Ultrasound imaging of carotid atherosclerosis in a normal population. The Tromsø Study

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ABSTRACT

In the Tromsø study, we have successfully used high resolution ultrasonography for assessing atherosclerosis development in a general population. The method is well suited for large epidemiological surveys. Methodological problems could be overcome by using highly standardized protocols, pre-study training of sonographers and readers, and off-line computer-based batch reading of digitized images. The Tromsø study has added new and important knowledge in the field of carotid atherosclerosis regarding prevalence, sex differences, risk factors and clinical significance.

INTRODUCTION

Atherosclerotic plaques are the underlying cause of the majority of myocardial infarction (MI) and ischemic strokes. The ability to both identify persons with atherosclerosis and quantify the extent of atherosclerosis is of great value in stratifying the future risk for cardiovascular diseases and also for monitoring ongoing treatment. High-resolution B-mode ultrasonography of the carotid arteries provides measures of intima-media thickness (IMT) and atherosclerotic plaques, both widely used as surrogate measures of cardiovascular disease. It is a cheap, mobile, and easily available method and has practically no complications or side effects. The ultrasound images show excellent accordance with the true arterial wall structures and wall thickness in histological studies (1), is highly correlated with known vascular risk factors (2,3) and with risk scores (4-6). The finding of carotid atherosclerosis correlates well with the extent of atherosclerotic disease elsewhere (7-9), and can predict future vascular events, like MI and stroke (3,10-12). The validity of B-mode ultrasound imaging to detect asymptomatic carotid artery atherosclerosis combined with high measurement reproducibility provides a powerful non-invasive scientific tool to test cross-sectional and prospective hypotheses related to disease epidemiology. Carotid ultrasound is used in several large population-based studies around the world (i.e ARIC, CHS, Rotterdam). Ultrasound examination of the carotid artery was implemented in the Tromsø Study as a part of the fourth survey in 1994-95.

IMT is made up of approximately 80% media (smooth muscle cells) and 20% intima (endothelial layer, basal lamina and subendothelial matrix). High resolution ultrasound can not differentiate between the two layers (Figure 1). Increased IMT can therefore be due to both medial hypertrophy and widening of the subintimal space. However, atherosclerosis is largely an intimal process with deposition of cholesterol and cell infiltration. Intima-media thickening, especially in its early development, reflects a hypertensive hypertrophic response of the medial cells related to changes in local shear stress and tensile stress, and is likely to reflect the influence of genes related to hypertension, such as angiotensin II, and angiotensin converting enzyme (13,14). In contrast, formed arterial plaques probably represent a later stage of atherogenesis related to inflammation, oxidation, endothelial dysfunction, and/or smooth muscle cell proliferation (15). The pathological processes leading to intima-media thickening and to plaque formation may therefore not be similar, and plaque and intima-media thickening may reflect different biological aspects of atherogenesis with distinctive relations to clinical vascular disease. IMT has usually been measured in the distal part of the common carotid artery (CCA) because high measurement precision is more easily obtained from this segment of the artery. However, plaques are rare in this arterial segment. Plaques usually occur at sites of non-laminar turbulent flow, such as in the carotid bulb and the proximal internal carotid segment (ICA) (16,17).

Whereas IMT is solely a quantitative measure that is measured continuously, carotid plaques can be assessed both quantitatively as a categorical variable (present/absent) and continuously (plaque numbers, plaque thickness, plaque area, plaque volume) and qualitatively, reflecting the composition of the plaque or plaque content (plaque echogenicity, plaque heterogeneity, plaque texture, measured either in categories or continuously) (Figure 2). Plaques that appear echolucent (low echogenicity) have a thin fibrous cap overlaying a lipid core with numerous macrophages, and are recognized as inflammatory plaques (18). The
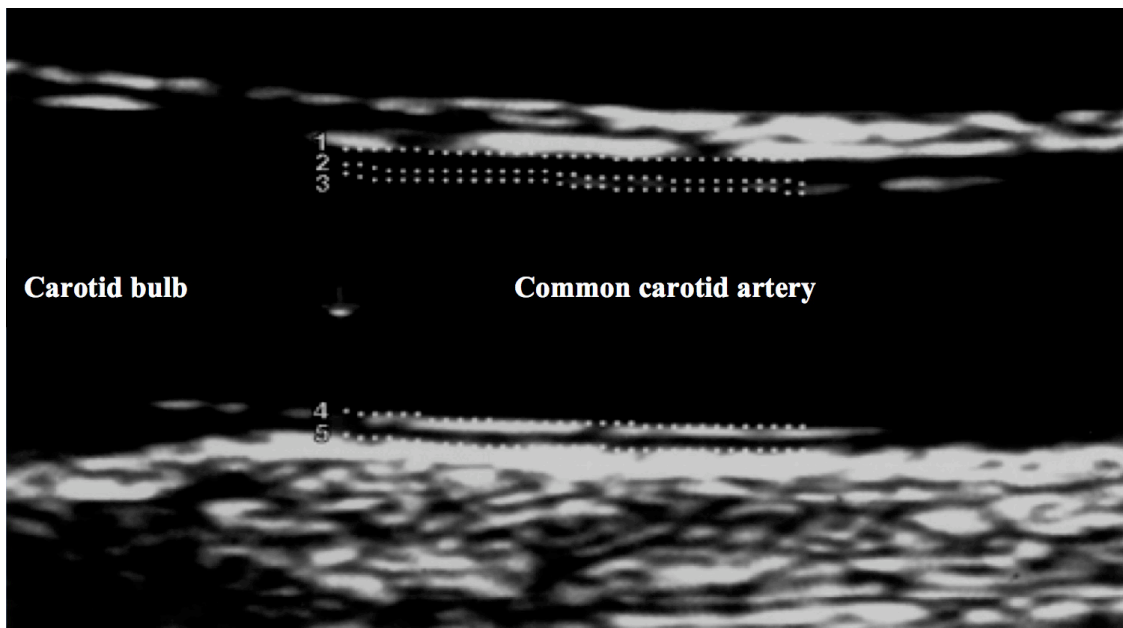
Figure 1. Marked interfaces of a characteristic longitudinal ultrasound image of the distal common carotid artery. In the near wall, the distance between the trailing edge of the periadventitia-adventitia interface (1) and the trailing edge of the intima-lumen interface (3) represents the intima-media thickness (IMT). In the far wall, the distance between the leading edge of the lumen-intima interface (4) and the leading edge of the media-adventitita interface (4) represents the IMT. The distance between 2 and 4 corresponds with the lumen diameter. The arrow marks the beginning of the carotid bifurcation (bulb).
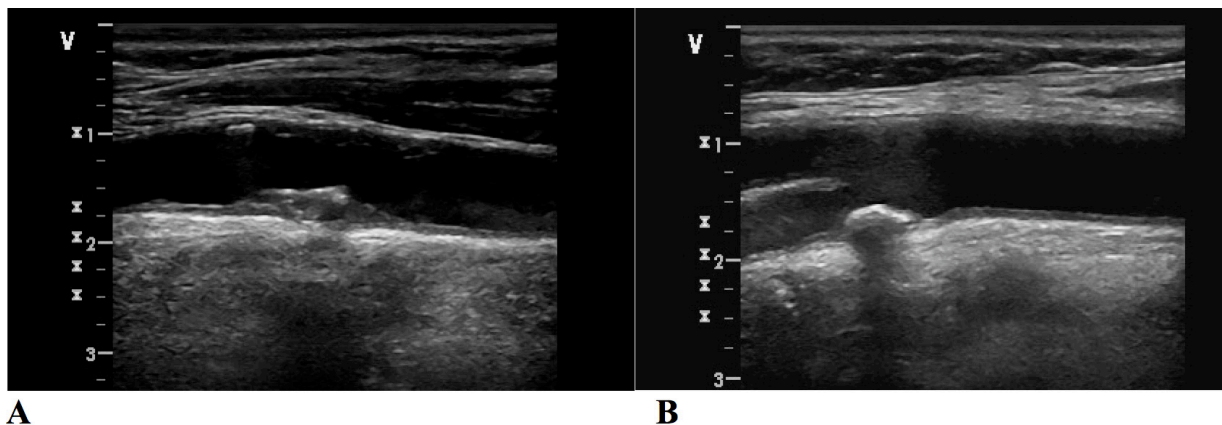

Figure 2. High resolution B-mode images of **A)** low-echogenic (echolucent) plaque and **B)** high-echogenic plaque.

presence of echolucent plaques is associated with higher risk of coronary and cerebrovascular ischemic events than echogenic plaques (18-20).

IMT measurements provide information on cardiovascular risk even when no plaque is present. This can be an advantage in population based studies where a significant proportion of the participants have no plaques. On the other hand, IMT is a relatively insensitive measure of plaque evolution since plaque grows longitudinally along the carotid axis of flow more than 2 times faster than it thickens (21). Because the annual rate of progression of IMT is below the resolution of carotid ultrasound (~0.3mm), large sample sizes are required to evaluate changes. Each dimension added (from one-dimensional IMT to 2D plaque area, and to 3D plaque volume) substantially lowers the sample size and duration of studies required to evaluate progression (22). Plaque area and volume measurements may therefore be more sensitive and representative measures of the atherosclerotic burden than plaque thickness or IMT. Total carotid plaque area is also a stronger predictor of events than IMT (12,23). However, IMT is often used as endpoint in efficacy studies and consensus on how to standardize measurements exists (24), whereas there is greater variety in plaque definitions across studies. Different investigators have proposed different definitions of plaque. Most characterize plaque as a localized protrusion into the vessel wall with a diameter that is at least 50% greater than the normal appearing nearby IMT segment (25).

METHODOLOGICAL CHALLENGES

Precise measurements are mandatory to make a good study. There are four important sources of variability in quantification of ultrasound assessed atherosclerosis: Choice of segments to measure, choice of equipment, sonographer error and reader error. Averages of several measurements taken from any single individual should provide greater reproducibility than any individual measurement (26). This provides the rationale for obtaining multiple IMT measurements from each segment of the artery (the near and far walls of the distal CCA, the bifurcation and the proximal ICA) and presenting results as the mean value of repeated consecutive measurements. A second source of variability is the ultrasound equipment. Many large multicenter epidemiologic studies have purchased uniform equipment and provided centralized standardisation across clinical sites to avoid this problem.

Different sonographers have potential to introduce significant variability into ultrasound readings. Ultrasound scanning is operator dependent, and the information obtained depends on the sonographers experience and technique. Plaques are small structures that easily can be overlooked. This is especially true for low-echogenic plaques located in the near wall of the artery and plaques located in the ICA, a segment which is technically more difficult to visualize. It is crucial to use the optimal insonation angle and visualize the artery in the diameter where the plaque is thickest. When beams of ultrasonic waves hit a structure (e.g vessel wall, a plaque) perpendicularly, the reflection will be optimal. Otherwise, some of the waves are scattered and reflected away from the transducer, and the quality of the B-mode image may become insufficient for analytical purposes, with unsharp contours, loss of small structures, and distortion of proportions within the image.

Variability between readers can also be substantial. If the scanning period is long, drift in both measurement and reading may be a problem as well. This can be reduced by "batch reading" by one single reader at the end of the study, automated image quantification, training and retraining of readers as well as sonographers. Repetitive reproducibility studies at fixed intervals should be implemented to ensure a high-quality classification practice during the whole survey period. Computer-assisted off-line classification represents a more objective method with better reproducibility (18,27).

Data on measurement agreement (at both inter- and intraobserver level) should be available when interpreting the results of ultrasound studies. In studies on progression, the measurement errors of at least two measurements are accumulated, giving substantially lower statistical power compared to baseline measurements only. Studies on progression require a higher level of reproducibility, more demanding techniques and highly standardized protocols that include the definition of anatomical landmarks, control of probe insonation angle, and careful circumferential scanning of segments to identify the maximum wall thickness. Random measurement errors will always tend to attenuate the true associations found in a study, whereas systematic errors (bias) may influence the association in either direction. The measurement errors in carotid ultrasonography are still too big to study progression of atherosclerosis between two points of time at an individual level. At a population level however, the large number will give enough power to overcome the measurement variability, and make it possible to detect even weak associations that may be of clinical importance.

ULTRASONOGRAPHY OF THE CAROTID ARTERIES IN THE TROMSØ STUDY

Subjects and methods

The Tromsø study is a population-based prospective study with repeated health surveys of inhabitants in the municipality of Tromsø, Norway. The main focus has been on cardiovascular diseases. In 1994 (baseline), all subjects aged 55-74 years and a random 5-10% sample in the other age groups > 24 years, were invited to ultrasound scanning of the carotid artery. In all, 6892 subjects (79% of the eligible population) attended for ultrasound screening. Of these persons, ultrasonography of the right carotid artery was performed in 6727 subjects. In the follow-up survey in 2001, all participants who were still alive and living in Tromsø were invited to a new examination. Ultrasound examination of the right carotid artery was performed in 5454 persons, in whom 4858 were examined for the second time. The right carotid artery was scanned longitudinally from the level of the clavicle, through the carotid bulb (bifurcation segment) and the ICA as far downstream as possible. The same ultrasound equipment (Acuson Xp10 128, ART upgraded, with a 7.5-MHz linear-array transducer, aperture size 38 mm) with standardized and fixed instrument set-up was used in both surveys. We used no fixed insonation angle, but recorded plaques and IMT at angles that gave technically best and most adequate view of IMT, plaque size and echogenicity. To ensure equal and standardized examination techniques and measurement procedures, all sonographers completed a 2-month pre-study training protocol.

A plaque was defined as a localised protrusion of the vessel wall into the lumen of at least 50% compared to the adjacent IMT. In each subject, a maximum of 6 plaques were registered in the near and far walls of CCA, bifurcation, and ICA, respectively. Plaque echogenicity was scored visually as echolucent, predominantly echolucent, predominantly echogenic, or echogenic. Digitalized longitudinal plaque images were transferred to and standardized in Adobe Photoshop, to calculate the plaque area and generate median values for the gray-scale pixel distribution of each plaque (the gray-scale median, GSM), an objective

measure of plaque echogenicity (28). In subjects with more than one plaque, the areas of all plaques were summarized to give the total plaque area. The GSM of the total plaque area was estimated as a weighted mean of the GSM value of each single plaque. Carotid stenosis was defined by one or both of the following criteria: 1) Peak systolic velocity in tightest stenotic part $\geq$ 0.2 m/sec higher than peak systolic velocity in the post-stenotic ICA segment, or $\geq$ 0.1 m/sec if the stenosis was located to the bifurcation/bulb. 2) A plaque thickness that constituted 35% or more of the lumen diameter at the plaque site. Subjects were referred to the Department of Neurology, University Hospital of North Norway if a suspected carotid stenosis or occlusion were found.

Automated R-triggered measurement of the right carotid IMT was performed in the near and far walls of the CCA and the far wall of the bulb (29). Measurements of IMT and CCA-lumen diameter were analyzed off-line by a an automated computerized edge-detection program developed by the Wallenberg Laboratory for Cardiovascular Research, Sahlgrenska University Hospital, Gothenburg University, Gothenburg, Sweden (30). The computer program estimates the mean IMT and lumen diameter from 100 measurements along a predefined 10 mm segment of the CCA and the carotid bulb. In the analyses, we used the average of the mean IMT of the 3 locations.

Aims of the study

Since atherosclerosis is an intermediate stage between risk factor exposition and clinical manifest cardiovascular disease, carotid atherosclerosis can be studied by two different approaches. If a measure of atherosclerosis (such as IMT, plaque presence, or plaque area) is used as surrogate endpoint, we can define this as the dependent variable in the statistical analyses and study which risk factors that independently predict development and progression of atherosclerosis. On the other hand, measures of atherosclerosis can be defined as independent variables in the study of atherosclerotic burden as predictor for myocardial infarction and ischemic stroke. In the Tromsø Study, we have used both approaches. Firstly, we estimated the distribution of and risk factors for IMT, and prevalence of and risk factors for atherosclerotic plaques and carotid stenosis in a general population. Secondly, we characterized risk factors for progression of carotid atherosclerosis. Finally, carotid atherosclerosis was evaluated as a predictor of myocardial infarction, stroke and death.

Results

Reproducibility

The intra-and interobserver agreement on plaque occurrence and visual assessed plaque echogenicity is displayed in Table 1. In the baseline survey in the Tromsø study, all plaque classification was performed off-line in one batch by one single reader at the end of

Table 1. Observer agreement (overall agreement in pooled analyses) on plaque detection and visual classification of plaque echogenicity. The Tromsø Study.

	Inter-observer agreement K (95% CI)	Intra-observer agreement K (95% CI)
Baseline (1994/95)		
Plaque detection	0.72 (0.60–0.84)	0.76 (0.63–0.89)
Plaque echogenicity	0.73 (0.59–0.87)	0.69 (0.55–0.83)
Follow-up (2001)		
Plaque detection	0.67 (0.58–0.76)	0.80 (0.70–0.91)
Plaque echogenicity	0.57 (0.51–0.62)	0.52 (0.44–0.60)

the study (31). This gave a uniform and consistent classification. In the follow-up survey, plaque assessment was performed on-line by four different readers (28). The rationale for doing this was the assumption that on-line reading would give a truer picture of plaque echogenicity. In spite of good agreement in the reproducibility studies at both baseline and follow-up, there were substantial differences in the relative distribution of plaque echogenicity classes in the two surveys. Subsequent analysis indicated that the classification practice in the follow-up survey had been modified over time, and that this variation would undermine a valid comparison between echogenicity in the two surveys. Therefore, all plaque images in the baseline- and follow-up survey were reclassified in one batch by two readers, using computer-assisted analysis (28). In Table 2, reproducibility data on computerized measures of plaque area and plaque GSM are given. Reproducibility of plaque area and GSM measurements was overall good with small inter-observer mean arithmetic and mean absolute differences. However, the limits of agreement indicate that a change in plaque area of as much as 8-9 mm^2 and a change in plaque GSM of as much as 19 can be attributed to measurement error when applying carotid ultrasound at an individual level. The mean degree of stenosis and median absolute difference between observers of the estimated degree of stenosis by the velocity method were 46.3 and 10.8%, respectively. The corresponding values were 51.0 and 5.8% for the diameter method and 57.1 and 7.2%, for the cross-sectional lumen method. The limits of agreement for intersonographer reproducibility varied between ±19.7 and 26.5% (32). For IMT, the mean inter- and intraobserver absolute differences were 0.21 mm and 0.22 mm respectively (29).

Distribution of and risk factors for IMT

In the baseline survey, IMT and cardiovascular risk factors were measured in 6408 men and women aged 25-84 years (33). The distribution of IMT is shown in Figure 3. Age, systolic blood pressure, total cholesterol, HDL cholesterol, body mass index, and smoking were independent predictors of IMT in both sexes. Fibrinogen levels and physical activity were associated with IMT in men only, whereas triglyceride levels

Table 2. Reproducibility of measurements of plaque area and grey-scale median (GSM). The Tromsø Study.

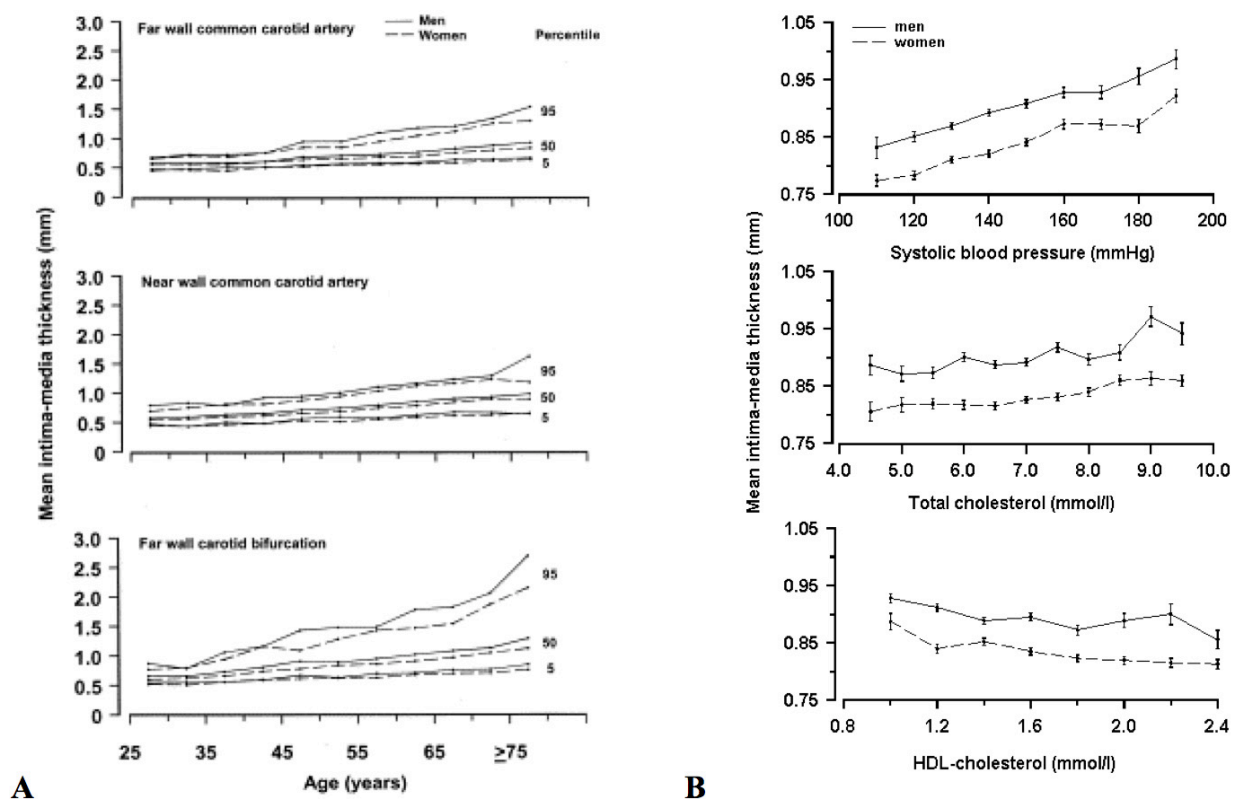|	Mean (SD)	Mean (95% CI) Arithmetic difference	Mean (SD) Absolute difference	Limits of agreement
Inter-observer				
Plaque GSM	50.9 (28.8)	−1.7 (−2.6, −0.7)	5.9 (8.0)	± 19.2
Plaque area (mm^2)	13.9 (9.0)	−1.0 (−1.4, −0.6)	2.9 (3.4)	± 8.6
Intra-observer				
Observer A				
Plaque GSM	50.2 (27.6)	−0.1 (−1.0, 0.8)	3.8 (4.8)	± 12.0
Plaque area (mm^2)	13.4 (7.9)	0.2 (−0.2, 0.7)	1.8 (2.5)	± 6.1
Observer B				
Plaque GSM	51.8 (30.2)	−0.9 (−2.2, 0.4)	5.3 (7.0)	± 17.1
Plaque area (mm^2)	13.8 (8.3)	0.0 (−0.5, 0.6)	2.1 (3.2)	± 7.5

Figure 3. **A)** Percentile distribution of mean IMT in the far wall of the common carotid artery (top panel), the near wall of the common carotid artery (middle panel) and the far wall of the carotid bifurcation (bottom panel) related to age. Men are shown in solid lines, women in dashed lines. Percentiles shown are from top to bottom the 95th, 50th and 5th percentile. **B)** Age-adjusted values of mean IMT (± SE) by systolic blood pressure (top panel), total cholesterol (middle panel) and HDL-cholesterol (bottom panel). Men are shown in solid lines, women in dashed lines. From Stensland-Bugge E, et al. *Atherosclerosis* 2001; **154**: 437-448. Reprinted with permission.

were associated with IMT independently of HDL cholesterol in women only. A family history of cardiovascular disease (CVD) was an independent predictor of IMT in both sexes, also when controlling for traditional CVD risk factors. The magnitude of the association between most risk factors and IMT did not differ depending on age, but the effects of physical activity and triglycerides were more pronounced at higher age. These data suggest that there are significant age and sex differences in the distribution and the determinants of subclinical atherosclerosis. We have also reported an inverse association between total testosterone levels and IMT in the carotid artery in men that was present also after excluding men with cardiovascular disease, but was not independent of BMI (34). The clinical relevance of this is uncertain and needs to be investigated in a clinical setting. No significant relationship between carotid IMT and serum TSH levels was

observed in normal, non thyroxine taking, subjects. Carotid IMT was increased in subjects taking thyroxine. Whether the increase in IMT is due to thyroxine ingestion or underlying thyroid disease cannot be answered from the study (35).

Prevalence of carotid plaques and carotid stenosis
At baseline, the prevalence and echogenicity of carotid plaques were registered in 3016 men and 3404 women (17). Plaque morphology was graded according to echogenicity. Atherosclerotic plaques were found in 55.4% of the men and 45.8% of the women. In men, there was a linear increase in plaque prevalence with age, whereas in women, there was a curvilinear age trend, with an inflection in the prevalence rate of women at approximately 50 years of age (Figure 4A). The male predominance in atherosclerosis declined after the age of 50 years, the plaque prevalence being similar in elderly men and women. Men had more low-echogenic plaques than women (Figure 4B); this sex difference in plaque echogenicity increased significantly (*P* = 0.005) with age. The sex difference in the prevalence of plaque and the female age trend in atherosclerosis showed significant changes at the age of approximately 50 years, suggesting an adverse effect of menopause on atherosclerosis. The higher proportion of soft low-echogenic plaques in men compared with women increased with age and may partly account for the prevailing male excess risk of coronary heart disease in the elderly despite a similar prevalence of atherosclerosis in elderly men and women.

The prevalence of carotid stenosis was 3.8% (95% CI 3.2–4.6%) in men and 2.7% (95% CI 2.2–3.3%) in women (*P* = 0.001) (36). The prevalence gradually increased by age in both genders. Cholesterol, HDL cholesterol (inverse), fibrinogen, systolic blood pressure levels and current smoking were independently associated with carotid artery stenosis in both women and men. The presence of carotid stenosis was significantly associated with a history of cerebrovascular disease, coronary heart disease and peripheral artery disease. Low levels of HDL cholesterol and increasing degree of stenosis were independently associated with an increased risk of having an echolucent plaque (37).

Predictors of plaque formation
In a follow-up study, we examined predictors of novel plaque development in 2610 persons who had no plaque present at the baseline survey in 1994 (38). At baseline, we measured all traditional cardiovascular risk factors as well as novel risk factors like monocyte count, white cell count and fibrinogen. At follow-up, the number of novel plaques was grouped as none, 1 plaque, and 2 or more plaques. In a multivariate ordinal logistic regression model, age, sex, total cholesterol, current smoking, systolic blood pressure, IMT and monocyte count were independent predictors of novel plaque formation (Table 3). No significant association was found between plaque formation and either white
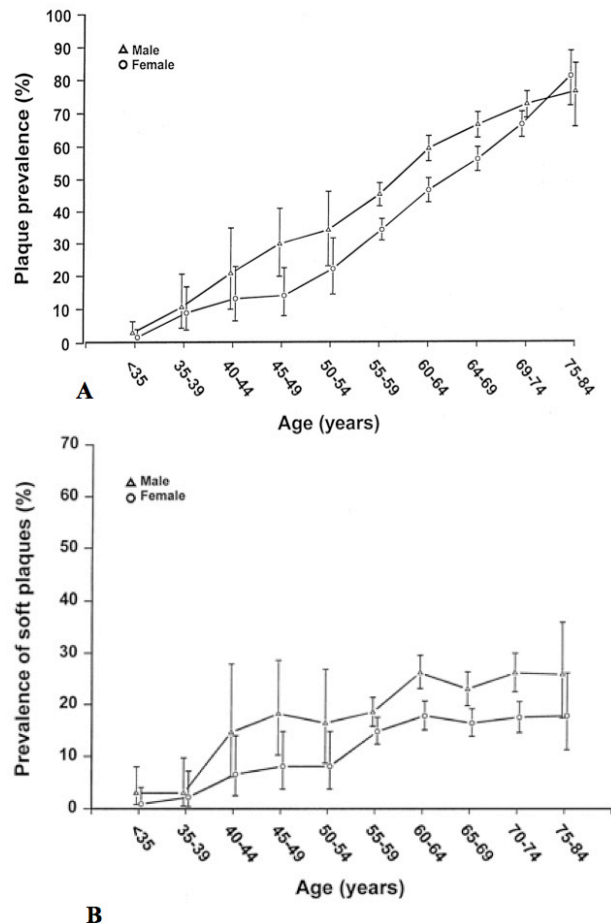

Figure 4. **A)** Prevalence of carotid plaque by age and sex. **B)** Prevalence of echolucent (soft) carotid plaques by age and sex. From Joakimsen O, et al. *Arterioscler Thromb Vasc Biol* 1999; **19**: 3007-3013. Reprinted with permission.

Table 3. Predictors of novel plaque formation. The Tromsø Study.

Risk factor	χ^2	OR (95% CI)
Monocyte count x10^9/L	14.1	1.18 (1.08–1.29)
Age, y	58.2	1.61 (1.42–1.81)
Male sex	9.2	1.33 (1.11–1.59)
Total cholesterol, mmol/L	25.2	1.26 (1.15–1.38)
Current smoking	28.4	1.73 (1.41–2.11)
Systolic blood pressure, mm Hg	23.2	1.25 (1.14–1.37)
Intima-media thickness, mm	46.8	1.43 (1.29–1.58)
R^2		0.19

The multivariate adjusted odds ratio predicts the probability of being in a higher category (more plaques) for 1 SD increase in the independent continuous variable or for being a male or current smoker. From Johnsen SH, et al. *Stroke* 2005; **36**: 715-719.

blood cell count or fibrinogen. This was the first prospective study demonstrating that elevated monocyte count in blood is an early marker of future plaque development. The findings fit well with the biologic model of atherogenesis where activation of monocytes and differentiation into lipid-laden macrophages are fundamental events in generation of atherosclerotic lesions. In the highest monocyte quartile, the risk for

Table 4. Predictors of plaque area progression. The Tromsø Study.

Risk factor	Model I		Model II		Model III		Model IV	
	ß* (SE)	P	ß* (SE)	P	ß* (SE)	P	ß* (SE)	P
HDL cholesterol, mmol/L	–0.95 (0.43)	0.02	–0.98 (0.44)	0.03	–0.93 (0.44)	0.03	–1.46 (0.48)	0.002
Age, year	...	...	1.47 (0.43)	0.0006	1.46 (0.45)	0.001	1.34 (0.48)	0.005
Male sex	...	...	1.07 (0.89)	0.2	1.13 (0.91)	0.2	1.25 (0.99)	0.2
Total cholesterol, mmol/L	...	...	...	...	–0.28 (0.44)	0.5	0.73 (0.51)	0.2
Systolic blood pressure, mm Hg	...	...	...	...	1.21 (0.44)	0.006	1.07 (0.48)	0.03
Smoking, yes vs. no	...	...	...	...	3.18 (0.92)	0.0005	3.20 (1.00)	0.001

*Values are regression coefficients (SE) expressed in mm^2 for a 1-SD change in continuous variables and for presence vs. absence of categorical variables.
Model I was unadjusted.
Model II was adjusted for age and sex.
Model III was adjusted for age, sex, total cholesterol, systolic blood pressure, and smoking.
Model IV excluded persons who had ever used lipid-lowering drugs (n = 442).
From Johnsen SH et al. *Circulation* 2005; **112**: 498-504.

having plaque compared with the lowest quartile was 1.85 (OR) (95% CI 1.41–2.43). Repeating the analysis without IMT did not change the monocyte estimate. Excluding subjects with known cardiovascular disease and diabetes mellitus from analysis neither changed this. Monocytes were not associated with IMT neither at baseline nor follow-up in persons who remained plaque free, suggesting that differences in monocyte activity is a determining factor for plaque formation but not for diffuse thickening of the intima-media layer. In cross-sectional analysis, women with late menopause and women who ever had used postmeno-pausal estrogens had significantly less plaques than women with early menopause and never users of estrogen (39). The ApoC-I content of VLDL particles was associated with plaque size in persons with carotid atherosclerosis (40). Our findings support the concept that the number of apoC-I per VLDL-particle may be of importance for initiation and progression of atherosclerosis. We found no evidence for persistent Chlamydia pneumoniae or cytomegalovirus infection in subjects with carotid plaque (41).

Risk factors associated with plaque echogenicity
In subjects with carotid stenosis, low levels of HDL cholesterol were independently associated with an increased risk of having an echolucent, rupture-prone atherosclerotic plaque. For 1-SD increase in HDL cholesterol, the risk of having lower plaque echo-genicity decreased by approximately 30% (OR 0.69, 95% CI 0.52–0.93) (37). Subjects with echolucent plaques had delayed postprandial clearance of chylo-micron triglycerides compared to controls. Low lipo-protein lipase (LPL) activity due to attenuated mobili-zation of LPL from capillary endothelium may play an important role in the formation of echolucent plaques by modulation of postprandial lipids and subsequent fat accumulation in the arterial wall (42). Glycated he-moglobin level was strongly related to the prevalence of carotid artery plaques with high echogenicity in

nondiabetic individuals (43). Serum osteoprotegerin was inversely associated with carotid plaque echogeni-city (44). Persons with carotid stenosis had significant-ly higher plasma tissue-plasminogen activator antigen (t-PA ag) and vonWillebrand factor concentrations than controls, and there was a significant inverse relationship between t-PA ag and plaque echogenicity (P = 0.034) (45). Echogenic plaques were associated with higher levels of thrombin-antithrombin com-plexes (TAT) and prothrombin fragment 1+2 (F1+2). TAT and F1+2 increased linearly with plaque echoge-nicity, suggesting that increasing plaque echogenicity is associated with thrombin generation in persons with carotid stenosis (46).

Predictors of plaque progression
We evaluated predictors for plaque progression in 1952 persons with pre-existing plaque at baseline (47). All plaque images were computer processed to yield a measure of plaque area and echogenicity, expressed as the GSM. After 7 years of follow-up, a new ultrasound screening was performed, and the changes in plaque area and echogenicity were assessed. In a multi-variable adjusted model, age, systolic blood pressure, current smoking and HDL-cholesterol were indepen-dent predictors of plaque growth (Table 4). For a 1-SD (0.41 mmol/L) lower HDL-cholesterol level, mean plaque area increased by 0.93 mm^2, P = 0.03. When users of lipid-lowering drugs were excluded from analysis, the HDL estimate was strengthened (β = 1.46 mm^2, P = 0.002). Although plaque area increased in 70% of cases, and most plaques became more echo-genic over the follow-up interval, the plaques that became more echolucent grew more in size than those that became more echogenic (P = 0.002). This study showed that a high level of HDL-cholesterol is protec-tive against plaque growth. Transformation of the plaque into higher echogenicity was associated with reduced growth. The findings of this study indicate that HDL-cholesterol stabilizes plaques and counteract
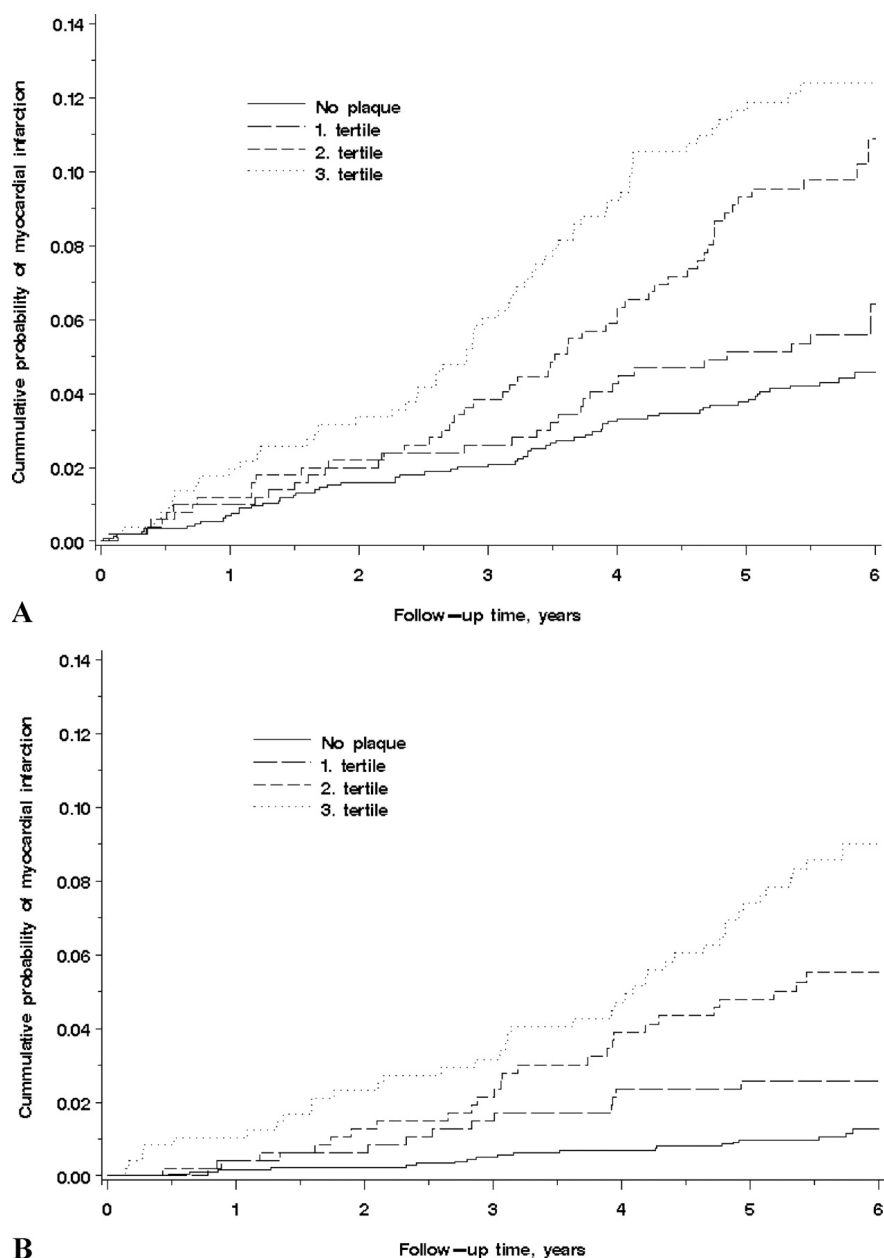
Figure 5. Proportion of MI in men (A) and women (B) according to total plaque area. From Johnsen SH, et al. *Stroke* 2007; **38**: 2873-2880. Reprinted with permission.

their growth by reducing their lipid content and inflammation. Albuminuria was positively related to plaque-initiation and plaque-growth (48). The proinsulin-to-insulin ratio was found to be associated with progressive carotid artery plaque size in women, but not in men (49).

Carotid plaque area as a predictor of first myocardial infarction
In this prospective study, we measured carotid IMT, total plaque area, and plaque echogenicity as predictors for first-ever MI (12). IMT, total plaque area, and plaque echogenicity were measured at baseline in 6226 men and women aged 25 to 84 years with no previous MI. The subjects were followed for 6 years and inci-

dent MI was registered. During follow-up, MI occurred in 6.6% of men and 3.0% of women. The adjusted relative risk (RR; 95% CI) between the highest plaque area tertile versus no plaque was 1.56 (1.04–2.36) in men and 3.95 (2.16–7.19) in women (Figure 5). In women, there was a significant trend toward a higher MI risk with more echolucent plaque. The adjusted RR (95% CI) in the highest versus lowest IMT quartile was 1.73 (0.98–3.06) in men and 2.86 (1.07–7.65) in women. When we excluded bulb IMT from analyses, IMT did not predict MI in either sex. This study showed that in a general population, carotid plaque area is a stronger predictor of first-ever MI than is IMT. Moreover, carotid atherosclerosis seems to be a stronger risk factor for MI in women than in men.

Carotid stenosis and plaque echogenicity as predictors of ischemic cerebrovascular events and death

The purpose of this study was to assess in a prospective design whether plaque morphology is associated with risk of ischemic stroke and other cerebrovascular events in subjects with carotid stenosis (19). A total of 223 subjects were identified with carotid stenosis and matched with 215 control subjects by age and sex. Follow-up time was 3 years. Plaque echogenicity was assessed at baseline and scored as echolucent, predominantly echolucent, predominantly echogenic, or echogenic. Forty-four subjects experienced ≥1 ischemic cerebrovascular events in the follow-up period. Plaque echogenicity, degree of stenosis, and white blood cell count were independent predictors of cerebrovascular events. The adjusted relative risk for cerebrovascular events in subjects with echolucent plaques was 4.6 (95% CI 1.1–18.9) compared to subject without stenosis, and there was a significant linear trend ($P = 0.015$) for higher risk with lower plaque echogenicity (Figure 6A). The adjusted relative risk for a 10% increase in the degree of stenosis was 1.2 (95% CI 1.04–1.40).

In a 4-year follow-up study of 248 subjects with suspected carotid stenosis and 496 age- and sex-matched controls, the number and causes of deaths were registered (50). The unadjusted relative risk for death was 2.72 (95% CI 1.57–4.75) for subjects with stenosis compared with control subjects. Adjusting for cardiovascular risk factors increased the relative risk to 3.47 (95% CI 1.47–8.19) (Figure 6B). The adjusted relative risk in persons with stenosis and no cardiovascular disease or diabetes was 5.66 (95% CI 1.53–20.90), which was higher than in subjects with stenosis and self-reported disease (1.79; 95% CI 0.75–4.27). There was a dose-response relationship between degree of stenosis and risk of death ($P = 0.002$ for linear trend). Carotid stenosis was a stronger predictor of death than self-reported cardiovascular disease or diabetes. The major cause of death was MI.

At the moment, we are analysing the ischemic stroke endpoints in relation to IMT and plaque status at baseline.

Carotid stenosis and cognitive function

Performance on several neuropsychological tests was compared in 189 subjects with ultrasound-assessed carotid stenosis and 201 control subjects without carotid stenosis (51). Subjects with a previous history of stroke were excluded. The test battery included tests of attention, psychomotor speed, memory, language, speed of information processing, motor functioning, intelligence, and depression. Sagittal T1-weighted and axial and coronal T2-weighted spin echo MRI was performed, and presence of MRI lesions (white matter hyperintensities, lacunar and cortical infarcts) was recorded. Subjects with carotid stenosis had significantly lower levels of performance in tests of attention, psychomotor speed, memory, and motor functioning,
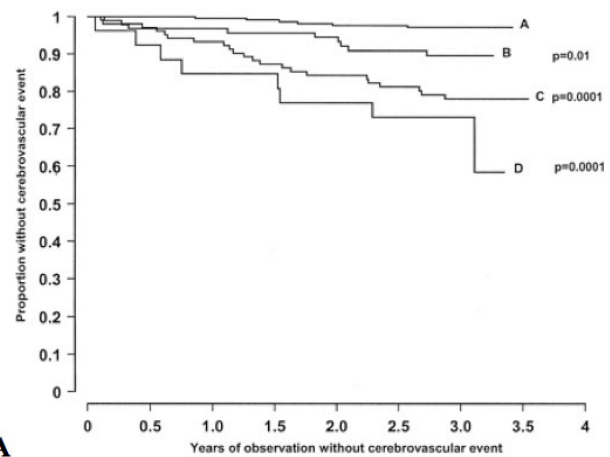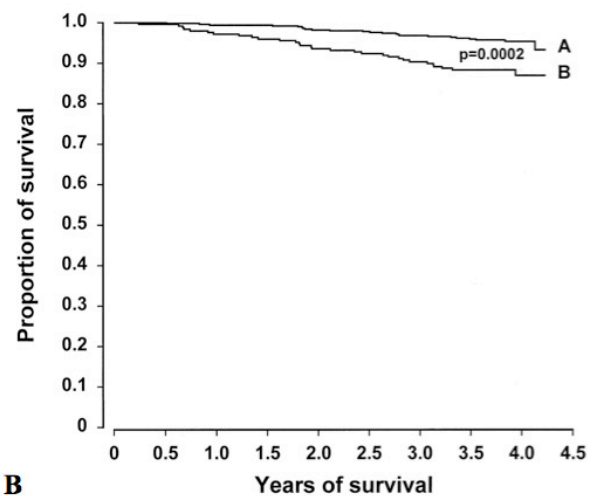
Figure 6. A) Graph of event-free survival for subjects without stenosis and subjects with stenosis according to plaque echogenicity. A, Subjects without stenosis; B, subjects with echogenic and predominantly echogenic plaques; C, subjects with predominantly echolucent plaques; and D, subjects with echolucent plaques. Probability values refer to comparison between group B, C, or D vs control subjects (A). From Mathiesen EB, et al. *Circulation* 2001; **103**: 2171-2175. Reprinted with permission.

B) The 4.2-year survival curves for all subjects with (B) and without (A) carotid stenosis. From Joakimsen O, et al. *Stroke* 2000; **31**: 1871-1876. Reprinted with permission.

independent of MRI lesions. There were no significant differences in tests of speed of information processing, word association, or depression. Cortical infarcts and white matter hyperintensities were equally distributed among persons with and without carotid stenosis. Lacunar infarcts were more frequent in the stenosis group ($P = 0.03$). Carotid stenosis was associated with poorer neuropsychological performance. This could not be explained by a higher proportion of silent MRI lesions in persons with asymptomatic carotid stenosis, making it less likely that the cognitive impairment was caused by silent emboli.

FUTURE POSSIBILITIES

The sixth Tromsø survey started in September 2007 and will be completed in December 2008. So far, more than 6000 persons have participated in the carotid ultrasound part of the study. The majority of these have participated at least once before. Thus, we will have unique long-term follow-up data of more than 13 years on the natural history of initiation and progression of atherosclerosis in a large, population-based sample. The value of the measurements of atherosclerosis is of course dependent on information on risk factors and clinical end-point information obtained in the Tromsø Study. Of special interest is the association between atherosclerosis and cognitive function. Cognitive tests were performed in the fifth survey in 2001 and are currently being repeated in the sixth survey. This will enable us to study the association between atherosclerosis, cognitive function and cognitive decline in a prospective design.

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