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Predictors of progression of ultrasound-assessed carotid artery atherosclerosis.

The Tromsø Study 1994-2008

Marit Herder

A dissertation for the degree of
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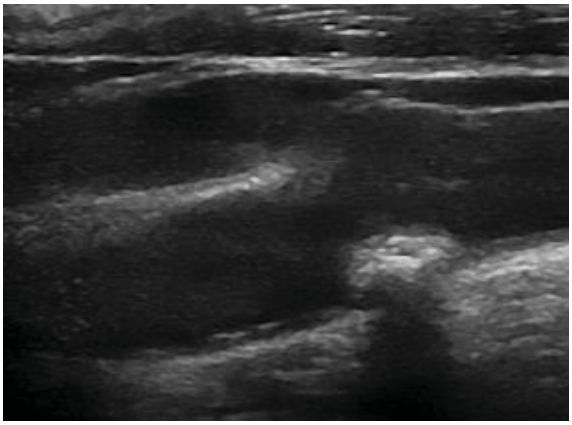

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Contents

Summary

Atherosclerosis is an important underlying cause of cardiovascular disease and death. According to the World Health Organization's Global Burden of Disease Study, ischemic heart disease and stroke combined killed 12.9 million people in 2010, or one in four deaths worldwide. Ultrasound of the carotid arteries can be used to assess the burden of atherosclerosis by measurements of intima-media thickness (IMT) and total plaque area (TPA). Age, male gender, serum cholesterol, blood pressure and smoking are well known risk factors for atherosclerosis, while factors that may influence the progression of atherosclerosis have been less extensively studied. In the longitudinal population-based Tromsø Study, ultrasound assessment of carotid atherosclerosis was performed at in 1994-5 and repeated in 2007-8. We found that age, male sex, total cholesterol, systolic blood pressure and smoking measured at baseline (1994-5) were associated with progression of TPA, whereas male sex, total cholesterol and systolic blood pressure (inverse) were predictors of progression of IMT. The metabolic syndrome, a cluster of metabolic and non-metabolic cardiovascular risk factors including impaired glucose tolerance, visceral adiposity, dyslipidemia, and hypertension, was not associated with progression of IMT or TPA in the total study population. Use of lipid-lowering drugs had a protective effect against progression of carotid atherosclerosis, most pronounced in subjects who had used LLD for 5 years or more.

Sammendrag

Aterosklerose er en viktig underliggende årsak til kardiovaskulær (hjerte-kar) sykdom og død. I følge WHO forårsaket ischemisk hjertesykdom og hjerneslag tilsammen 12.9 millioner dødsfall i 2010, eller 1 av 4 dødsfall i verden. Ultralyd av hovedpulsårene på halsen (arteria carotis) kan brukes for å vurdere grad av aterosklerose i halskarene, ved bruk av å målinger av intima-media-tykkelse (IMT) og totalt plakkareal (TPA). Alder, mannlig kjønn, totalkolesterol, blodtrykk og røyking er velkjente risikofaktorer for aterosklerose. Risikofaktorer for progresjon av aterosklerose har i mindre grad vært studert. I den longitudinelle, populasjonsbaserte Tromsøundersøkelsen ble ultralydsmålinger av aterosklerose i halspulsåren (arteria carotis) målt i 1994-5 og gjentatt i 2007-8. Vi fant at alder, kjønn (mannlig), totalkolesterol, systolisk blodtrykk og røyking var assosiert med progresjon av TPA, mens kjønn (mann), totalkolesterol og systolisk blodtrykk (inverst) var uavhengige risikofaktorer for progresjon i IMT. Metabolsk syndrom, en ansamling av metabolske så vel som ikke-metabolske kardiovaskulære risikofaktorer som inkluderer nedsatt glukosetoleranse, økt livvidde, dyslipidemi og forhøyet blodtrykk, var ikke assosiert med progresjon av IMT aller TPA i studiepopulasjonen som helhet. Bruk av kolesterolsenkende medikamenter hadde en beskyttende effekt på progresjon av aterosklerose, og dette var mest uttalt hos de som hadde brukt slike medikamenter mer enn 5 år.

List of papers

I. Herder M, Johnsen SH, Arntzen KA, Mathiesen EB. **Risk factors of progression of carotid intima-media thickness and total plaque area: A 13 year follow-up study: The Tromsø Study.** *Stroke* 2011; 43:1818-1823

II. Herder M, Arntzen KA, Johnsen SH, Mathiesen EB. **The metabolic syndrome and progression of carotid atherosclerosis over 13 years. The Tromsø Study.** *Cardiovasc Diabetol* 2012;11:77

III. Herder M, Arntzen KA, Johnsen SH, Eggen AE, Mathiesen EB. **Long-term use of lipid-lowering drugs slows progression of carotid atherosclerosis: the Tromsø Study 1994-2008**. *Arterioscler Thromb Vasc Biol* 2013; 33:858-62

Abbreviations

BMI-body mass index

CCA-common carotid artery

CHD-coronary heart disease

CT-computer tomography

CRP-C-reactive protein

CVD-cardiovascular disease

FW-far wall

GSM-grey scale median

HDL-high density lipoprotein

ICA-internal carotid artery

IGT-impaired glucose tolerance

IMT-intima media thickness

LDL-low density lipoprotein

LLD-lipid lowering drugs

MetS-metabolic syndrome

MRI-magnetic resonance imaging

NW-near wall

RCT-randomized controlled trial

SD-standard deviation

TPA-total plaque area

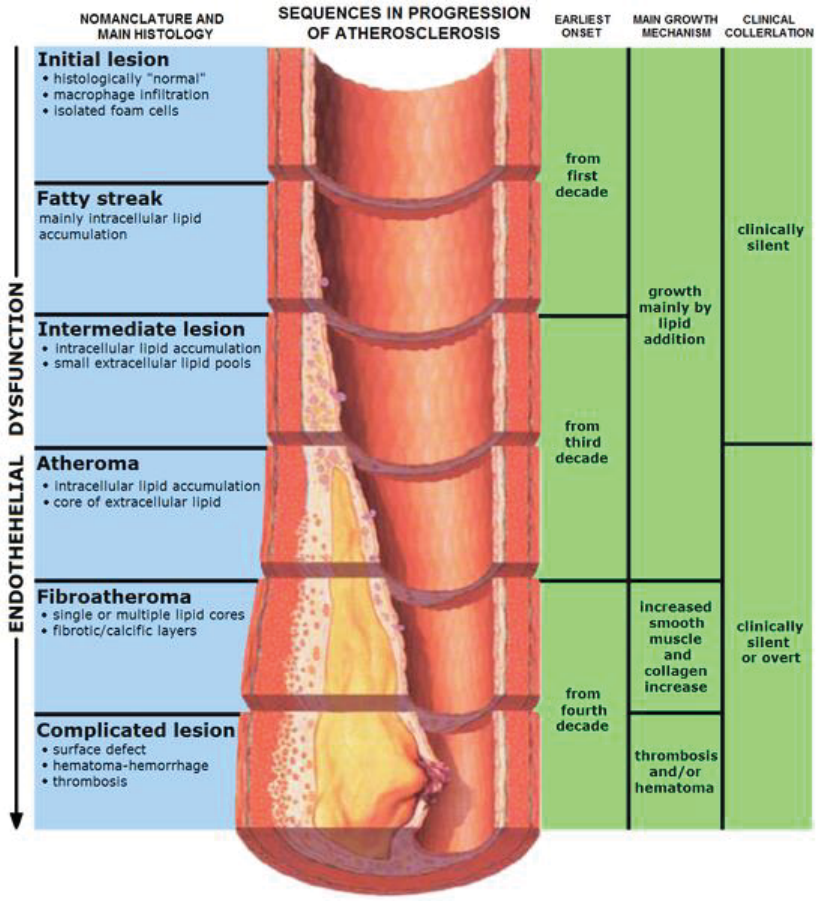
1. Introduction

1.1 Atherosclerosis

Atherosclerosis is the underlying cause of the majority of cardiovascular diseases (CVD) stroke and myocardial infarction.[1-3] Although incidence rates of both coronary heart disease and stroke have been declining in the Western world in the last decades, mortality rates of ischemic heart disease and stroke are still increasing worldwide.[4, 5] Ischemic heart disease and stroke combined killed 12·9 million people in 2010, or one in four deaths worldwide. While the majority of cardiovascular disease events do not occur until middle age, atherosclerosis develops early in life.[6, 7]

The artery wall consists of three layers; the intima, the media and the adventitia. The intima layer or tunica intima is the innermost towards the lumen of the vessel, and consists of endothelial cell and the internal basement membrane. The tunica media consists of smooth muscle cells, and the adventitial layer of connective tissue with elastic fibers and the external basement layer. Atherosclerosis is a condition in which the artery wall thickens as a result of accumulation of fatty deposits within the sub-intimal layer of the vessel wall. Early atherosclerotic changes are fatty streaks or intimal thickening due to accumulation of smooth muscle cells. Intimal thickening may be the beginning of clinically significant lesions.[8] Biochemical, inflammatory and immune-modulating reactions which involve multiple cell types are initiated by the accumulation and oxidation of low-density proteins within the arterial wall. This leads eventually to the development of the raised atherosclerotic lesion – the plaque (Figure 1).

Figure 1. Development of atherosclerosis



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Inflammation is modulated by macrophages that enter the arterial wall. They promote continued recruitment of immune cells and continued accumulation of LDL-cholesterol within the arterial wall. As a part of the immune response, T-lymphocytes enter the intima layer of the arterial wall and are activated following interaction with macrophages. T-cells secrete pro-inflammatory cytokines that contribute to additional atherosclerotic lesion progression. In response to secreted growth factors from activated immune cells, smooth muscle cells produce collagen, thus further promoting the inflammatory process. This results in a pathological thickening of the intima. As a necrotic core develops, continued activation and proliferation of smooth muscle cells contributes to a weakening of the fibrous cap, and

the risk of plaque rupture.[9] Certain sites are predisposed for atherosclerotic lesion formation. Most prone are areas with turbulent rather than laminar flow, such as branching points of arteries. Hence, plaques are much more common in the area of the carotid bifurcation than in the common carotid artery.[9]

1.2. Measurement of atherosclerosis

1.2.1 Imaging modalities

Various imaging modalities can be used to assess atherosclerosis in the arterial wall, where ultrasonography, magnetic resonance imaging (MRI), and computer tomography (CT) and are the most commonly used. Multislice CT is suited for detection of carotid plaques, as well as measurements of remaining lumen diameter. The resolution of a CT scan and the fact that it mainly highlights calcified tissue makes it not suited for detection of the different layers of the vessel wall,[10] and its use in population-based studies is limited by the use of contrast media and radiation exposure. MRI has a high spatial resolution, and the emergence of larger field strengths in MRI holds promise for better quality on imaging studies of small areas, such as the carotid vessel wall. However, the high associated costs limit the use of MRI in epidemiological trials.

B-mode ultrasound is a simple tool, which enables us to visualize the vessel walls of the carotid artery at relatively low costs and without any risk. B-mode ultrasound has been used as imaging modality in the Tromsø Study, and will be thoroughly discussed later in this thesis. Intravascular sonography offers information on both plaque burden and coronary atheroma volume as it depicts the arterial lumen and the arterial wall with high resolution. It is an invasive technique and as such not useful as a tool in large population based studies. Contrast-enhanced ultrasound is a novel and minimally invasive imaging technique that can

be used in assessing atherosclerotic lesions at risk of rupturing, but it has only been tested in limited clinical settings so far.[11] In addition, there is no radiation hazard associated with ultrasonography, which also makes it suited for repeated measurements.

1.2.2. Ultrasonographic measures of the atherosclerosis in the carotid artery

Carotid IMT is widely used as a measure of atherosclerosis. The intima-media thickness is depicted as the "double-line" pattern of the near- and far wall of the vessel, and represents the boundaries of the intima-media layers seen on artery specimens.[12] IMT increases by age and grows more rapidly in the presence of vascular risk factors. Earlier studies have found that IMT progresses approximately 0.015 mm annually.[13]

Because atherosclerosis is so strongly related to both cardiovascular risk factors and CVD, it is widely used as a surrogate endpoint in studies on CVD. There is an ongoing debate as to whether IMT is a valid measurement of atherosclerosis,[14] or merely reflects hypertrophic adaptive response to high shear stress due to hypertension. Plaques are depicted on ultrasonography as focal protrusions into the lumen. As plaques develop at sites prone to atherosclerosis development, i.e. low shear stress and non-laminar turbulent flow,[15] as in the carotid bifurcation or internal carotid artery, they may be more representative of the real atherosclerotic process, compared to the IMT. Atherosclerotic plaque formation represents a stage of atherogenesis related to oxidation of lipids, infiltration and transmigration of lymphocytes and monocytes, inflammation and smooth muscle cell proliferation, and represent a more advanced atheromatous stage.[16] Plaque echogenicity is related to the contents of the plaque, where structures with higher echogenicity have a higher content of dense fibrous tissue and calcification, whereas structures with lower echogenicity (echolucency) have a higher content of lipids. Different scoring systems have been developed

for assessing the plaque burden. The San Daniele study used a plaque score based on degree of stenosis, echogenicity, texture (homogeneity) and surface characteristics in stroke risk prediction.[17] The Rotterdam Study used a plaque score based on the number of sites with ultrasonographically detected plaques in the carotid arteries.[18] The Northern Manhattan Study used maximum plaque thickness as a marker of plaque burden.[19] Spence and coworkers used total plaque area (TPA) and total plaque volume to assess plaque response on treatment in a clinical observational study between 1997 and 2007.[20] Barnett and coworkers found that the average change of plaque area during 2 years was double that of plaque thickness.[21]

1.3. Risk factors for CVD and atherosclerosis

Risk factors for CVD and atherosclerosis have been studied through both population-based as well as clinical studies. Since the first publications from the Framingham study in the 1960s, it was shown that age, gender, cholesterol, hypertension, and smoking were the most important risk factors for coronary heart disease,[22] later often referred to as the traditional cardiovascular risk factors. As these risk factors do not explain all cardiovascular risk, efforts have been made to identifying additional biomarkers for CVD. High density lipoprotein (HDL) cholesterol was identified as a risk factor for myocardial infarction in 1977.[23] Later, several biomarkers have been suggested, such as markers of inflammation (CRP), diabetes mellitus and impaired glucose tolerance and others.[24] In the Emerging Risk Factors Collaboration, a collaborative study on over 1.1 million participants from 104 prospective population-based studies, elevated blood pressure, tobacco use, raised blood glucose, elevated fibrinogen, CRP, diabetes, physical inactivity and obesity/overweight were important risk factors for CVD.[25-28]

There are few studies on risk factors for progression of atherosclerosis. In the Rotterdam Study atherosclerosis was measured at multiple sites in the arterial tree and carotid atherosclerosis was measured as IMT in the common carotid artery. Plaque progression was calculated on the basis of a weighted plaque score ranging from 0-6, based on the number of sites a plaque was detected, divided by possible sites with a ultrasonic picture available.[18] The observation time was 6.5 years, and age, smoking, total cholesterol and systolic blood pressure and/or hypertension were strong, independent risk factors of progression of atherosclerosis. In the Atherosclerosis Risk in Community-study (ARIC), diabetes, current smoking, HDL-cholesterol levels and pulse pressure predicted IMT progression.[29] In a Finnish population based study on men only, age, LDL-cholesterol, smoking, blood leucocyte count and platelet aggregability were the strongest predictors of CCA-IMT progression.[30]

1.4. The metabolic syndrome

The metabolic syndrome (MetS) is a cluster of metabolic and non-metabolic risk factors associated with increased risk of CVD and diabetes.[31-34] Although the concept of the metabolic syndrome has been widely investigated in basic, epidemiological and clinical research for several decades, there is still considerable uncertainty and controversy about the pathophysiology, its definition and prognostic relevance. The underlying pathophysiology is thought to be related to insulin resistance, reflected in the use of the term “insulin resistance syndrome”. Recent evidence indicates that central obesity is a precursor to the development of MetS.[35] Recently, the International Diabetes Federation (IDF), the American Heart Association/National Heart, Lung, and Blood Institute (AHA/NHLBI), the World Heart Federation, the International Atherosclerosis Society, and the International Association for the Study of Obesity joined forces to develop one unified definition of MetS.[28, 35] This

consensus defined the MetS as increased waist circumference (population- and country-specific thresholds), increased fasting triglycerides levels (≥7.1 mmol/L) or drug treatment for elevated triglyceride glucose levels, reduced HDL-cholesterol level (<1.0 mmol/L in men, <1.3 mmol/L in women) or drug treatment for reduced HDL levels, increased blood pressure (systolic ≥130 and/or diastolic ≥85 mm Hg) or antihypertensive drug treatment in a patient with a history of hypertension, and increased fasting glucose (>5.5 mmol/L) or drug treatment of increased glucose levels.[28, 35]

There is considerable doubt about whether the MetS predicts CVD better than the sum of its components. The majority of published reports have failed to prove the added value of MetS in CVD risk prediction.[36] However, a recent systematic review and meta-analysis of 37 longitudinal studies showed that MetS was associated with future cardiovascular events and death with a relative risk (RR) of 1.78 (95% confidence interval (CI) 1.58- 2.00). The association remained after adjusting for traditional cardiovascular risk factors (RR 1.54, 95% CI 1.32 - 1.79).[37]

1.5 Carotid atherosclerosis as predictor of CVD

The Cardiovascular Health Study was one of the first studies to show that increased IMT was associated with increased risk of myocardial infarction and stroke.[38] This has later been reproduced in numerous studies. A meta-analysis by Lorentz and coworkers in 2007 on IMT as predictor of myocardial infarction and stroke in general populations showed that an absolute carotid IMT difference of 0.1 mm increased the future risk for MI of 10-15% and for stroke 13-18%.[39] Measurements of IMT have later been included as a risk stratification tool for CVD prevention in clinical guidelines both in Europe and USA.[40, 41]

Whereas single measurements of IMT at baseline are consistently predictive of CVD, progression of IMT has been used as a surrogate endpoint for CVD outcomes in several clinical trials. However, it is unclear whether progression of IMT is associated with CVD endpoints. In the Multi-Ethnic Study of Atherosclerosis (MESA) with 5082 participants and an observation time of 3.2 years, IMT progression was associated with incident stroke in a cohort free of prevalent CVD and atrial fibrillation at baseline.[42] In a systematic review and meta-regression analysis of IMT as a surrogate endpoint in RCTs of cardiovascular therapies, Goldberger et al found that less progression of IMT was associated with a lower likelihood of nonfatal MI in selected RCTs; however, these findings were inconsistent, suggesting caution in using IMT as a surrogate end point.[43] Costanzo et al showed that regression or slowed progression of carotid IMT did not reflect reduction in cardiovascular events in a meta-analysis on 41 RCTs on different cardiovascular drug therapies.[44]

In the last years, there has been increasing interest in the contribution of plaques in cardiovascular risk assessment. In the Northern Manhattan Study, maximum carotid plaque thickness was associated with increased risk of vascular outcomes.[19] A publication from the Tromsø Study (n=6584) showed that total plaque area in the carotid artery predicted 10 years risk of ischemic stroke in both men and women, while IMT in the far wall of the common carotid artery was not associated with future ischemic stroke.[45] Another publication from the Tromsø study showed that carotid plaque area was a stronger predictor of first-ever MI than was IMT.[46] Spence et.al shoved that carotid plaque area and progression of carotid plaque identified patients with high cardiovascular risk.[47] The Atherosclerosis Risk In Communities (ARIC) study recently showed that adding plaque to IMT and traditional risk factors improved CHD risk prediction.[48] A recent review by Inaba

et al suggests that ultrasound assessment of carotid plaque compared to that of IMT have higher diagnostic accuracy for prediction of future myocardial infarction and detection of coronary artery disease.[49]

The increasing interest in plaque measurements is reflected in the European guidelines for CVD prevention, where both IMT and plaque measurements are recommended in risk assessment in asymptomatic individuals at moderate risk. The latest Mannheim consensus on IMT as a surrogate endpoint of cardiovascular outcomes in clinical trials evaluating the efficacy of cardiovascular risk factor modification has acknowledged that incorporating carotid plaque measurements adds to the cardiovascular risk assessment.[50, 51]

1.6 Lipid lowering medication in relation to carotid artery disease

High cholesterol level is a strong risk factor for atherosclerosis, cardiovascular morbidity and mortality. Statins are the most important lipid-lowering drugs (LLD) in both primary and secondary prevention of CVD. Their main action is on reducing the LDL-cholesterol. Several randomized controlled trials have showed marked effect of statins in reducing risk of myocardial infarction and stroke.[52-57] A review of clinical studies found a significant beneficial effect of statins on IMT progression as well as stroke event rates.[58] Other meta-analyses and RCTs have also shown that statins slow the progression of IMT.[59]
Makris et al performed a meta-analysis on 17 prospective observation studies and 9 RCTs that had assessed the effect of LLD on plaque morphology (size and composition). These studies were small; the largest study included 149 and the smallest 8 participants. Statin treatment was associated with a beneficial effect on plaque morphology, and slower progression, remodeling or even regression of the plaques.[60] In an RCT that compared rovustatin vs. placebo in 492 low risk patients, plaque progression was significantly lower in the statin

group.[61] In study on 654 patients who were randomized to 80 mg atorvastatin vs. 40 mg atorvastatin, more intensively treated patients had no change in atheroma burden, whereas patients with moderate dosage showed progression.[62]

The generalizability of randomized controlled trials may sometimes be limited, and there is little knowledge on whether the effect of statins seen in RCTs applies to progression of atherosclerosis in the general population. In a prospective clinical study on 4378 patients who were referred to a stroke and atherosclerosis prevention clinic, a halt in plaque progression was observed after the implementation of more intensive medical therapy which included increase of statin dosage.[20]

2. Aims of the thesis

The main objective of this thesis was to study different risk factors that may influence progression of carotid atherosclerosis in a general population over 13 years.

2.1. Specific aims:

I. To assess the role of traditional cardiovascular risk factors in progression of carotid IMT and TPA, and to assess whether the association between risk factors and the markers of atherosclerosis were different for IMT and TPA.

II. To study the impact of the metabolic syndrome and its components on progression of carotid atherosclerosis.

III. To study the effect of lipid-lowering drugs on progression of carotid atherosclerosis.

3. Material and methods

3.1 Study population – The Tromsø Study

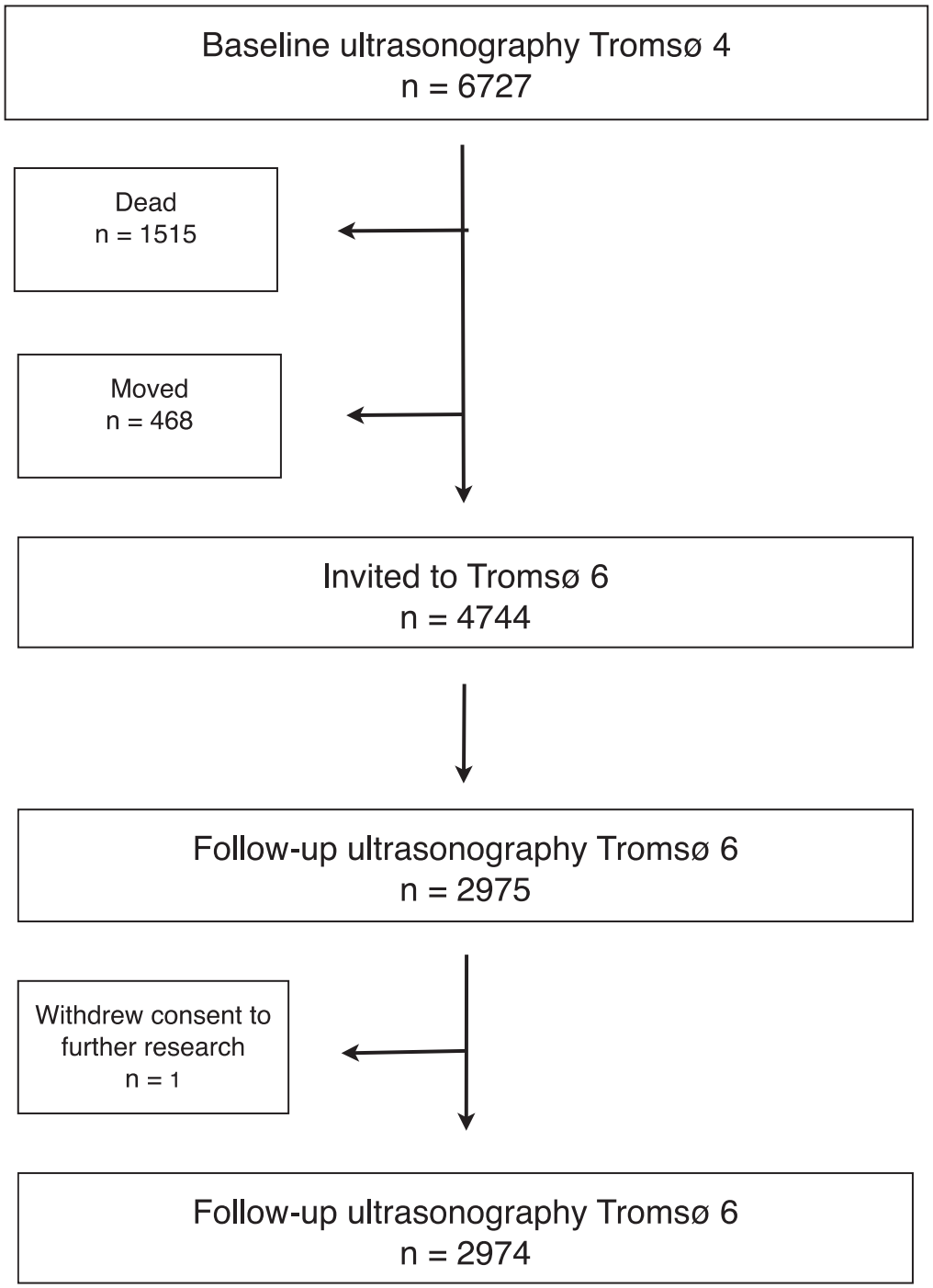
The study population in all three papers consisted of attendees in the Tromsø Study who participated in the carotid ultrasound screenings in both the 4th and 6th survey. The Tromsø Study cohort has been recruited among the inhabitants of the municipality of Tromsø, Norway, situated at 69° N. Among the current 70,000 inhabitants, about 60,000 people live in the city-like town-center, while the rest is scattered throughout the whole municipality. Tromsø is a center of education, research, administration and fishing-related activities. The population is growing and is dominated by Caucasians of mainly Norwegian origin, but also includes a Sami minority. The Tromsø population may be considered as representative of a Northern European, white, urban population.[63, 64]

Since 1974, a total of 6 cross-sectional screening surveys (Tromsø 1-6, 1974-2008) have taken place, 6-7 years apart. The primary focus of the study is on cardiovascular disease, but over the years, the study has gradually expanded to include many other diseases and health related topics. Ultrasonography of the right carotid artery was initiated in the 4th survey (1994-1995), and was repeated in the 5th (2001-2002) and 6th (2007-2008) survey. All subjects were to give written consent to medical research. This consent can be withdrawn or reinstated at any point in time. Hence, the number of participants with valid medical consent can vary over time. In the 4th study, all participants who were between 55-74 years old and 5-10% samples of remaining birth cohorts were invited to a second visit (4-12 weeks after the first visit) with ultrasonography of the carotid artery. A total of 6727 (76 %) of eligible subjects attended the 2nd visit. The study participants in the three papers were all participants in the carotid ultrasound examination of the 4th (1994-1995; baseline) and the 6th (2007-2008; follow-up) survey, with a mean follow-up time of 13 years. During follow-up, 1515 persons

died and 468 persons moved out of the municipality. Of the remaining 4744 subjects who were invited to participate in the 6th survey, 2975 subjects (63 % of the eligible population, 42 % of the baseline population) attended the follow-up carotid ultrasound examination, leaving 2975 subjects and these formed the basis for the study population of Paper 1. Later, one participant withdrew the consent to use the data for research purposes, leaving 2974 subjects to be included in Paper 2 and Paper 3 (Figure 2). Due to lack of information on deaths and emigration for the forty-one participants who had attended Tromsø 4, but who did not have valid written consent at the time the dataset was generated, we made erroneous assumptions about the numbers of participants who died or moved from Tromsø between baseline and follow-up. As a result of this, incorrect numbers of subjects who died, moved, and were invited to the second visit in Tromsø 6 were reported in Paper 2. The correct numbers are reported in Paper 3 and in Figure 2. A correction of the numbers reported in Paper 2 has been submitted to the journal Cardiovascular Diabetology.

The Tromsø Study is approved by the Regional Committee for Medical and Health Research Ethics and the Norwegian Data Protection Authority.

Figure 2. Description of the participation in the ultrasound examination in the 6th survey of the Tromsø Study (2007-2008, follow-up) in those who participated in the carotid ultrasound examination in the 4th survey (1994-1995, baseline).



3.2 Carotid ultrasonography

High-resolution B-mode ultrasonography of the right carotid artery was at baseline performed with a duplex scanner (Acuson Xp10 128, ART-upgraded) equipped with a 7.5 MHz linear array transducer and at follow-up with a duplex scanner GE Vivid 7 with a linear 12 MHz transducer and followed the same scanning and reading procedures and reproducibility as published previously.[65-67] Different sonographers did the baseline and follow-up scanning, and to ensure equal and standardized examination techniques and measurement procedures, all sonographers completed a 2-month pre-study training protocol (Appendix V).

A plaque was defined as a localised protrusion of the vessel wall into the lumen of at least 50% compared to the adjacent IMT. Six locations of the carotid artery were examined for plaque presence; the far (FW) and near walls (NW) of the CCA, the bifurcation (bulb) and the ICA. If more than one plaque was present in a predefined location, the biggest plaque was chosen. The area of each plaque was outlined manually with automatic calculation of plaque area. The areas of all plaques were summarized to give the total plaque area (TPA). Plaque echogenicity was assessed as the standardized median of the gray scale distribution of each plaque (GSM). In subjects with more than one plaque, the GSM of the total plaque area was estimated as a weighted mean of the GSM value of each single plaque.

Automated R-triggered measurement of IMT was performed in the far wall and near wall of the distal CCA,[66] as well as the far wall of the carotid bifurcation and was not limited to plaque-free segments. Final reading of both IMT and plaque data was done off line by the researchers. Measurements of IMT were analysed off line by a semi-automated computerized edge-detection program.[68] In Paper 1, only measurements from the FW of the distal CCA were used. To ensure that the CCA-FW-IMT measurements were done in plaque-free

segments only,[69] we excluded subjects with plaque in the distal CCA (n=145).[70] In Papers 2 and 3, we used the average of the mean IMT in three separate recordings from the three predefined locations in the analyses. Progression of atherosclerosis (ΔIMT and ΔTPA) was calculated subtracting values of IMT or TPA measurements in the 4th survey from the corresponding values in the 6th survey.

3.3 Cardiovascular risk factors

Height and weight were measured in participants wearing light clothing and no footwear. BMI was calculated as weight (kg) divided by height (m). Blood pressure was recorded three times at one-minute intervals after two minutes of seated resting with the use of an automatic device (Dinamap Vital Signs Monitor 1846 Criticon in Tromsø 4, and Dinamap ProCare 300 Monitor in Tromsø 6) and by specially trained technicians. The mean of the last two recordings was used in the report. Analyses of non-fasting serum total cholesterol, HDL-cholesterol and triglycerides were done by enzymatic colorimetric methods. In the 4th survey, lipid levels were measured twice with an interval of 4–12 weeks and the averages of these values were used in the analyses presented in this report. As serum low density lipoprotein (LDL) concentration was not measured in the 4th survey, we calculated LDL levels according to Friedewald's formula: LDL-cholesterol = Total cholesterol – HDL-cholesterol – (0.45 x triglycerides) in subjects with triglyceride levels below 4.52 mmol/L. LDL was analyzed by homogeneous enzymatic colorimetric method in the 6th survey. Serum uric acid in Tromsø 4 was measured by photometry with COBAS® instruments (Roche diagnostics, Switzerland) using an enzymatic colorimetric test, the uricase/PAP method. Glycosylated hemoglobin (HbA1C) levels were measured with a liquid chromatographic procedure. All analyses were performed at the Department of Laboratory Medicine, University Hospital of North Norway.

Information on diabetes mellitus, use of insulin and/or oral anti-diabetic drugs, smoking habits (current daily smoking; yes/no), history of myocardial infarction, angina pectoris, stroke (yes/no) and treated hypertension (never/previous/current) were obtained from self-administered questionnaires (Appendices II-IV). CVD was defined as self-reported prevalent angina pectoris and/or previous myocardial infarction and/or hemorrhagic or non-hemorrhagic stroke. Diabetes was defined as self-reported prevalent diabetes and/or use of anti-diabetic medication.

All variables used in the prediction models in this thesis were obtained at the 4th survey in 1994. An important exception is self-reported use of lipid-lowering drugs, where we in Paper 3 used information obtained in the 4th, 5th and 6th surveys (see below, chapter 3.5). In Paper 2, we also used self-reported information on use of lipid-lowering, anti-platelet and antihypertensive drugs at baseline and follow-up as adjustment variables in supplementary analyses of the relationship between the metabolic syndrome and atherosclerosis. For lipid-lowering and antihypertensive drugs, we used available information from questionnaires and from individual written lists of the brand names of all current medication that the participant had used the previous week (4th survey) or the preceding four weeks (6th survey). For anti-platelet drugs, we used information from the brand name lists only, as the questionnaires did not include information on this item. In Paper 3, we performed additional analyses where participants with CVD at follow-up were excluded (Paper 3, page 859, Results section). We used the same definition for CVD at follow-up as for CVD at baseline.

3.4 Definition of the metabolic syndrome

MetS was defined according to a modified version of the National Cholesterol Education Program Adult Treatment Panel III (NCEP, ATPIII).[34] According to this definition, the MetS is present when three or more of the following five criteria are fulfilled; abdominal obesity, hypertriglyceridemia, low HDL-cholesterol, hypertension, or elevated fasting glucose. Abdominal obesity was defined as waist circumference $\geq$ 102 cm in men and $\geq$ 88 cm in women. Hypertriglyceridemia was defined as elevated triglycerides $\geq$ 150 mg/dL (1.7 mmol/L) or self-reported lipid-lowering drug treatment. Low HDL cholesterol was defined as $<$ 40 mg/dL (1.0 mmol/L) for men and $<$ 50 mg/dL (1.30 mmol/L) for women or self-reported lipid-lowering drug treatment. As fasting glucose was not measured in the Tromsø Study, HbA1c $\geq$ 6.1% and/or non-fasting plasma glucose $\geq$11.1 mmol/L and/or self-reported diabetes and/or use of anti-diabetic medication was defined as impaired glucose tolerance. Hypertension was defined as elevated systolic blood pressure $\geq$ 130 mmHg, or diastolic blood pressure $\geq$ 85 mmHg, or self-reported current antihypertensive drug treatment.

3.5 Lipid-lowering drugs (LLD)

To assess the use of LLD over time, we used all available information on use of medication from the 4th, 5th and 6th surveys. Information was based on questionnaire data and self-reported written lists of all current medication (Appendix II-IV). In the 4th survey (baseline), participants below the age of 70 were asked 'Have you used cholesterol lowering drugs during the last 14 days?'. In the 5th survey in 2001-2002, all participants were asked about current or previous use of LLD ('Do you use cholesterol lowering drugs?', answer categories: currently/previously/never). Information on the use of LLD from the 5th survey was available for 2895 of the 2974 participants (97% of study population). In the 6th survey, all participants were asked about current or previous use of LLD ('Do you use, or have you used cholesterol

lowering drugs?', answer categories: currently/previously/never) and their age when they started with LLD ('If you use or have used cholesterol lowering drugs, how old were you the first time?').

Approximately 60% of those who answered that they used lipid-lowering drugs did answer the follow-up question on how old they were when they started. In addition, the participants were asked to write a list of the brand names of all current medication they had used the previous week (4th survey) or the preceding four weeks (5th and 6th survey) and/or bring the medication with them to the study center. A trained technician at the study site checked the questionnaire and lists of brand names, and participants were asked to confirm if no use of medication was reported. Based on data from all three surveys, we calculated the duration of LLD use. Long-term use of LLD was defined as use either for more than 5 years (current age minus age at start), or use in at least two of the three surveys (each conducted more than 5 years apart). Any-time use of LLD was defined as use in any of the three surveys, with the exclusion of long-term users.

3.6 Statistical methods

Stata SE 11 (StataCorp LP, College Station, TX, USA) and the SAS software, version 9, were used for all analyses. Differences between groups were analyzed with *t*-test or Wilcoxon rank sum tests (continuous variables) and χ^2 (dichotomous variables). In Paper 3, within-group changes between baseline and follow-up were tested by paired (repeated) *t*-test for continuous variables and McNemar's test for categorical variables. Within-group change is presented as unadjusted values. Values are presented as means (SD), median (interquartile range) or numbers (%). TPA was square-root-transformed to approximate normal distribution. Change in IMT (ΔIMT) and square-root-transformed TPA (ΔTPA) was calculated subtracting the

values obtained in the 4th survey from the values from the 6th survey. The independent relationship between the different explanatory variables (cardiovascular risk factors, components of the metabolic syndrome and use of lipid lowering drugs) and the outcome variables (TPA, IMT, ΔTPA and ΔIMT) was assessed in multiple linear regression models, with two-sided p-values < 0.05 considered as statistically significant. In Paper 1, the main objective was to compare the effect of each independent variable on the outcome variables and to assess whether the effects were different for IMT and TPA. We therefore chose a complete case analysis and standardized all dependent and independent variables by use of z-scores. In Paper 2 and 3, we allowed for missingness in both explanatory and response variables. In Paper 1 and 2, the explanatory variables were entered stepwise using the forward selection method. In Paper 2, the multivariable models included LDL cholesterol. Unfortunately, this was incorrectly typed as total cholesterol in two instances in the paper (the Statistical analysis section in Paper 2). A correction has been submitted to the journal.

Interaction by age and sex was examined by adding cross-product terms between sex (or age) and each explanatory variable to the models. In Paper 2, there was significant interaction between sex and MetS in the IMT models, and all analyses were therefore stratified by sex. In Paper 1, the only consistent interaction was between sex and cardiovascular disease.
In paper 3, we found no interaction between use of LLD and age or sex. We therefore chose to perform non-stratified analyses of the total cohort in Paper 1 and 3.

4. Results

4.1. Paper 1

Risk Factors for Progression of Carotid Intima-Media Thickness and Total Plaque Area. A 13-Year Follow-Up Study: The Tromsø Study.

In this study we assessed cardiovascular risk factors of 13-years progression of carotid atherosclerosis in a middle-aged population (mean age at baseline 55.8 years for men and 56.6 years for women). Mean yearly progression of IMT was 0.012 mm in men and 0.011 in women. Mean yearly progression of TPA was 0.82 mm^2 in men and 0.56 mm^2 in women. Plaque growth progressed more rapidly in both men and women after the age of 50, whereas the progression rate of IMT was constant over time.

Sex, age, total cholesterol, systolic blood pressure and smoking were significant predictors of both follow-up IMT and TPA. BMI and HDL-cholesterol were predictors of follow-up IMT only. Use of LLD at baseline and prevalent CVD predicted follow-up TPA but not IMT.

Age, sex, total cholesterol, systolic blood pressure, smoking and use of LLD predicted progression of TPA, whereas sex, total cholesterol and systolic blood pressure predicted IMT progression. Systolic blood pressure was negatively associated IMT progression. The variance explained by traditional cardiovascular risk factors in general was modest, but somewhat greater for ΔTPA (summarized model $R^2 = 0.038$) than for ΔIMT (summarized model $R^2 = 0.010$).

4.2. Paper 2

The metabolic syndrome and progression of carotid atherosclerosis over 13 years. The Tromsø Study.

In this study, we assessed the associations between the MetS and the different components of the MetS (exposure variables) and follow-up levels and progression of IMT and TPA

(outcome variables). MetS was an independent predictor of follow-up IMT and TPA in women, and of follow-up IMT, but not TPA in men. MetS did not predict progression of IMT or TPA in the total cohort, but was associated with progression of IMT and TPA progression in subjects below 50 years of age. In analyses where the components of MetS were entered separately to the models, hypertension predicted follow-up IMT in both men and women and progression of TPA in women. Impaired glucose tolerance was associated with follow-up levels of IMT and TPA and with progression of IMT in men. Low HDL level predicted follow-up IMT in women, and hypertriglyceridemia was associated with follow-up IMT in men and women. Abdominal obesity was not significantly associated with IMT or TPA.

4.3. Paper 3

Long-term use of lipid-lowering drugs slows progression of carotid atherosclerosis.

The Tromsø Study 1994-2008

In this study, we assessed whether long-term use and any-time use of LLD predicted 13-years progression of atherosclerosis. Of the 2974 participants, 443 persons were long-term users and 419 persons were any-time users of LLD.

Both long-term use and any-time use of LLD protected against progression of carotid atherosclerosis. In long-term users, the beta coefficients (β) for ΔIMT and ΔTPA was -0.0387 mm (p=0.0002) and -0.400mm (p=0.006), respectively. In any-time users, the protective effect was weaker; β= -0.024 mm, (p=0.046) for ΔIMT and β= -0.318 mm^2 (p=0.06 for ΔTPA), indicating a dose-response relationship. The estimates remained significant after exclusion of participants with CVD either at baseline and/or at follow-up (n=649).

5. Discussion

5.1 Methodological considerations

5.1.1 Study design

A major strength of the Tromsø Study is the prospective design and the large sample size. The longitudinal design allows for repeated standardized measurements of carotid ultrasound variables as well as cardiovascular risk factors, relevant for adjustment. Our study is one of few studies that have assessed risk factors for progression of atherosclerosis measured as both IMT and plaque in the same individuals.

5.1.2 Internal validity

Internal validity is defined as validity of inference for the source population of study subjects, or in other words, whether obtained results are representative or true for the population under study. Three types of error may threaten the internal validity: selection bias, information bias and confounding.

Selection bias

Selection biases are distortions that occur as a result of procedures used to select subjects and from factors that influence study participation,[71] and the main concern is that association between exposure and outcome among those selected for analysis differs from the association among those eligible.[72] Healthy persons could be more prone to volunteer in population studies. This is known as the healthy participant bias or volunteer/self-selection bias and may dilute true associations between risk factors and outcome by underestimating the true associations between exposure and outcome at follow up. In another Norwegian population-based study (the HUNT Study), the prevalence of common chronic diseases

among non-participants was higher than in participants,[73] and it is likely that the same is true for the Tromsø Study. In prospective cohort studies, selection bias is usually not a major problem since information on exposure is obtained before the development of the outcome of interest. However, selection bias must be considered when the loss to follow-up is high, like in our study.

Of the 6727 persons who participated at baseline, only 2975 attended the follow-up examination. Non-attendance at follow-up was due to migration in 468 and to death in 1515 of the participants at baseline. Those who attended both surveys were healthier than those who were lost to follow-up. In Tromsø 4, 14.3% of all those who attended carotid ultrasonography had self-reported cardiovascular disease, compared to 19.5 % among those who attended ultrasonography in Tromsø 4 but not in Tromsø 6. In contrast, only 7.8% of those participating in both Tromsø 4 and Tromsø 6 had self-reported CVD. In Tromsø 4, 4.6 % had self-reported diabetes, compared to 1.4% among those who attended both Tromsø 4 and Tromsø 6. The total mortality was higher in the MetS group than the non-MetS group; 28.8 vs 19.6%, $p < 0.0001$. It is possible that those with more severe baseline atherosclerosis and progression of atherosclerosis could be more prone to non-attendance due to cardiovascular disease or death and that the use of statins may have been more frequent in this group. We have no specific reason to assume that the relationship between risk factors and atherosclerosis progression would be differential in attendees and non-attendees, but this cannot be ruled out. The considerable loss to follow-up is likely to have affected the effect estimates, and the magnitude of this effect is unknown.

Information bias and misclassification

Information bias is the systematically inaccurate measurement of either the exposure or outcome variable. It can be non-differential (not dependent of the outcome variable) or differential (dependent of the outcome variable). In cohort studies, the information bias tends to be non-differential (not affecting any groups more than others), and this might dilute or underestimates the effect estimate.[71] Sources of error in estimation may be random (lack of precision) or systematic (inaccuracy, bias). The term accuracy refers to how close the measured values are to the true values, while precision refers to the magnitude of the differences between replicated measurements of the same material (reproducibility). Standard protocols and standard operational procedures were used to minimize errors.

In our study, both the exposure variables and the outcome variables could have been misclassified. Important possible sources of information bias are the assessment of IMT and TPA (outcome variables) and the definition and classification of MetS and of use of LLD (exposure variables), which in the following will be discussed in more detail.

Reproducibility of the ultrasound measurements

Several measures were taken to standardize measurements and thereby avoid bias. Technicians underwent a two-month training program prior to study start, and standard operational procedures were used to minimize errors. In order to estimate measurement variability, we conducted studies of between and within observer reproducibility in all surveys.[65-67] Ultrasound equipment was changed between the 5th and the 6th survey, and the inter-equipment variability was therefore also tested.

Reproducibility of ultrasound measurements from Tromsø 4 and 5 has been described earlier and are summarized in Table 1 and 2.[65-67] In Tromsø 4, reproducibility for IMT

measurements was assessed by inviting 111 participants to a second ultrasound scan within 3 weeks of the first scan. On each occasion three sonographers examined the subjects.[66]

In the 6th survey, a consecutive sample of participants was selected for a reproducibility study. Two or three sonographers scanned seventy-six participants on the same day, and 71 of them were rescanned 1-2 weeks later. The sonographers had no knowledge of each other's results, or results from previous examinations. The inter- and intra-observer reproducibility of IMT measurements was similar in Tromsø 4 and 6 (Table 1).

Table 1. Inter-observer and intra-observer variability of pairwise measurements of mean* intima-media thickness in the 4th and 6th surveys of the Tromsø Study.

	Mean (SD)	Mean arithmetic difference (95% CI)	Mean absolute difference (SD)	Limits of agreement
Inter-observer				
Tromsø 4	0.84 (0.28)	-0.01	0.11	±0.29
Tromsø 6	0.96 (0.21)	0.01 (-0.37,0.37)	0.08	±0.21
Intra-observer				
Tromsø 4	0.84 (0.28)	-0.01	0.10	±0.33
Tromsø 6	0.97 (0.203)	0.02 (-0.018, 0.048)	0.08 (0.07)	±0.20

*Average of the mean of three measurements in each of the three locations; the far and near wall of the common carotid and the far wall of the bifurcation.

Reproducibility for plaque measurements and plaque detection was assessed in Tromsø 6 (in the same subjects as described above for IMT) and in combined data from Tromsø 4 and 5. There were 107 paired observations in the baseline study (Tromsø 4), and 83 in the follow-up study (Tromsø 5) (Table 2).[67]

Table 2. Inter-observer and intra-observer variability of pairwise plaque area measurements in the 4th, 5th and 6th surveys of the Tromsø Study.

	Mean (SD)	Mean arithmetic difference (95% CI)	Mean absolute difference (SD)	Limits of agreement
Inter-observer				
Tromsø 4/5*	13.9 (9.0)	-1.0 (-1.4,-0.6)	2.9 (3.4)	±8.6
Tromsø 6†	24.6 (15.0)	-0.8 (-0.01,0.04)	6.1 (5.5)	±16.0
Intra-observer				
Tromsø 4/5 – observer 1	13.4 (7.9)	0.2 (-0.2, 0.7)	1.8 (2.5)	±6.1
Tromsø 4/5 – observer 2	13.8 (8.3)	0.0 (-9.5, 0.7)	2.1 (3.2)	±7.5
Tromsø 6†	23.8 (12.7)	9.6 (-2.6, 5.3)	6.7 (7.0)	± 18.9

*Single plaque measurements.
†Total plaque area measurements.

The arithmetic differences between paired observations were plotted against their average to examine whether the differences were constant over the range of measurements (Figure 3).[74] Any systematic differences between observers would result in the mean of the differences being significantly different from zero. The wider the scatter between the points in the direction of the y-axis, the worse will be the agreement. If the differences are normally distributed, 95 % of the differences will lie within a range of ± 1.96 SDs of the mean arithmetic difference, referred to as the limits of agreement. The mean or median absolute difference represents the typical magnitude, although not the "direction" of the differences. Reproducibility of plaque detection was analyzed with the use of the kappa statistic (κ).[75]

The reproducibility of single plaque area measurements from Tromsø 4 and 5 and TPA measurements from Tromsø 6 are shown in Table 2. As expected, the variability was higher for TPA than for single plaque measurements. More surprising was that the intra-observer reproducibility in Tromsø 6 was similar to or even slightly lower than the inter-observer reproducibility. This is also reflected in the kappa values for plaque detection, which was 0.65 in the inter-observer study and 0.63 in the intra-observer study.

The variability study between the GE Vivid 7 and the Acuson XP10 was performed in January 2012 on 79 subjects, of whom 38 had ≥ 1 plaques. Subjects were examined with the Acuson XP10 first, and all examinations were performed by one person. All readings of IMT and plaques were done by a second person, blinded to the identity of the participants. The results are shown in Table 3. The variability shoved higher IMT values when measured with GE Vivid 7 compared to Acuson XP10, making it likely that the progression of IMT was overestimated due to change of machinery. There was no systematic bias between the ultrasound equipment for the TPA measurements.

Table 3. Inter-equipment variability of pairwise measurements of mean* intima-media thickness and total plaque area in the 4th and 6th surveys of the Tromsø Study.

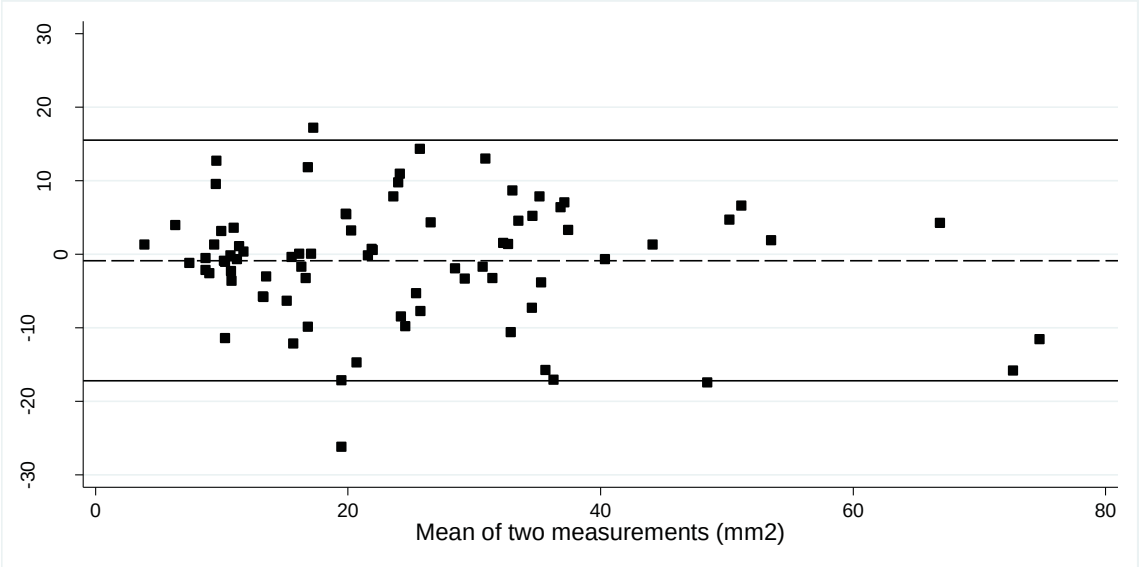
	Mean (SD)	**Mean arithmetic difference (95% CI)**	**Mean absolute difference (SD)**	**Limits of agreement**
Intima- media thickness	0.87 (0.13)	0.15 (0.13, 0.17)	0.15	±0.16
Total plaque area	24.1 (18.2)	2.4 (-0.5, 5.4)	6.5 (5.7)	-

*Average of the mean of three measurements in each of the three locations; the far and near wall of the common carotid and the far wall of the bifurcation.

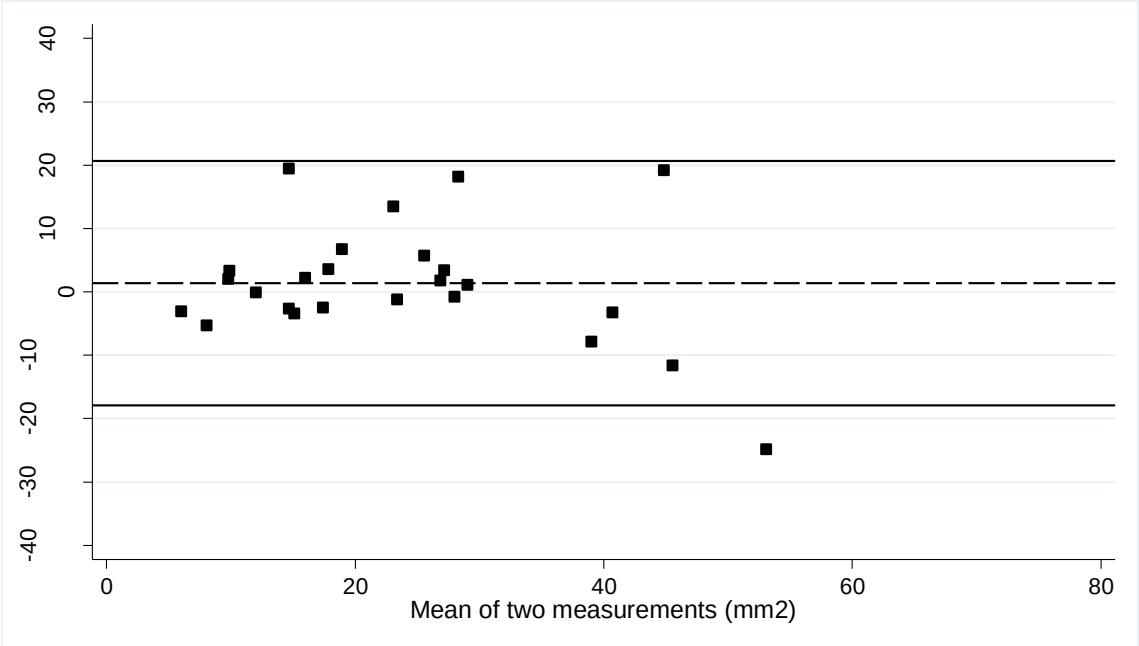
Figure 3. Bland-Altman plots of inter- and intra-observer reproducibility of measurements of total plaque area (TPA) and intima media thickness (IMT).

All panels (a-d) show the difference between pairwise measurements plotted against the average of pairwise measurements. Dotted lines denote the average difference between paired measurements and solid lines denotes the limits of agreement.

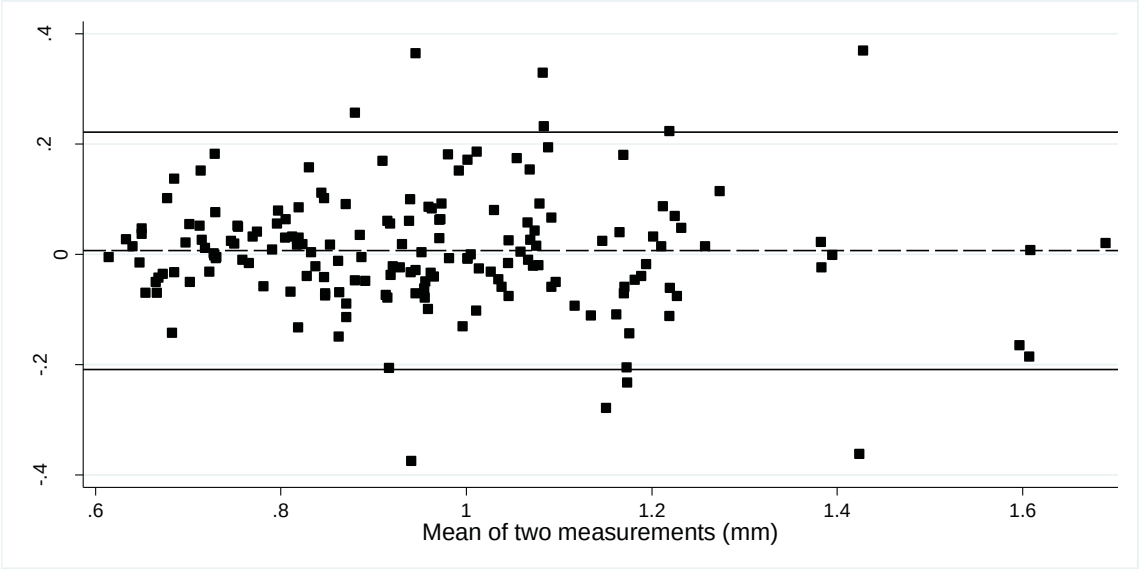
3a: Inter-observer reproducibility of TPA



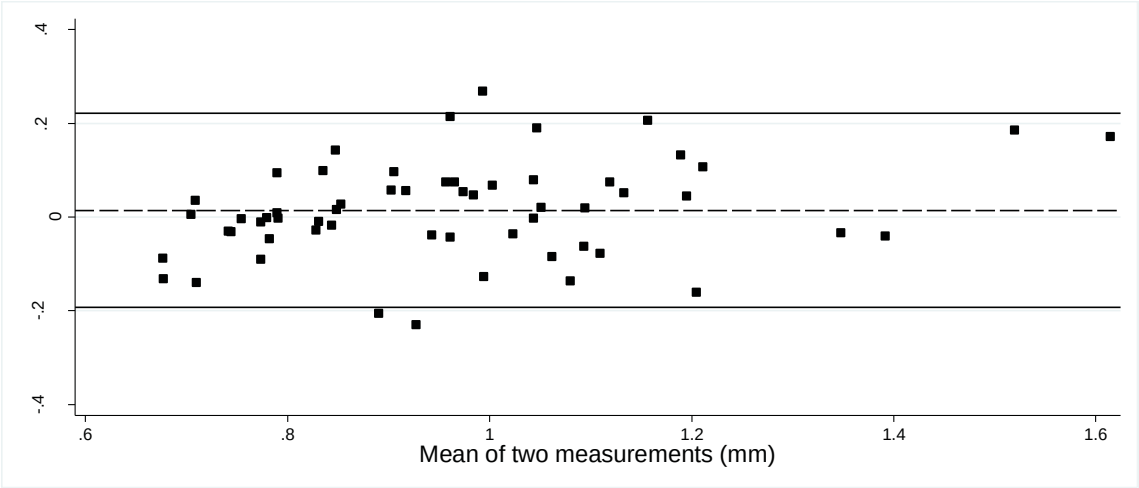
3b: Intra-observer reproducibility of TPA



3c: Inter-observer reproducibility of IMT



3d: Intra-observer reproducibility of IMT



Random measurement errors at baseline and follow-up tend to accumulate and hence attenuate the differences we seek to detect. Imprecision in the measurements of carotid atherosclerosis in our study is likely to have weakened the true relationship between risk factors and the measurements of atherosclerosis. Previous reproducibility data on IMT from Tromsø 4 showed that the variability was not completely at random, but increased with

increasing IMT,[58] indicating that the estimates will be weaker in those with highest IMT levels.

Another source of misclassification of an individual's true atherosclerotic burden is the fact that only the right carotid artery was examined. Including the left carotid artery could have yielded better estimates of the actual atherosclerotic burden and stronger estimates of the relationships between risk factors and atherosclerosis.

The carotid ultrasound protocol in Tromsø 4 was not originally set up to monitor change in IMT or TPA over time. Although several effort were undertaken to standardize measurements, further standardization could have reduced measurement errors. Use of standardized uptake angles could have secured that participants were repeatedly scanned in the same angles as in former surveys. However, scanning at identical angles does not ensure that the areas with the most progression are captured. More intensive training and use of fewer sonographers could also have improved reproducibility. The use of multiple sonographers is prone to yield more imprecise results in longitudinal studies, but is difficult to avoid, especially when the examination volumes are large and the time span long.

Definition of the metabolic syndrome

One of the main components of the metabolic syndrome, impaired glucose tolerance, could not be assessed according to recommended criteria, as fasting glucose was not measured in the Tromsø Study. Instead, we defined HbA1c ≥6.1% and/or non-fasting plasma glucose ≥11.1 mmol/L and/or self-reported diabetes and/or use of anti-diabetic medication as impaired glucose tolerance. The 6.1% cutoff for HbA1c was based on previous studies.[76, 77] The use of HbA1c ≥6.1% as a substitute for fasting plasma glucose ≥5.6 mmol /L may result in

misclassification of subjects with impaired glucose tolerance. However, the HbA1C 6.1% cutoff is supported by a recent report from the Tromsø Study. [78] Those invited were all subjects without self-reported diabetes and with HbA1c in the range 5.8–6.9% and a random sample of approximately 200 subjects with HbA1c 5.3% and 5.4% and 100 subjects with HbA1c 5.5%, 5.6%, and 5.7%, respectively. Of the 4393 who were invited, 3476 participants completed an oral glucose tolerance test (OGTT).The best sensitivity (69.8%) and specificity (81.8%) for diabetes (n=199) were found at HbA1c 6.2%., while the best cut-off points for impaired fasting glucose (n=314) and impaired glucose tolerance (n=404) were found at HbA1c 5.9% and 6.0%, respectively.

Another important source of error was the use of non-fasting lipid levels and the definition of hypertriglyceridemia. While HDL cholesterol is less influenced by non-fasting state, non-fasting triglyceride levels are problematic because of the large variation in pre- and postprandial levels of triglycerides.[79] Furthermore, we included use of LLD (all types) in the definition of the triglyceridemia, while the standard criterion is use of drugs aimed specifically at reduction of triglycerides (fibrates and nicotinic acids).[31, 34] As our definition of MetS differs from the most common definitions, the results cannot be directly compared to other studies that have used standard definitions.

Use of lipid-lowering drugs

The use of LLD in our population increased considerably during the observation period, from 1.6% in 1994, to 27% in 2008. Duration of LLD use was estimated on information obtained from both questionnaires as well as lists of current medication at three points in time.

Data on medication use is prone to recall bias. Although previous studies have shown that repeated self-reported use of drugs that are used regularly, such as statins, reflect chronic

exposure,[80, 81] participants may have failed to report use of LLD because they were not aware of the nature of the drug they were taking, or they could have forgotten to fill in all brand names in the medication lists. After the publication of Paper 3, we have validated the data on drug use obtained in Tromsø 6 against data from the Norwegian Prescription Database. The database was established in January 2004 and receives monthly data on drug prescriptions from all Norwegian pharmacies. Self-reported use of LLD in Tromsø 6 against data from the prescription database 6 months prior to the survey shows a kappa value of 0.94, sensitivity of 98% and specificity of 99% (Anne Elise Eggen, personal communication).

Another form of bias relevant for pharmaco-epidemiological studies is immortal time bias. Immortal time refers to a period of follow-up during which the study outcome cannot occur.[82, 83] In our study, this bias is avoided by the fact that the outcome variable is progression of atherosclerosis over time, which can be measured equally in exposed and non-exposed individuals.

Change in cardiovascular risk factor levels over time

In our studies, we used risk factor measured at baseline as exposure variables. This could be regarded as a source of misclassification, as risk factors levels are likely to change throughout the follow-up period. This was indeed shown for several risk factors in our study (Paper 3, Table 1). Risk factors measurements at several points point in time could have reduced measurement error and better reflected the true exposure levels over time.

Confounding

Confounding, unlike bias, is not an error in the study itself, but is the effect of additional variables that might be responsible for the observed observation. The confounder is an

independent factor for the outcome variable that is also associated or correlated with one or more of the exposure variables. In our studies, we have adjusted for known confounders such as age, sex and various cardiovascular risk factors. In Paper 3, we performed supplementary analyses where subjects with CVD were excluded. Nevertheless, we cannot exclude that the results may have been confounded by unknown variables that we were unable to account for.

5.1.3. External validity/generalizability

The external validity applies to the ability to generalize the results to other populations than the study population. For a study to hold external validity, it must be internally valid. The Population Registry was the source for the invitation issued. The age and sex distribution of the Tromsø Study reflects the Tromsø population in general and are not substantially different from other Western populations with regards to prevalence of CVD and risk factor levels. The IMT levels and plaque prevalence are comparable to those in other European and American populations. Hence, our results are likely to be applicable to similar Caucasian, Northern European populations.[63, 64]

5.2 Some statistical considerations

In all three papers included in this thesis, the outcome of interest was change in a continuous variable (IMT or TPA). We measured IMT and TPA at baseline and then again at follow-up. Both follow-up levels and a change score, calculated as the difference between the follow-up value and the baseline value, was used as outcome variables. In studies of change over time, it is important to consider the regression toward the mean (RTM) phenomenon in order to separate real change from the effect of natural variation. RTM describes the phenomenon where extreme measurements at one measurement point will tend to reverse against a less extreme value upon subsequent measurement. This occurs when values observed with random

error; i.e. a non-systematic variation in the observed values around a true population mean.[84, 85] The variation may be caused by random measurement error or random fluctuation in a subject. The magnitude of the regression effect can be determined from the correlation between pre-and post-measurements.[86] In general, within the same subject, extreme values (high or low) are likely to be followed by less extreme values closer to the subject's true mean. The effect of RTM is not restricted to individual measurements, but also applies to the group level, and is especially important to take into consideration when comparisons are done in groups that are categorized on the basis on the initial values.[86, 87]

Both the use of change scores (also referred to as growth score) and RTM have been subject to much debate within the scientific community. While some authors warn against use of change scores and find them unreliable,[88, 89] others argue that difference scores are very reliable in situations where individual variations in true change exists.[90, 91] There is also an on-going debate how to deal with RTM in studies of change. The most widespread statistical technique is probably analysis of covariance (ANCOVA) with adjustment for baseline values of the outcome of interest.[87, 92] Many authors recommend adjustment for baseline values in all longitudinal studies of change to avoid the effect of any random differences in initial levels across the groups that are being compared.[86, 92].

Other authors have argued against adjustment for baseline values. One of the strongest opponent against the view that RTM is unavoidable in longitudinal research, and that change scores are unreliable, is David Rogosa. Rogosa argues that rather than a law of nature, RTM is a statistical tautology arising from the use of standard deviation as a metric of change, and may not occur if a non-standardized metric is used.[93] Rogosa's view has been supported by others who argue that although adjustment for baseline values of the dependent variable may

ameliorate certain biases, it introduces others that often will exceed the bias eliminated.[94, 95] When the outcome variable is measured with error, inclusion of baseline values as a covariate may result in the finding of a relationship between the observed change and the explanatory variables even when no such association exist between the true outcome and the explanatory variables.[94, 96] Several alternative statistical techniques for the analysis of change have been proposed.[97, 98]

In our studies, we chose not adjust for baseline values of IMT or TPA. This was in line with our previous choice in studies on change in ultrasound-assessed atherosclerosis based on the Tromsø Study.[99] An alternative approach could have been to adjust for both baseline and follow-up levels, or the mean of baseline and follow-up, as this variable is independent of the change score, which the baseline value is not. This approach is used in an individual data meta-analysis on predictors of change of IMT, the Individual progression of carotid intima media thickness as a surrogate of vascular risk (PROG-IMT) study, in which the Tromsø Study is one of the participating centers (Simon G. Thompson, personal communication). However, although the baseline levels of TPA and IMT were not used to define groups for later comparison, adjustment for baseline levels to avoid RTM could have been considered appropriate due to differences in the baseline levels of atherosclerosis in subjects with and without MetS (Paper 2) and in users and non-users of LLD (Paper 3). In Supplementary Tables 1 through 5, the analyses from Paper 1, 2, and 3 have been repeated in models with adjustment for baseline values in analyses with follow-up levels as outcome, and for baseline and the mean of baseline and follow-up values in analysis with the change score as outcome. In addition, the analyses originally presented in Paper 2 were done with variables standardized by use of z-scores for better to be able to compare the strength impact of the variables on the outcome. The results show substantial differences depending on the choice of

model. Overall, adjustment for baseline values in analyses with follow-up levels as the outcome variable tended to weaken the estimates originally presented in Paper 1 (Supplementary Table 1). The explained variance increased and was mostly explained by the baseline values of IMT and TPA, respectively. In analyses of change scores as dependent variable, adjustment for baseline values strengthened the estimates, while the mean of baseline and follow-up values weakened the estimates. The overall explained variance increased in both models.

Similarly, the analyses in Paper 2 were substantially influenced by choice of model. In analyses with MetS, age, LDL cholesterol, smoking and baseline IMT /TPA as predictor variables and follow-up levels of IMT/TPA as outcome, the baseline levels explained most of the variance while the other estimates were weakened. As opposed to this, adjustment for baseline variables tended to strengthen the estimates in analyses with change scores, and in these analyses, MetS was a significant predictor of change in both IMT and TPA in men, but not in women. Adjustment for mean of baseline and follow-up did not influence the relationship between MetS and the outcome variables. Analyses where each component of MetS was entered as separate variables showed similar results as described for MetS, with a tendency toward weakening of the estimates. Age became negatively associated with change in IMT after adjustment for mean of baseline and follow-up values, and the same did LDL cholesterol in men and hypertension in women. These associations are counter-intuitive and difficult to explain, and seem to be a result of this particular model.

In Paper 3, the relationship between use of LLD and progression of atherosclerosis was no longer significant after adjustment for baseline values, while the estimates were strengthened after adjustment for the mean of baseline and follow-up values.

The tables illustrate the complexity of analyses of change and that the choice of model has substantial impact on the obtained results. It is recommended that researchers should decide upon the analytical strategy prior to performing the statistical analyses, and thereafter adhere to the initial analysis plan.[92] Therefore, in the further discussion of the results in this thesis, I have chosen to refer to the original analyses (as presented in the papers).

5.3 Discussion of main results

5.3.1 Cardiovascular risk factors and progression of atherosclerosis

We found that age, male sex, total cholesterol, HDL-cholesterol (inversely), systolic blood pressure, body mass index and smoking predicted follow-up levels of IMT. In contrast, systolic blood pressure was negatively associated with progression of IMT. These apparently conflicting results are however partly supported by previous studies. In the Rotterdam Study, systolic blood pressure was a predictor of severe progression of IMT, but not for mild or moderate progression.[18] In the ARIC study, hypertension was not an independent risk factor for yearly progression of IMT.[29] Salonen and Salonen did not find an association between hypertension or current blood pressure level and a two year progression of IMT.[30] One possible explanation might be that there is larger within-person variance of progression of IMT than of cross-sectional measurements, and this can result in stronger estimates for cross-sectional analyses compared to longitudinal studies with several measurements.

The annual progression of IMT was 0.012mm in men and 0.011mm in women, which is higher than the annual progression of 0.009 mm in the ARIC cohort.[29] As discussed above, we may have overestimated the progression of IMT due to change of equipment. However, in

a pooled analysis on annual progression of IMT in control subjects who participated in RCTs, the annual change of IMT was 0.015 mm.[13]

We found that the progression rate of TPA increased by age, whereas progression of IMT was constant across age groups. Also, the explained variance of CVD risk factors on IMT and TPA progression was low to moderate; 10% for ΔIMT, and 38% for ΔTPA. This might be due to different qualitative aspects of these two measures of atherosclerosis, with medial thickening increasing at a more constant level, while plaque size increases more rapidly over the years. There is an ongoing debate as to what ultrasonographic measures most correctly describe the atherosclerotic process.[47, 49, 100-103] Plaques and IMT are highly correlated, but may not reflect the same biological aspects of atherogenesis, and these entities may have different relations to cardiovascular risk factors as well as to clinical vascular disease. IMT mainly represents hypertensive medial hypertrophy, whereas TPA represents the intimal thickening constituting atherosclerosis.[104] TPA has been stronger correlated with traditional risk factors than IMT in previous studies.[14, 101]

The IMT is a small structure, only fractions of a millimeter and the resolution of the B-mode ultrasonography is below the quantities being measured. This makes the method less suitable for repeated measurements, as random measurements errors at baseline and follow-up are accumulated, thus attenuating the differences we aim to detect.[105] The TPA measures a larger quantity, and may thus being more robust against measurement errors. This may be one of the reasons for lack of association between progression of IMT and cardiovascular endpoints in Paper 1.

There was significant interaction between cardiovascular disease and sex for all outcome variables. In forward stepwise multivariable models in women, CVD was negatively associated with follow-up levels of IMT (standardized β= -0.089, p=0.006) and with change in TPA (standardized β= -0.074, p=0.009), while there was no association between CVD and change in IMT or follow-up levels of TPA. In men, CVD was positively associated with follow-up levels of TPA in men (standardized β=0.077, p=0.002), but not with follow-up levels of IMT or progression of IMT or TPA. There was no interaction between cardiovascular risk factors and age.

5.3.2 Metabolic syndrome and progression of carotid atherosclerosis

We found that the MetS was an independent predictor of follow-up levels of IMT and TPA, for men, and for IMT in women. There was no overall association between MetS and progression of IMT and TPA. Among the components of the MetS, hypertension predicted TPA progression in women and impaired glucose tolerance predicted IMT progression in men.

There is an ongoing debate as to whether the MetS is a better predictor of cardiovascular disease than the sum of its components. Hypertension was the component of MetS most consistently associated with follow-up levels of IMT and TPA among men and women. Hypertension was also associated with progression of TPA in women. Impaired glucose tolerance (IGT) was associated with follow-up levels and progression of IMT in men only. The underlying pathophysiology of MetS is thought to be related to IGT, and associations between levels of IMT and IGT has been shown previously in cross-sectional studies.[106, 107]

Previous cross-sectional studies have found increasing IMT in subjects with MetS.[108-110] Few studies have assessed the relationship between MetS and progression of IMT. The European Lacidipine Study on Atherosclerosis (ELSA) found that progression of IMT was slightly higher in persons with MetS, but this association was not significant after adjustment for cardiovascular risk factors.[111]

In our study, MetS was associated with progression of atherosclerosis in subjects below 50 years of age. This is in line with the findings in a Finnish study where progression of IMT was associated with MetS in subjects aged 27-37 years.[112] As the atherosclerotic process accelerates through the 4^{th} and 5^{th} decade, this might imply that the MetS is more important as a risk factor in the early stages of the atherosclerotic process. Caution must be taken to this hypothesis, as longitudinal data on this field are scarce.

In a cross-sectional study from the multiethnic Northern Manhattan Study (NOMAS), MetS and the number of MetS components was significantly associated with plaque presence.[113] The proportion of subjects with MetS was high in NOMAS (49%). The NOMAS study also showed a significant association between MetS and arterial stiffness, independent of the presence of carotid plaque and intima media thickness.[114] In the Bruneck Study, persons with MetS had higher rates of progression of carotid atherosclerosis, measured as formation of new plaques and carotid stenosis.[115] We cannot exclude that the lack of overall association between MetS and atherosclerosis progression in our study may be due to imprecise measurements of both the predictor and the outcome variables, as discussed above. Our definition of MetS may have led to some misclassification. Imprecision in measurement of outcome variables of progression may be due to accumulation of random measurement

error at baseline and follow-up, and this can attenuate the differences and inability to detect a true relationship between MetS and change in carotid atherosclerosis.

Supplemental Tables 3 and 4 show the results of the multivariable-adjusted analyses in Paper 2 repeated with all variables standardized by use of z-scores. These show that for both men and women, age was the strongest predictor of follow-up levels of IMT and TPA. Of the components of the MetS, hypertension is also in men by far the strongest predictor of follow-up levels of IMT and TPA. For ΔIMT, neither the Mets nor its components have significant associations in neither men nor women. Hypertension was significantly associated with ΔTPA in men.

We found that MetS predicted progression of IMT and TPA in men below 50 years of age, but not in the total cohort. However, we found no significant interaction between age and MetS, and in retrospect, we find that we may have put too much emphasis on this finding as presented in the paper.

5.3.3 Effect of lipid-lowering drugs on progression of atherosclerosis

We found that long term as well as any-time use of LLD protected against progression of IMT and TPA. Similar results have been shown in RCTs [54, 116, 117] and clinical patient series.[20, 118] Our study indicates that this also applies to subjects belonging to the general population. As statins are related to slower progression of atherosclerosis, it has been assumed that the protective effect of statins on CVD is at least partly mediated through the effect on atherosclerosis. In a meta-analysis of 28 RCTs with 15 598 patients, Goldberger et al. found that change in IMT was a significant predictor for myocardial infarction. Surprisingly, and as acknowledged by the authors, counter-intuitively, no significant relationship was found

between mean change in IMT and nonfatal myocardial infarction when the analysis was limited to RCTs which evaluated statin therapy. The authors conclude that this may implicate that the protective effect of statins on cardiovascular disease is not mediated through IMT.[43]

The protective effect of statins was present also when we excluded subjects with prevalent CVD at baseline and/or follow-up. The benefit of taking statins in primary CVD prevention has been much debated.[119] A recent Cochrane update on statins for the primary prevention of cardiovascular disease, showed reductions in all-cause mortality, major vascular events and revascularization among people without evidence of CVD treated with statins, and without any excess of adverse events.[120, 121] The results of our study should not be taken in favor of use of statins in primary prevention, as no analyses of net positive effects (whether the beneficial effects of statins outweighed the possible detrimental effects) nor of cost-effectiveness could be done.

In the Results section in Paper 3, the change in IMT and square-root-transformed TPA levels in non-users and long-term users of LLD was unintentionally mixed up. An erratum has been submitted to the journal. In Figure panel B, square-root-transformed TPA levels were shown.

6. Conclusions

We found that progression of TPA was independently predicted by age, sex, total cholesterol, systolic blood pressure and smoking. Total cholesterol, sex and systolic blood pressure (inversely) were associated with IMT progression.

Plaque growth progressed more rapidly after the age of 50, while the progression rate of IMT was constant over time.

MetS was an independent predictor of follow-up IMT and TPA in men and of IMT in women. MetS was not an independent predictor of progression of TPA and IMT in the total cohort.

Use of LLD had a protective effect on both TPA and IMT progression, most pronounced in long-term users.

References

1. Strong JP SL, Restrepo C: **Atherosclerosis in persons with coronary heart disease.** *Lab Invest* 1968, 18(5):527-537.
2. Restrepo C Morgogni M, Solberg LA: **Atherosclerosis in persons with selected diseases.** *Lab Invest* 1968, 18(5):552-559.
3. Libby P, Ridker PM, Maseri A**: Inflammation and atherosclerosis.** *Circulation* 2002, 105(9):1135-1143.
4. Lozano R NM, Foreman K, Lim S, Shibuya K, Aboyans V et al: **Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010.** *Lancet* 2012, 380(9859):2095-2128
5. Feigin VL, Lawes CM, Bennett DA, Barker-Collo SL, Parag V: **Worldwide stroke incidence and early case fatality reported in 56 population-based studies: a systematic review.** *Lancet Neurol* 2009, 8(4):355-369.
6. Duff GL McMillan GC: **Pathology of atherosclerosis**. *Am J Med* 1951, 1:92-108.
7. Holman RL MHJ, Strong JP, Geer JC: **The natural history of atherosclerosis. The early aortic lesions as seen in New Orleans in the middle of the 20th century.** *Am J Pathol* 1958, 2:209-235.
8. Finn AV, Kolodgie FD, Virmani R: **Correlation between carotid intimal/medial thickness and atherosclerosis: a point of view from pathology.** *Arterioscler Thromb Vasc Biol* 2010, 30(2):177-181.
9. Libby P: **Inflammation in atherosclerosis.** *Nature* 2002, 420(6917):868-874.
10. de Groot E, van Leuven SI, Duivenvoorden R, Meuwese MC, Akdim F, Bots ML, Kastelein JJ: **Measurement of carotid intima-media thickness to assess progression and regression of atherosclerosis.** *Nat Clin Pract Cardiovasc Med* 2008, 5(5):280-288.
11. Partovi S, Loebe M, Aschwanden M, Baldi T, Jager KA, Feinstein SB, Staub D: **Contrast-enhanced ultrasound for assessing carotid atherosclerotic plaque lesions.** *Am J Rad* 2012, 198(1): 13-19.
12. Pignoli P, Tremoli E, Poli A, Oreste P, Paoletti R: **Intimal plus medial thickness of the arterial wall: a direct measurement with ultrasound imaging.** *Circulation* 1986, 74(6):1399-1406.
13. Bots ML, Evans GW, Riley WA, Grobbee DE: **Carotid intima-media thickness measurements in intervention studies: design options, progression rates, and sample size considerations: a point of view.** *Stroke* 2003, 34(12):2985-2994.
14. Al-Shali K, House AA, Hanley AJ, Khan HM, Harris SB, Mamakeesick M, Zinman B, Fenster A, Spence JD, Hegele RA: **Differences between carotid wall morphological phenotypes measured by ultrasound in one, two and three dimensions.** *Atherosclerosis* 2005, 178(2):319-325.
15. Beere PA, Glagov S, Zarins CK: **Experimental atherosclerosis at the carotid bifurcation of the cynomolgus monkey. Localization, compensatory enlargement, and the sparing effect of lowered heart rate.** *Arterioscler Thromb* 1992, 12(11):1245-1253.
16. Bui QT, Prempeh M, Wilensky RL: **Atherosclerotic plaque development.** *Int J Biochem Cell Biol* 2009, 41(11):2109-2113.
17. Prati P, Tosetto A, Casaroli M, Bignamini A, Canciani L, Bornstein N, Prati G, Touboul PJ: **Carotid plaque morphology improves stroke risk prediction: usefulness of a new ultrasonographic score.** *Cerebrovasc Dis* 2011, 31(3):300-304.

18. van der Meer IM, Iglesias del Sol A, Hak AE, Bots ML, Hofman A, Witteman JC: **Risk factors for progression of atherosclerosis measured at multiple sites in the arterial tree: the Rotterdam Study.** *Stroke* 2003, 34(10):2374-2379.
19. Rundek T, Arif H, Boden-Albala B, Elkind MS, Paik MC, Sacco RL: **Carotid plaque, a subclinical precursor of vascular events: the Northern Manhattan Study.** *Neurology* 2008, 70(14):1200-1207.
20. Spence JD, Hackam DG: **Treating arteries instead of risk factors: a paradigm change in management of atherosclerosis.** *Stroke* 2010, 41(6):1193-1199.
21. Barnett PA, Spence JD, Manuck SB, Jennings JR: **Psychological stress and the progression of carotid artery disease.** *J Hypertens* 1997, 15(1):49-55.
22. Truett J CJ, Kannel W: **A multivariate analysis of the risk of coronary heart disease in Framingham.** *J Chronic Dis* 1967, 20:511-524.
23. Miller NE ,Thelle D, Førde OH, Mjøs OD: **The Tromsø heart-study. High-density lipoprotein and coronary heart-disease: a prospective case-control study.** *Lancet* 1977, 1(8019):965-968.
24. Fruchart JC, Nierman MC, Stroes ES, Kastelein JJ, Duriez P: **New risk factors for atherosclerosis and patient risk assessment.** *Circulation* 2004, 109(23):15-19.
25. Kaptoge S, Di Angelantonio E, Pennells L, Wood AM, White IR, Gao P, Walker M, Thompson A, Sarwar N, Caslake M et al: **C-reactive protein, fibrinogen, and cardiovascular disease prediction.** *New Engl J Med* 2012, 367(14):1310-20
26. Di Angelantonio E, Gao P, Pennells L, Kaptoge S, Caslake M, Thompson A, Butterworth AS, Sarwar N, Wormser D, Saleheen D et al: **Lipid-related markers and cardiovascular disease prediction.** *JAMA* 2012, 307(23):2499-2506.
27. Wormser D, Kaptoge S, Di Angelantonio E, Wood AM, Pennells L, Thompson A, Sarwar N, Kizer JR, Lawlor DA, Nordestgaard BG et al: **Separate and combined associations of body-mass index and abdominal adiposity with cardiovascular disease: collaborative analysis of 58 prospective studies.** *Lancet* 2011, 377(9771):1085-1095.
28. Sarwar N, Gao P, Seshasai SR, Gobin R, Kaptoge S, Di Angelantonio E, Ingelsson E, Lawlor DA, Selvin E, Stampfer M et al: **Diabetes mellitus, fasting blood glucose concentration, and risk of vascular disease: a collaborative meta-analysis of 102 prospective studies.** *Lancet* 2010, 375(9733):2215-2222.
29. Chambless LE, Folsom AR, Davis V, Sharrett R, Heiss G, Sorlie P, Szklo M, Howard G, Evans GW: **Risk factors for progression of common carotid atherosclerosis: the Atherosclerosis Risk in Communities Study, 1987-1998.** *Am J Epidemiol* 2002, 155(1):38-47.
30. Salonen R, Salonen JT: **Progression of carotid atherosclerosis and its determinants: a population-based ultrasonography study.** *Atherosclerosis* 1990, 81(1):33-40.
31. Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, Gordon DJ, Krauss RM, Savage PJ, Smith SC, Jr. et al: **Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement.** *Circulation* 2005, 112(17):2735-2752.
32. Eckel RH, Alberti KG, Grundy SM, Zimmet PZ**: The metabolic syndrome.** *Lancet* 2010, 375(9710):181-183.

33. Alberti KG, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, Fruchart JC, James WP, Loria CM, Smith SC, Jr.: **Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity.** *Circulation* 2009, 120(16):1640-1645.

34. **Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report.** *Circulation* 2002, 106(25):3143-3421.

35. Cameron AJ, Boyko EJ, Sicree RA, Zimmet PZ, Soderberg S, Alberti KG, Tuomilehto J, Chitson P, Shaw JE: **Central obesity as a precursor to the metabolic syndrome in the AusDiab study and Mauritius.** *Obesity* 2008, 16(12):2707-2716.

36. Kahn R, Buse J, Ferrannini E, Stern M: **The metabolic syndrome: time for a critical appraisal: joint statement from the American Diabetes Association and the European Association for the Study of Diabetes.** *Diabetes Care* 2005, 28(9):2289-2304.

37. Gami AS, Witt BJ, Howard DE, Erwin PJ, Gami LA, Somers VK, Montori VM: **Metabolic syndrome and risk of incident cardiovascular events and death: a systematic review and meta-analysis of longitudinal studies.** *J Am Coll Cardiol* 2007, 49(4):403-414.

38. 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.** *New Engl J Med* 1999, 340(1):14-22.

39. Lorenz MW, Markus HS, Bots ML, Rosvall M, Sitzer M: **Prediction of clinical cardiovascular events with carotid intima-media thickness: a systematic review and meta-analysis.** *Circulation* 2007, 115(4):459-467.

40. Greenland P, Alpert JS, Beller GA, Benjamin EJ, Budoff MJ, Fayad ZA, Foster E, Hlatky MA, Hodgson JM, Kushner FG et al: **2010 ACCF/AHA guideline for assessment of cardiovascular risk in asymptomatic adults: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines**. *Circulation* 2010, 122(25):e584-636.

41. Perk J, De Backer G, Gohlke H, Graham I, Reiner Z, Verschuren M, Albus C, Benlian P, Boysen G, Cifkova R et al: **European Guidelines on cardiovascular disease prevention in clinical practice (version 2012). The Fifth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice (constituted by representatives of nine societies and by invited experts).** *Eur Herat J* 2012, 33(13):1635-1701.

42. Polak JF, Pencina MJ, O'Leary DH, D'Agostino RB: **Common carotid artery intima-media thickness progression as a predictor of stroke in multi-ethnic study of atherosclerosis.** *Stroke* 2011, 42(11):3017-3021.

43. Goldberger ZD, Valle JA, Dandekar VK, Chan PS, Ko DT, Nallamothu BK: **Are changes in carotid intima-media thickness related to risk of nonfatal myocardial infarction? A critical review and meta-regression analysis.** *Am Heart J* 2010, 160(4):701-714.

44. Costanzo P, Perrone-Filardi P, Vassallo E, Paolillo S, Cesarano P, Brevetti G, Chiariello M: **Does carotid intima-media thickness regression predict reduction of cardiovascular events? A meta-analysis of 41 randomized trials.** *J Am Coll Cardiol* 2010, 56(24):2006-2020.

45. Johnsen SH, Mathiesen EB, Joakimsen O, Stensland E, Wilsgaard T, Løchen ML, Njølstad I**: Carotid atherosclerosis is a stronger predictor of myocardial infarction in women than in men: a 6-year follow-upstudy of 6226 persons: the Tromsø Study.** *Stroke* 2007, 38(11):2873-2880**.**

46. Mathiesen EB, Johnsen SH, Wilsgaard T, Bønaa KH, Løchen ML, Njølstad I**: Carotid plaque area and intima-media thickness in prediction of first-ever ischemic stroke: a 10-year follow-up of 6584 men and women: the Tromsø Study.** *Stroke* 2011, 42(4):972-978.

47. Spence JD, Eliasziw M, DiCicco M, Hackam DG, Galil R, Lohmann T: **Carotid plaque area: a tool for targeting and evaluating vascular preventive therapy.** *Stroke* 2002, 33(12):2916-2922.

48. Nambi V, Chambless L, Folsom AR, He M, Hu Y, Mosley T, Volcik K, Boerwinkle E, Ballantyne CM: **Carotid intima-media thickness and presence or absence of plaque improves prediction of coronary heart disease risk: the ARIC (Atherosclerosis Risk In Communities) study.** *J Am Coll Cardiol* 2010, 55(15):1600-1607.

49. Inaba Y, Chen JA, Bergmann SR: **Carotid plaque, compared with carotid intima-media thickness, more accurately predicts coronary artery disease events: a meta-analysis.** *Atherosclerosis* 2012, 220(1):128-133.

50. Rundek T, Salameh MJ: **Carotid plaque assessment: a bumpy road to improved risk prediction.** *J Am Coll Cardiol y* 2010, 56(13):1069; author reply 1069-1070.

51. Touboul PJ, Hennerici MG, Meairs S, Adams H, Amarenco P, Bornstein N, Csiba L, Desvarieux M, Ebrahim S, Hernandez Hernandez R et al: **Mannheim carotid intima-media thickness and plaque consensus (2004-2006-2011). An update on behalf of the advisory board of the 3rd, 4th and 5th watching the risk symposia, at the 13th, 15th and 20th European Stroke Conferences, Mannheim, Germany, 2004, Brussels, Belgium, 2006, and Hamburg, Germany, 2011.** *Cerebrovasc Dis* 2012, 34(4):290-296.

52. **Randomised trial of cholesterol lowering in 4444 patients with coronary heart disease: the Scandinavian Simvastatin Survival Study (4S).** *Lancet* 1994, 344(8934):1383-1389.

53. Bots ML, Palmer MK, Dogan S, Plantinga Y, Raichlen JS, Evans GW, O'Leary DH, Grobbee DE, Crouse JR, 3rd: **Intensive lipid lowering may reduce progression of carotid atherosclerosis within 12 months of treatment: the METEOR study.** *J Internal Med* 2009, 265(6):698-707.

54. Shepherd J, Cobbe SM, Ford I, Isles CG, Lorimer AR, MacFarlane PW, McKillop JH, Packard CJ: **Prevention of coronary heart disease with pravastatin in men with hypercholesterolemia. West of Scotland Coronary Prevention Study Group.** *N Engl J Med* 1995, 333(20):1301-1307.

55. Hebert PR, Gaziano JM, Chan KS, Hennekens CH: **Cholesterol lowering with statin drugs, risk of stroke, and total mortality. An overview of randomized trials.** *JAMA* 1997, 278(4):313-321.

56. **S**ever PS, Dahlof B, Poulter NR, Wedel H, Beevers G, Caulfield M, Collins R, Kjeldsen SE, Kristinsson A, McInnes GT et al: **Prevention of coronary and stroke events with atorvastatin in hypertensive patients who have average or lower-than-average cholesterol concentrations, in the Anglo-Scandinavian Cardiac Outcomes Trial--Lipid Lowering Arm (ASCOT-LLA): a multicentre randomised controlled trial.** *Drugs* 2004, 64(Suppl 2):43-60.

57. Amarenco P, Labreuche J: **Lipid management in the prevention of stroke: review and updated meta-analysis of statins for stroke prevention.** *Lancet Neurol* 2009, 8(5):453-463.

58. Paraskevas KI, Hamilton G, Mikhailidis DP: **Statins: an essential component in the management of carotid artery disease.** *J Vasc Surg* 2007, 46(2):373-386.

59. Crouse JR: **Effects of statins on carotid disease and stroke.** *Curr Opin Lip* 1999, 10(6):535-541.

60. Makris GC, Lavida A, Nicolaides AN, Geroulakos G: **The effect of statins on carotid plaque morphology: a LDL-associated action or one more pleiotropic effect of statins?** *Atherosclerosis* 2010, 213(1):8-20.

61. Nissen SE: **Halting the progression of atherosclerosis with intensive lipid lowering: results from the Reversal of Atherosclerosis with Aggressive Lipid Lowering (REVERSAL) trial.** *Am J Med* 2005, 118(Suppl 12A):22-27.

62. Nissen SE: **Effect of intensive lipid lowering on progression of coronary atherosclerosis: evidence for an early benefit from the Reversal of Atherosclerosis with Aggressive Lipid Lowering (REVERSAL) trial.** *Am J Cardiol* 2005, 96(5A):61F-68F.

63. Eggen AE, Mathiesen EB, Wilsgaard T, Jacobsen BK, Njølstad I: **The sixth survey of the Tromsø Study (Tromsø 6) in 2007-08: collaborative research in the interface between clinical medicine and epidemiology: study objectives, design, data collection procedures, and attendance in a multipurpose population-based health survey.** *Scand J Publ Health* 2013, 41(1):65-80.

64. Jacobsen BK, Eggen AE, Mathiesen EB, Wilsgaard T, Njølstad I: **Cohort profile: the Tromsø Study.** *Int J Epidemiol* 2012, 41(4):961-967.

65. Joakimsen O, Bønaa KH, Stensland-Bugge E: **Reproducibility of ultrasound assessment of carotid plaque occurrence, thickness, and morphology. The Tromsø Study.** *Stroke* 1997, 28(11):2201-2207.

66. Stensland-Bugge E, Bønaa KH, Joakimsen O: **Reproducibility of ultrasonographically determined intima-media thickness is dependent on arterial wall thickness. The Tromsø Study.** *Stroke* 1997, 28(10):1972-1980.

67. Fosse E, Johnsen SH, Stensland-Bugge E, Joakimsen O, Mathiesen EB, Arnesen E, Njølstad I: **Repeated visual and computer-assisted carotid plaque characterization in a longitudinal population-based ultrasound study: the Tromsø Study.** *Ultrasound Med Biol* 2006, 32(1):3-11.

68. Wendelhag I, Liang Q, Gustavsson T, Wikstrand J: **A new automated computerized analyzing system simplifies readings and reduces the variability in ultrasound measurement of intima-media thickness.** *Stroke* 1997, 28(11):2195-2200.

69. Touboul PJ, Hennerici MG, Meairs S, Adams H, Amarenco P, Bornstein N, Csiba L, Desvarieux M, Ebrahim S, Fatar M et al: **Mannheim carotid intima-media thickness consensus (2004-2006). An update on behalf of the Advisory Board of the 3rd and 4th Watching the Risk Symposium, 13th and 15th European Stroke Conferences, Mannheim, Germany, 2004, and Brussels, Belgium, 2006.** *Cerebrovasc Dis* 2007, 23(1):75-80.

70. Herder M, Johnsen SH, Arntzen KA, Mathiesen EB: **Risk factors for progression of carotid intima-media thickness and total plaque area: a 13-year follow-up study: the Tromsø Study.** *Stroke* 2012, 43(7):1818-1823.
71. Rothman K: **Modern Epidemiology,** 2nd Edition: Lippincott-Raven; 1998.
72. Hernan MA, Hernandez-Diaz S, Robins JM: **A structural approach to selection bias.** *Epidemiology* 2004, 15(5):615-625.
73. Langhammer A, Krokstad S, Romundstad P, Heggland J, Holmen J: **The HUNT study: participation is associated with survival and depends on socioeconomic status, diseases and symptoms.** *BMC Med Res Met* 2012, 12:143.
74. Bland JM, Altman DG: **Statistical methods for assessing agreement between two methods of clinical measurement.** *Lancet* 1986, 1(8476):307-310.
75. Fleiss J: **Statistical Methods for Rates and Proportions**, 2nd edn. New York, NY: John Wiley and sons, Inc.; 1981.
76. Ginde AA, Cagliero E, Nathan DM, Camargo CA, Jr.: **Value of risk stratification to increase the predictive validity of HbA1c in screening for undiagnosed diabetes in the US population.** *J Gen Int Med* 2008, 23(9):1346-1353.
77. Bennett CM, Guo M, Dharmage SC: **HbA(1c) as a screening tool for detection of Type 2 diabetes: a systematic review.** *Diabetic Med* 2007, 24(4):333-343.
78. Hutchinson MS JR, Njølstad I, Schirmer H, Figenschau Y, Svartberg J, Jorde R: **Effects of Age and Sex on Estimated Diabetes Prevalence Using Different Diganostic Criteria: The Tromsø OGTT Study.** *Int J Endocrinol* 2013.
79. Cooper GR, Myers GL, Smith SJ, Schlant RC: **Blood lipid measurements. Variations and practical utility.** *JAMA* 1992, 267(12):1652-1660.
80. Nielsen MW, Sondergaard B, Kjoller M, Hansen EH: **Agreement between self-reported data on medicine use and prescription records vary according to method of analysis and therapeutic group.** *J Clin Epidemiol* 2008, 61(9):919-924.
81. Noize P, Bazin F, Pariente A, Dufouil C, Ancelin ML, Helmer C, Moore N, Fourrier-Reglat A: **Validity of chronic drug exposure presumed from repeated patient interviews varied according to drug class.** *J Clin Epidemiol* 2012, 65(10):1061-1068.
82. Suissa S: **Immortal time bias in observational studies of drug effects.** *Pharmacoepidemiol Drug Safe* 2007, 16(3):241-249.
83. Suissa S: **Immortal time bias in pharmaco-epidemiology.** *Am J Epidemiol* 2008, 167(4):492-499.
84. Nesselroade JRS, S.M; Baltes, P.B: **Regression toward the mean and the study of change.** *Psychological Bulletin* 1980, 88(3):622-636.
85. Barnett AG, van der Pols JC, Dobson AJ: **Regression to the mean: what it is and how to deal with it.** *Int J Epidemiol* 2005, 34(1):215-220.
86. Weeks DL: **The regression effect as a neglected source of bias in nonrandomized intervention trials and systematic reviews of observational studies.** *Eval Health Prof* 2007, 30(3):254-265.
87. Bland JM, Altman DG**: Regression towards the mean.** *BMJ* 1994, 308(6942):1499.
88. Lord FM: **Elementary models for measuring change.** In: *Problems in measuring change*. Edited by Harris CW. Madison: University of Wisconsin Press; 1967.
89. Chronbach L, Furby J**: How should we measure change-or should we?** *Psychol Bul* 1970(74):68-80.
90. Rogosa RD, Willett JB: **Demonstrating the reliability of the difference score in the measurement of change.** *J Edu Measur* 1984, 20(4):335-343.
91. Willett JB: **Questions and answer in the measurement of change.** In: *Review of Research in Education*. Edited by E.Z R. Washington: AERA; 1989: 345-422.

92. Vickers AJ, Altman DG: **Statistics notes: Analysing controlled trials with baseline and follow up measurements.** *BMJ* 2001, 323(7321):1123-1124.
93. Rogosa D: Myths about longitudinal research. In: *The analysis of change,* vol. 2. New Jersey: Lawrence Erlbaum Associates Inc.; 1995.
94. Vollmer WM: **Comparing change in longitudinal studies: adjusting for initial value.** *J Clin Epidemiol* 1988, 41(7):651-657.
95. Glymour MM, Weuve J, Berkman LF, Kawachi I, Robins JM: **When is baseline adjustment useful in analyses of change? An example with education and cognitive change.** *Am J Epidemiol* 2005, 162(3):267-278.
96. Yanez ND, 3rd, Kronmal RA, Shemanski LR: **The effects of measurement error in response variables and tests of association of explanatory variables in change models.** *Stat Med* 1998, 17(22):2597-2606.
97. Mee RW, Chua T.: **Regression Towards the Mean and the Paired sample t test.** *Am Stat* 1991, 45(1):39-42.
98. Ostermann T, Willich SN, Lüdtke R: **Regression toward the mean--a detection method for unknown population mean based on Mee and Chua's algorithm.** *BMC Med Res* 2008, 8:52.
99. Johnsen SH, Mathiesen EB, Fosse E, Joakimsen O, Stensland-Bugge E, Njølstad I, Arnesen E: **Elevated high-density lipoprotein cholesterol levels are protective against plaque progression: a follow-up study of 1952 persons with carotid atherosclerosis the Tromso study.** *Circulation* 2005, 112(4):498-504.
100. Spence JD, Hegele RA: **Non-invasive assessment of atherosclerosis risk.** *Curr Drug Targets Cardiovasc Haematol Disord* 2004, 4(2):125-128.
101. Spence JD, Hegele RA: **Noninvasive phenotypes of atherosclerosis: similar windows but different views.** *Stroke* 2004, 35(3):649-653.
102. Spence JD: **Is carotid intima-media thickness a reliable clinical predictor?** *Mayo Clin Proc* 2008, 83(11):1299-1300; author reply 1300-1291.
103. Rundek T, Brook RD, Spence JD: **Letter by Rundek et al regarding article, "Prediction of clinical cardiovascular events with carotid intima-media thickness: a systematic review and meta-analysis".** *Circulation* 2007, 116(9):e317; author reply e318.
104. Spence JD: **Measurement of intima-media thickness vs. carotid plaque: uses in patient care, genetic research and evaluation of new therapies.** *Int J Stroke* 2006, 1(4):216-221.
105. Lorenz MW, Polak JF, Kavousi M, Mathiesen EB, Volzke H, Tuomainen TP, Sander D, Plichart M, Catapano AL, Robertson CM et al: **Carotid intima-media thickness progression to predict cardiovascular events in the general population (the PROG-IMT collaborative project): a meta-analysis of individual participant data.** *Lancet* 2012, 379(9831):2053-2062.
106. Henry RM, Kostense PJ, Dekker JM, Nijpels G, Heine RJ, Kamp O, Bouter LM, Stehouwer CD: **Carotid arterial remodeling: a maladaptive phenomenon in type 2 diabetes but not in impaired glucose metabolism: the Hoorn study.** *Stroke* 2004, 35(3):671-676.
107. Temelkova-Kurktschiev TS, Koehler C, Leonhardt W, Schaper F, Henkel E, Siegert G, Hanefeld M: **Increased intimal-medial thickness in newly detected type 2 diabetes: risk factors.** *Diabetes Care* 1999, 22(2):333-338.
108. Ballantyne CM, Hoogeveen RC, McNeill AM, Heiss G, Schmidt MI, Duncan BB, Pankow JS: **Metabolic syndrome risk for cardiovascular disease and diabetes in the ARIC study.** *Int J Obes* 2008, 32 Suppl 2:S21-24.

109. Kawamoto R, Tomita H, Ohtsuka N, Inoue A, Kamitani A: **Metabolic syndrome, diabetes and subclinical atherosclerosis as assessed by carotid intima-media thickness.** *J Arteroscler Thromb* 2007, 14(2):78-85.
110. Pollex RL, Al-Shali KZ, House AA, Spence JD, Fenster A, Mamakeesick M, Zinman B, Harris SB, Hanley AJ, Hegele RA: **Relationship of the metabolic syndrome to carotid ultrasound traits.** *Cardiovasc Ultrasound* 2006, 4:28.
111. Zanchetti A, Hennig M, Baurecht H, Tang R, Cuspidi C, Carugo S, Mancia G: **Prevalence and incidence of the metabolic syndrome in the European Lacidipine Study on Atherosclerosis (ELSA) and its relation with carotid intima-media thickness.** *J Hypertens* 2007, 25(12):2463-2470.
112. Koskinen J, Kahonen M, Viikari JS, Taittonen L, Laitinen T, Ronnemaa T, Lehtimaki T, Hutri-Kahonen N, Pietikainen M, Jokinen E et al: **Conventional cardiovascular risk factors and metabolic syndrome in predicting carotid intima-media thickness progression in young adults: the cardiovascular risk in young Finns study.** *Circulation* 2009, 120(3):229-236.
113. Rundek T, White H, Boden-Albala B, Jin Z, Elkind MS, Sacco RL: **The metabolic syndrome and subclinical carotid atherosclerosis: the Northern Manhattan Study.** *J Cardiometabol Syndr* 2007, 2(1):24-29.
114. Della-Morte D, Gardener H, Denaro F, Boden-Albala B, Elkind MS, Paik MC, Sacco RL, Rundek T: **Metabolic syndrome increases carotid artery stiffness: the Northern Manhattan Study.** *Int J Stroke* 2010, 5(3):138-144.
115. Bonora E, Kiechl S, Willeit J, Oberhollenzer F, Egger G, Bonadonna RC, Muggeo M: **Carotid atherosclerosis and coronary heart disease in the metabolic syndrome: prospective data from the Bruneck study.** *Diabetes Care* 2003, 26(4):1251-1257.
116. Crouse JR, 3rd, Raichlen JS, Riley WA, Evans GW, Palmer MK, O'Leary DH, Grobbee DE, Bots ML: **Effect of rosuvastatin on progression of carotid intima-media thickness in low-risk individuals with subclinical atherosclerosis: the METEOR Trial.** *JAMA* 2007, 297(12):1344-1353.
117. MacMahon S, Sharpe N, Gamble G, Hart H, Scott J, Simes J, White H: **Effects of lowering average of below-average cholesterol levels on the progression of carotid atherosclerosis: results of the LIPID Atherosclerosis Substudy. LIPID Trial Research Group.** *Circulation* 1998, 97(18):1784-1790.
118. Bogiatzi C, Spence JD**: Ezetimibe and regression of carotid atherosclerosis: importance of measuring plaque burden.** *Stroke* 2012, 43(4):1153-1155.
119. Blumenthal RS: **Should healthy people take cholesterol drugs to prevent heart disease.** In: *Wall Street Journal*. New York: WSJ; 2012.
120. **Taylor F, Ward K, Moore TH, Burke M, Davey Smith G, Casas JP, Ebrahim S: Statins for the primary prevention of cardiovascular disease.** *Cochrane Database Syst Rev* 2011(1):CD004816.
121. Taylor F, Huffman MD, Macedo AF, Moore TH, Burke M, Davey Smith G, Ward K, Ebrahim S: **Statins for the primary prevention of cardiovascular disease.** *Cochrane Database Syst Rev* 2013, 1:CD004816.

Paper I

Paper II

Paper III

Appendix I

Supplementary Tables 1-5

Supplementary Table 1. Analysis of predictors of intima-media thickness (IMT) and total plaque area (TPA) at follow-up in two different models* of stepwise multivariable regression analysis: The Tromsø Study 1994-2008

	Intima-media thickness					
	Model 1			**Model 2**		
	β†	r^2	P value	β†	r^2	P value
Age, years	0.359	0.145	<0.0001	0.182	0.020	<0.0001
Male sex	0.177	0.039	<0.0001	0.126	0.015	<0.0001
Total cholesterol, mmol/L	0.066	0.004	0.0001	0.037	0.001	0.03
HDL cholesterol, mmol/L	-0.049	0.002	0.011	-	-	-
Systolic blood pressure, mm Hg	0.041	0.001	0.003	-	-	-
Body mass index, kg/m^2	0.081	0.011	<0.0001	0.053	0.003	0.001
Daily smoking	0.054	0.003	0.002	0.045	0.002	0.005
Use of lipid-lowering drugs	-	-	-	-	-	-
Cardiovascular disease	-	-	-	-	-	-
Diabetes	-	-	-	-	-	
Baseline value of IMT				0.393	0.269	<0.0001
Summarized model R^2		0.206			0.311	
	Total plaque area‡					
	Model 1			**Model 2**		
	β†	r^2	P value	β†	r^2	P value
Age, years	0.263	0.099	<0.0001	0.157	0.026	<0.0001
Male sex	0.1136	0.020	<0.0001	0.082	0.006	<0.0001
Total cholesterol, mmol/L	0.125	0.019	<0.0001	0.084	0.006	<0.0001
HDL cholesterol, mmol/L	-	-	-	-	-	-
Systolic blood pressure, mm Hg	0.126	0.014	<0.0001	0.077	0.069	<0.0001
Body mass index, kg/m^2	-	-	-	-	-	-
Daily smoking	0.0165	0.003	<0.0001	0.119	0.013	<0.0001
Use of lipid-lowering drugs	0.075	0.007	<0.0001	0.054	0.003	0.0005
Cardiovascular disease	0.045	0.002	<0.0001	-	-	-
Diabetes	-	-	-	-	-	-
Baseline value of TPA				0.448	0.297	<0.0001
Summarized model R^2		0.186			0.358	

HDL; high density lipoprotein

*The variables included in the models were the following: Model 1: age, sex, total cholesterol, HDL cholesterol, systolic blood pressure, body mass index, daily smoking, use of lipid-lowering drugs, prevalent self-reported cardiovascular disease, prevalent self-reported diabetes (all assessed at baseline). Model 2: Model 1 + baseline value of IMT or TPA (as indicated).

†Standardized regression β coefficients; z-scores for all independent and independent variables

‡Square root transformed

Supplementary Table 2. Analysis of predictors of change in intima-media thickness (IMT) and total plaque area (TPA) in three different models* of stepwise multivariable regression analysis: The Tromsø Study 1994-2008

	ΔIntima-media thickness								
	Model 1			Model 2			Model 3		
	β†	r^2	P value	β†	r^2	P value	β†	r^2	P value
Age, years	-	-	-	0.142	0.014	<0.0001	-	-	-
Male sex	0.067	0.004	0.0002	0.119	0.012	<0.0001	0.050	0.003	0.009
Total cholesterol, mmol/L	0.050	0.002	0.0002	0.061	0.003	0.002	-	-	-
HDL cholesterol, mmol/L	-	-	-	-	-	-	-	-	
Systolic blood pressure, mm Hg	- 0.076	0.004	0.002	-	-	-	- 0.089	0.004	<0.0001
Body mass index, kg/m^2	-	-	-	-	-	-	-	-	-
Daily smoking	-	-	-	-	-	-	-	-	-
Use of lipid-lowering drugs	-	-	-	-	-	-	-	-	-
Cardiovascular disease	-	-	-	-	-	-	-	-	-
Diabetes	-	-	-	-	-	-	-	-	-
Baseline value of IMT				- 0.294	0.037	<0.0001			
Mean of baseline and follow-up IMT							0.075	0.006	0.0002
Summarized model R^2		0.010			0.067			0.013	
	ΔTotal plaque area								
	Model 1			Model 2			Model 3		
	β†	r^2	P value	β†	r^2	P value	β†	r^2	P value
Age, years	0.103	0.015	<0.0001	0.183	0.036	<0.0001	-	-	-
Male sex	0.054	0.004	0.004	0.096	0.008	<0.0001	-	-	-
Total cholesterol, mmol/L	0.067	0.005	0.0006	0.098	0.009	<0.0001	-	-	-
HDL cholesterol, mmol/L	-	-	-	-	-	-	-	-	-
Systolic blood pressure, mm Hg	0.056	0.003	0.006	0.089	0.009	<0.0001	-	-	-
Body mass index, kg/m^2	-		-	-	-	-	-	-	-
Daily smoking	0.106	0.011	<0.0001	0.139	0.017	<0.0001	0.048	0.002	0.008
Use of lipid-lowering drugs	0.041	0.002	0.03	0.062	0.004	0.0005	-	-	-
Cardiovascular disease	-	-	-	-	-	-	- 0.038	0.001	0.04
Diabetes	-	-		-	-	-	-	-	-
Baseline value of TPA				- 0.319	0.042	<0.0001			
Mean of baseline and follow-up TPA							0.369	0.135	<0.0001
Summarized model R^2		0.038			0.126			0.139	

HDL; high density lipoprotein

*The variables included in the models were the following: Model 1: age, sex, total cholesterol, HDL cholesterol, systolic blood pressure, body mass index, daily smoking, use of lipid-lowering drugs, prevalent self-reported cardiovascular disease, prevalent self-reported diabetes (all assessed at baseline). Model 2: Model 1 + baseline value of IMT or TPA (as indicated). Model 3: Model 1 + the mean value of baseline and follow-up IMT and TPA (as indicated).
†Standardized regression β coefficients; z-scores for all independent and independent variables
‡Square root transformed

Supplementary Table 3a. Analysis of associations between MetS and follow-up values of intima-media thickness (IMT) in two different models* of stepwise multivariable regression analysis: The Tromsø Study 1994-2008

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	Intima-media thickness					
	Model 1			Model 2		
	β†	r^2	P value	β†	r^2	P value
Men						
MetS	0.100	0.010	<0.0001	0.043	0.002	0.044
Age, years	0.359	0.107	<0.0001	0.080	0.003	0.002
LDL-chol (mmol/L)	0.079	0.005	0.004	-		-
Daily smoking	0.077	0.007	0.0002	0.051	0.002	0.02
Baseline value of IMT				0.548	0.357	<0.0001
Summarized r^2		0.129			0.364	
Women						
MetS	0.071	0.046	0.018	-	-	-
Age, years	0.369	0.212	<0.0001	0.169	0.0278	<0.0001
LDL-chol (mmol/L)	0.118	0.018	<0.0001	0.062	0.005	0.002
Daily smoking	0.047	0.035	0.04	0.058	0.003	0.04
Baseline value of IMT				0.495	0.362	<0.0001
Summarized r^2		0.237			0.397	

HDL; high density lipoprotein

*The variables included in the models were the following: Model 1: age, sex, total cholesterol, HDL cholesterol, systolic blood pressure, body mass index, daily smoking, use of lipid-lowering drugs, prevalent self-reported cardiovascular disease, prevalent self-reported diabetes (all assessed at baseline). Model 2: Model 1 + baseline value of IMT

†Standardized regression β coefficients; z-scores for all independent and independent variables

Supplementary Table 3b. Analysis of associations between MetS and follow-up values of total plaque area (TPA) in two different models* of stepwise multivariable regression analysis: The Tromsø Study 1994-2008

	Total plaque area‡					
	Model 1			Model 2		
	β†	r^2	P value	β†	r^2	P value
Men						
MetS	0.074	0.005	0.005	0.047	0.002	0.044
Age, years	0.358	0.090	<0.0001	0.203	0.023	<0.0001
LDL-chol (mmol/L)	0.102	0.008	0.0005	-		-
Daily smoking	0.171	0.027	<0.0001	0.117	0.011	<0.0001
Baseline value of TPA	-		-	0.452	0.275	<0.0001
Summarized r^2		0.131			0.312	
Women						
MetS	-		-	-		-
Age, years	0.270	0.132	<0.0001	0.166	0.040	<0.0001
LDL-chol (mmol/L)	0.177	0.041	<0.0001	0.114	0.017	<0.0001
Daily smoking	0.138	0.021	<0.0001	0.103	0.011	<0.0001
Baseline value of TPA				0.452	0.299	<0.0001
Summarized r^2		0.194			0.3676	

HDL; high density lipoprotein

*The variables included in the models were the following: Model 1: age, sex, total cholesterol, HDL cholesterol, systolic blood pressure, body mass index, daily smoking, use of lipid-lowering drugs, prevalent self-reported cardiovascular disease, prevalent self-reported diabetes (all assessed at baseline). Model 2: Model 1 + baseline value of TPA

†Standardized regression β coefficients; z-scores for all independent and independent variables

‡Square root transformed

Supplementary Table 4a. Analysis of associations between MetS and change in values of intima-media thickness (IMT) from baseline to follow-up in three different models* of stepwise multivariable regression analysis: The Tromsø Study 1994-2008

	ΔIntima-media thickness								
	Model 1			Model 2			Model 3		
	β†	r^2	P value	β†	r^2	P value	β†	r^2	P value
Men									
MetS	-	-	-	0.054	0.003	0.04	-	-	-
Age, years	-	-	-	0.100	0.005	0.002	-0.23	0.038	<0.0001
LDL-chol (mmol/L)	-	-	-	-		-	-	-	-
Daily smoking	-	-	-	0.063	0.004	0.02	-	-	-
Baseline value of IMT	-	-	-	-0.238	0.036	<0.0001	-	-	-
Mean of baseline and follow-up IMT							0.438	0.114	<0.0001
Summarized r^2		-			0.047			0.152	
Women									
MetS	-		-	-	-	-	-	-	-
Age, years	0.103	0.009	<0.0001	0.211	0.043	<0.0001	-0.134	0.019	<0.0001
LDL-chol (mmol/L)	-	-	-	0.077	0.007	0.002	-	-	-
Daily smoking	0.086	0.007	0.0008	0.073	0.005	0.004	0.072	0.005	0.002
Baseline value of IMT				-0.304	0.024	<0.0001			
Mean of baseline and follow-up IMT							0.474	0.143	<0.0001
Summarized r^2		0.016			0.079			0.167	

*The variables included in the models were the following: Model 1: age, sex, total cholesterol, HDL cholesterol, systolic blood pressure, body mass index, daily smoking, use of lipid-lowering drugs, prevalent self-reported cardiovascular disease, prevalent self-reported diabetes (all assessed at baseline). Model 2: Model 1 + baseline value of IMT or TPA (as indicated). Model 3: Model 1 + the mean of baseline and follow-up IMT values (as indicated).
†Standardized regression β coefficients; z-scores for all independent and independent variables

Supplementary Table 4b. Analysis of associations between MetS and change in values of total plaque area (TPA) from baseline to follow-up in three different models* of stepwise multivariable regression analysis: The Tromsø Study 1994-2008

	ΔTotal plaque area‡								
	Model 1			Model 2			Model 3		
	β†	r^2	P value	β†	r^2	P value	β†	r^2	P value
Men									
MetS	-	-	-	0.055	0.003	0.044	-	-	-
Age, years	0.124	0.010	<0.0001	0.236	0.031	<0.0001	-		-
LDL-chol (mmol/L)	-	-	-	-	-	-	-	-	-
Daily smoking	0.089	0.007	0.002	0.135	0.015	<0.0001	-	-	-
Baseline value of ITPA	-	-	-	-0.313	0.056	<0.0001			
Mean of baseline and follow-up TPA							0.357	0.122	<0.0001
Summarized r^2		0.016			0.104			0.122	
Women									
MetS		-	-	-	-	-	-	-	-	-
Age, years		0.122	0.011	<0.0001	0.193	0.055	<0.0001	-	-	-
LDL-chol (mmol/L)		0.089	0.027	0.0002	0.133	0.023	<0.0001	-	-	-
Daily smoking		0.093	0.010	<0.0001	0.119	0.016	<0.0001	-	-	-
Baseline value of TPA					-0.312	0.038	<0.0001			
Mean of baseline and follow-up TPA								0.379	0.145	<0.0001
Summarized r^2			0.047			0.132			0.145	

*The variables included in the models were the following: Model 1: age, sex, total cholesterol, HDL cholesterol, systolic blood pressure, body mass index, daily smoking, use of lipid-lowering drugs, prevalent self-reported cardiovascular disease, prevalent self-reported diabetes (all assessed at baseline). Model 2: Model 1 + baseline value of IMT or TPA (as indicated). Model 3: Model 1 + the mean of baseline and follow-up TPA values (as indicated).

†Standardized regression β coefficients; z-scores for all independent and independent variables

‡Square root transformed

Supplementary Table 5. Multivariable adjusted regression analysis of the effect of use of lipid-lowering drugs (LLD) and cardiovascular risk factors on progression of atherosclerosis in three different models*. The Tromsø Study 1994-2008.

	ΔIntima-media thickness					
	Model 1		Model 2		Model 3	
	β†	P value	β†	P value	β	P value
Age, years	0.0005	0.2	0.003	<0.0001	-0.003	<0.0001
Male sex	0.019	0.006	0.032	<0.0001	-0.008	0.2
Total cholesterol, mmol/L	0.011	0.0005	0.012	<0.0001	0.006	0.04
HDL cholesterol, mmol/L	-0.009	0.3	-0.018	0.04	0.008	0.3
Systolic blood pressure, mm Hg	0.0002	0.4	0.0005	0.004	-0.0005	0.003
Daily smoking	0.024	0.001	0.026	0.0003	0.014	0.04
Cardiovascular disease	0.017	0.2	0.017	0.2	0.014	0.2
Baseline value of IMT			-0.295	<0.0001		
Mean of baseline and follow-up IMT					0.499	<0.0001
Use of LLD						
Any-time use of LLD	-0.024	0.046	-0.018	0.1	-0.029	0.01
Long-term use of LLD	-0.039	0.0002	-0.021	0.04	-0.059	<0.0001
	ΔTotal plaque area†					
	Model 1		Model 2		Model 3	
	β	P value	β	P value	β	P value
Age, years	0.027	<0.0001	0.048	<0.0001	-0.001	0.8
Male sex	0.347	0.0004	0.502	<0.0001	0.106	0.3
Total cholesterol, mmol/L	0.149	0.0005	0.186	<0.0001	0.077	0.06
HDL cholesterol, mmol/L	0.073	0.6	-0.019	0.9	0.157	0.2
Systolic blood pressure, mm Hg	0.008	0.001	0.012	<0.0001	0.002	0.4
Daily smoking	0.568	<0.0001	0.778	<0.0001	0.221	0.03
Cardiovascular disease	0.211	0.24	0.389	0.02	0.389	0.8
Baseline value of TPA			-0.393	<0.0001		
Mean of baseline and follow-up TPA					0.422	<0.0001
Use of LLD						
Any-time use of LLD	-0.318	0.06	-0.256	0.1	-0.319	0.049
Long-term use of LLD	-0.400	0.006	-0.107	0.4	-0.631	<0.0001

HDL; high density lipoprotein

*The variables included in the models were the following: Model 1: age, sex, total cholesterol, HDL cholesterol, systolic blood pressure, daily smoking, prevalent self-reported cardiovascular disease, prevalent self-reported diabetes (all assessed at baseline), and use of lipid-lowering drugs. Model 2: Model 1 + baseline value of IMT or TPA (as indicated). Model 3: Model 1 + the mean value of baseline and follow-up IMT and TPA (as indicated).

† Square root transformed

Appendix II

Questionnaires and invitations to the 4th Tromsø Study

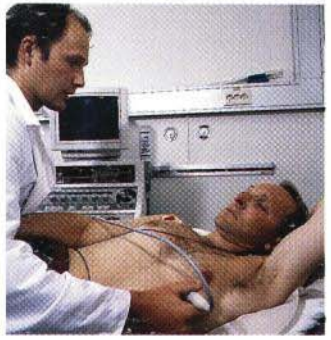
English versions

YOU ARE INVITED TO THE SPECIAL STUDY

The health study in Tromsø invites some of the participants for a free special study.

The special study

The Special Study uses advanced technology which makes images of blood vessels and the heart, and provides information on skeletal structure and fatty tissue. X-ray technology is not used, but rather ultrasound or light-waves which are reflected against a small device held to the skin (pictured). These tests do not penetrate the skin, are not painful and have no known side-effects. The Special Study also involves blood- and urine samples, as well as registering heart activity (ECG).



Why are you invited?

We do not have the opportunity to offer the Special Study to everyone. We invite all men and women born between 1920 and 1939 and some randomly picked from other age-groups.

What is the purpose?

Many diseases evolve gradually over long periods of time without people's awareness, but with advanced methods it is possible to detect changes early. In certain cases prevention or treatment can be initiated even before the disease develops. In other cases we are not sure what the changes signify and further research is necessary. The Special Study is therefore a unique offer which not only has value to you personally; the results are used in medical research which breeds increased knowledge about how diseases initiate and how they can be prevented and treated.

The Special Study involves

✓ Ultrasound of blood vessels and the heart

The arteries in the neck and stomach are studied. This gives information whether the arteries are clogged or whether they are diluted/contracted. The shape of the heart and its functionality is looked at in 50 per cent of the participants.

✓ Study of bone density and amount of fat

The measurements are used to determine risks of osteoporosis and fractures, and whether there is a correlation between body fat and disease.

✓ ECG

ECG is registering heart activity which also provides information concerning heart disease.

✓ Urine sample

The urine samples are used to indicate kidney function through measuring the amount of protein and creatinine substances. The result is most accurate if urine from the separate days are examined.

✓ Blood sample

Blood samples are examined for fatty substances and substances which indicate how the kidneys work, metabolism (calcium and sugar) and blood clotting. The blood sample is frozen so it can be used for later research.

✓ Further follow up

- If we think further examination or treatment is required, it will be offered to you.
- Some participants may be asked to take part in later studies for further research.

Practical information

Place and time

The examination will take place in the second floor at Elisabeth center; the old maternity hospital (Mellomveien 50) - at the floor above the Tromsø study. The examination takes 1 to 1.5 hours and is free of charge.

We hope you can use the time appointed.
Date and time is given in the brochure. If you need to change appointment, we ask that you notify us by calling 77 64 59 00

Urine sample

You have been given three urine glasses marked 1, 2 and 3. We wish that you take a morning urine sample in each glass in the last three days before the special study. You have therefore got a glass for every morning. Note the following:

1. Please urinate a small amount of urine in the toilet before you take the urine sample. Last morning sample is taken on the day you come to the survey.

2. State the date on each urine glass.

3. It is an advantage if samples can stay cold.

4. Deliver all three glasses when you come to the survey.

Use of medicine

On the next page please make a note which medications you've used the past week. This can be important when interpreting the results.

Clothing

Because of the blood pressure measuring, we ask you to wear clothes that are not tight on the arm. When examining the heart, it is necessary to undress the upper body. At examination of the aorta some clothes must be pulled down so that the abdominal region is exposed.

About consent

The information about you will be treated confidentially. The information will be stored and used according to the rules set by the Data Inspectorate and Norwegian law. The study has been recommended by The Regional Committee for Research Ethics. Should further examinations be required, we ask your consent to forward relevant data to your doctor or the Regional Hospital in Tromsø. We also request that you upon arrival give your consent to:

- ✘ that we forward your results to your doctor or the Regional Hospital in Tromsø if you need further examination.
- ✘ that your results may be used for medical research through combining them with other health- and disease registries as well as information from previous health studies in Tromsø. Prior to analysing the results your name and social security number will be removed.
- ✘ that your blood sample may be stored and used for medical research.
- ✘ that the Health Examination in Tromsø may contact you later with a request to participate in other studies.

Even if you give your consent now, you may later reconsider and deny the use of your results.

The special study

is part of the health survey in Tromsø, and organized by the University of Tromsø, Faculty of Medicine in cooperation with the Regional Hospital in Tromsø

UNIVERSITETET I TROMSØ

Use of medicine

To interpret the results we want information about medication use in the last week. Please state name, strength and dose of all medications that you are using. If in doubt about filling, bring the drugs. We will then be able to help you.

Name of medicine	Strength	Dose

Welcome

THE SPECIAL STUDY '94 – 95

SPESIALUNDERSØKELSEN '94 - 95

You are invited to the special study in Tromsø

LUNDBLAD GRAFISK AS, TROMSØ

HEALTH SURVEY
Invitation

"THIS IS YOUR CHANCE"

Date of birth **Social security No.** **Municipality** **Electoral ward No.**

Welcome to the Tromsø Health Survey!

The Health Survey is coming to Tromsø. This leaflet will tell you when and where. You will also find information about the survey in the enclosed brochure.

We would like you to fill in the form overleaf and take it with you to the examination.

The more people take part in the survey, the more valuable its results will be. We hope, therefore, that you will be able to come. Attend even if you feel healthy, if you are currently receiving medical treatment, or if you have had your cholesterol and blood pressure measured recently.

Yours sincerely,
Municipal Health Authorities
Faculty of Medicine - University of Tromsø
National Health Screening Service

"THIS IS A REAL OPPORTUNITY- TAKE IT!"

YOUR OWN HEALTH

What is your current state of health? *Tick one box only.*

Poor	12	1
Not so good		2
Good		3
Very good		4

Do you have, or have you had:

		Yes	No	Age first time
A heart attack	13			years
Angina pectoris (heart cramp)	16			years
A cerebral stroke/ brain haemorrhage	19			years
Asthma	22			years
Diabetes	25			years

Do you use blood pressure lowering drugs?

Currently	28	1
Previously, but not now		2
Never used		3

Have you during the last year suffered from pains and/or stiffness in muscles and joints that have lasted continuously for at least 3 months? 29 — Yes / No

Have you in the last two weeks felt:

		No	A little	A lot	Very much
Nervous or worried?	30				
Anxious?	31				
Confident and calm?	32				
Irritable?	33				
Happy and optimistic?	34				
Down/depressed?	35				
Lonely?	36				
		1	2	3	4

SMOKING

Did any of the adults at home smoke while you were growing up? 37 — Yes / No

Do you currently, or did you previously, live together with daily smokers after your 20th birthday? 38 — Yes / No

If "YES", for how many years in all? 39 — Years

How many hours a day do you normally spend in smoke-filled rooms? 41 — Hours

Put 0 if you do not spend time in smoke-filled rooms.

Do you yourself smoke:

		Yes	No
Cigarettes daily?	43		
Cigars/ cigarillos daily?	44		
A pipe daily?	45		

If you previously smoked daily, how long is it since you quit? 46 — Years

If you currently smoke, or have smoked previously:

How many cigarettes do you or did you usually smoke per day? 48 — cigarettes

How old were you when you began daily smoking? 52 — Age years

How many years in all have you smoked daily? 54 — Years

EXERCISE

How has your physical activity in leisure time been during this last year? *Think of your weekly average for the year.*

Time spent going to work counts as leisure time.

		Hours per week: None	Less than 1	1-2	3 or more
Light activity *(not sweating/out of breath)*	56				
Hard activity *(sweating/ out of breath)*	57				
		1	2	3	4

COFFEE

How many cups of coffee do you drink daily?

Put 0 if you do not drink coffee daily.

Coarsely ground coffee for brewing	58	Cups
Other coffee	60	Cups

ALCOHOL

Are you a teetotaller? 62 — Yes / No

How many times a month do you normally drink alcohol? *Do not count low-alcohol beer.*

Put 0 if less than once a month. 63 — Times

How many glasses of beer, wine or spirits do you normally drink in a fortnight? 65

Do not count low-alcohol beer.

Put 0 if less than once a month.

Beer	Wine	Spirits
Glasses	Glasses	Glasses

FAT

What type of margarine or butter do you usually use on bread? *Tick one box only.*

Don't use butter/margarine	71	1
Butter		2
Hard margarine		3
Soft margarine		4
Butter/margarine mixtures		5
Light margarine		6

EDUCATION/WORK

What is the highest level of education you have completed?

7-10 years primary/secondary school, modern secondary school	72	1
Technical school, middle school, vocational school, 1-2 years senior high school		2
High school diploma (3-4 years)		3
College/university, less than 4 years		4
College/university, 4 or more years		5

What is your current work situation?

Paid work	73	
Full-time housework	74	
Education, military service	75	
Unemployed, on leave without payment	76	

How many hours of paid work do you have per week? 77 — No. of hours

Do you receive any of the following benefits?

Sickness benefit (sick leave)	79	
Rehabilitation benefit	80	
Disability pension	81	
Old-age pension	82	
Social welfare benefit	83	
Unemployment benefit	84	

ILLNESS IN THE FAMILY

Have one or more of your parents or siblings had a heart attack or had angina (heart cramp)? 85 — Yes / No / Don't know

The Tromsø Health Survey

The main aim of the Tromsø Study is to improve our knowledge about cardiovascular diseases in order to aid prevention. The survey is also intended to improve our knowledge of cancer and other general conditions, such as allergies, muscle pains and mental conditions. We would therefore like you to answer some questions about factors that may be relevant for your risk of getting these and other illnesses.

This form is a part of the Health Survey, which has been approved by the Norwegian Data Inspectorate and the Regional Board of Research Ethics. The answers will only be used for research purposes and will be treated in strict confidence. The information you give us may later be stored along with information from other public health registers in accordance with the rules laid down by the Data Inspectorate and the Regional Board of Research Ethics.

If you are in doubt about what to answer, tick the box that you feel fits best.

The completed form should be sent to us in the enclosed pre-paid envelope.

Thank you in advance for helping us.

Yours sincerely,

Faculty of Medicine
University of Tromsø

National Health
Screening Service

If you do not wish to answer the questionnaire, tick the box below and return the form. Then you will not receive reminders.

I do not wish to answer the questionnaire ..... 17 ☐

Day Month Year

Date for filling in this form:..... 18 ...../...../.....

CHILDHOOD/YOUTH

In which Norwegian municipality did you live at the age of 1 year?

..... 24 - 28

If you did not live in Norway, give country of residence instead of municipality.

How was your family's financial situation during your childhood?

- Very good ..... 29 ☐
- Good ..... ☐
- Difficult ..... ☐
- Very difficult ..... ☐

How many of the first three years of your life

- did you live in a town/city? ..... 30 ____years
- did your family have a cat or dog in the home? ..... 31 ____years

How many of the first 15 years of your life

- did you live in a town/city? ..... 32 ____years
- did your family have a cat or dog in the home? ..... 34 ____years

HOME

Who do you live with?

Tick once for each item and give the number .		Yes	No	Number
Spouse/partner	36	☐	☐	
Other people over 18 years	37	☐	☐	_____
People under 18 years	40	☐	☐	_____

How many of the children attend day care/kindergarten? .... 43 _____

What type of house do you live in?

- Villa/detached house ..... 45 ☐ 1
- Farm ..... ☐ 2
- Flat /apartment ..... ☐ 3
- Terraced /semi-detached house ..... ☐ 4
- Other ..... ☐ 5

How big is your house? ..... 46 _____ m^2

Approximately what year was your house built? ..... 49 _____

		Yes	No
Has your house been insulated after 1970?	53	☐	☐
Do you live on the lower ground floor/basement?	54	☐	☐
If "Yes", is the floor laid on concrete?	55	☐	☐

What is the main source of heat in your home?

- Electric heating ..... 56 ☐
- Wood-burning stove ..... ☐
- Central heating system using:
  - Paraffin ..... ☐
  - Electricity ..... ☐

		Yes	No
Do you have fitted carpets in the living room?	60	☐	☐
Is there a cat in your home?	61	☐	☐
Is there a dog in your home?	62	☐	☐

WORK

If you have paid or unpaid work, how would you describe your work?

- Mostly sedentary work? ..... 63 ☐ 1
  (e.g. office work, mounting)
- Work that requires a lot of walking? ..... ☐ 2
  (e.g. shop assistant, light industrial work, teaching)
- Work that requires a lot of walking and lifting? ..... ☐ 3
  (e.g. postman, nursing, construction)
- Heavy manual work? ..... ☐ 4
  (e.g. forestry, heavy farm-work, heavy construction)

Can you decide yourself how your work should be organised?

- No, not at all ..... 64 ☐ 1
- To a small extent ..... ☐ 2
- Yes, to a large extent ..... ☐ 3
- Yes, I decide myself ..... ☐ 4

		Yes	No
Are you on call, do you work shifts or nights?	65	☐	☐

Do you do any of the following jobs (full- or part-time)?

Tick one box only for each item.		Yes	No
Driver	66	☐	☐
Farmer		☐	☐
Fisherman		☐	☐

YOUR OWN ILLNESSES

Have you ever had:
Tick one box only for each item. Give your age at the time.
*If you have had the condition several times, how old were you **last** time?*

		Yes	No	Age
Hip fracture	69	☐	☐	_____
Wrist/forearm fracture	72	☐	☐	_____
Whiplash	75	☐	☐	_____
Injury requiring hospital admission	78	☐	☐	_____
Gastric ulcer	81	☐	☐	_____
Duodenal ulcer	84	☐	☐	_____
Gastric/duodenal ulcer surgery	87	☐	☐	_____
Neck surgery	90	☐	☐	_____

Have you you ever had, or do you still have:
Tick one box only for each item.

		Yes	No
Cancer	93	☐	☐
Epilepsy		☐	☐
Migraine		☐	☐
Chronic bronchitis		☐	☐
Psoriasis		☐	☐
Osteoporosis	98	☐	☐
Fibromyalgia/fibrositis/chronic pain syndrome		☐	☐
Psychological problems for which you have sought help		☐	☐
Thyroid disease		☐	☐
Liver disease		☐	☐
Kidney disease	103	☐	☐
Appendectomy		☐	☐
Allergy and hypersensitivity:			
Atopic eczema (e.g. childhood eczema)		☐	☐
Hand eczema		☐	☐
Hay fever		☐	☐
Food allergy	108	☐	☐
Other hypersensitivity (not allergy)		☐	☐

How many times have you had a cold, influenza (flu), vomiting/diarrhoea, or similar in the last six months? ____ times

		Yes	No
Have you had this in the last 14 days?	112	☐	☐

ILLNESS IN THE FAMILY

Tick for the relatives who have or have ever had any of the following diseases:
Tick "None" if none of your relatives have had the disease.

		Mother	Father	Brother	Sister	Child	None
Cerebral stroke or brain haemorrhage	113	☐	☐	☐	☐	☐	☐
Heart attack before age 60	119	☐	☐	☐	☐	☐	☐
Cancer	125	☐	☐	☐	☐	☐	☐
Asthma	131	☐	☐	☐	☐	☐	☐
Gastric/duodenal ulcer	137	☐	☐	☐	☐	☐	☐
Osteoporosis	143	☐	☐	☐	☐	☐	☐
Psychological problems	149	☐	☐	☐	☐	☐	☐
Allergy	155	☐	☐	☐	☐	☐	☐
Diabetes	161	☐	☐	☐	☐	☐	☐
– age when they got diabetes	167	___	___	___	___	___	

SYMPTOMS

		Yes	No
Do you cough about daily for some periods of the year?	177	☐	☐
If "Yes":			
Is your cough productive ?	178	☐	☐
Have you had this kind of cough for as long as 3 months in each of the last two years?	179	☐	☐
Have you had episodes of wheezing in your chest?	180	☐	☐
If "Yes", has this occurred:			
Tick one box only for each item.			
At night	181	☐	☐
In connection with respiratory infections		☐	☐
In connection with physical exertion		☐	☐
In connection with very cold weather		☐	☐
Have you noticed sudden changes in your pulse or heart rhythm in the last year?	185	☐	☐

How often do you suffer from sleeplessness?

Never, or just a few times a year	186	☐ 1
1-2 times a month		☐ 2
Approximately once a week		☐ 3
More than once a week		☐ 4

If you suffer from sleeplessness, what time of the year does it affect you most?

No particular time of year	187	☐ 1
Especially during the polar night		☐ 2
Especially during the midnight sun season		☐ 3
Especially in spring and autumn		☐ 4

		Yes	No
Have you in the last year suffered from sleeplessness to the extent that it has affected your ability to work?	188	☐	☐

How often do you suffer from headaches?

Rarely or never	189	☐ 1
Once or more a month		☐ 2
Once or more a week		☐ 3
Daily		☐ 4

Does the thought of getting a serious illness ever worry you?

Not at all	190	☐ 1
Only a little		☐ 2
Some		☐ 3
Very much		☐ 4

USE OF HEALTH SERVICES

How many visits have you made during the past year due to your own health or illness:
*Tick **0** if you have **not** had such contact*

		Number of times the past year
To a general practitioner (GP)/Emergency GP	191	_____
To a psychologist or psychiatrist		_____
To an other medical specialist (not at a hospital)		_____
To a hospital out-patient clinic	197	_____
Admitted to a hospital		_____
To a medical officer at work		_____
To a physiotherapist	203	_____
To a chiropractor		_____
To an acupuncturist		_____
To a dentist	209	_____
To an alternative practitioner (homoeopath, foot zone therapist, etc.)		_____
To a healer, faith healer, clairvoyant		_____

MEDICATION AND DIETARY SUPPLEMENTS

Have you for any length of time in the past year used any of the following medicines or dietary supplements daily or almost daily? Indicate how many months you have used them.
*Put **0** for items you have **not** used.*

Medicines

Painkillers	215	_____ months
Sleeping pills		_____ months
Tranquillizers		_____ months
Antidepressants	221	_____ months
Allergy drugs		_____ months
Asthma drugs		_____ months

Dietary supplements

Iron tablets	227	_____ months
Calcium tablets or bonemeal		_____ months
Vitamin D supplements		_____ months
Other vitamin supplements	233	_____ months
Cod liver oil or fish oil capsules		_____ months

Have you in the last 14 days used the following medicines or dietary supplements?
*Tick **one** box only for **each** item.*

		Yes	No
Medicines			
Painkillers	237	❑	❑
Antipyretic drugs (to reduce fever)		❑	❑
Migraine drugs		❑	❑
Eczema cream/ointment		❑	❑
Heart medicines (not blood pressure)		❑	❑
Cholesterol lowering drugs		❑	❑
Sleeping pills		❑	❑
Tranquillizers		❑	❑
Antidepressants		❑	❑
Other drugs for nervous conditions		❑	❑
Antacids	247	❑	❑
Gastric ulcer drugs		❑	❑
Insulin		❑	❑
Diabetes tablets		❑	❑
Drugs for hypothyroidism (Thyroxine)		❑	❑
Cortisone tablets	252	❑	❑
Other medicine(s)		❑	❑
Dietary supplements			
Iron tablets		❑	❑
Calcium tablets or bonemeal		❑	❑
Vitamin D supplements		❑	❑
Other vitamin supplements	257	❑	❑
Cod liver oil or fish oil capsules		❑	❑

FRIENDS

How many good friends do you have whom you can talk confidentially with and who give you help when you need it? 259 _____ good friends
Do not count people you live with, but do include other relatives!

How many of these good friends do you have contact with at least once a month? ..... 261 _____

		Yes	No
Do you feel you have enough good friends?	263	❑	❑

How often do you normally take part in organised gatherings, e.g. sewing circles, sports clubs, political meetings, religious or other associations?

Never, or just a few times a year	264	❑ 1
1-2 times a month		❑ 2
Approximately once a week		❑ 3
More than once a week		❑ 4

FOOD HABITS

If you use butter or margarine on your bread, how many slices does a small catering portion normally cover? By this, we mean the portion packs served on planes, in cafés, etc. (10-12g)

A catering portion is enough for about ..... 265 _____ slices

What kind of fat is normally used in **cooking** (not on the bread) in your home?

Butter	266	❑
Hard margarine		❑
Soft margarine		❑
Butter/margarine blend		❑
Oils	270	❑

What kind of bread (bought or home-made) do you usually eat?
Tick one or two boxes!

	White bread	Light textured	Ordinary brown	Coarse brown	Crisp bread
The bread I eat is most similar to:	❑ 271	❑	❑	❑	❑ 275

How much (in **number** of glasses, cups, potatoes or slices) do you usually eat or drink **daily** of the following foodstuffs?
*Tick one box for **each** foodstuff.*

		0	Less than 1	1-2	3-4	5-6	More than 6
Full milk (ordinary or curdled) (glasses)	276	❑	❑	❑	❑	❑	❑
Semi-skimmed milk (ordinary or curdled) (glasses)		❑	❑	❑	❑	❑	❑
Skimmed milk (ordinary or curdled) (glasses)		❑	❑	❑	❑	❑	❑
Tea (cups)		❑	❑	❑	❑	❑	❑
Orange juice (glasses)		❑	❑	❑	❑	❑	❑
Potatoes	281	❑	❑	❑	❑	❑	❑
Slices of bread in total (incl. crisp-bread)		❑	❑	❑	❑	❑	❑
Slices of bread with - fish (e.g. mackerel in tomato sauce)		❑	❑	❑	❑	❑	❑
- lean meat (e.g. ham)		❑	❑	❑	❑	❑	❑
- fat meat (e.g. salami)		❑	❑	❑	❑	❑	❑
- cheese *(e.g. Gouda/ Norvegia)*	286	❑	❑	❑	❑	❑	❑
- brown cheese		❑	❑	❑	❑	❑	❑
- smoked cod caviare		❑	❑	❑	❑	❑	❑
- jam and other sweet spreads		❑ 1	❑ 2	❑ 3	❑ 4	❑ 5	❑ 6

How many **times per week** do you normally eat the following foodstuffs?
*Tick a box for **all** foodstuffs listed.*

		Never	Less than 1	1	2-3	4-5	almost daily
Yoghurt	290	❑	❑	❑	❑	❑	❑
Boiled or fried egg		❑	❑	❑	❑	❑	❑
Breakfast cereal/ oat meal, etc.		❑	❑	❑	❑	❑	❑
Dinner with - unprocessed meat		❑	❑	❑	❑	❑	❑
- sausage/meatloaf/ meatballs		❑	❑	❑	❑	❑	❑
- fatty fish (e.g. salmon/redfish)	295	❑	❑	❑	❑	❑	❑
- lean fish (e.g. cod)		❑	❑	❑	❑	❑	❑
- fishballs/fishpudding/fishcakes		❑	❑	❑	❑	❑	❑
- vegetables		❑	❑	❑	❑	❑	❑
Mayonnaise, remoulade		❑	❑	❑	❑	❑	❑
Carrots	300	❑	❑	❑	❑	❑	❑
Cauliflower/cabbage/ broccoli		❑	❑	❑	❑	❑	❑
Apples/pears		❑	❑	❑	❑	❑	❑
Oranges, mandarins		❑	❑	❑	❑	❑	❑
Sweetened soft drinks		❑	❑	❑	❑	❑	❑
Sugar-free ("Light") soft drinks		❑	❑	❑	❑	❑	❑
Chocolate		❑	❑	❑	❑	❑	❑
Waffles, cakes, etc.	307	❑ 1	❑ 2	❑ 3	❑ 4	❑ 5	❑ 6

ALCOHOL

How often do you usually drink	beer?	wine?	spirits?
Never, or just a few times a year	☐	☐	☐ 1
1-2 times a month	☐	☐	☐ 2
About once a week	☐	☐	☐ 3
2-3 times a week	☐	☐	☐ 4
More or less daily	☐	☐	☐ 5
	308		310

Approximately how often during the last year have you consumed alcohol corresponding to at least 5 small bottles of beer, a bottle of wine, or 1/4 bottle of spirits?

- Not at all the last year ☐ 1
- A few times ☐ 2
- 1-2 times a month ☐ 3
- 1-2 times a week ☐ 4
- 3 or more times a week ☐ 5

For approximately how many years has your alcohol consumption been as you described above? 312 ____years

WEIGHT REDUCTION

About how many times have you deliberately tried to lose weight? Write **0** if you never have.

- before age 20 314 _____ times
- later 316 _____ times

If you have lost weight deliberately, about how many kilos have you ever lost at the most?

- before age 20 318 _____ kg
- later 320 _____ kg

What weight would you be satisfied with (your "ideal weight")? 322 _____ kg

URINARY INCONTINENCE

How often do you suffer from urinary incontinence?

- Never 325 ☐ 1
- Not more than once a month ☐ 2
- Two or more times a month ☐ 3
- Once a week or more ☐ 4

Your comments:

TO BE ANSWERED BY WOMEN ONLY

MENSTRUATION

How old were you when you started menstruating? 326 _____years

If you no longer menstruate, how old were you when you stopped menstruating? 328 _____years

Apart from pregnancy and after giving birth, have you ever stopped having menstruation for 6 months or more? 330 Yes ☐ No ☐

If "Yes", how many times? 331 _____ times

If you still menstruate or are pregnant:

What date did your last menstruation period begin? 333 day/month/year ___/___/___

Do you usually use painkillers to relieve period pains? 339 Yes ☐ No ☐

PREGNANCY

How many children have you given birth to? 340 _____ children

Are you pregnant at the moment? 342 Yes ☐ No ☐ Don't know ☐

Have you during pregnancy had high blood pressure and/or proteinuria? 343 Yes ☐ No ☐

If "Yes", during which pregnancy?

	Pregnancy First	Later
High blood pressure 344	☐	☐
Proteinuria 346	☐	☐

If you have given birth, fill in for each child the year of birth and approximately how many months you breastfed the child.

Child	Year of birth:	Number of months breastfed:
1	348 _____	_____
2	_____	_____
3	356 _____	_____
4	_____	_____
5	364 _____	_____
6	_____	_____

CONTRACEPTION AND ESTROGEN

Do you use, or have you ever used:	Now	Before	Never
Oral contraceptive pills (incl. minipill) 372	☐	☐	☐
Hormonal intrauterine device	☐	☐	☐
Estrogen (tablets or patches) 374	☐	☐	☐
Estrogen (cream or suppositories)	☐	☐	☐
	1	2	3

If you use oral contraceptive pills, hormonal intrauterine device, or estrogen, what brand do you currently use?

376 .....

If you use or have ever used oral contraceptive pills:

Age when you started to take the pill? 380 _____years

How many years in total have you taken the pill? 382 _____years

If you have given birth, how many years did you take the pill before your first delivery? 384 _____years

If you have stopped taking the pill:

Age when you stopped? 386 _____years

Thank you for the help! Remember to mail the form today!

The Tromsø Health Survey

Appendix III

Questionnaires and invitations to the 5th Tromsø Study

Health survey

Personal Invitation

Don't write here

5.3 (Municipality) (County) (Country)

9.3 (Business) 9.4 (Occupation) 14.7 (Mark)

1. YOUR OWN HEALTH

1.1 What is your current state of health? *(Tick one only)*

Poor ☐ 1 | Not so good ☐ 2 | Good ☐ 3 | Very good ☐ 4

1.2 Do you have, or have you had?:

	Yes	No	Age first time
Asthma	☐	☐	
Hay fever	☐	☐	
Chronic bronchitis/emphysema	☐	☐	
Diabetes	☐	☐	
Osteoporosis	☐	☐	
Fibromyalgia/chronic pain syndrome	☐	☐	
Psychological problems for which you have sought help	☐	☐	
A heart attack	☐	☐	
Angina pectoris (heart cramp)	☐	☐	
Cerebral stroke/brain haemorrhage	☐	☐	

1.3 Have you noticed attacks of sudden changes in your pulse or heart rhythm in the last year? ☐ Yes ☐ No

1.4 Do you get pain or discomfort in the chest when:
Walking up hills, stairs or walking fast on level ground? ☐ Yes ☐ No

1.5 If you get such pain, do you usually:

Stop? ☐ 1 | Slow down? ☐ 2 | Carry on at the same pace? ☐ 3

1.6 If you stop, does the pain disappear within 10 minutes? ☐ Yes ☐ No

1.7 Can such pain occur even if you are at rest? ☐ Yes ☐ No

2. MUSCULAR AND SKELETAL COMPLAINTS

2.1 Have you suffered from pain and/or stiffness in muscles and joints during the last 4 weeks?
(Give duration only if you have had problems)

	No complaint	Some complaint	Severe complaint	Duration: Up to 2 weeks	Duration: 2 weeks or more
Neck/shoulders	☐	☐	☐	☐	☐
Arms, hands	☐	☐	☐	☐	☐
Upper part of your back...	☐	☐	☐	☐	☐
Lumbar region	☐	☐	☐	☐	☐
Hips, legs, feet	☐	☐	☐	☐	☐
Other places	☐	☐	☐	☐	☐
	1	2	3	1	2

2.2 Have you ever had:

	Yes	No	Age last time
Fracture in the wrist/forearm	☐	☐	
Hip fracture?	☐	☐	

3. OTHER COMPLAINTS

3.1 Below is a list of various problems. Have you experienced any of this during the last week (including today)?
(Tick once for each complaint)

	No complaint	Little complaint	Pretty much	Very much
Sudden fear without reason	☐	☐	☐	☐
Felt afraid or anxious	☐	☐	☐	☐
Faintness or dizziness	☐	☐	☐	☐
Felt tense or upset	☐	☐	☐	☐
Tend to blame yourself	☐	☐	☐	☐
Sleeping problems	☐	☐	☐	☐
Depressed, sad	☐	☐	☐	☐
Feeling of being useless, worthless	☐	☐	☐	☐
Feeling that everything is a struggle	☐	☐	☐	☐
Feeling of hopelessness with regard to the future	☐	☐	☐	☐
	1	2	3	4

4. USE OF HEALTH SERVICES

4.1 How many times in the last 12 months have you been to/used:
(Tick once for each line)

	None	1-3 times	4 or more
General practitioner (GP)	☐	☐	☐
Medical officer at work	☐	☐	☐
Psychologist or psychiatrist *(private or out-patient clinic)*	☐	☐	☐
Other specialist *(private or out-patient clinic)*	☐	☐	☐
Emergency GP *(private or public)*	☐	☐	☐
Hospital admission	☐	☐	☐
Home nursing care	☐	☐	☐
Physiotherapist	☐	☐	☐
Chiropractor	☐	☐	☐
Dentist	☐	☐	☐
Alternative practitioner	☐	☐	☐

5. CHILDHOOD/YOUTH AND AFFILIATION

5.1 How long altogether have you lived in the county? ☐☐ year
(Put 0 if less than half a year)

5.2 How long altogether have you lived in the municipality? ☐☐ year
(Put 0 if less than half a year)

5.3 Where did you live most of the time before the age of 16?
(Tick one option and specify)

Same municipality .... ☐ 1

Another municipality in the county ..... ☐ 2 Which one: _____

Another county in Norway ☐ 3 Which one: _____

Outside Norway ..... ☐ 4 Country:: _____

5.4 Have you moved within the last five years?

No ☐ 1 | Yes, one time ☐ 2 | Yes, more than once ☐ 3

6. BODY WEIGHT

6.1 Estimate your body weight when you were 25 years old: ☐☐☐ kg

7. FOOD AND BEVERAGES

7.1 How often do you usually eat these foods?
(Tick once per line)

	Rarely /never	1-3 times /month	1-3 times /week	4-6 times /week	1-2 times /day	3 times or more /day
Fruit, berries	☐	☐	☐	☐	☐	☐
Cheese *(all types)*	☐	☐	☐	☐	☐	☐
Potatoes	☐	☐	☐	☐	☐	☐
Boiled vegetables	☐	☐	☐	☐	☐	☐
Fresh vegetables/salad	☐	☐	☐	☐	☐	☐
Fatty fish *(e.g. salmon, trout, mackerel, herring)*	☐	☐	☐	☐	☐	☐
	1	2	3	4	5	6

7.2 What type of fat do you usually use? *(Tick once per line)*

	Don't use	Butter	Hard margarine	Soft/light margarine	Oils	Other
On bread	☐	☐	☐	☐	☐	☐
For cooking	☐	☐	☐	☐	☐	☐
	1	2	3	4	5	6

7.3 Do you use the following dietary supplements:

	Yes, daily	Sometimes	No
Cod liver oil, fish oil capsules	☐	☐	☐
Vitamins and/or mineral supplements?	☐	☐	☐

7.4 How much of the following do you usually drink?
(Tick once per line)

	Rarely /never	1-6 glasses /week	1 glass /day	2-3 glasses /day	4 glasses or more /day
Full milk, full-fat curdled milk, yoghurt	☐	☐	☐	☐	☐
Semi-skimmed milk, semi-skimmed curdled milk,low-fat yoghurt	☐	☐	☐	☐	☐
Skimmed milk, skimmed curdled milk	☐	☐	☐	☐	☐
Extra semi-skimmed milk	☐	☐	☐	☐	☐
Juice	☐	☐	☐	☐	☐
Water	☐	☐	☐	☐	☐
Mineral water (e.g. Farris, Ramløsa etc)	☐	☐	☐	☐	☐
Cola-containing soft drink	☐	☐	☐	☐	☐
Other soda/soft drink	☐	☐	☐	☐	☐
	1	2	3	4	5

7.5 Do you usually drink soft drink: with sugar ☐ 1 without sugar ☐ 2

7.6 How many cups of coffee and tea do you drink daily? *Number of cups*
(Put 0 for the types you don't drink daily)

Filtered coffee ..... ☐☐

Boiled coffee/coarsely ground coffee for brewing ..... ☐☐

Other type of coffee ..... ☐☐

Tea ..... ☐☐

7.7 Approximately how often have you during the last year consumed alcohol? *(Do not count low-alcohol and alcohol-free beer)*

Never consumed alcohol ☐ 1	Have not consumed alcohol last year ☐ 2	A few times last year ☐ 3	About 1 time a month ☐ 4
2-3 times per month ☐ 5	About1 time a week ☐ 6	2-3 times a week ☐ 7	4-7 times a week ☐ 8

To those who have consumed the last year:

7.8 When you drink alcohol, how many glasses or drinks do you normally drink? *number* ☐☐

7.9 Approximately how many times during the last year have you consumed alcohol equivalent to 5 glasses or drinks within 24 hours? *Number of times* ☐☐

7.10 When you drink, do you normally drink: *(Tick one or more)*

Beer ☐ Wine ☐ Spirits ☐

8. SMOKING

8.1 How many hours a day do you normally spend in smoke-filled rooms? *Number of total hours* ☐☐

	Yes	No
8.2 Did any of the adults smoke at home while you were growing up?	☐	☐
8.3 Do you currently, or did you previously live together with a daily smoker after your 20th birthday?	☐	☐

	Yes, now	Yes, previously	Never
8.4 Do you/did you smoke daily?	☐	☐	☐

If NEVER: Go to question 9 : (EDUCATION AND WORK)

8.5 If you smoke daily now, do you smoke:	Yes	No
Cigarettes?	☐	☐
Cigars/cigarillos?	☐	☐
A pipe?	☐	☐

8.6 If you previously smoked daily, how long is it since you quit? *Number of years* ☐☐

8.7 If you currently smoke, or have smoked previously:

How many cigarettes do you or did you normally smoke per day? *Number of cigarettes* ☐☐

How old were you when you began daily smoking? *Age in years* ☐☐

How many years in all have you smoked daily? *Number of years* ☐☐

9. EDUCATION AND WORK

9.1 How many years of education have you completed? *Number of years* ☐☐
(Include all the years you have attended school or studied)

9.2 Do you currently have paid work?

Yes, full-time ☐ 1 Yes, part-time ☐ 2 No ☐ 3

9.3 Describe the activity at the workplace where you had paid work for the longest period in the last 12 months. *(e.g. Accountancy firm, school, paediatric department, carpentry workshop, garage, bank, grocery store, etc.)*

Business: _____
If retired, enter the former business and occupation. Also applies to 9.4

9.4 Which occupation/title have or had you at this workplace?
(e.g. Secretary, teacher, industrial worker, nurse, carpenter, manager, salesman, driver, etc.)

Occupation: _____

9.5 In your main occupation, do you work as self-employed, as an employee or family member without regular salary?

Self-employed ☐ Employee ☐ Family member ☐

	Yes	No
9.6 Do you believe that you are in danger of losing your current work or income within the next two years?	☐	☐

9.7 Do you receive any of the following benefits?	Yes	No
Sickness benefit (are on sick leave)	☐	☐
Old age pension, early retirement (AFP) or survivor pension	☐	☐
Rehabilitation/reintegration benefit	☐	☐
Disability pension (full or partial)	☐	☐
Unemployment benefits during unemployment	☐	☐
Social welfare benefits	☐	☐
Transition benefit for single parents	☐	☐

10. EXERCISE AND PHYSICAL ACTIVITY

10.1 How has your physical activity in leisure time been during this last year?

Think of a weekly average for the year.
Time spent going to work is count as leisure time. Answer both questions.

	Hours per week: None	Less than 1	1-2	3 or more
Light activity *(not sweating/out of breath)...*	☐	☐	☐	☐
Hard physical activity *(sweating/out of breath).....*	☐ 1	☐ 2	☐ 3	☐ 4

10.2 Describe exercise and physical exertion in your leisure time. If your activity varies much e.g. between summer and winter, then give an average. The question refers only to the last year.

(Tick the most appropriate box)

Reading, watching TV or other sedentary activity? ..... ☐ 1

Walking, cycling or other forms of exercise at least 4 hours a week? ..... ☐ 2
(Include walking or cycling to work, Sunday walk/stroll,etc.)

Participation in recreational sports, heavy gardening, etc.? ☐ 3
(Note: duration of activity at least 4 hours a week)

Participation in hard training or sports competitions, regularly several times a week? ..... ☐ 4

11. FAMILY AND FRIENDS

11.1 Do you live with:

	Yes	No
Spouse/partner?.....	☐	☐

11.2 How many good friends do you have? *Number of friends* ☐☐

Count the ones you can talk confidentially with and who can give you help when you need it. Do not count people you live with, but do include other relatives.

11.3 How much interest do people show for what you do?
(Tick only once)

Great interest	Some interest	Little interest	No interest	Uncertain
☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

11.4 How many associations, sport clubs,groups, religious communities or similar do you take part in? *Number* ☐☐
(Write 0 if none)

11.5 Do you feel that you can influence what happening in your local community where you live? *(Tick only once)*

Yes, a lot	Yes, some	Yes, a little	No	Never tried
☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

12. ILLNESS IN THE FAMILY

12.1 Have one or more of your parents or siblings had a heart attack (heart wound) or angina pectoris (heart cramp)? .....

	Yes	No	Don't know
	☐	☐	☐

12.2 Tick for the relatives who have or have had any of the illnesses: *(Tick for each line)*

	Mother	Father	Brother	Sister	Child	None of these
Cerebral stroke or brain haemorrhage	☐	☐	☐	☐	☐	☐
Heart attack before age of 60 years	☐	☐	☐	☐	☐	☐
Asthma.....	☐	☐	☐	☐	☐	☐
Cancer	☐	☐	☐	☐	☐	☐
Diabetes	☐	☐	☐	☐	☐	☐

12.3 If any relatives have diabetes, at what age did they get diabetes (if for e.g. many siblings, consider the one who got it earliest in life):

Don't know, not applicable	Mother's age	Father's age	Brother's age	Sister's age	Child's age
☐	☐☐	☐☐	☐☐	☐☐	☐☐

13. USE OF MEDICINES

With medicines, we mean drugs purchased at pharmacies. Supplements and vitamins are not considered here.

13.1 Do you use:

	Now	Previously, but not now	Never used
Blood pressure lowering drugs	☐	☐	☐
Cholesterol-lowering drugs	☐	☐	☐

13.2 How often have you during the last 4 weeks used the following medicines?
(Tick once for each line)

	Not used in the last 4 weeks	Less than every week	Every week but not daily	Daily
Painkillers non-prescription	☐	☐	☐	☐
Painkillers on prescription	☐	☐	☐	☐
Sleeping pills	☐	☐	☐	☐
Tranquillizers	☐	☐	☐	☐
Antidepressants	☐	☐	☐	☐
Other prescription medicines ...	☐ 1	☐ 2	☐ 3	☐ 4

13.3 For those medicines you have checked in points 13.1 and 13.2, and that you've used during the last 4 weeks:

State the name and the reason that you are taking/have taken these (disease or symptom):
(Tick for each duration you have used the medicine)

Name of the medicine: *(one name per line)*	Reason for use of the medicine	How long have you used the medicine: Up to 1 year	1 year or more
		☐	☐
		☐	☐
		☐	☐
		☐	☐
		☐	☐
		☐	☐
		☐	☐

If there is not enough space here, you may continue on a separate sheet that you attach

14. THE REST OF THE FORM IS TO BE ANSWERED BY WOMEN ONLY

14.1 How old were you when you started menstruating? *Age in years* ☐☐

14.2 If you no longer menstruating, how old were you when you stopped menstruating? *Age in years* ☐☐

14.3 Are you pregnant at the moment?

Yes	No	Uncertain	Above fertile age
☐ 1	☐ 2	☐ 3	☐ 4

14.4 How many children have you given birth to? *Number of children* ☐☐

14.5 Do you use, or have you ever used?
(Tick once for each line)

	Now	Before, but not now	Never
Oral contraceptive pills/mini pill/ contraceptive injection	☐	☐	☐
Hormonal intrauterine device (IUD) *(not ordinary IUD)*..	☐	☐	☐
Estrogen *(tablets or patches)*	☐	☐	☐
Estrogen *(cream or suppositories)*	☐	☐	☐

14.6 If you use/have used prescription estrogen:
How long have you used it? *Number of years* ☐☐

14.7 If you use contraceptive pills, mini pill, contraceptive injection, hormonal IUD or estrogen, what brand do you use?

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Appendix IV

Questionnaires and invitations to the 6th Tromsø Study

English versions

The Tromsø Study

The form will be read electronically. Please use a blue or black pen
You can not use comas, use upper-case letters.

2007 - 2008 Confidential

HEALTH AND DISEASES

1 **How do you in general consider your own health to be?**

- ☐ Very good
- ☐ Good
- ☐ Neither good nor bad
- ☐ Bad
- ☐ Very bad

2 **How is your health compared to others in your age?**

- ☐ Much better
- ☐ A little better
- ☐ About the same
- ☐ A little worse
- ☐ Much worse

3 **Do you have, or have you had?**

	Yes	No	Age first time
A heart attack	☐	☐	
Angina pectoris *(heart cramp)*	☐	☐	
Cerebral stroke/brain hemorrhage	☐	☐	
Atrial fibrillation	☐	☐	
High blood pressure	☐	☐	
Osteoporosis	☐	☐	
Asthma	☐	☐	
Chronic bronchitis/Emphysema/COPD	☐	☐	
Diabetes	☐	☐	
Psychological problems *(for which you have sought help)*	☐	☐	
Hypothyroidism	☐	☐	
Kidney disease, *not including urinary tract infection (UTI)*	☐	☐	
Migraine	☐	☐	

4 **Do you have persistent or constantly recurring pain that has lasted for 3 months or more?**

- ☐ Yes ☐ No

5 **How often have you suffered from sleeplessness during the last 12 months?**

- ☐ Never, or just a few times
- ☐ 1-3 times a month
- ☐ Approximately once a week
- ☐ More that once a week

6 **Below you find a list of various problems. Have you experienced any of this during the last week (including today)?** (Tick once for each complaint)

	No complaint	Little complaint	Pretty much	Very much
Sudden fear without reason	☐	☐	☐	☐
Felt afraid or anxious	☐	☐	☐	☐
Faintness or dizziness	☐	☐	☐	☐
Felt tense or upset	☐	☐	☐	☐
Tend to blame yourself	☐	☐	☐	☐
Sleeping problems	☐	☐	☐	☐
Depressed, sad	☐	☐	☐	☐
Feeling of being useless, worthless	☐	☐	☐	☐
Feeling that everything is a struggle	☐	☐	☐	☐
Feeling of hopelessness with regard to the future	☐	☐	☐	☐

USE OF HEALTH SERVICES

7 **Have you during the last 12 months visited:** If YES; how many times?

	Yes	No	No. of times
General practitioner (GP)	☐	☐	
Psychiatrist/psychologist	☐	☐	
Medical specialist outside hospital *(other than general practitioner/psychiatrist)*	☐	☐	
Physiotherapist	☐	☐	
Chiropractor	☐	☐	
Alternative practitioner *(homeopath, acupuncturist, foot zone therapist, herbal medicine practitioner, laying on hands practitioner, healer, clairvoyant, etc.)*	☐	☐	
Dentist/dental service	☐	☐	

8 **Have you during the last 12 months been to a hospital?**

	Yes	No	No. of times
Admitted to a hospital	☐	☐	
Had consultation in a hospital without admission;			
At psychiatric out-patient clinic	☐	☐	
At another out-patient clinic	☐	☐	

9 **Have you undergone any surgery during the last 3 years?**

- ☐ Yes ☐ No

USE OF MEDICINES

10 **Do you currently use, or have you used some of the following medicines?** (Tick once for each line)

	Never used	Now	Earlier	Age first time
Blood pressure lowering drugs	☐	☐	☐	
Cholesterol lowering drugs	☐	☐	☐	
Drugs for heart disease	☐	☐	☐	
Diuretics	☐	☐	☐	
Drugs for osteoporosis	☐	☐	☐	
Insulin	☐	☐	☐	
Tablets for diabetes	☐	☐	☐	
The drugs for hypothyroidism Thyroxine/levaxin	☐	☐	☐	

11 **How often have you during the last 4 weeks used the following medicines?** (Tick once for each line)

	Not used in the last 4 weeks	Less than every week	Every week, but not daily	Daily
Painkillers on prescription	☐	☐	☐	☐
Painkillers non-prescription	☐	☐	☐	☐
Sleeping pills	☐	☐	☐	☐
Tranquillizers	☐	☐	☐	☐
Antidepressants	☐	☐	☐	☐

12 **State the name of all medicines -both those on prescription and non-prescription drugs- you have used regularly during the last 4 weeks.** Do not include vitamins, minerals, herbs, natural remedies, other nutritional supplements, etc.

If there is not enough space for all medicines, continue on a separate sheet.

When attending you will be asked whether you have used antibiotics or painkillers the last 24 hours. If you have, you will be asked to provide the name of the drug, strength, dose and time of use.

FAMILY AND FRIENDS

13 **Who do you live with?** (Tick for each question and give the number)

	Yes	No	Number
Spouse/partner	☐	☐	
Other people older than 18 years	☐	☐	
People younger than 18 years	☐	☐	

14 **Tick for the relatives who have or have had**

	Parents	Children	Siblings
A heart attack	☐	☐	☐
A heart attack before age of 60	☐	☐	☐
Angina pectoris *(heart cramp)*	☐	☐	☐
Cerebral stroke/brain haemorrhage	☐	☐	☐
Osteoporosis	☐	☐	☐
Gastric/duodenal ulcers	☐	☐	☐
Asthma	☐	☐	☐
Diabetes	☐	☐	☐
Dementia	☐	☐	☐
Psychological problems	☐	☐	☐
Substance abuse	☐	☐	☐

15 **Do you have enough friends who can give you help when you need it?**

☐ Yes ☐ No

16 **Do you have enough friends whom you can talk confidentially with?**

☐ Yes ☐ No

17 **How often do you normally take part in organised gatherings, e.g. sport clubs, political meetings, religious or other associations?**

☐ Never, or just a few times a year
☐ 1-2 times a month
☐ Approximately once a week
☐ More than once a week

WORK, SOCIAL SECURITY AND INCOME

18 **What is the highest level of education you have completed?** (Tick once)

☐ Primary/secondary school, modern secondary school
☐ Technical school, vocational school, 1-2 years senior high school
☐ High school diploma
☐ College/university less than 4 years
☐ College/university 4 years or more

19 **What is your main activity?** (Tick once)

☐ Full time work ☐ Housekeeping
☐ Part time work ☐ Retired/benefit recipient
☐ Unemployed ☐ Student/military service

20 **Do you receive any of the following benefits?**

- ☐ Old-age, early retirement or survivor pension
- ☐ Sickness benefit (on sick leave)
- ☐ Rehabilitation benefit
- ☐ Full disability pension
- ☐ Partial disability pension
- ☐ Unemployment benefits
- ☐ Transition benefit for single parents
- ☐ Social welfare benefits

21 **What was the household's total taxable income last year?** Include income from work, pensions, benefits and similar

- ☐ Less than 125 000 NOK
- ☐ 125 000-200 000 NOK
- ☐ 201 000-300 000 NOK
- ☐ 301 000-400 000 NOK
- ☐ 401 000-550 000 NOK
- ☐ 551 000-700 000 NOK
- ☐ 701 000 -850 000 NOK
- ☐ More than 850 000 NOK

22 **Do you work outdoor at least 25% of the time, or in cold buildings (e.g. storehouse/industry buildings)?**

- ☐ Yes
- ☐ No

PHYSICAL ACTIVITY

23 **If you have paid or unpaid work, which statement describes your work best?**

- ☐ Mostly sedentary work
  (e.g. office work, mounting)
- ☐ Work that requires a lot of walking
  (e.g. shop assistant, light industrial work, teaching)
- ☐ Work that requires a lot of walking and lifting
  (e.g. postman, nursing, construction)
- ☐ Heavy manual labour

24 **Describe your exercise and physical exertion in leisure time. If your activity varies much, e.g. between summer and winter, then give an average. The question refers only to the last year.** (Tick the most appropriate box)

- ☐ Reading, watching TV, or other sedentary activity.
- ☐ Walking, cycling, or other forms of exercise at least 4 hours a week *(include walking or cycling to work, Sunday-walk/stroll, etc.)*
- ☐ Participation in recreational sports, heavy gardening, etc. *(note:duration of activity at least 4 hours a week)*
- ☐ Participation in hard training or sports competitions, regularly several times a week.

25 **How often do you exercise?** (With exercise we mean for example walking, skiing, swimming or training/sports)

- ☐ Never
- ☐ Less than once a week
- ☐ Once a week
- ☐ 2-3 times a week
- ☐ Approximately every day

26 **How hard do you exercise on average?**

- ☐ Easy- do not become short-winded or sweaty
- ☐ You become short-winded and sweaty
- ☐ Hard- you become exhausted

27 **For how long time do you exercise every time on average?**

- ☐ Less than 15 minutes
- ☐ 15-29 minutes
- ☐ 30-60 minutes
- ☐ More than 1 hour

ALCOHOL AND TOBACCO

28 **How often do you drink alcohol?**

- ☐ Never
- ☐ Monthly or less frequently
- ☐ 2-4 times a month
- ☐ 2-3 times a week
- ☐ 4 or more times a week

29 **How many units of alcohol** (a beer, a glass of wine or a drink) **do you usually drink when you drink alcohol?**

- ☐ 1-2
- ☐ 3-4
- ☐ 5-6
- ☐ 7-9
- ☐ 10 or more

30 **How often do you drink 6 units of alcohol or more in one occasion?**

- ☐ Never
- ☐ Less frequently than monthly
- ☐ Monthly
- ☐ Weekly
- ☐ Daily or almost daily

31 **Do you smoke sometimes, but not daily?**

- ☐ Yes
- ☐ No

32 **Do you/did you smoke daily?**

- ☐ Yes, now
- ☐ Yes, previously
- ☐ Never

33 **If you previously smoked daily, how long is it since you quit?**

Number of years ☐☐

34 **If you currently smoke, or have smoked previously: How many cigarettes do you or did you usually smoke per day?**

Number of cigarettes ☐☐

35 **How old were you when you began daily smoking?**

Age in years ☐☐

36 **How many years in all have you smoked daily?**

Number of years ☐☐

37 **Do you use or have you used snuff or chewing tobacco?**

- ☐ No, never
- ☐ Yes, previously
- ☐ Yes, sometimes
- ☐ Yes, daily

DIET

38 **Do you usually eat breakfast every day?**

☐ Yes ☐ No

39 **How many units of fruit or vegetables do you eat on average per day?** (units means for example a fruit, a cup of juice, potatoes, vegetables)

Number of units ☐☐

40 **How many times a week do you eat warm dinner?**

Number ☐☐

41 **How often do you usually eat these foods?**
(Tick once for each line)

	0-1 times/ mth	2-3 times/ mth	1-3 times/ week	4-6 times/ week	1-2 times/ day
Potatoes	☐	☐	☐	☐	☐
Pasta/rice	☐	☐	☐	☐	☐
Meat *(not processed)*	☐	☐	☐	☐	☐
Processed meat *(sausages, hamburger, etc.)*	☐	☐	☐	☐	☐
Fruits, vegetables, berries	☐	☐	☐	☐	☐
Lean fish	☐	☐	☐	☐	☐
Fatty fish *(e.g. salmon, trout, mackerel, herring, halibut, redfish)*	☐	☐	☐	☐	☐

42 **How much do you usually drink the following?**
(Tick once for each line)

	Rarely/ never	1-6 glasses /week	1 glass /day	2-3 glasses /day	4 or more glasses /day
Milk, curdled milk, yoghurt	☐	☐	☐	☐	☐
Juice	☐	☐	☐	☐	☐
Soft drinks with sugar	☐	☐	☐	☐	☐

43 **How many cups of coffee and tea do you drink daily?** (Put 0 for the types you do not drink daily)

	Number of cups
Filtered coffee	☐☐
Boiled coffee (coarsely ground coffee for brewing)	☐☐
Other types of coffee	☐☐
Tea	☐☐

44 **How often do you usually eat cod liver and roe?** (i.e. "mølje")

☐ Rarely/never ☐ 1-3 times/year ☐ 4-6 times/year
☐ 7-12 times/year ☐ More than 12 times/year

45 **Do you use the following nutritional supplements?**

	Daily	Sometimes	No
Cod liver oil or fish oil capsules	☐	☐	☐
Omega 3 capsules *(fish oil, seal oil)*	☐	☐	☐
Calcium tablets	☐	☐	☐

QUESTIONS FOR WOMEN

46 **Are you pregnant at the moment?**

☐ Yes ☐ No ☐ Uncertain

47 **How many children have you given birth to?**

Number ☐☐

48 **If you have given birth, fill in for each child: birth year, birth weight and months of breastfeeding** (Fill in the best you can)

Child	Birth year	Birth weight in grams	Months of breastfeeding
1	☐☐☐☐	☐☐☐☐	☐☐
2	☐☐☐☐	☐☐☐☐	☐☐
3	☐☐☐☐	☐☐☐☐	☐☐
4	☐☐☐☐	☐☐☐☐	☐☐
5	☐☐☐☐	☐☐☐☐	☐☐
6	☐☐☐☐	☐☐☐☐	☐☐

49 **Have you during pregnancy had high blood pressure?**

☐ Yes ☐ No

50 **If yes, during which pregnancy?**

☐ The first ☐ Second or later

51 **Have you during pregnancy had proteinuria?**

☐ Yes ☐ No

52 **If yes, during which pregnancy?**

☐ The first ☐ Second or later

53 **Were any of your children delivered prematurely (a month or more before the due date) because of preeclampsia?**

☐ Yes ☐ No

54 **If yes, which child?**

1st child ☐ 2nd child ☐ 3rd child ☐ 4th child ☐ 5th child ☐ 6th child ☐

55 **How old were you when you started menstruating?**

Age ☐☐

56 **Do you currently use any prescribed drug influencing the menstruation?**

Oral contraceptives, hormonal intrautrine or similar ☐ Yes ☐ No

Hormone treatment for menopausal problems ☐ Yes ☐ No

When attending you will get supplementary questions about menstruation and any use of hormones. Write down on a sheet of paper the names of all the hormones you have used and bring it with you. You will also be asked whether your menstruation have ceased and possibly when and why.

Appendix V

Protocols for ultrasound measurements

Tromsø 4, 5 and 6

English versions

PROCEDURES FOR MEASUREMENTS OF INTIMA-MEDIA THICKNESS AND RECORDING AND MEASUREMENTS OF PLAQUE OF THE RIGHT CAROTID ARTERY. THE TROMSØ-STUDY 1994/95 AND 2001

by Oddmund Joakimsen
Revised March 2001

1. The Acuson ultrasound instrument is switched on.

2. A videocassette is inserted in the video recorder.

3. Check that the videotape has been wound to the right position, do not overwrite previous recordings. The videocassette should not be removed from the recorder during the day.

4. Cassettes are marked with serial numbers, uneven numbers for Acuson I, even numbers for Acuson II.

5. The initials and the identity numbers of the participant and the sonographer number (Einar = 1, Stein Harald = 2, Technician = 3) are written on each ultrasound image recorded. Labels with the ID-number of the participants are attached to the registration form, in which all ultrasound data obtained from the participants are filled (plaque localization, size, "missing measures" coding, etc.).

6. A RES-field, appropriately adjusted to a maximum width of the screen and a depth of a little more than the preset size (> 2 cm) is positioned on the screen (This makes off-line calibration easier).

7. The subject is examined in a supine position with the head slightly rotated to the left (15-45 degrees). ECG-pads are attached to both arms and the right leg (or abdomen) (lead I), and the right carotid is insonated by a 7.5 MHz ultrasound transducer.

8. The examination starts with identification of crossectional B-mode images of the carotid artery, and, if necessary for identifying purposes in combination with colour-Doppler and/or pulsed wave Doppler 5 MHz. The examination starts caudally in the neck, normally just above the clavicle, then moving the probe upstream with simultaneous rotation movements to search for plaques also at the circumference of the vessel. Thus, the carotid artery is searched from the proximal part of the common carotid artery (CCA), upstream to the bifurcation (BULB), and as far up in the internal carotid artery (ICA) as technically possible. A PLAQUE is defined as a presumed atherosclerotic lesion of the intima layer of the vessel wall presenting a focal protrusion of more than 50% of the intima-media thickness (IMT**)** of the surrounding vessel wall, often with deviating echogenicity compared to other part of the artery wall. Whether a plaque is present or not is a decision taken by the sonographer during the examination. Live crossectional imaging of the whole carotid artery is recorded on the videotape.

9. An ultrasound examination sequence is then performed in the TRIPLEX -mode (i.e., combination of B-mode examination, pulsed wave Doppler, colour Doppler) 3-4 cm proximally to the bifurcation and upstream 2-3 cm distally the bifurcation in the ICA. The objective of this part of the examination is to look for stenotic areas along the artery that causes hemodynamic disturbances. However, if plaques later during the B-mode scanning procedure are found suspicious of a hemodynamic significant stenosis, a new TRIPLEX examination is performed to re-evaluate the flow conditions. A LIVE TRIPLEX-sequence of the relevant part of the carotid artery is recorded on the videotape if a stenosis is suspected.

10. B-mode longitudinal ultrasound scanning of the carotid artery is then performed. To get an optimal topographic reference, the examination is starting as proximally as possible in CCA. The probe is then moving upstream with simultaneous rotating movements to look for plaques in all segments, both the near and the far wall. If a plaque is found, a frozen image of the vessel-wall is taken – either directly by using the "FREEZE"- key, or by choosing on of the pictures from the cine-loop. It is important that the plaque is presented as distinctly as possible and after the guidelines according to elementary ultrasound principles such as vertical propagation of the ultrasound beam, presentation of the plaque in the full diameter of the vessel and not in chord, not cutting the plaque skew causing a falsely too large thickness of the plaque. To ensure the quality of plaque registration, some technical points may be of help: The plaque should be "attached" at its both ends to the typical double-lined intima-media structures visible on the B-mode image, and these double-lined structures should best be visible both in the near and the far wall at the same time. When the echogenicity obtained is as high as possible (as bright as possible), this is an indication that the ultrasound waves have cut the plaque optimally. An electronic calliper is put on the top of the plaque (at the interface between the surface of the plaque and the vessel lumen), and another calliper in the presumed transition zone between the media and the adventitia layer. The distance between the callipers is the thickness of the plaque, and that value is put on the registration form in the appropriate box. The B-mode image of the plaque is identified correctly by marking on the display what has been found, and where: PLAQUE ICA FW (a plaque in the far wall of the internal carotid artery), PLAQUE BULB NW (a plaque in the near wall of the bifurcation), etc. A short recording of approximately 5 sec. is videotaped. If more than one plaque is present at a site (e.g., in the far wall of ICA), the largest is chosen and recorded.

    After identifying and recording of plaques, imaging procedures to get optimal measures of IMT from CCA and the BULB are performed. Optimal images are available when distinct double contours of the vessel wall typical for the intima-media complex can be seen. It is important that the longitudinal axis of the insonated vessel wall is perpendicular to the ultrasound beam direction. To avoid falsely too thick intima-media layer, the IMT should be measured in the full diameter of the artery and not in a chord. When satisfactory images are achieved, R-wave triggered IMT-registrations are recorded on a cine-loop containing more than 20 images. Afterwards, the images stored in the cine-loop are scrutinized and 3 of most representative images, and each at least 10 images apart, are selected for recording on the videotape.

    Regarding IMT measurements in the BULB, the start of the BULB is first identified and then marked with an arrow. This is the point where the parallel walls of the CCA are starting to diverge. If the probe throughout the recording process in the cine-loop has changed position, the placing of the arrow marker must be adjusted accordingly. It is important to underline that it is the sonographer who places the marker and not the off-

line reader of the IMT-measurements. The arrow setting has to be as precise as possible, particularly when a plaque is located in the border zone between BULB and CCA to avoid over-or underestimating of IMT.

The target site for IMT measurements of BULB is the 1 cm area from the start of the BULB and upstream, distally. If only a part of this distance is measurable, a recording may, however, be performed on this shorter distance if the live sequence shows that this part of the vessel wall is representative of the rest of the 1 cm area. This shorter, measurable distance is marked with an electronic star. The 3 chosen images are marked BULB1, BULB2 and BULB3 and recorded on the videotape. If no measurable image is possible to obtain, an image from the BULB is still recorded and marked MB, i.e., "missing bulb". IMT measurements from the near wall of the BULB are not performed.

11. Then a B-mode scanning of the CCA is performed, starting at the BULB and downstream as far as possible. Registration and measurements of plaque are done in the same way as mentioned above. The images with plaques are marked PLAQUE CCA FW and PLAQUE CCA NW, video recording is performed of both the live sequence and the frozen, marked images. R-wave triggered CCA IMT-registrations are recorded and the 3 optimal images are chosen from the cine-loop as described in paragraph 10. It is important to get representative images also from the near wall since IMT-measurements from the CCA-NW will be done off-line. The arrow-marker is placed in the same position as for the BULB measurements. The target site for IMT measurements of CCA is the 1 cm area from the start of the BULB and 1 cm downstream, proximally. The three images chosen to be recorded are marked CCA1, CCA2 and CCA3. If no measurable image is possible to obtain, an image from the CCA is still recorded and marked MC ("missing CCA"). All measurements on the far wall refer to the so-called "leading edge" principle (or "upper demarcation line"). These structures are not being different in thickness when the emitted power (mW/cm^2) or of the ultrasound instrument's gain setting are changed (nor are biological different conditions of subjects examined).

Near wall measurements, however, are performed on "far edge" principles, which means that IMT to some degree may be dependent on some of the technical conditions mentioned above (e.g., gain setting). Standardized examination conditions therefore are particularly important for the near wall measurements. It is, however, not possible, in technical terms, to obtain such ideal conditions because individually instrument adjusting alternatives always are more or less involved in processing optimal B-mode images. However, setting of functions such, as emitted power of ultrasound, preprocession, postprocession, gainsetting, etc. should be standardized as much as possible. Biologic inter-individual differences (obesity, position of the neck arteries, short or long necks, etc.) causing need of some different adjustments, however, are not possible to standardize. If the visibility of IMT and plaques is not optimal, the gainsetting (both the general and the segmental) should first be adjusted to improve the quality of the image. The gain should all the time be set high enough to identify soft, echolucent plaques but not too high to conceal small plaques due to "ultrasound noise". Only as an exception, adjustments of the other functions should be done.

12. **<u>Scoring of plaque-echogenicity</u>.** We aim at the highest echogenicity as possible since false too low echogenicity is a common problem due to several reasons: The plaque is cut too skew by the ultrasound beam, the longitudinal axis of the insonated vessel wall is not parallel to the ultrasound probe surface causing sub-optimal reflection of ultrasound energy (scattering), a far wall plaque is located within a ultrasound shadow from a calcified near wall plaque due to sub-optimal insonation angel. We therefore use the ultrasound signals from the media-adventitia interface as a reference of echogenicity to enhance precision on morphology scoring. This structure is easy to identify and is always presenting as high-echogenic, and is also localized close to the target, the atherosclerotic plaque.

    In a 4-step scale from 1 to 4, the media-adventitia echogenicity and plaques of similar echogenicity is given a value of 4. On a grey-scale, such objects appear white or close to white. A plaque of grade 1 consequently reflects no or almost no ultrasound signals and appears black or dark grey on images. Flowing blood appearing black on ultrasound images is the reference structure on this end of the scale. Grade 2 and 3 represent intermediary echogenicity: grade 1, the plaque consisting of more echolucent than echogenic material (≤ 50% echogenic material); grade 3, more echogenic than echolucent (> 50% echogenic material). Apart from the ultrasound reference structures used in this protocol, the echogenicity scoring is similar to previous reports in the literature.[1, 2]

    Grade 5 represents plaques that are not possible to classify on ultrasound of technical reasons (e.g., plaques in the far wall concealed by the echo shadow from calcified near wall plaques, not possible to angling of the probe to obtain representative images, plaque localized to high upstream to get high-quality images, etc.)

    When a plaque is heterogeneous and consists partly of high-echogenic and partly of low-echogenic material, the scoring of echogenicity is based of an overall impression of the dominating plaque echogenicity. When more than 80% of the plaque is of a given echogenicity, the echogenicity is scored as if the whole plaque consisted of this echogenicity although the rest of the plaque echogenicity was differing 2 or 3 grades from the dominating class of echogenicity. If the percentage is below 80%, interpolating is performed by judgement.

Thus, plaque echogenicity is classified as follows:

Grade 1: Echolucent (0- 20 % of plaque material is high-echogenic).
Grade 2: Predominant echolucent (21-50 % of plaque material is high-echogenic).
Grade 3: Predominant echogenic (51-79 % of plaque material is high-echogenic).
Grade 4: Echogenic (80-100 % of plaque material is high-echogenic).
Grade 5: Missing, not classifiable

In the same way, a total echogenicity status for an artery is determined if more than one plaque is present. The same limit of 80% is the basis of scoring of total plaque area.

AFTER EXAMINATION:

13. Do not remove the cassette from the video recorder before the end of the day, or when the cassette is full.

14. Check that the registration form is completed appropriately. In the ”Remarks” box, coding for reasons for missing of measurable images should be done:

    MB 1= missing images from BULB due to obesity.

    MB 2= missing images from BULB due to a steep angle between CCA and BULB.

    MB 3= missing images from BULB due to technically difficult examinations.

    MB 4= missing images from BULB due to previous surgery or radiation.

    MB 5= other reasons

    In the same way, missing coding for CCA and ICA is performed: MC 1, MC 2, etc.

A referral form to Department of Neurology, University Hospital, Tromsø is completed when a suspected carotid stenosis or occlusion are found. Two criteria for defining a stenosis are used. Either a velocity increase across an atherosclerotic plaque in BULB of 0.1 m/sec. or more or 0.2 m/sec. in ICA, compared to the reference velocity distally in ICA; or a plaque thickness that constitutes 35% or more of the lumen diameter at the plaque site. The velocities should be manually angle-corrected for the angle at which Doppler-beams are emitted into the vessel. Occlusion is suspected when the open lumen of the artery is not visible on B-mode or if there is a visible occluding plaque in the artery, and there is no detectable flow in the artery by pulsed Doppler or by colour-Doppler. The referral threshold should be low to avoid false negative stenosis cases. The person, who is referred, should be given a written and verbal information of the finding and clinical implications before living the room.

References:

1: Geroulakos G. et al. *Br J Surg.* 1993;80:1274-1277
2: Steffen CM. et al. *Aust. NZ J Surg.* 1989;59:529-534

English version June 2005 Stein Harald Johnsen

”Grabbing”-protocol

(Digitizing plaque images from SVHS-cassette)

- PC + monitor, and Panasonic 7560 video recorder + monitor are switched on. The video screen is preset to PAL.

- The videocassette is inserted in the video recorder. Wind on to the plaque image of interest. Check continuously the plaque registration form (Excel-sheet) to ensure that no plaque images are missed. The frame on the video screen should be smoothly adjusted until it is stationary, without any “snow”.

- Start **Matrox Intellicam** on the PC desktop.

- If this is the ***first ‘grab’*** from the videocassette, the GSM-value for the background colour of the recorded image should be calibrated according to the following procedure (1-5). If not, proceed to the next step.

    1. Grab an image (see the procedure for GRAB!).
    2. Save the image in the catalogue C:\My documents\Plaque\Test as test.tif.
    3. Export the image to Adobe Photoshop 3.0.
    4. Press the Image **m**ode – **G**reyscale, and then ’**OK**’ on the mini-menu ’Discard colour information?’
    5. Delimit a ’black’ area outside the B-mode picture on the screen with the squared tool function, and press **I**mage – **H**istogram. The mean value should be between 1-3. If it is <1 or >3, the ’BLACK LEVEL’-button on the Panasonic 7650’s ’TBC CONTROL’ is turned a little clockwise/counter-clockwise respectively, and the procedure is repeated until the mean value is between 1-3 (but not 0, then the ’BLACK LEVEL’ is too low!). This procedure should be repeated at every start-up, and each time a new videocassette is inserted, to ensure that background-black really *is* black.

GRAB!

- Press **Ctrl + M**, or press the **camera-icon** in the menu (nr 6 from left) to grab the image. Repeat until you have an optimal image. Every time Matrox Intellicam is started, the ‘Digitizer Configuration Format’ menu will appear on the screen – Choose ’PAL’ in the box and press ‘OK’.

- Save the image by pressing **Alt+F – A**, or **F**ile – Save **A**s, (but ***not*** **Ctrl+S**, or **Alt+F – S**, then the previous image will be erased!) and use the file name from the plaque registration form. *PS – check for writing error.* The file is saved in the catalogue corresponding to the tape number (Tape 01 etc…) under Tr4 or Tr5 respectively. (To simplify the file name routine, the file can be copied from Excel, and pasted in the file name column in Intellicam, and thereafter press <Enter> to save…but still check for writing error!!). Fill in ’grab’ – date (format: ddmmyy, f. ex 011102, 150103) on the Excel sheet as a ‘receipt’.

- Wind on to the next plaque recording, adjust smoothly and repeat the procedure…11000 times!

Procedures for measuring intima-media thickness and plaques in the right carotid artery. The Tromsø Study 2007-8.

1. Switch on Vivid 7
2. Select ***New Exam*** and log in using your user credentials.
3. For every new participant: Select ***New Exam***, then ***Search/Create patient***. Place cursor in Patient ID. Scan participant barcode using scanner. Select ***Create patient***.
4. The participant's personal code will appear on the upper left hand side of the screen, your user credentials will appear to the right of date and time, followed by application mode *"Carotid"*.
5. Attach ECG electrodes to both arms and left leg of participant. Red on right arm, yellow on left arm and green on left leg. Select "*Physio*" to activate ECG function at multifunction buttons right beneath the two rectangular screen displays. Select ECG to display ECG readings on screen.
6. Participant should be placed in the supine position, with head/neck tilted backwards and slightly to the left. Cover clothes in the neck with tissue paper. Apply gel at probe or at participant's neck.
7. Start examination by acquiring transversal scans of carotid artery. Start at the level of the clavicle and proceed distally along common carotid artery. If necessary, use color Doppler (select Color) to identify the artery. From the bifurcation, proceed along the internal carotid artery to the level of the jawbone as far as technically possible. The purpose is to identify the common carotid artery, the bifurcation and the internal carotid artery as well as identifying possible plaques in these locations. (See pt. 9 for identification of plaques).
8. Switch to longitudinal examination of carotid artery. Start as proximal as possible and proceed slowly distally. Be sure to tilt the probe as to cover the largest sector possible of the neck, so that the arteries are viewed in different angles. Adjust *Gain* by turning knobs marked *2D* for optimization of view.
9. Plaque detection: Plaques are defined as a supposed atherosclerotic lesion in the intima with focal protrusion towards the lumen of the artery, and with the focal protrusion comprising more than 50% of the adjacent intima media thickness.
10. Plaques are registered in the following locations:

    Far wall of common carotid artery
    Near wall of common carotid artery
    Far wall of bifurcation
    Near wall of bifurcation
    Far wall of internal carotid artery
    Near wall of internal carotid artery

To obtain good pictures, it is important that the segment were the plaque is to be measured is depicted as horizontally oriented in the picture as possible. Avoid taking pictures were the artery is bending upwards or downwards at the screen. A plaque picture should be obtained with a full diameter of the artery. The ideal is that the "double line" of the IMT is seen as a continuity of the plaque both proximally and distally. The "double line" IMT should be detected in both the near and far wall.

Take pictures of plaques in every location. If there is more than one plaque in each segment, choose the greater one for the picture. When god, representative pictures are depicted on the screen, select Freeze. Select the best picture by turning the trackball. Name picture with correct label (i.e. PLAQUE_CCA_FAR_WALL) by selecting HOME at keyboard, hit select several times to choose right label. Save picture by selecting IMG store. Select Freeze once more to remove freeze of cine loop.

Plaque pictures should be used for detection of plaque thickness, plaque area and plaque echogenicity (GSM). As a main rule, one representative picture should be used for all measurements. If you think that the most representative thickness and/or area is best shown in one projection, and the echogenicity in another projection, capture and freeze two pictures of the same plaque. Label plaque with the right localization adding what should be measured. For instance: Picture 1: PLAQUE_CCA_FAR_WALL AREAL,
Picture 2: PLAQUE_CCA_FAR_WALL EKKO. If there are no plaques in in any part of the examined artery, capture one representative picture of the artery and label as following: NO_PLAQUES.

Then do examinations of the intima-media thickness of the distal part of common carotid artery (far wall and near wall) and in the bifurcation (far wall). It is important that this segment of the artery is depicted so that the ultrasound beam is perpendicular on the longitudinal axis of the artery. It is important that IMT is measured in a full diameter of the artery. Ideally, the artery should be depicted horizontally on the screen so that the "double line" contour of the intima media complex is visualized in both near and far wall.

IMT-pictures to be saved shall be R-triggered. Select *Physio* to activate ECG-function in the display. When a good depiction of IMT is obtained, select *ECG TRIG*. Record a cine-loop of at least 30 pictures. Select Freeze and choose the three most representative pictures, which should be at least 10 pictures apart and save. Each picture is labeled according to location (for instance IMT_CCA_1). The transition between the CCA and bifurcation is marked with a **+** in the lumen of the artery, using the trackball and Caliper. The origin of the bifurcation is defined as the beginning of divergence of the near and far wall. It is important to place the **+** as precisely as possible.
ECG trigging is removed by once more selecting ECG TRIG (knob light turns off).

Then do uptakes of the IMT in the bifurcation. IMT in the bifurcation should be measured from the beginning of the bifurcation and 1 cm distally. If the sonographer finds the quality of the pictures not good enough for measuring 1 cm, but is god enough for a shorter segment, this should be marked by inserting an exclamation mark at the distal measuring point (select **!** at the keyboard and place with trackball). Uptakes, marking of start of bifurcation and labeling follows same procedure as for IMT in CCA.

If the quality of the IMT-uptakes in CCA and /or bifurcation is of low quality and not suitable for measurements, the pictures should be labeled IMT_CCA_MISSING or IMT_BULB_MISSING.

11. Some participants should be referred to neurological outpatient clinics.

    The criteria are:

    a. Plaques in the CCA, in the bifurcation or the ICA with maximum thickness ≥50% of lumen diameter measured at the same point or suspect lesion of this size. This is defined as stenosis.
    b. Occlusion or suspect occlusion of the CCA, in the bifurcation or the ICA.
    c. Technical difficulties which arises doubt as to whether the above mentioned criteria are fulfilled.

    The participant should be informed about referral to outpatient clinic before he/she leaves the examination, with correct information about the reason for referral. Emphasis should be placed on non-dramatization of the condition. Information should be given about the fact that plaques are very common in middle-aged and older age-groups, and that with most persons they will not give any symptoms. The referral will for most persons act as a safety precaution, ensuring that preventive measures can be installed.

    Make an uptake that shows the reason why you want to refer the participant, label it correctly (REFERRED_STENOSIS, REFERRED_OCCLUSION, REFERRED_TECHNICAL). Fill in referral papers, and make sure they are handled by the right person at the end of the day.

12. When the uptake of one participant is ended, select ***Archive***, then ***END EXAM*** in the Patient information sheet. You will be asked to select save all pictures (***Save all***), select pictures for saving (***Select***) or not to save pictures (***None***). Normally select ***Save All***, or ***Select*** if there are pictures that can be deleted.
13. Clean probe with soft tissue paper after examination.
14. Next participant is registered by selecting ***New exam***.
15. At the end of the day: Turn off VIVID 7. Clean keyboard and probe with moist tissue paper. Dry off with tissue paper.

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