid and femoral arteries as assessed by the ultrasound method [30, 31]. However, endothelial dysfunction, especially reduction in the bioavailability of endothelium derived nitric oxide is a key early event in atherogenesis, appearing long before the formation of structural atherosclerotic changes [1, 2]. The regular use of smokeless tobacco results in blood levels of nicotine similar to those observed in cigarette smokers [32] and animal studies have shown an impairment of endothelium-dependent dilation of arterioles after acute infusion of nicotine at a concentration similar to that observed in smokers [33]. The mechanism for altered responses of peripheral arterioles during exposure to nicotine appears to be related to the production of oxygen radicals as treatment with superoxide dismutase significantly restores impaired vasodilatation [34]. However, the precise cellular pathway that accounts for the formation of the oxygen radicals remains unclear. The acute effect of oral snuff on endothelial function has not been studied yet.

Fig. 4 Oral moist snuff (portion bag)

Prednisolone and atherosclerosis

The role of glucocorticoids (GC) in the occurrence of cardiovascular disease (CVD) in patients with rheumatoid arthritis (RA) has been a subject of controversy for decades. To date, there are only a few studies of the effects of GC on CVD and atherosclerosis in RA. Thus, multivariate analyses show that use of prednisone is a risk factor for mortality in RA [35]. Furthermore, in a retrospective study, GC treatment early in the disease increased the risk of CVD, possibly indicating an aggressive disease, whereas in patients with any CVD event during follow up, GC treatment for at least one year seemed to delay the event [36]. Possibly

the deleterious effects of GC on lipids, glucose metabolism, and blood pressure could be balanced by its control of inflammation. In a cross-sectional study, lifetime GC treatment was associated with presence of carotid plaque and arterial incompressibility in patients with RA; however, treatment was given by indication [37] and therefore the results are difficult to interpret. The net CVD effect of low-dose glucocorticoid therapy, as well as high-dose therapy targeted toward severe inflammatory manifestations in RA patients, remains unknown. To determine the issue of GC treatment and its risk for atherosclerosis the need for study of inception cohorts has been addressed, including patients with early RA [38].

Aims

- To determine whether intake of oral moist snuff causes acute endothelial dysfunction in the brachial artery of long-term snuff users.

- To assess the strength of any relation between walls changes in the thoracic aorta and carotid arteries and the angiographic severity and extent of coronary artery lesions in patients with angiographically verified CAD.

- To examine the relationship between morphological and functional parameters of the brachial and carotid arteries and the angiographic extent and severity of coronary artery stenosis in patients with severe CAD.

- To determine the influence of low-dose prednisolone on atherosclerosis, endothelial function, and risk factors for atherosclerosis in patients with early rheumatoid arthritis (RA).

Subjects and Procedures

Study I: Twenty healthy snuff users were enrolled in the study (18 males and two females), mean age 34 years (24 ± 6). No participant had a history of cardiovascular disease or diabetes. None of the participants took any drug. The studies were always conducted early in the morning after 10-h fasting. FMD measurements were performed in duplicate at baseline, and then 20 and 35 min after the administration of 1 g portion-bag-packed moist snuff of the same brand or placebo. Ten of the subjects were studied twice according to a randomized cross-over procedure, once with snuff and once with placebo within a week of the first study. Amongst these 10 subjects, half of them were examined with placebo first and the other five subjects began with the snuff examination. Figure 5 shows the flow chart of the study.

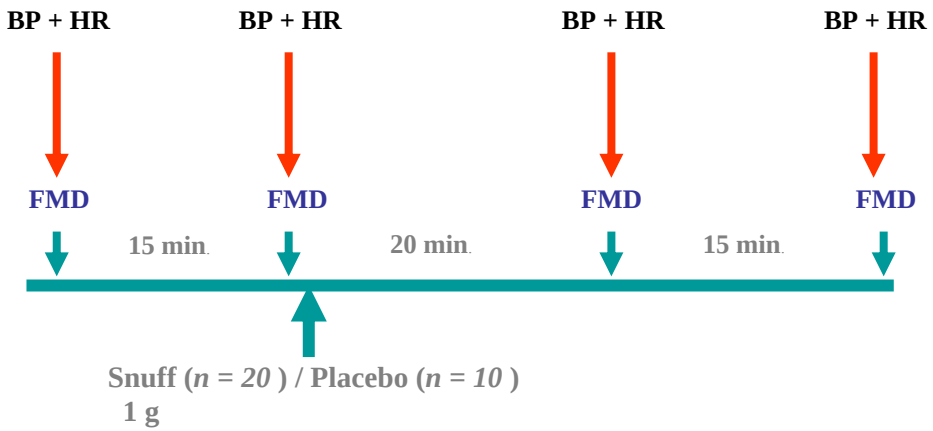


Fig. 5 Flow-mediated dilation at baseline was calculated as a mean value of the two measurements. Ten subjects were studied once with snuff and the other ten subjects were randomized to placebo or snuff and studied twice by a cross-over procedure within a week of the first study.

Study II: Thirty-seven consecutive patients (29 males and 8 females), age 65 ± 10 years, referred to coronary bypass surgery due to verified coronary artery disease were included in the study. All patients had already undergone coronary arteriography. Carotid B-mode ultrasonography was performed in all patients 2 weeks before the operation. Transesophageal echocardiography was performed peroperatively for evaluation of atherosclerotic changes in the ascending aorta, the aortic arch and the descending aorta.

Study III: Fifty-eight patients (46 males and 12 females), age 67 ± 9, referred to coronary bypass surgery because of verified CAD were included in the study. All patients had already undergone coronary arteriography. FMD in the brachial artery as well as carotid B-mode ultrasonography were performed on all patients approximately 2 weeks before the operation. The patients were examined in the morning after an overnight fast.

Study IV: Sixty-seven patients with RA were recruited from Karolinska University Hospital, Huddinge, as part of the 6-center BARFOT (Better Anti-Rheumatic FarmacO-Therapy) study, a Swedish multicenter study designed to investigate clinical and therapeutic aspects of early RA. Of the 67 participating patients, 34 (13 males and 21 females), age 55 ± 25 years, had been randomized to treatment with prednisolone 7.5 mg daily for the first 2 years of the disease, the prednisolone group. At the 2-year control, 13 among these were randomized according to the trial protocol to continue the prednisolone treatment for at least another 2 years, in fact the whole study period, and 21 to stop this treatment. Thirty-three of the 67 patients (11 males and 22 females), age 55 ± 10 years, were randomized at baseline to nontreatment with prednisolone during the whole study period, the no-prednisolone group. Disease modifying antirheumatic drug (DMARD) was chosen by the treating physicians, in accord with the treatment strategy recommended in Sweden at the time of the study. Treatment with nonsteroidal anti-inflammatory drugs (NSAID) was permitted and intraarticular steroid treatment was allowed. Four patients randomized to prednisolone treatment were taking antihypertensive drugs compared to 6 patients randomized to no-prednisolone. None had lipid-lowering therapy. After a mean of 5 years intima-media thickness (IMT) and calculated intima-media area (IMa) of the carotid arteries were determined by B-mode ultrasound. Endothelial function was determined by flow-mediated dilatation (FMD) of the brachial artery. The patients were examined in the morning after an overnight fast.

The Ethics

The Ethics Committee of the Karolinska Institute, Huddinge University Hospital approved all studies, and all subjects gave their informed consent to participate.

Methods

Assessment of flow-mediated dilatation, intima–media thickness and calculated intima–media area in the brachial artery

The ultrasound procedures for assessing endothelium dependent FMD were performed as described in the international guidelines by Corretti et al. [39]. The patients were told not to use long-acting nitroglycerin or calcium antagonist drugs 36 h before the examination.

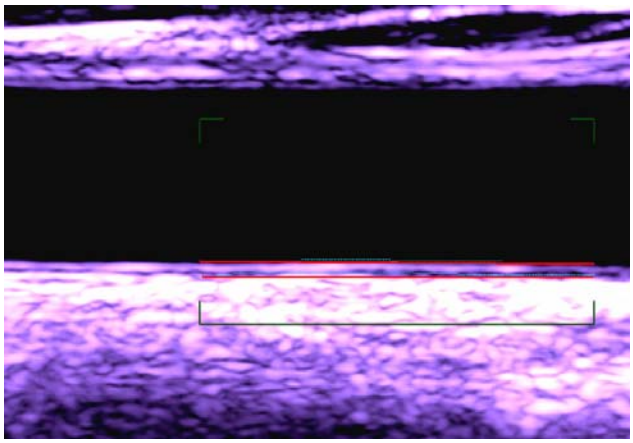


Fig. 6 Measurement of brachial artery IMT

A high-resolution ultrasound scanner (System Five; GE Vingmed, Horten, Norway) with a 10.0 MHz linear array transducer was used. After a 10-min equilibration period at rest in the recumbent position, a single dedicated ultrasonographer performed measurements of the left brachial artery FMD. Scans of the brachial artery were taken proximal to the antecubital fossa and saved on videotape. Baseline diameter recordings were obtained after which arterial occlusion was performed by inflating a forearm blood pressure cuff (12.5 cm wide) to 250 mmHg for 4.5 min. After cuff release, diameter recordings were repeated during the post occlusive increase in brachial artery blood flow (Fig. 1). The complete experimental sequence was performed twice at 30-min intervals. Images were digitally acquired from the videotape and measured in random order by a single observer blinded to the conditions under which the ultrasonic images were obtained. The brachial artery lumen diameter was defined as the distance from the leading edge of the near wall intima–lumen echo to the leading edge of the far wall lumen–intima echo along a line perpendicular to the artery's long axis. A computer system [40] with automated tracing of echo interfaces and measurements of distances between the wall echoes within a 5 mm long section of the brachial artery was used. The brachial artery diameter was calculated in diastolic frames taken coincidentally with the R wave on the electrocardiogram twice at rest and then 45, 60 and 75 s after cuff deflation. A mean of the diameters after 45, 60 and 75 s

was calculated. Diameter changes were expressed as the percentage change relative to the mean baseline value. The mean of two FMD examinations was used. The IMT of the brachial artery was defined as the distance between the leading edge of the lumen–intima echo and the leading edge of the media–adventitia echo in the far wall (Fig. 6). The cross-sectional intima–media area of the brachial artery was calculated using the same formula as for the calculated common carotid intima–media area [41]. The differences between repeated measurements of IMT, lumen diameter and FMD, by using the automated analysing system, were 3.0, 1.4 and 5.5% (coefficient of variation), respectively.

Assessment of carotid atherosclerosis

The right and left carotid arteries were examined with a duplex scanner (Aspen, Acuson, Mountain View, California, USA) using a 7 MHz linear array transducer. The patients were investigated in the supine position with the head slightly turned from the sonographer. The same trained sonographer performed all scans. The carotid arteries were carefully examined with regard to wall changes. The far wall of the common carotid artery (CCA), 0.5–1.0 cm proximal to the beginning of the carotid bulb, was used for measurements of the IMT. The IMT was defined as the distance between the leading edge of the lumen–intima echo and the leading edge of the media–adventitia echo. The lumen diameter was defined as the distance between the leading edge of the intima–lumen echo of the near wall and the leading edge of the lumen–intima echo of the far wall. The examinations were videotaped for subsequent analyses by a computer system [40] with automated tracing of echo interfaces and measurements of distances between the wall echoes within a 10 mm long section of CCA in late diastole, defined by a simultaneous electrocardiographic recording. The mean values of the IMT and lumen diameter within the 10 mm long section were calculated. Carotid plaque was defined as a localized intima–media thickening of greater than

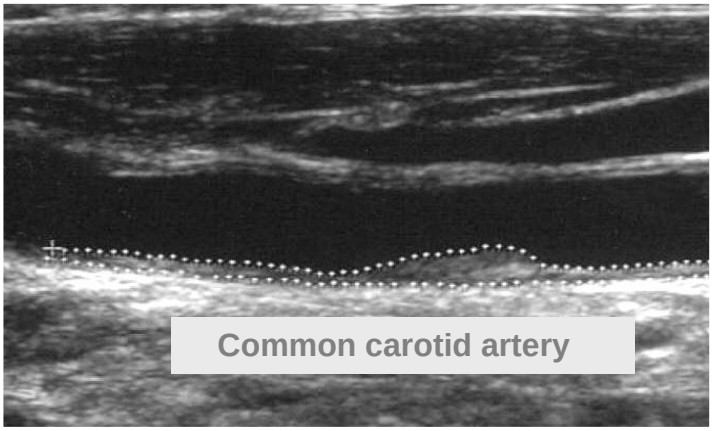


Fig. 7 Carotid plaque = IMT > 1 mm and at least a 100% increase in thickness compared with adjacent wall segment

1 mm and at least a 100% increase in thickness compared with adjacent wall segments (Fig. 7). Plaque was screened for in the CCA, and the internal and external carotid arteries. Plaque occurrence was scored as the absence of plaque, the presence of unilateral plaque, and the presence of bilateral plaque. The differences between repeated measurements of IMT and lumen diameter, by using the automated analysing system, were 3.2 and 0.6% (coefficient of variation), respectively (with an IMT of 0.48–1.04 mm and a lumen diameter of 4.34–7.91 mm). To compensate for the stretching effect of arterial distension (secondary to increased arterial pressure) on the wall thickness, the cross-sectional IMa was calculated by using the formula 3.14 [(lumen diameter/2 + intima–media thickness) ^{2} - (lumen diameter/2) ^{2}] (Fig. 8). This calculated IMa, but not the IMT, has been shown to be unaffected by variations in artery distension secondary to changes in blood pressure [41]. The ultrasonographic methods used have been described in detail previously [14, 42].

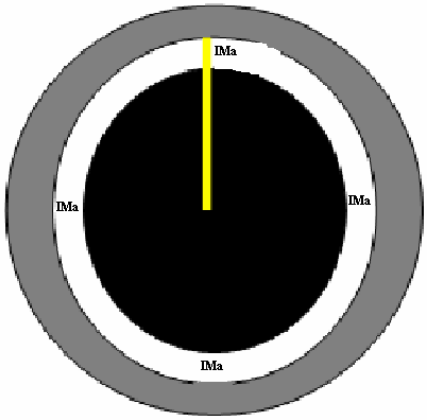


Fig. 8 Calculated IMa = 3.14 [(lumen diameter/2 + intima-media thickness)^2 − (lumen diameter/2)^2]

Assessment of aortic atherosclerosis and scoring

Transesophageal echocardiography was performed preoperatively after induction of anaesthesia by an experienced anaesthesiologist using a multiplane 3.5–7.0 MHz probe (Vingmed System Five, GE) according to a standard protocol. Special attention was paid to the aortic arch and the descending aorta with regard to atherosclerotic lesions.

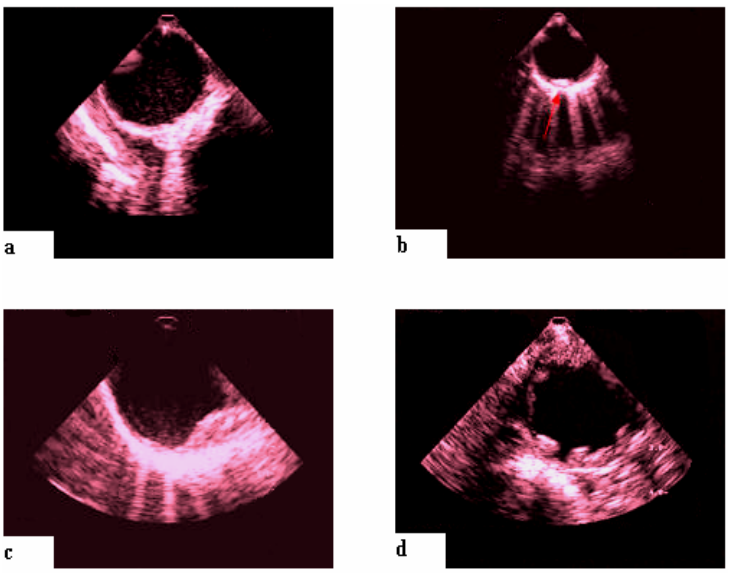


Fig. 9
a) Grade I: < 1 mm, smooth intimal
b) Grade II: 1- 3 mm, plaque
c) Grade III: 3 - 5 mm, plaque
d) Grade IV: > 5 mm, plaque

Atherosclerotic lesions of the thoracic aorta were graded using a modification of a previously described classification [20, 22] (Fig. 9). The thoracic aorta was considered normal (grade I) when the intimal surface was smooth and continuous without lumen irregularities or increased echo density, with an intimal thickness less than 1.0 mm. Grade II was defined as a simple atherosclerotic plaque with increased echo density of the intima extending less than 3.0 mm into the aortic lumen. Grade III was defined as an atherosclerotic plaque extending 3.0 mm or greater and less than 5.0mm into the aortic lumen. In grade IV, the atherosclerotic plaque was 5.0 mm or greater in thickness.

Assessment of coronary atherosclerosis and scoring

All patients were catheterized percutaneously via the femoral vessels using the standard Judkins technique, or via the right brachial artery using the Sones technique. An interventional cardiologist, who was blinded to the FMD and the carotid and brachial artery IMT and TEE, performed angiographic scoring. Coronary angiograms were interpreted visually and were always analysed in two orthogonal views, and were scored by two techniques, as follows. For the severity score, the number of major vessels with luminal stenoses equalling 70% (lumen diameter reduction) were scored from 0 to 3 (for right, left anterior descending, and circumflex arteries). Left main stenosis ≥ 70% was scored as one-vessel disease if there was no lesion equal to 70% in the other vessels. The extent score was defined by using a modification of a previously described method [43, 44] (Fig. 10). The extent score was defined by counting the number of segments showing stenosis of 50 – 75%. This definition does not introduce any weight related either to the haemodynamic importance of the segment or to the degree of stenosis within the 50–75%

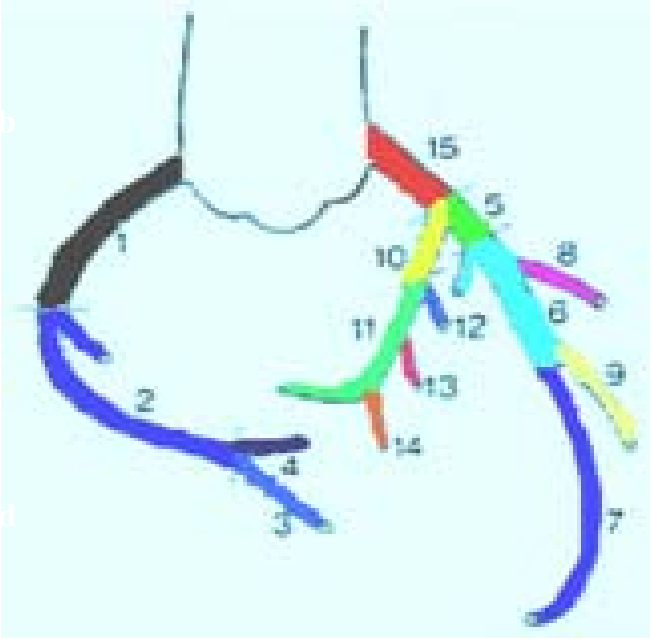


Fig. 10 The coronary extent score was defined by counting the number of segments showing stenosis of 50 – 75%.

range. The extent score definition is not related to a haemodynamic concept, but to a pathophysiological concept: it attempts to summarize the moderate lesions. These lesions have a high potential for progression, whereas the more severe (> 75%) lesions are probably secondary to thrombotic, occlusive modifications than to a primary atherogenic progression.

Assessment of disease and risk factors for atherosclerosis

RA disease activity was measured by the Disease Activity Score composite index (DAS28), calculated on 28 joints [45], which includes number of swollen joints, number of tender joints, patient's assessment of global disease activity, and erythrocyte sedimentation rate (ESR). Functional disability was assessed using the Swedish version of the Stanford Health Assessment Questionnaire (HAQ) [46]. The HAQ score ranges from 0 to 3, a higher score indicating a higher degree of disability. Blood pressure was measured in both arms after 10 minutes of rest by a specially trained nurse. Hypertension was defined as blood pressure > 140/90 or treatment with antihypertensive drugs. The questionnaire and interview, for which the research nurse was responsible, included information about smoking history, previous and present cardiovascular morbidity, and presence of diabetes mellitus. Smokers were defined as patients consuming at least one cigarette daily. Diabetes mellitus was defined as present if the patient had a history of diabetes or if the fasting plasma glucose exceeded 126 mg/dl or if the plasma glucose exceeded 200 mg/dl 2 h after a meal.

Blood samples were taken between 8:00 and 9:30 AM, after 8 to 12 hours of fasting. Levels of serum total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglycerides were measured enzymatically (Modular Analytics P, Roche Diagnostics). Serum low-density lipoprotein (LDL) cholesterol was calculated using the Friedewalds equation (LDH = total cholesterol – HDL cholesterol – 0.45 × triglyceride). Lipoprotein(a) [Lp(a)] levels were determined by a turbidimetric method (ModularAnalytics P, Roche Diagnostics). Hypercholesterolemia was defined as a total serum cholesterol level >220 mg/dl or documented hypercholestrolemia requiring lipid-lowering drug therapy. Plasma homocysteine was determined by fluorescence- polarization immunoassay (Abbot). Fibrinogen, homocysteine, ESR, and high-sensitivity C-reactive protein (CRP) levels were determined by routine techniques at the Department of Clinical Chemistry, Karolinska University Hospital.

Statistical analyses

Study I: Results are presented as mean and standard deviation (SD). Paired t-test was used to compare continuous variables. All tests were two-sided and $p < 0.05$ was regarded as statistically significant.

Study II: All data are presented as mean ± SD. Nonparametric Spearman's rank correlation test was used in the correlation analyses. A p-value <0.05 was considered statistically significant.

Study III: All data are presented as mean $\pm$ SD or SEM. Analysis of variance was used to analyse differences among groups with regard to the coronary score. Non-parametric Spearman's rank correlation test was used in the correlation analyses. Multiple regression analyses, including all variables significantly associated with the variable in focus in univariate analyses as an independent variable were performed. A p value < 0.05 was considered statistically significant.

Study IV: Skewed continuous variables were logarithmically transformed to attain normal distribution. Study groups were compared using ANOVA for continuous variables and chi-square for categorical variables. Fisher's PLSD was used as post-hoc test. Correlation analysis was performed using simple and multiple regression for normally distributed variables and Spearman correlation analysis for non-normally distributed variables. The significance level was put at $p < 0.05$.

Results

Study I:

Mean blood pressure was 109/74 mmHg and heart rate was 55 beats per minute at baseline (Table 1). Basal vessel diameter (before and 20 and 35 min after snuff administration: 3.80 ± 0.34, 3.78 ± 0.35 and 3.81 ± 0.30 mm) remained unchanged (Table 2). Heart rate, systolic and diastolic blood pressure increased significantly after snuff administration (Fig.11), but remained unchanged after placebo. The percentage increase in blood flow during reactive hyperaemia increased slightly but not significantly 20 min after snuff administration (before and 20 and 35 min after snuff administration: 338 ± 138, 365 ± 125 and 319 ± 105%) (Table 2) and remained unchanged after placebo (baseline 431 ± 140, 446 ± 144 and 441 ± 117%). FMD values declined significantly (Table 2 and Fig. 12), but remained unchanged after placebo.

Table 1. Baseline characteristics (*n = 20*)

SBP (mmHg)	109 ± 10
DBP (mmHg)	74 ± 5
HR (bpm)	55 ± 9

SBP, systolic blood pressure;
DBP, Diastolic blood pressure;
HR, heart rate

Table 2. Brachial artery diameter, peak hyperemic blood flow, blood flow increase and FMD before and 20 and 35 minutes after the administration of moist snuff (*n = 20*)

	Before snuff	**20 min after snuff**	**35 min after snuff**
Baseline brachial artery diameter (mm)	3,80 ± 0,34	3,78 ± 0,35	3,81 ± 0,30
Peak hyperemic blood flow (ml/min)	438 ± 140	465 ± 125	419 ± 105
Blood flow increase (%)	338 ± 138	365 ± 125	319 ± 105
FMD (%)	3,4 ± 2,0	3,1 ± 2,4	2,2 ± 1,3*

Means ±SD, $p < 0.05$*

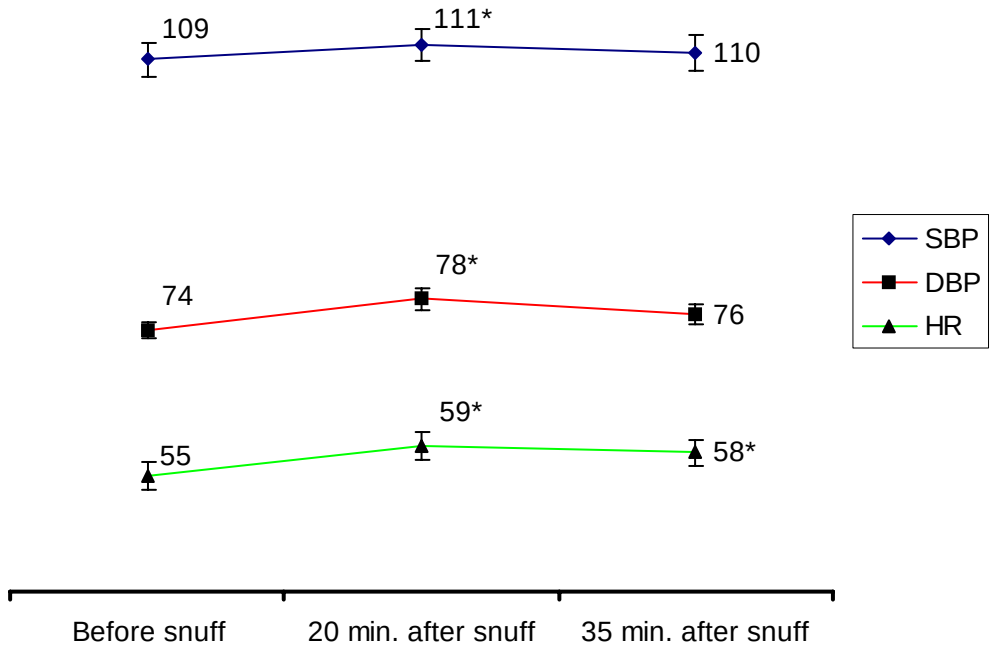


Fig. 11 Effect of oral snuff on heart rate (HR) (beats/min), systolic (SBP) and diastolic (DBP) blood pressure (mmHg). Oral snuff significantly increased HR 20 and 35 minutes after snuff, SBP and DBP 20 minutes after snuff. (*p <0.05)

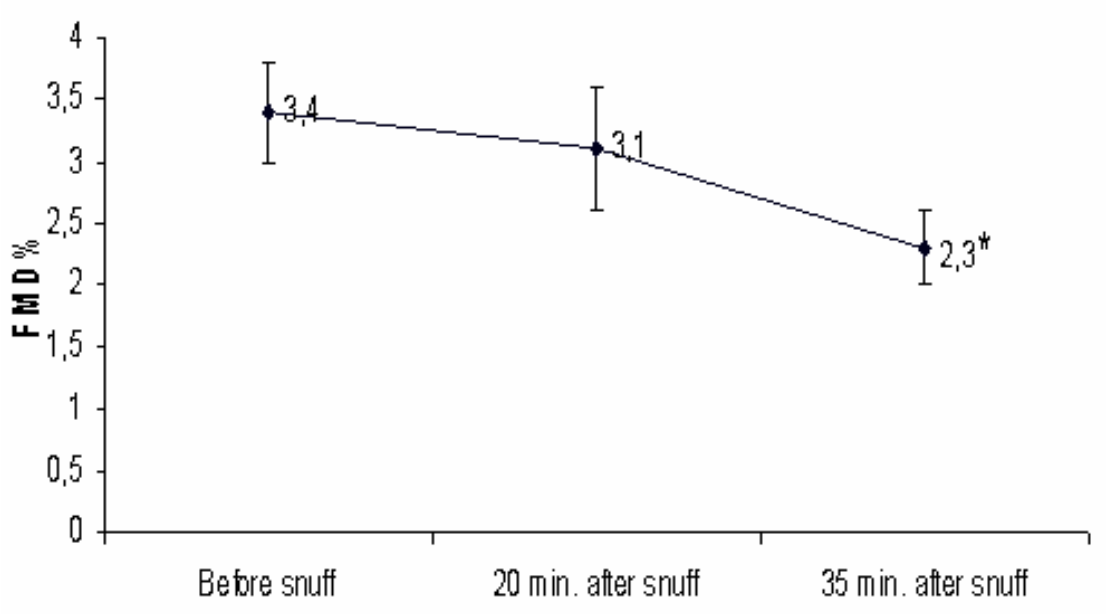


Fig. 12 Effect of oral moist snuff on flow-mediated dilation. FMD decreased significantly 35 min. after administration of snuff in healthy snuff users (*p = 0.0037).

StudyII:

The demographic characteristics of the patients are presented in Table 3. The average of right and left sided carotid IMT and IMa was 0.87±0.21mm and 20.4±6.9 mm^2, respectively (Table 4). All patients had carotid plaques. TEE detected grades II–IV atherosclerotic plaques in the thoracic aorta in 32 of the 37 (86%) patients (Table 5). The plaques were most frequent in descending aorta. Coronary angiography detected 1-vessel disease in 4 patients (11%), 2-vessels disease in 10 patients (27%) and 3-vessels disease in 23 (62%) of the 37 patients.

Table 3. Demographic characteristics of the patients (n = 37)

Gender (men/women), *n*	29/8
Age (years)	65 ± 10
Blood pressure, mm Hg	
Systolic	142 ± 22
Diastolic	83 ± 10
Body mass index, kg/m^2	27 ± 3
Smoking status	
Current *n* (%)	4 (11)
Former *n* (%)	17 (46)
Hypertension, *n* (%)	12 (32)
Hyperlipidemia, *n* (%)	32 (86)
Diabetes mellitus, *n* (%)	2 (5)
Myocardial infarction, *n* (%)	25 (68)
Stroke, *n* (%)	1 (3)
Intermittent claudication, *n* (%)	6 (16)

Values are means ± SD

Table 4. Severity of atherosclerotic wall changes in the carotid arteries of 37 patients

Common carotid artery	IMT (mm)	mean ± SD
	Left side	0.87 ± 0.23
	Right side	0.86 ± 0.20
Common carotid artery	IMa (mm^2)	
	Left side	20.1 ± 7.1
	Right side	20.3 ± 6.9
Plaque		*n* (%)
	Absent	0 (0)
	Unilateral	10 (27)
	Bilateral	27 (73)

Table 5. Severity of atherosclerotic plaque in the thoracic aorta of 37 patients, *n* (%)

Location of aortic plaque	Grade I	Grade II	Grade III	Grade IV
Descending aorta	6 (16)	17 (46)	10 (27)	4 (11)
Aortic arch	25 (68)	5 (13,5)	5 (13,5)	2 (5)

Correlation of aortic plaques with extent of CAD

A significant correlation was seen between extent of coronary artery stenosis, defined as coronary angiographic extent score, and aortic wall changes, defined as plaques score (r = 0.46, p = 0.008) (Fig. 13). The correlation remained significant (r = 0.44, p = 0.02) after excluding patients with diabetes mellitus. There was a weak correlation (r = 0.22, p = 0.04) between coronary artery severity score, defined as 1-, 2-, 3- vessel disease and aortic plaque score.

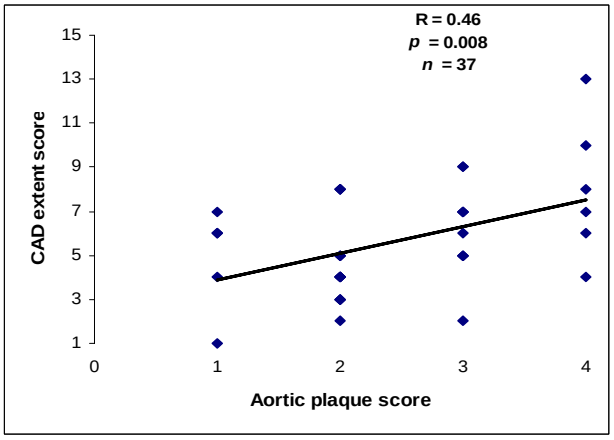


Fig. 13 Scatter plot showing the relationship between CAD, as measured by extent score, and plaque status in the thoracic aorta.

Correlation of carotid wall changes with extent of CAD

A significant correlation was seen between the coronary angiography extent score and the common carotid artery mean IMT (r = 0.44, p = 0.007) (Fig. 14). The correlation remained significant (r = 0.40, p = 0.02) after excluding patients with diabetes mellitus. There were no significant correlations between extent of CAD and IMa, and carotid plaque, respectively. There was however a significant correlation (r = 0.33, p = 0.02) between severity of CAD, defined as 1-, 2- or 3-vessel disease, and mean IMT.

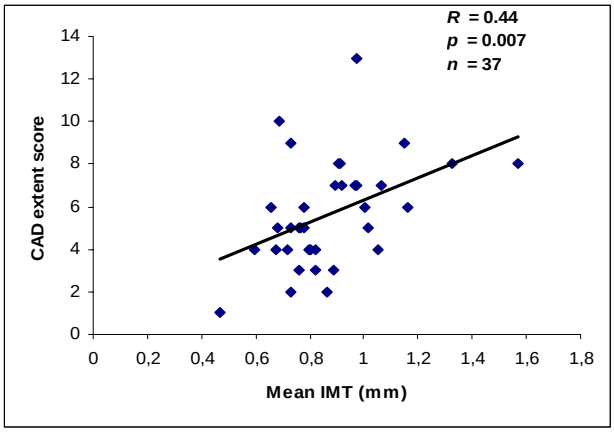


Fig. 14 Scatter plot showing the relationship between CAD, as measured by extent score, and mean IMT for the combined left and right common carotid arteries.

Correlation of aortic plaques with carotid wall changes

A significant correlation was seen between aortic plaque score and the common carotid artery mean IMT (r = 0.39, p = 0.02) (Fig. 15). The correlation remained significant (r = 0.35, p = 0.04) after excluding patients with diabetes mellitus. However, there were no significant correlations between aortic plaque score, IMa or carotid plaque.

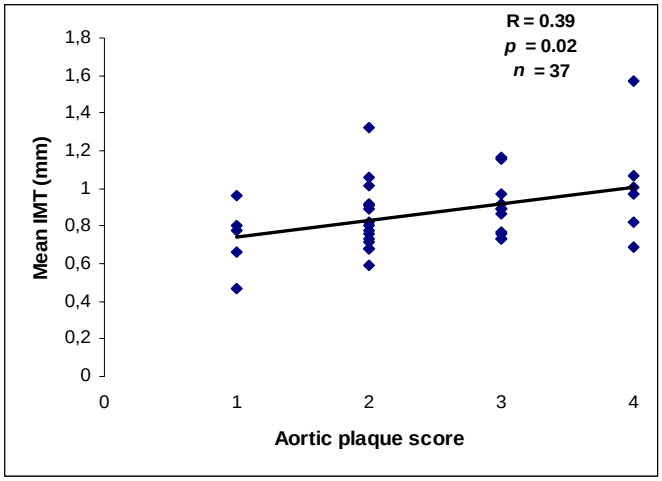


Fig. 15 Scatter plot showing the relationship between aortic plaque score and mean IMT for the combined left and right common carotid arteries.

Study III:

The demographic characteristics of the patients are presented in Table 6. The mean values of right and left-sided carotid IMT and IMa were 0.84 ± 0.20 mm and 19.75 ± 6.72 mm^2, respectively (Table 7). Fifty-five out of 58 patients (95%) had carotid plaques. Mean brachial artery FMD and IMT were 5.4 ± 5.7% and 0.35 ± 0.05 mm, respectively (Table 8). Coronary angiography detected one vessel disease in eight patients (14%), two-vessel disease in 13 patients (22%) and three-vessel disease in 37 of the 58 patients (64%).

Table 6. Demographic characteristics of the patients (n = 58)

Sex (men/women), *n*	46/12
Age (years)	67 ± 9
Blood pressure, (mm Hg)	
Systolic	140 ± 30
Diastolic	80 ± 9.8
Body mass index, kg/m^2	27 ± 3.4
Cholesterol (mmol/l)	5.3 ± 1.2
Smoking status	
Current *n* (%)	6 (10)
Former *n* (%)	26 (45)
Hypertension, *n* (%)	22 (38)
Diabetes mellitus, *n* (%)	3 (5)
Myocardial infarction, *n* (%)	30 (52)
Stroke, *n* (%)	7 (12)
Claudicatio intermittent, *n* (%)	11 (19)
Coronary angiogram	
One-vessel disease, *n* (%)	8 (14)
Two-vessel disease, *n* (%)	13 (22)
Three-vessel disease, *n* (%)	37 (64)

Values are mean ± SD

Table 7. Severity of atherosclerotic wall changes in the carotid arteries of 58 patients

Common carotid artery IMT (mm)		mean ± SD
	Left side	0.85 ± 0.25
	Right side	0.80 ± 0.18
Common carotid artery IMa (mm^2)		
	Left side	19.4 ± 7.6
	Right side	18.1 ± 6.1
Plaque		*n* (%)
	Absent	3 (5)
	Unilateral	12 (21)
	Bilateral	43 (74)

IMT, intima-media thickness; IMa, calculated intima-media area

Table 8. Flow-mediated dilatation, intima-media thickness and calculated intima-media area in the brachial artery of 58 patients

	Mean ± SD
Baseline brachial artery diameter (mm)	3,6 ± 0,7
Peak hyperemic blood flow (ml/min)	440 ± 140
Blood flow increase (%)	340 ± 138
FMD (%)	5,4 ± 5,7
IMT (mm)	0,35 ± 0,05
IMa (mm^2)	4,45 ± 1,33

FMD, Flow-mediated dilatation; ; IMT, intima-media thickness; IMa, calculated intima-media area

Correlation of flow-mediated dilatation, intima–media thickness and calculated intima-media area in the brachial artery with extent of coronary artery disease

A significant correlation was seen between the extent of coronary artery stenosis defined as the coronary angiographic score and both the brachial artery IMT and IMa (r = 0.38, P = 0.006; Fig. 16 and r = 0.31, P = 0.02, respectively). Both brachial artery IMT and brachial artery IMa were significantly associated with body mass index (BMI), systolic blood pressure and age (P < 0.05). The relationship between IMa in the brachial artery and coronary angiographic score remained significant when adjustments for BMI, systolic blood pressure and age were made in a multiple regression analysis (P < 0.05), whereas the relationship between brachial IMT and the coronary angiographic score became non-significant after the same adjustments. There was also a significant correlation between the coronary artery severity score, defined as one-, two-, or three-vessel disease, and both the brachial artery IMT and IMa (*P* = 0,01; and P = 0.04, respectively). There was no significant correlation between FMD and the extent or severity of coronary artery stenosis.

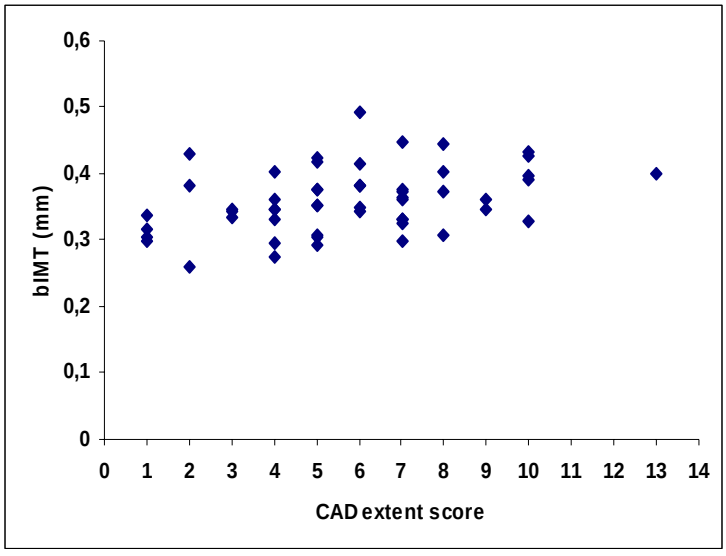


Fig. 16 Relationship between intima–media thickness in the brachial artery (bIMT) and coronary artery disease (CAD) as measured by extent score.

Correlation of flow-mediated dilatation, intima–media thickness and calculated intima-media area in the brachial artery with carotid wall changes

A significant correlation was seen between the mean common carotid artery IMT and the brachial artery IMT (r = 0.30, P = 0.03) (Fig. 17). Furthermore, there was a significant correlation between the mean common carotid artery IMa and the brachial artery IMa (r = 0.30, P = 0.03. The relationship between IMT:s in the brachial and carotid arteries did not remain significant when adjustments for BMI, systolic blood pressure and age were made in a multiple regression analysis. There was no significant correlation between FMD and the mean common carotid artery IMT or IMa (r = 0.16, P = 0.23 and r = 0.17, P = 0.24, respectively).

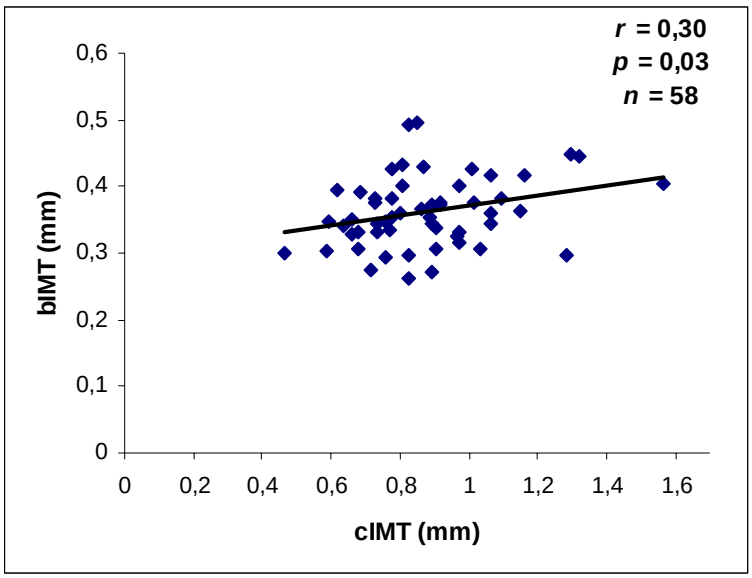


Fig. 17 Linear relationship between intima–media thickness in the brachial artery (bIMT) and mean intima–media thickness for the combined left and right carotid arteries (cIMT).

Study IV:

Baseline characteristics of the study group are given in Table 9.

Table 9. Baseline characteristics of the study group at enrolment separated into those randomized to prednisolone or to no prednisolone from onset of the disease

	Prednisolone (N=34)	No Prednisolone (N=33)	p-values
Age, years	55 (25)	55 (10)	Ns
Sex, % males	38.2	33.3	Ns
Patient with nodules, n	1	4	Ns
HAQ score	1.1 (0.58)	1.2 (0.56)	Ns
DAS28	5.4 (1.0)	5.3 (1.1)	Ns
CRP	38.6 (36.9)	43.4 (45.1)	Ns
ESR, mm	45.1 (25.2)	45.6 (28.9)	Ns
Blood pressure, mmHg	136 (20)/79 (10)	142 (21)/82 (9)	Ns
Smoking status, n			Ns
Non-smoker	14	8	Ns
Current smoker	12	15	Ns
Ex-smoker	8	10	Ns
Diabetes mellitus, n	2	2	Ns
Ischemic cardiovascular disease, n	5	4	Ns

Results are presented as means (SD) or percentage (%).
Ns= not significant; N = number.

There were no significant differences between treatment groups at enrolment into the BARFOT program. Of note, disease activity determined as DAS28 and HAQ score did not differ between groups. These baseline characteristics for 67 patients did not differ from the rest of the patients in the main study (data not shown). There were no significant differences in smoking habits or incidence of previous ischemic cardiovascular diseases between the 2 groups. All 13 patients randomized to continue prednisolone at Year 2 were taking that drug

at completion of the study. Of those randomized to stop prednisolone treatment after 2 years, one started that treatment at Year 3. No patient in the no-prednisolone group started with prednisolone during the study. In both treatment groups, no patient received more than 5 intraarticular steroid injections per year. Following the study protocol, DMARD were given to all patients at baseline; most patients were prescribed methotrexate including folic acid supplementation followed by sulfasalazine, with no difference between the study groups. At 1, 2, and 4 years as well as at the time for the present atherosclerosis assessments, treatment with DMARD was comparable between groups (Table 10).

Table 10. Number of patients treated with DMARD, biologics and NSAID in the two groups at the time of atherosclerosis assessment for this study

	Prednisolone N=34	No prednisolone N=33	p
DMARD			
Methotrexate	14	11	Ns
Sulfasalazine	2	4	Ns
Others	5	6	Ns
Methotrexate-combinations	5	7	Ns
Other combinations	1	1	Ns
No DMARD	7	3	Ns
TNF-inhibitors	0	1	Ns
NSAID	12	20	0.05

Ns: no significance

Those treated with prednisolone were more often not treated with NSAID (12 vs. 20; p = 0.05). A few patients in each group started treatment with antihypertensive drugs during the study, but none started lipid-lowering drugs. As described in Table 11, there were no significant differences between the prednisolone group and the no-prednisolone group in common carotid artery IMT, lumen diameter, IMa or prevalence of plaques in the carotid arteries. Nor was there any difference between the two groups in FMD. There were no differences in the mentioned variables between patients treated with prednisolone for the whole study period and those not treated at all (Table 11).

Table 11. Atherosclerosis measurements of the prednisolone and no prednisolone patients, after a mean of five years after enrolment into the study

	All Patients, N = 34*	Prednisolone Patients Treated 2 yrs, N = 21	Patients Treated Whole Study Period, N = 13	No Prednisolone, All Patients, N = 33*	p, All Pred vs No-pred	p, Pred Whole Period vs no-pred
IMT left, mm	0.690 (0.54–0.91)	0.640 (0.54–0.95)	0.710 (0.59–0.91)	0.696 (0.61–0.87)	NS	NS
IMT right, mm	0.636 (0.57–0.74)	0.650 (0.57–0.80)	0.600 (0.57–0.72)	0.653 (0.58–0.80)	NS	NS
IMT mean, mm	0.675 (0.58–0.82)	0.660 (0.56–0.86)	0.681 (0.58–0.80)	0.673 (0.62–0.80)	NS	NS
Carotid artery lumen, left diameter, mm	6.16 (5.60–6.64)	6.17 (5.70–6.80)	6.12 (5.60–6.60)	6.00 (5.84–6.59)	NS	NS
Carotid artery lumen, right diameter, mm	6.01 (5.57–6.68)	6.06 (5.49–6.57)	6.00 (5.57–6.86)	5.89 (5.49–6.48)	NS	NS
Carotid artery lumen, mean diameter, mm	6.14 (5.57–6.63)	6.20 (5.58–6.53)	6.06 (5.68–6.76)	5.89 (5.55–6.55)	NS	NS
cIMa left, mm^2	14.0 (11.61–23.16)	13.2 (11.14–23.50)	15.3 (11.61–21.86)	14.4 (12.25–17.25)	NS	NS
cIMa right, mm^2	13.6 (11.05–18.43)	14.3 (10.94–20.37)	12.2 (11.40–17.31)	13.3 (11.86–17.24)	NS	NS
cIMa mean, mm^2	13.7 (11.45–20.37)	13.7 (11.48–21.56)	14.9 (11.45–18.43)	14.1 (12.34–17.38)	NS	NS
Plaque prevalence, %	82.3	85.1	71.4	81.9	NS	NS
Brachial artery diameter, mm	3.68 ± 1.2	3.50 ± 1.2	3.96 ± 1.5	3.53 ± 0.93	NS	NS
FMD %	3.88 ± 2.8	4.17 ± 3.2	3.44 ± 2.08	3.74 ± 2.9	NS	NS

Data are mean ± SD for normally distributed variables, median (interquartile range) for nonparametric variables. * Brachial artery diameter and FMD% were only measured in 33 patients in the prednisolone group and 26 patients in the no-prednisolone group. IMT: intima-media thickness, IMa: calculated intima-media area, FMD: flow-mediated dilatation of brachial artery.

Similarly, there was no difference in levels of biomarkers including lipoproteins, CRP, ESR, creatinine, and glucose or in blood pressure between the 2 treatment groups (Table 12). However, patients treated for at least 4 years (and currently treated) with prednisolone had a trend to higher systolic blood pressure (157 ± 29 mm Hg) as compared with those never treated with prednisolone (141 ± 28 mm Hg; p = 0.060). Further, in this subgroup of patients treated with prednisolone the whole time, the systolic but not the diastolic blood pressure increased from disease onset to the time of our investigation, from 136/79 to 155/85 mm Hg (p = 0.011). Also, the patients treated constantly with prednisolone had significantly higher total cholesterol levels (5.6 mmol/L ± 1.39) compared to no treated patients (4.9 mmol/L ± 1.28; p = 0.03). Other factors did not differ between these subgroups. In a simple regression

model, mean IMT was significantly associated with systolic blood pressure ($R = 0.59$, $p < 0.001$), HDL ($R = –0.34$, $p = 0.008$), triglycerides ($R = 0.37$, $p = 0.005$), creatinine ($R = 0.39$, $p < 0.02$), age ($R = 0.65$, $p < 0.001$), and cigarette pack-years ($R = 0.40$, $p < 0.004$). In contrast, LDL, glucose, homocysteine, ESR, CRP, DAS28, fibrinogen, Lp(a), and body mass index (BMI) were found not to be significantly associated with IMT. However, in a stepwise multiple regression model, only age and HDL remained significant in the model if the whole group was analyzed ($F = 46.2$ and 19.9, respectively; $p < 0.05$). In a simple regression model, mean IMa was significantly associated with systolic blood pressure ($R = 0.60$, $p < 0.001$), HDL ($R = –0.36$, $p = 0.004$), triglycerides ($R = 0.39$, $p = 0.0012$), age ($R = 0.59$, $p < 0.001$), and BMI ($R = 0.26$, $p < 0.0034$). In contrast, LDL, glucose, homocysteine, ESR, CRP, DAS28, fibrinogen, Lp(a), and cigarette pack-years were not significantly associated with IMa. In a stepwise multiple regression model, age, systolic blood pressure, and HDL remained significant in the model ($F = 8.5$, 10.6, and 13.2, respectively; $p < 0.05$). In the whole study group, only age and creatinine were associated with presence of atherosclerotic plaques in a univariate analysis, but only age remained significant in a logistic regression model (chi-square 5.1, $p = 0.02$). FMD% was not associated with any of the variables tested. During the whole observation period only one patient, in the prednisolone group, had a CVD event, namely, a coronary revascularization procedure.

Table 12. Atherosclerosis risk factors, and disease activity in the two treatment groups at time of atherosclerosis measurements

	Prednisolone (N=34)	No prednisolone (N=33)	p- values
BMI	25.7 ±4.2	25.6 ±4.8	Ns
Total Cholesterol	5.2±1.2	4.9±0.9	Ns
HDL	1.6±0.57	1.6±0.42	Ns
LDL	3.1±1.0	2.8±0.7	Ns
Triglycerides	1.1 (0.67)	1.0 (0.50)	Ns
Lp(a)	281 (598)	184.5 (323)	Ns
Homocystein	12.9±4.9	12.7±3.7	Ns
Fibrinogen	3.4±0.75	3.7±1.0	Ns
Creatinine	71 (21.7)	69 (27.2)	Ns
Glucose	4.9 (0.7)	5.05 (1.0)	Ns
Blood pressure (systolic)	143.8±25.3	140.8±27.8	Ns
Blood pressure (diastolic)	80.0±6.9	80.0±14.0	Ns
HAQ score	0.45±0.49	0.81±0.62	0.012
ESR	15.0 (18)	16.0 (23.2)	Ns
CRP	4.0 (10.3)	4.7 (18.6)	Ns
DAS28	3.0±1.3	3.3±1.5	Ns

Data is presented as mean ± SD for normally distributed variables and as median (Inter-quartile range) for non-parametric variables. BMI= body mass index; HDL= high-density lipoprotein; LDL= low-density lipoprotein; Lp(a)= Lipoprotein(a).

Discussion

Relationship between angiographic extent and severity of coronary artery stenosis and morphological and functional parameters of the brachial artery, the carotid arteries and the thoracic aorta

The measurement of brachial artery FMD as a non-invasive test of endothelial function was described by Celermajer et al. [1], who demonstrated that FMD of the systemic arteries is impaired in children and adults with familial hypercholesterolemia, in adult smokers and in patients with CAD. They suggested that impaired FMD might indicate an early functional abnormality of the arterial system in the preclinical phase of vascular disease [3, 4]. Our study is consistent with this finding in that FMD in CAD patients was impaired (5.4 $\pm$ 5.7%). However, conflicting results have been obtained regarding the relationship between FMD and the extent and severity of CAD [5, 47, 48]. Probable explanations for this difference might be that FMD can be affected by several different confounding factors such as age, sex and brachial lumen diameter as well as the presence of different risk factors for CAD such as diabetes mellitus, hyperlipidemia, hypertension and smoking. It seems that the blunted response of the brachial artery to reactive hyperemia, with increasing age or in patients with severe CAD is so strong that it can be difficult to discriminate between patients in different stages of CAD. These facts could explain the lack of relationship between FMD and extent and severity of coronary artery stenosis in our study.

The FMD technique thus appears to be better suited for studies of younger individuals or possibly the prediction of the presence or absence of CAD in a low-prevalence population rather than the extent of coronary lesions in a high-prevalence population. Contrary to the brachial artery FMD, the B-mode ultrasonographic measurements of the brachial artery IMT and IMa reflect the extent of coronary artery stenosis in this population with advanced atherosclerosis. However, we observed an independent relationship between IMa in the brachial artery and FMD, indicating a parallel process in the development of morphological and functional changes in the vessel.

The result of our study indicates that the thickening process of the intima–media in the brachial artery occurs parallel to the atherosclerotic process of the carotid artery. Furthermore, we observed that the morphological parameters of the brachial artery (IMT, IMa), but not FMD were correlated with the atherosclerotic wall changes in the carotid arteries. This is in

line with the results from other investigators, who have shown no significant correlation between the common carotid IMT and the brachial artery FMD in CAD patients or in hypertensive patients [48, 49, 50]. However, Hashimoto et al. [49] showed an inverse correlation between carotid IMT and FMD when both CAD and non-CAD patients were included in the calculation, but not in the CAD patients alone. This finding suggests that carotid IMT is well correlated with endothelial function in the early stage of the atherosclerosis process but not in the later phase. However, our study shows that the brachial artery IMT correlates with the carotid IMT in patients with advanced stages of atherosclerosis.

Since the common carotid artery is an elastic artery and the brachial artery is a muscular artery one may speculate that the increases in IMT in the two arteries represent different pathological processes. However, in our study we could show that IMa:s in the brachial artery and the carotid artery were significantly correlated to the coronary angiographic score and to each other after the adjustments for BMI, systolic blood pressure and age indicating a parallel process in the development of morphological changes in different arterial sites.

Carotid IMT has been shown to be a good surrogate marker of clinical and generalized atherosclerosis [9, 12, 51]. Conflicting results have been obtained regarding the role of common carotid IMT as a marker of the extent of CAD [12-14, 52-54]. The discrepancy between the results may be explained by the different and more intricate methods used for scoring the extent of carotid atherosclerosis. Hulthe et al. showed a strong association between extent of coronary atherosclerosis and carotid plaque score by using a semi quantitative subjective scale to grade the size of plaques [13]. However, this scoring system is less suitable for the carotid arteries than for the thoracic aorta because of the anatomical position and the presence of acoustic shadows that may occur at sites with lesions. Moreover, we could not show a correlation between IMa and angiographic extent of CAD, contradicting a previous study from our research group where IMa of the left side provided a statistically significant discrimination of patients with CAD [14]. We believe that this difference can be explained by a selection bias of the comparatively small study population. We observed a high prevalence of carotid plaques and grades II–IV atherosclerotic plaques in the thoracic aorta (100 and 86%, respectively) in CAD patients. This finding is in line with the results of a previous study by Kallikazaros et al., which showed a close relationship between carotid and ascending aortic atherosclerosis in cardiac patients [55]. They reported that only age was significantly related to carotid and aortic atherosclerosis and suggested that the other common

cardiovascular risk factors such as diabetes mellitus loose some of their importance at increasing age. We did not find a significant correlation between carotid plaque score and the extent of coronary atherosclerosis since all subjects had carotid plaques probably due to the selection of patients with severe CAD. Moreover, atherosclerosis in the thoracic aorta and the common carotid artery IMT were clearly and significantly associated with the extent of coronary atherosclerosis in a group of patients with severe CAD. Although plaque thickness in the thoracic aorta and IMT in the common carotid artery were significantly higher in subjects with more severe CAD, there was a considerable overlap.

We found that the scoring of CAD into 0-, 1-, 2- and 3- vessel disease, was less correlated with atherosclerosis in the carotid artery, brachial artery and the thoracic aorta than the 15-segment coding system of the American Heart Association [44]. The latter extent score was defined by counting the number of segments with stenosis of 50–75%, thus adding up the number of moderate lesions. These lesions are of importance since they have a high potential for primary progression, whereas more severe (>75%) lesions are more likely to have secondary, thrombotic and occlusive modifications.

In summary, we have shown a significant correlation between the morphological changes in brachial and carotid arterial walls and the extent of coronary atherosclerosis. A corresponding correlation was not found for the functional parameter of the brachial artery as defined by FMD.

These findings indicate a potential of B-mode ultrasonography of the carotid and brachial arteries and of transesophageal echocardiography of the aorta as valuable complements to the conventional diagnostic methods in patients with suspected CAD. The present studies were performed in a symptomatic, high prevalence population undergoing coronary bypass surgery. The selection of patients may thus limit the applicability of the results in other populations.

Accordingly, we could not separate endothelium-independent dilatation from endothelium-dependent dilatation since we did not use nitroglycerine in our study. However, we measured a variable (FMD) that has been demonstrated to predict cardiovascular events [7, 8] and is correlated to risk factors for CAD [4].

Relationship between atherosclerosis risk factors and morphological and functional parameters of the carotid and brachial arteries

In our study of patients with rheumatoid arthritis and low prevalence of symptomatic atherosclerosis we could with simple regression analysis show a significant association between both mean common carotid artery IMT and IMa and conventional risk factors such as age, systolic blood pressure, HDL and triglycerides. We also found a significant association between brachial artery IMT and IMa and BMI, systolic blood pressure and age in patients with CAD. These results are in line with previous population-based studies on relationship between the conventional CAD risk factors and early atherosclerosis [see e. g. 9, 56].

In contrast to morphological variables FMD was not associated with any of the risk factors tested in the studied patient populations. This finding is in line with the result of a previous study by Witte DR et al., which showed in a meta- regression analysis that only in populations at low risk, endothelial function measured by FMD is related to the principal cardiovascular risk factors, and to the estimated 10-year risk of coronary heart disease [57].

Our results indicate that the morphological variables IMT and IMa, but not the functional variable FMD, identify risk factors of importance for the development of atherosclerosis in middle-aged and elderly subjects.

Oral snuff and endothelial function

We have shown that the administration of 1 g oral moist snuff causes acute endothelial dysfunction in the brachial artery of chronic snuff users. However, the precise mechanisms by which oral snuff leads to this endothelial dysfunction are unknown. Application of nasal nicotine spray causes endothelial dysfunction [58]. Endothelial dysfunction is present in chronic smokers as well as after acute cigarette smoking [3, 59, 60]. Thus, it is most likely that the acute impairment of endothelial function after the use of cigarettes, nicotine spray and snuff is induced by nicotine. Furthermore, we found the same haemodynamic responses to oral snuff as previously demonstrated by other investigators [25, 61]. The predominant haemodynamic effects of nicotine result from activation of the sympathetic nervous system mediated by release of noradrenaline and adrenaline [62]. Sympathetic stimulation evoked by baroreceptor unloading markedly reduces FMD, possibly by decreasing NO availability [63].

In addition to studies of the sympathetic effect on FMD, several investigators have examined the role of oxygen radicals and tetrahydrobiopterin (BH4) in impaired FMD both in humans and animals [33, 64, 65]. Qin et al. have shown that application of superoxide dismutase or BH4 during infusion of nicotine could prevent impaired NOS-dependent vasodilatation in an animal model, suggesting that the formation of oxygen radicals, possibly via an alternation in the utilization of BH4, contributes to this impairment [65]. In line with previous studies the present study indicates that oral moist snuff is associated with higher ambulatory blood pressure, increased heart rate, an increased risk of type 2 diabetes and endothelial dysfunction [61, 66]. Thus, these factors might contribute to an increased cardiovascular risk in snuff users. Flow-mediated dilation is a surrogate variable, which predicts cardiovascular events [7, 8]. Based on the results of the present study and the known haemodynamic effects of snuff we suggest oral snuff should be considered as a public health risk. However, further investigations are needed to find out whether long-term snuff consumption is associated with higher risk of cardiovascular events. Otherwise, the health care system will most likely face a new, growing, harmful habit in the near future. Smokeless tobacco may not be as harmful as smoking cigarettes but it involves a large number of the population, as there are almost one million snuff users in Sweden alone. A proportionately small increase in cardiovascular morbidity or developing insulin resistance due to this habit has obvious effects on the public health level. Many of the snuff consumers were recruited from smokers who intended to quit smoking. Use of snuff results in blood levels of nicotine similar to those observed in cigarette smokers [32] and therefore many subjects find it very difficult to quit the use of snuff later on. There are numerous studies regarding smoking cessation whereas we lack snuff use cessation studies [67]. Taken together, we do believe there is a significant health risk if snuff is marketed as a harmless substitute for smoking. In conclusion oral moist snuff significantly impairs endothelial function and has negative haemodynamic effects. As endothelial dysfunction predicts cardiovascular morbidity, use of oral snuff should be discouraged.

Prednisolone and atherosclerosis

We report that low-dose prednisolone treatment in patients with rheumatoid arthritis did not influence atherosclerosis, determined as IMT, IMa, and presence of atherosclerotic plaques in carotid arteries, or endothelial function determined as FMD in the brachial artery. The role of prednisolone in development of atherosclerosis in patients with autoimmune disorders has

been much discussed, as prednisolone (or other types of GC) is — in addition to other side effects — atherogenic, due to its well-known effects on metabolic factors, for example, by reducing insulin sensitivity in RA [68] and by triggering diabetes [69]. The beneficial short-term effect of GC in the treatment of RA has been known for a long time, but it is only recently that low doses of prednisolone for 2 years have been found to have a positive effect on outcome in RA [70]. It is therefore even more important to determine the effects of prednisolone on atherosclerosis in order to prevent negative side effects. Since atherosclerosis is an inflammatory disease and the main cause of CVD, it could be hypothesized that prednisolone may ameliorate disease development by inhibiting inflammation in the artery wall. This possibility is supported by experimental studies in atherosclerosis-prone rabbit models, where cortisone acetate decreased the progression of atherosclerosis by 60%, but also raised cholesterol levels [71]. Dexamethasone had a similar effect in another rabbit model, by decreasing atherosclerosis in parallel with induction of hyperlipidemia [72]. On the other hand, when high-dose GC was used in another rabbit model, atherogenesis was increased in the treatment group [73]. It is plausible to assume that the different effects of GC on risk factors such as hyperlipidemia, diabetes, and hypertension on the one hand and the antiinflammatory effects on the other have opposite effects on atherogenesis, and the final outcome may depend on which of these effects dominates. The dose of prednisolone may therefore be a crucial factor that determines the outcome of treatment in atherosclerosis and CVD. We recently reported a weak but significant association between total prednisolone dose and CVD in patients with SLE in a nested case-control study to investigate risk factors and underlying mechanisms of the very high risk of CVD in SLE [74]. However, prednisolone is given to patients with active disease and signs of systemic inflammation and an association between prednisolone dose and CVD may also reflect the role of inflammation for development of CVD. Doses in patients with active SLE are typically higher than those used in our present study. Furthermore, the SLE study was not prospective [74]. To our knowledge the role of prednisolone in atherosclerosis and CVD in rheumatic diseases has not been studied in randomized prospective studies before.

The evidence from other diseases is also very scarce. However, one study indicates that low-dose inhaled steroids in patients with asthma is associated with a decreased risk of myocardial infarction [75]. In Cushing's disease, where cortisone levels are high, the risk of CVD is elevated, and this increased risk also persists when the underlying disease is cured [76].

Available evidence is therefore compatible with the possibility that the effects of GC on

atherosclerosis and CVD may depend on dose, and that lower doses as in our study are at least not atherogenic. It is still possible that the treatment with prednisolone for 2 or at least 4 years was not enough to elicit increased atherosclerosis that would have developed at a later stage. This possibility is not supported by our findings, as flow-mediated dilatation of the brachial artery, an early sign of endothelial dysfunction, was not different between those who received prednisolone and those who did not. Endothelial dysfunction precedes development of atherosclerosis and has been reported by several investigators to be a sign of early changes that may later lead to increased atherosclerosis and CVD [77]. Endothelial dysfunction is an early measurement of arterial changes, and is more prevalent in patients with RA compared to controls [78]. Our data indicate that low-dose prednisolone does not promote a further increase in endothelial dysfunction. Mechanisms other than the metabolic and anti-inflammatory properties of GC may influence atherosclerosis and risk for CVD, since GC has proapoptotic, antiproliferative, and antifibrotic effects. These could theoretically inhibit plaque growth, but on the other hand could also destabilize plaque, leading to plaque rupture and ensuing myocardial infarction or stroke. Further, functional resistance to GC may contribute to excessive wound repair in atherosclerosis and restenosis [79].

We observed that current low-dose prednisolone, in the subgroup that was treated for at least 4 years, was associated with higher systolic blood pressure, a reported risk factor for atherosclerosis in patients with RA [80]. This finding indicates that close monitoring of blood pressure is important during low-dose prednisolone treatment. This potentially atherogenic effect of prednisolone could be counterbalanced by treatment with antihypertensive drugs. Prednisolone was also associated with higher total cholesterol levels in the smaller group treated for at least 4 years. This may indicate that monitoring of lipids is important during low-dose prednisolone treatment.

The exact role of atherosclerosis as determined by IMT and IMa in RA-related CVD is not clear. Most studies report greater IMT or at least increased prevalence of atherosclerotic plaques in RA patients compared to controls; but negative findings have also been reported [81-87]. It may be that despite the fact that ultrasound does not always allow discrimination between lesions in RA patients and controls — there may still be important differences in the local inflammatory process leading to plaque rupture [88].

Our findings indicate that low-dose prednisolone does not appear to have an adverse effect on atherosclerosis and endothelial function, at low concentrations (7.5 mg). However, prednisolone still may have an adverse effect on systolic blood pressure and cholesterol level

in the group treated for at least 4 years. Larger, prospective studies are needed to investigate whether low-dose prednisolone influences clinical events like myocardial infarction and stroke.

Conclusions:

- Intake of oral moist snuff significantly impaired endothelial function, measured as FMD of the brachial artery, in long-term snuffers. As endothelial dysfunction predicts cardiovascular morbidity, use of oral snuff should be discouraged.

- Morphological parameters of the carotid arteries and the thoracic aorta were related to severity and extent of coronary artery stenosis in patients with severe CAD. B-mode ultrasonography of the carotid arteries and transesophageal echocardiography of the aorta therefore have a potential for diagnostic evaluation of patients with suspected CAD.

- Morphological but not functional parameters of the brachial artery were associated with the extent of coronary artery stenosis and atherosclerotic wall changes in the carotid arteries in patients with severe CAD. These findings indicate a potential of B-mode ultrasonography of morphological parameters in the brachial artery in the diagnostic evaluation of patients with suspected CAD.

- Low-dose prednisolone did not influence endothelial function or subclinical atherosclerosis in patients with RA. The study verifies the relation between risk factors for atherosclerosis and carotid IMT and IMa.

Acknowledgements

I am sincerely grateful to all those involved in the completion of this work, including all my colleagues, staff of the Karolinska Hospital in Huddinge and Stockholm Heart Center. I am especially grateful to:

Professor Stefan Agewall, my supervisor for your continuous support in the Ph.D. program. You were always there to listen and give advice. You were the single one responsible for involving me in research program at the cardiology department. You showed me different approaches to research problems and stressed the importance of being persistent to accomplish goals. Not only are you an excellent supervisor but also a good friend. Thank you for your kindness, helpfulness and great sense of responsibility. I am deeply grateful for your support.

Professor Tomas Jogestrand, my co-supervisor for teaching me vascular imaging and providing me with the necessary working circumstances to complete my studies. I was privileged working with such a great scientist with excellent knowledge in vascular medicine. I do not know how I would have achieved it without your and Stefan's intensive feedback, particularly during the last sex months.

Professor Ingegäld Hafström, for engaging me in your study on patients with Rheumatoid arthritis, it gave me a new viewpoint of the cardiologist field.

Associate professor Jan van Linden, co-author, for helping me with transesophageal echo examinations and your friendly support.

Risto Jussila, MD, co author, for helping me with collecting data on coronary angiography.

Margareta Ekberg, MTc, for helping me with vascular imaging examinations.

Göran Källner, PhD, co-author and thoracic surgeon, for smooth co-operation with paper II-III .

Stefan Deneberg, Margareta Wörnert and Johan Frostegård, co-authors, for co-operating with paper IV.

Faris al-Khalili, PhD, colleague at Stockholm Heart Center, for encouraging me to take the last step in my PhD when I was almost giving up. Not only could I discuss my problems with you but you also made me look at the bright side of things. I will never forget your expression about “necessary evil”. No wonder that you won the price of best tutor of the year. Thank you for believing in me and encouraging me to achieve new goals.

Jan Ulfberg, associate professor and colleague at Avesta Hospital, for introducing me to the world of research. You have been my role model as a doctor, scientist and a good human being with a great heart.

Ray Thuman, colleague at Avesta Hospital. Thank you for reviewing my first study.

Lars Åke Brodin, colleague and professor. Thank you for motivating me to take the chance to do research in Cardiff. You made it all possible.

Alan G Fraser, the former president of European Association of Echocardiography. Researching by your side was a big dream coming true. Thank you for six months full of knowledge and exciting experience. It was a great adventure to do research with you.

My parents, Zahra and Mozafar, for giving me life and providing conditions for education.

Naghmeh, my wonderful wife, for your patience and endless support. I will never forget your engagement, valuable inputs in my research program and for recruiting snuff users for my first study. Thanks for providing me with the perfect environment and encouraging me to take the last step in my PhD. You facilitated my revision by taking care of our twins in Iran on your own. I will make it up to you.

Kiana and Niki, my daughters for your patience in early childhood when I was absent.

Shahla, my mother-in-law, for being there, letting us rest and keeping the project going.

Gretel Holmström and Jonas Sköldberg, heads of Stockholm Heart Center. Thank you for great working conditions and for supporting me during the entire research process. I promise to make up for all the Fridays I was unavailable the last two years. I doubt I would have been given the same generous conditions elsewhere.

And the final thanks to Swedish Heart and Lung foundation, The Swedish Rheumatism Association, King Gustav V 80 Year Foundation, Ugglas Foundation and The Swedish Science Fund for giving me grants for the project.

References

1. Celermajer DS, Sorensen KE, Gooch VM, Spiegelhalter DJ, Miller OI, Sullivan ID, Lloyd JK, Deanfield JE. Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. Lancet 1992;340:1111–1115.

2. Reddy KG, Nair RN, Sheehan HM, Hodgson JM. Evidence that selective endothelial dysfunction may occur in the absence of angiographic or ultrasound atherosclerosis in patients with risk factors for atherosclerosis. J Am Coll Cardiol 1994;23:833–843.

3. Celermajer DS, Sorensen KE, Georgakopoulos D, Bull C, Thomas O, Robinson J, Deanfield JE. Cigarette smoking is associated with dose-related and potentially reversible impairment of endothelium-dependent dilation in healthy young adults. Circulation 1993;88:2149–2155.

4. Celermajer DS, Sorensen KE, Bull C, Robinson J, Deanfield JE. Endothelium-dependent dilatation in the systemic arteries of asymptomatic subjects relates to coronary risk factors and their interaction. J Am Coll Cardiol 1994;24:1468–1474.

5. Neunteufl T, Katzenschlager R, Hassan A, Klaar U, Schwarzacher S, Glogar D, Bauer P, Weidinger F. Systemic endothelial dysfunction is related to the extent and severity of coronary artery disease. Atherosclerosis 1997;129:111–118.

6. Takase B, Uehata A, Akima T, Nagai T, Nishioka T, Hamabe A, Satomura K, Ohsuzu F, Kurita A. Endothelium-dependent flow-mediated vasodilation in coronary and brachial arteries in suspected coronary artery disease. Am J Cardiol 1998;82:1535–1539 (A7–8).

7. Gokce N, Keaney JF Jr, Hunter LM, Watkins MT, Menzoian JO, Vita JA. Risk stratification for postoperative cardiovascular events via non-invasive assessment of endothelial function: a prospective study. Circulation 2002;105:1567–1572.

8. Neunteufl T, Heher S, Katzenschlager R, Wolfl G, Kostner K, Maurer G. Late prognostic value of flow-mediated dilatation in the brachial artery of patients with chest pain.
Am J Cardiol 2000;86:207–210.

9. Salonen R, Salonen JT. Determinants of carotid intima-media thickness: a population-based ultrasonography study in eastern Finnish men. J Intern Med 1991;229:225–231.

10. O'Leary DH, Polak JF, Wolfson SK Jr, Bond MG, Bommer W, Sheth S, Psaty BM, Sharrett AR, Manolio TA. Use of sonography to evaluate carotid atherosclerosis in the elderly. The Cardiovascular Health Study. CHS Collaborative Research Group. Stroke 1991;22:1155–1163.

11. Bots ML, Witteman JC, Grobbee DE. Carotid intima-media wall thickness in elderly women with and without atherosclerosis of the abdominal aorta. Atherosclerosis 1993;102:99–105.

12. Wofford JL, Kahl FR, Howard GR, McKinney WM, Toole JF, Crouse JR 3rd. Relation of extent of extracranial carotid artery atherosclerosis as measured by B-mode ultrasound to the extent of coronary atherosclerosis. Arterioscler Thromb 1991;11:1786–1794.

13. Hulthe J, Wikstrand J, Emanuelsson H, Wiklund O, de Feyter PJ, Wendelhag I. Atherosclerotic changes in the carotid artery bulb as measured by B-mode ultrasound are associated with the extent of coronary atherosclerosis. Stroke 1997;28:1189–1194.

14. Nowak J, Nilsson T, Sylven C, Jogestrand T. Potential of carotid ultrasonography in the diagnosis of coronary artery disease: a comparison with exercise test and variance ECG. Stroke 1998;29:439–446.

15. O'Leary DH, Polak JF, Kronmal RA, Manolio TA, Burke GL, Wolfson SK Jr. Carotid-artery intima and media thickness as a risk factor for myocardial infarction and stroke in older adults. Cardiovascular Health Study Collaborative Research Group. N Engl J Med 1999;340:14–22.

16. Witteman JC, Kannel WB, Wolf PA, Grobbee DE, Hofman A, D'Agostino RB, Cobb JC. Aortic calcified plaques and cardiovascular disease (the Framingham Study). Am J Cardiol 1990;66:1060–1064.

17. Nihoyannopoulos P, Joshi J, Athanasopoulos G, Oakley CM. Detection of atherosclerotic lesions in the aorta by transesophageal echocardiography. Am J Cardiol 1993;71:1208–1212.

18. Tribouilloy C, Peltier M, Colas L, Rida Z, Rey JL, Lesbre JP. Multiplane transoesophageal echocardiographic absence of thoracic aortic plaque is a powerful predictor for absence of significant coronary artery disease in valvular patients, even in the elderly. A large prospective study. Eur Heart J 1997;18:1478–1483.

19. Solberg LA, Strong JP. Risk factors and atherosclerotic lesions. A review of autopsy studies. Arteriosclerosis 1983;3:187–198.

20. Fazio GP, Redberg RF, Winslow T, Schiller NB. Transesophageal echocardiographically detected atherosclerotic aortic plaque is a marker for coronary artery disease. J Am Coll Cardiol 1993;21:144–150.

21. Tribouilloy C, Shen WF, Peltier M, Lesbre JP. Noninvasive prediction of coronary artery disease by transesophageal echocardiographic detection of thoracic aortic plaque in valvular heart disease. Am J Cardiol 1994;74:258–260.

22. Khoury Z, Gottlieb S, Stern S, Keren A. Frequency and distribution of atherosclerotic plaques in the thoracic aorta as determined by transesophageal echocardiography in patients with coronary artery disease. Am J Cardiol 1997;79:23–27.

23. Matsumura Y, Takata J, Yabe T, Furuno T, Chikamori T, Doi YL. Atherosclerotic aortic plaque detected by transesophageal echocardiography: its significance and limitation as a marker for coronary artery disease in the elderly. Chest 1997;112:81–86.

24. Amanullah AM, Artel BJ, Grossman LB, Espioneza A, Chaudhry FA. Usefulness of complex atherosclerotic plaque in the ascending aorta and arch for predicting cardiovascular events. Am J Cardiol 2002;89:1423–1426.

25. Benowitz NL, Porchet H, Sheiner L, Jacob P 3rd. Nicotine absorption and cardiovascular effects with smokeless tobacco use: comparison with cigarettes and nicotine gum. Clin Pharmacol Ther 1988;44:23–28.

26. Pittilo RM, Wolf N. Cigarette smoking and endothelial injury: a review. Adv Exp Med Biol 1990;273:61–78.

27. Huhtasaari F, Asplund K, Lundberg V, Stegmayr B, Wester PO. Tobacco and myocardial infarction: is snuff less dangerous than cigarettes? BMJ 1992;305:1252–1256.

28. Bolinder G, Alfredsson L, Englund A, de Faire U. Smokeless tobacco use and increased cardiovascular mortality among Swedish construction workers. Am J Public Health 1994;84:399–404.

29. Huhtasaari F, Lundberg V, Eliasson M, Janlert U, Asplund K. Smokeless tobacco as a possible risk factor for myocardial infarction: a population-based study in middle-aged men. J Am Coll Cardiol 1999;34:1784–1790.

30. Bolinder G, Norén A, de Faire U, Wahren J. Smokeless tobacco use and atherosclerosis: an ultrasonographic investigation of carotid intima media thickness in healthy middle-aged men. Atherosclerosis 1997;132:95–103.

31. Wallenfeldt K, Hulthe J, Bokemark L, Wikstrand J, Fagerberg B. Carotid and femoral atherosclerosis, cardiovascular risk factors and C-reactive protein in relation to smokeless tobacco use or smoking in 58-year-old men. J Intern Med 2001;250:492–501.

32. Benowitz NL. Nicotine and smokeless tobacco. CA Cancer J. Clin 1988;38:244–247.

33. Mayhan WG, Patel KP. Effect of nicotine on endotheliumdependent arteriolar dilatation in vivo. Am J Physiol 1997;272:H2337–2342.

34. Mayhan WG, Sharpe GM. Superoxide dismutase restores endothelium-dependent arteriolar dilatation during acute infusion of nicotine. J Appl Physiol 1998;85:1292–1298.

35. Wolfe F, Mitchell DM, Sibley JT, Fries JF, Bloch DA, Williams CA, Spitz PW, Haga M, Kleinheksel SM, Cathey MA. The mortality of rheumatoid arthritis. Arthritis Rheum 1994;37:481-494.

36. Wållberg-Jonsson S, Johansson H, Ohman ML, Rantapää-Dahlqvist S. Extent of inflammation predicts cardiovascular disease and overall mortality in seropositive rheumatoid arthritis. A retrospective cohort study from disease onset. J Rheumatol 1999;26:2562-2571.

37. del Rincón I, O'Leary DH, Haas RW, Escalante A. Effect of glucocorticoids on the arteries in rheumatoid arthritis. Arthritis Rheum 2004;50:3813-3822.

38. Raynauld JP. Cardiovascular mortality in rheumatoid arthritis: how harmful are corticosteroids? J Rheumatol 1997;24:415-416.

39. Corretti MC, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau F, Creager MA, Deanfield J, Drexler H, Gerhard-Herman M, Herrington D, Vallance P, Vita J, Vogel R; International Brachial Artery Reactivity Task Force. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. J Am Coll Cardiol 2002;39:257–265.

40. Wendelhag I, Liang Q, Gustavsson T, Wikstrand J. A new automated computerized analyzing system simplifies readings and reduces the variability in ultrasound measurement of intimamedia thickness. Stroke 1997;28:2195–2200.

41. Jogestrand T, Nowak J, Sylven C. Improvement of common carotid intima-media complex measurements by calculating the cross-sectional area.
J Vasc Invest 1995;1:193–195.

42. Lemne C, Jogestrand T, de Faire U. Carotid intima-media thickness and plaque in borderline hypertension. Stroke 1995;26:34–39.

43. Moise A, Clement B, Saltiel J. Clinical and angiographic correlates and prognostic significance of the coronary extent score. Am J Cardiol 1988;61:1255–1259.

44. Austen WG, Edwards JE, Frye RL, Gensini GG, Gott VL, Griffith LS, McGoon DC, Murphy ML, Roe BB. A reporting system on patients evaluated for coronary artery disease. Report of the Ad Hoc Committee for Grading of Coronary Artery Disease, Council on Cardiovascular Surgery American Heart Association. Circulation 1975;51:5–40.

45. Prevoo ML, van 't Hof MA, Kuper HH, van Leeuwen MA, van de Putte LB, van Riel PL. Modified disease activity scores that include twenty-eight-joint counts. Development and validation in a prospective longitudinal study of patients with rheumatoid arthritis.
Arthritis Rheum 1995;38:44-48.

46. Ekdahl C, Eberhardt K, Andersson SI, Svensson B. Assessing disability in patients with rheumatoid arthritis. Use of a Swedish version of the Stanford Health Assessment Questionnaire. Scand J Rheumatol 1988;17:263-271.

47. Enderle MD, Schroeder S, Ossen R, Meisner C, Baumbach A, Haering HU, Karsch KR, Pfohl M. Comparison of peripheral endothelial dysfunction and intimal media thickness in patients with suspected coronary artery disease. Heart 1998;80:349–354.

48. Frick M, Schwarzacher SP, Alber HF, Rinner A, Ulmer H, Pachinger O, Weidinger F. Morphologic rather than functional or mechanical sonographic parameters of the brachial artery are related to angiographically evident coronary atherosclerosis. J Am Coll Cardiol 2002;40:1825–1830.

49. Hashimoto M, Eto M, Akishita M, Kozaki K, Ako J, Iijima K, Kim S, Toba K, Yoshizumi M, Ouchi Y. Correlation between flow-mediated vasodilatation of the brachial artery and intima-media thickness in the carotid artery in men. Arterioscler Thromb Vasc Biol 1999; 19:2795–2800.

50. Barenbrock M, Hausberg M, Kosch M, Golubev SA, Kisters K, Rahn KH. Flow-mediated vasodilatation and distensibility in relation to intima-media thickness of large arteries in mild essential hypertension. Am J Hypertens 1999;12:973–979.

51. Bots ML, Hoes AW, Koudstaal PJ, Hofman A, Grobbee DE. Common carotid intima-media thickness and risk of stroke and myocardial infarction: the Rotterdam Study. Circulation 1997;96:1432–1437.

52. Craven TE, Ryu JE, Espeland MA, Kahl FR, McKinney WM, Toole JF, McMahan MR, Thompson CJ, Heiss G, Crouse JR 3rd. Evaluation of the associations between carotid artery stenosis: a case-controll study. Circulation 1990;82:1230–1242.

53. Adams MR, Nakagomi A, Keech A, Robinson J, McCredie R, Bailey BP, Freedman SB, Celermajer DS. Carotid intima-media thickness is only weakly correlated with the extent and severity of coronary artery disease. Circulation 1995;92:2127–2134.

54. Lekakis JP, Papamichael CM, Cimponeriu AT, Stamatelopoulos KS, Papaioannou TG, Kanakakis J, Alevizaki MK, Papapanagiotou A, Kalofoutis AT, Stamatelopoulos SF. Atherosclerotic changes of extracoronary arteries are associated with the extent of coronary atherosclerosis. Am J Cardiol 2000;85:949– 952.

55. Kallikazaros IE, Tsioufis CP, Stefanadis CI, Pitsavos CE, Toutouzas PK. Closed relation between carotid and ascending aortic atherosclerosis in cardiac patients. Circulation 2000;102(III):263–268.

56. Ebrahim S, Papacosta O, Whincup P, Wannamethee G, Walker M, Nicolaides AN, Dhanjil S, Griffin M, Belcaro G, Rumley A, Lowe GD. Carotid plaque, intima media thickness, cardiovascular risk factors, and prevalent cardiovascular disease in men and women: the British Regional Heart Study. Stroke 1999;30(4):841-850.

57. Witte DR, Westerink J, de Koning EJ, van der Graaf Y, Grobbee DE, Bots ML. Is the association between flow-mediated dilation and cardiovascular risk limited to low-risk populations? J Am Coll Cardiol. 2005 ;45:1987-1993.

58. Neunteufl T, Heher S, Kostner K, Mitulovic G, Lehr S, Khoschsorur G, Schmid RW, Maurer G, Stefenelli T. Contribution of nicotine to acute endothelial dysfunction in long-term smokers. J Am Coll Cardiol 2002;39:251–256.

59. Zeiher AM, Scha¨chinger V, Minners J. Long-term cigarette smoking impairs endothelium-dependent coronary arterial vasodilator function.
Circulation 1995;92:1094–1100.

60. Lekakis J, Papamichael C, Vemmos C, Nanas J, Kontoyannis D, Stamatelopoulos S, Moulopoulos S. Effect of acute cigarette smoking on endothelium-dependent brachial artery dilation in healthy individuals. Am J Cardiol 1997;79:529–531.

61. Bolinder G, de Faire U. Ambulatory 24-h blood pressure monitoring in healthy, middle-aged smokeless tobacco users, smokers and non-tobacco users.
Am J Hypertens 1998;11:1153–1163.

62. Cryer PE, Haymond MW, Santiago JV, Shah SD. Norepinephrine and epinephrine release and adrenergic mediation of smoking-associated hemodynamic and metabolic events. N Engl J Med 1976;295:573–577.

63. Hijmering ML, Stroes ES, Olijhoek J, Hutten BA, Blankestijn PJ, Rabelink TJ. Sympathetic activation markedly reduces endothelium-dependent, flow-mediated vasodilatation. J Am Coll Cardiol 2002;39:683–688.

64. Higman DJ, Strachan AM, Buttery L, Hicks RC, Springall DR, Greenhalgh RM, Powell JT. Smoking impairs the activity of endothelial nitric oxide synthase in saphenous vein. Arterioscler Thromb 1996;16:546–552.

65. Qin F, Hong S, Mayhan WG. Impairment of nitric oxide synthase-dependent dilatation of cerebral arterioles during infusion of nicotine. Am J Physiol 2002;284:H528–534.

66. Persson PG, Carlsson S, Svanström L, Ostenson CG, Efendic S, Grill V. Cigarette smoking, oral moist snuff use and glucose intolerance. J Intern Med 2000;248:103–110.

67. Benowitz NL. Nicotine addiction. Prim Care 2001;24:277– 322.

68. Dessein PH, Joffe BI, Stanwix AE, Christian BF, Veller M. Glucocorticoids and insulin sensitivity in rheumatoid arthritis. J Rheumatol 2004;31:867-874.

69. Frostegard J. Atherosclerosis in patients with autoimmune disorders. Arterioscler Thromb Vasc Biol 2005;25:1776-1785.

70. Svensson B, Boonen A, Albertsson K, van der Heijde D, Keller C, Hafström I. Low-dose prednisolone in addition to the initial disease-modifying antirheumatic drug in patients with early active rheumatoid arthritis reduces joint destruction and increases the remission rate: a two-year randomized trial. Arthritis Rheum 2005;52:3360-3370.

71. Makheja AN, Bloom S, Muesing R, Simon T, Bailey JM. Anti-inflammatory drugs in experimental atherosclerosis. 7. Spontaneous atherosclerosis in WHHL rabbits and inhibition by cortisone acetate. Atherosclerosis 1989;76:155-161.

72. Asai K, Funaki C, Hayashi T, Yamada K, Naito M, Kuzuya M, Yoshida F, Yoshimine N, Kuzuya F. Dexamethasone-induced suppression of aortic atherosclerosis in cholesterol-fed rabbits. Possible mechanisms. Arterioscler Thromb 1993;13:892-899.

73. Janiak P, Villeneuve N, Pillon A, Jacquemin C, Breugnot C, Gransagne D, Vilaine JP. Non-hypertensive deoxycorticosterone-salt treatment accelerates atherosclerosis progression in Watanabe heritable hyperlipidemic rabbit. J Vasc Res 1994;31:347-358.

74. Svenungsson E, Jensen-Urstad K, Heimbürger M, Silveira A, Hamsten A, de Faire U, Witztum JL, Frostegård J. Risk factors for cardiovascular disease in systemic lupus erythematosus. Circulation 2001;104:1887-1893.

75. Huiart L, Ernst P, Ranouil X, Suissa S. Low-dose inhaled corticosteroids and the risk of acute myocardial infarction in COPD. Eur Respir J 2005;25:634-639.

76. Colao A, Pivonello R, Spiezia S, Faggiano A, Ferone D, Filippella M, Marzullo P, Cerbone G, Siciliani M, Lombardi G. Persistence of increased cardiovascular risk in patients with Cushing's disease after five years of successful cure. J Clin Endocrinol Metab 1999;84:2664-2672.

77. Goligorsky MS. Endothelial cell dysfunction: can't live with it, how to live without it. Am J Physiol Renal Physiol 2005;288:F871-880.

78. Hänsel S, Lässig G, Pistrosch F, Passauer J. Endothelial dysfunction in young patients with long-term rheumatoid arthritis and low disease activity.
Atherosclerosis 2003;170:177-180.

79. Bray PJ, Du B, Mejia VM, Hao SC, Deutsch E, Fu C, Wilson RC, Hanauske-Abel H, McCaffrey TA. Glucocorticoid resistance caused by reduced expression of the glucocorticoid receptor in cells from human vascular lesions.
Arterioscler Thromb Vasc Biol 1999;19:1180-1189.

80. Dessein PH, Joffe BI, Veller MG, Stevens BA, Tobias M, Reddi K, Stanwix AE. Traditional and nontraditional cardiovascular risk factors are associated with atherosclerosis in rheumatoid arthritis. J Rheumatol 2005;32:435-442.

81. Del Rincón I, Williams K, Stern MP, Freeman GL, O'Leary DH, Escalante A.Association between carotid atherosclerosis and markers of inflammation in rheumatoid arthritis patients and healthy subjects. Arthritis Rheum 2003;48:1833-1840.

82. Jonsson SW, Backman C, Johnson O, Karp K, Lundström E, Sundqvist KG, Dahlqvist SR. Increased prevalence of atherosclerosis in patients with medium term rheumatoid arthritis. J Rheumatol 2001;28:2597-2602.

83. Park YB, Ahn CW, Choi HK, Lee SH, In BH, Lee HC, Nam CM, Lee SK. Atherosclerosis in rheumatoid arthritis: morphologic evidence obtained by carotid ultrasound. Arthritis Rheum 2002;46:1714-1719.

84. Del Rincon I, O'Leary DH, Freeman GL, Escalante A. Acceleration of atherosclerosis during the course of rheumatoid arthritis. Atherosclerosis 2007;195(2):354-360.

85. Solomon DH, Karlson EW, Rimm EB, Cannuscio CC, Mandl LA, Manson JE, Stampfer MJ, Curhan GC. Cardiovascular morbidity and mortality in women diagnosed with rheumatoid arthritis. Circulation 2003;107:1303-1307.

86. Wolfe F, Freundlich B, Straus WL. Increase in cardiovascular and cerebrovascular disease prevalence in rheumatoid arthritis. J Rheumatol 2003;30:36-40.

87. Kumeda Y, Inaba M, Goto H, Nagata M, Henmi Y, Furumitsu Y, Ishimura E, Inui K, Yutani Y, Miki T, Shoji T, Nishizawa Y. Increased thickness of the arterial intima-media detected by ultrasonography in patients with rheumatoid arthritis. Arthritis Rheum 2002;46:1489-1497.

88. Geng YJ, Libby P. Progression of atheroma: a struggle between death and procreation. Arterioscler Thromb Vasc Biol 2002;22:1370-1380.

Papers I-IV