UiT
THE ARCTIC UNIVERSITY OF NORWAY

Faculty of Health Sciences
Department of Clinical Medicine

C-reactive protein and other circulating biomarkers in carotid atherosclerosis and cardiovascular disease

The Tromsø Study 1994-2013

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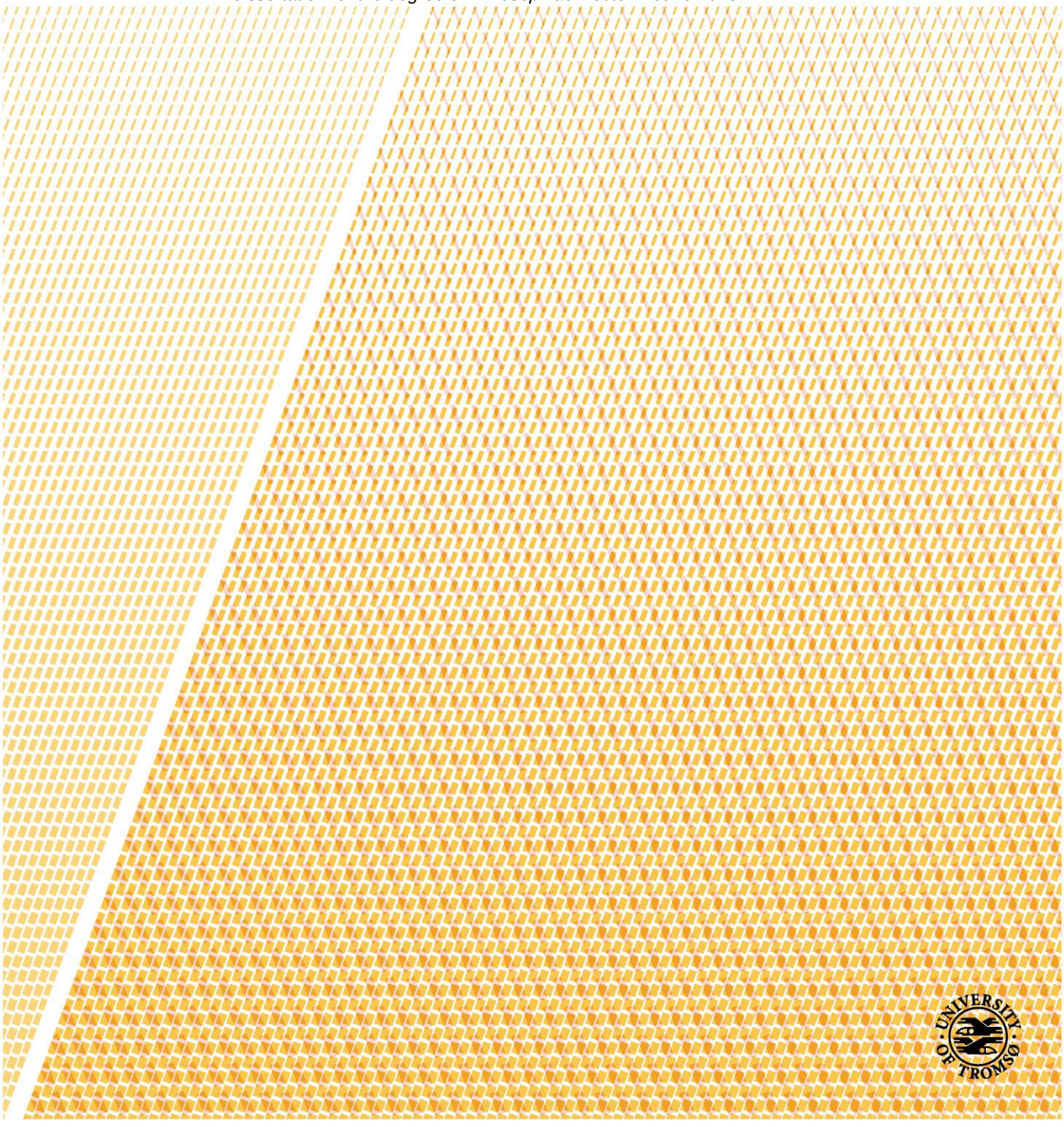
Agnethe Eltoft

A dissertation for the degree of Philosophiae Doctor – June 2018



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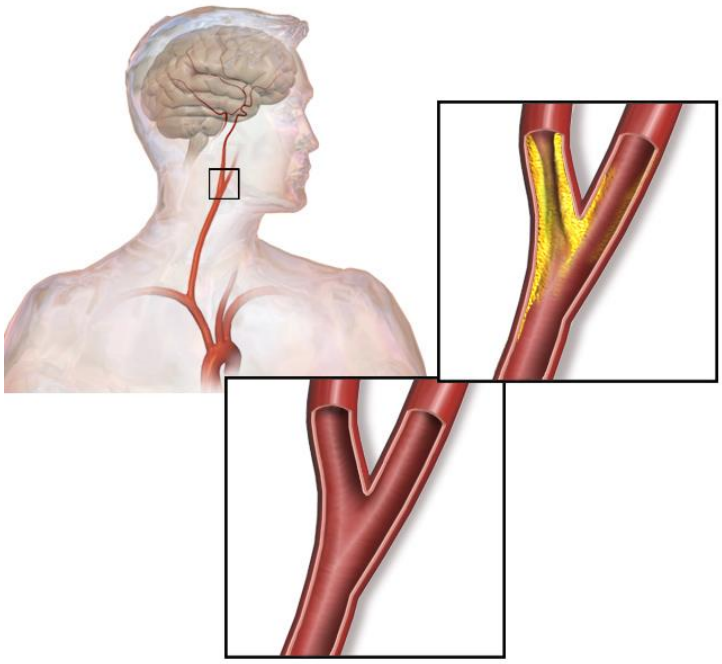

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"The purpose of thinking about the future is not to predict it but to raise people's hopes."

Freeman Dyson

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Agnethe Eltoft

Tromsø, May 2018

Contents

Summary

Cardiovascular disease (CVD) is the leading cause of death and morbidity worldwide. In Norway there are approximately 13,000 heart attacks and 12,000 strokes each year. Despite the fact that preventive and acute treatment have improved significantly over the last 30 years, CVD rates are expected to increase globally. The traditional risk factors (age, sex, blood pressure, cholesterol, diabetes and smoking) have limited ability to single out individuals at increased risk of CVD. It is therefore important to identify novel markers of disease activity in the preclinical phase which may improve identification of individuals at risk and refine individualized preventive treatment. Atherosclerosis is the underlying cause of most CVD events. Deposits of lipids and inflammation (plaques) in the arteries may rupture and initiate blood clot formation which subsequently occludes arteries and compromises blood flow to organs such as the heart and brain. The degree of atherosclerosis can be measured by ultrasound of the carotid arteries. Previous research indicates that plaques which increase in size pose a higher risk of CVD than atherosclerosis that remains stable over time. The aim of this study was to investigate the relationship between the inflammatory marker C-reactive protein (CRP) and other markers in blood with the progression of atherosclerosis, as well as clinical events such as myocardial infarction (MI) and ischemic stroke (IS).

The Tromsø Study is a population-based cohort study where participants have been invited to repeated health surveys. Repeated assessments of traditional risk factors, blood samples and ultrasound of the right carotid artery have been performed in the period 1994-2008. In addition, clinical events such as MI and IS have been recorded. This has given us a unique opportunity to investigate the association between blood markers and the progression of carotid atherosclerosis over time, as well as the association to clinical events.

We found that CRP was associated with the presence of carotid plaque and total plaque area in cross sectional examinations. The associations were strongest in men. CRP did not predict future plaque formation or plaque progression adjusted for traditional risk factors. Both CRP and carotid total plaque area were associated with higher risk of future MI and IS. Individuals who had both elevated CRP and large carotid plaques had highest risk of MI and IS. Carotid ultrasound examination and determination of CRP levels in the blood added predictive value beyond traditional risk factors for identification of individuals with increased CVD risk. The inflammatory cytokine interleukin-6 was associated with plaque progression after six years of follow-up, suggesting that interleukin-6 may be a useful marker to identify patients with unstable plaque in a middle-aged general population.

Sammendrag

Hjerte- og karsykdom er ledende årsak til død og sykelighet på verdensbasis. I Norge er det årlig ca. 13 000 hjerteinfarkt og 12 000 hjerneslag. Til tross for at den forebyggende og akuttmedisinske behandlingen er betydelig forbedret i løpet av de siste 30 år, forventes en fortsatt økning i hjerte- og karsykdommer på verdensbasis. De tradisjonelle risikofaktorene (alder, kjønn, blodtrykk, kolesterol, diabetes og røyking) har begrenset evne til å forutsi hvilke individer som har økt risiko for hjerte- og karsykdom. Det er derfor viktig å identifisere nye markører som er assosiert med økt risiko for sykdom, for å kunne tilby personer med økt risiko en bedre tilpasset forebyggende behandling. Aterosklerose er bakenforliggende årsak til de fleste kliniske hendelser og er en sykdomsprosess som fører til avleiring (plakk) av fett, kalk og betennelsesceller i arterier. Aterosklerotiske plakk som sprekker kan føre til at det dannes blodpropper som tetter til pulsårer og hemmer blodtilførselen til viktige organer som hjerte og hjerne. Grad av aterosklerose kan måles med ultralyd av halskar. Mye tyder på at plakk som øker i størrelse utgjør en høyere risiko for hjerte- og karsykdom enn aterosklerose som forblir stabil over tid. Målet med denne studien var å undersøke sammenhengen mellom betennelsesmarkøren C-reaktivt protein (CRP) og andre markører i blod med utvikling av aterosklerose, samt kliniske hendelser som hjerteinfarkt og hjerneslag.

Tromsøundersøkelsen er en pågående helseundersøkelse av befolkningen i Tromsø hvor deltakerne har blitt invitert til gjentatte undersøkelser. Denne avhandlingen bygger på repeterte målinger av tradisjonelle risikofaktorer, blodprøver samt ultralyd av halskar hos deltakerne i perioden 1994-2008. I tillegg er det registrert kliniske hendelser som hjerteinfarkt og hjerneslag til og med 2013. Dette har gitt oss en unik mulighet til å undersøke sammenhengen mellom markører i blodet og utviklingen av aterosklerose i halskar, samt kliniske hendelser.

Vi fant at nivå av CRP i blodet var assosiert med tilstedeværelse av plakk i halskar og totalt plakkareal i tverrsnittsundersøkelse. Sammenhengen var sterkest hos menn. CRP kunne ikke forutsi fremtidig utvikling av plakk eller økning av plakkstørrelse i analyser justert for tradisjonelle risikofaktorer. Både CRP i blod og plakkstørrelse i halskar var assosiert med høyere risiko for fremtidig hjerteinfarkt og hjerneslag. De som hadde både forhøyet CRP og store plakk hadde den høyeste risiko for hjerteinfarkt og hjerneinfarkt. Ultralydundersøkelse av halskar og nivå av CRP i blodet ga tilleggseffekt utover tradisjonelle risikofaktorer når det gjaldt å identifisere individer med økt risiko for hjerte- og karsykdom. Nivå av betennelsesmarkøren interleukin-6 var forbundet med plakkvekst seks år senere. Dette tyder på at interleukin-6 kan være en nyttig markør for å identifisere pasienter med ustabile plakk.

List of papers

This thesis is based on the following papers, referred to in the text by their Roman numerals:

I. C-reactive protein in atherosclerosis - A risk marker but not a causal factor? A 13-year population-based longitudinal study: The Tromsø study.
Eltoft A, Arntzen KA, Hansen JB, Wilsgaard T, Mathiesen EB, Johnsen SH.
Atherosclerosis. 2017 Aug; 263:293-300.

II. Joint effect of carotid plaque and C-reactive protein on first-ever ischemic stroke and myocardial infarction?
Eltoft A, Arntzen KA, Wilsgaard T, Hansen JB, Mathiesen EB, Johnsen SH.
J Am Heart Assoc. 2018 May; 7: e008951

III. Interleukin-6 is an independent predictor of progressive atherosclerosis in the carotid artery: The Tromsø Study.
Eltoft A, Arntzen KA, Wilsgaard T, Mathiesen EB, Johnsen SH.
Atherosclerosis. 2018 Apr; 271:1-8.

Abbreviations

ApoA1: Apolipoprotein-A1
ApoB100: Apolipoprotein-B100
AUC: Area under the receiver operating characteristic curve
BMI: Body mass index
BNP: B-type natriuretic peptide
CAC: Coronary artery calcium
CHD: Coronary heart disease
CI: Confidence interval
CKMB: MB fraction of creatine kinase
CRP: C-reactive protein
CT: Computer tomography
CT-proAVP: Copeptin (C-terminal part of the arginine vasopressin prohormone)
Cu/Zn SOD: Copper/zinc superoxide dismutase
CV: Coefficient of variation
CVD: Cardiovascular disease
EC: Endothelial cell
ECG: Electrocardiogram
FDR: False discovery rate
HbA1c: Glycosylated hemoglobin
HDL-C: High density lipoprotein cholesterol
HR: Hazard ratio
IDI: Integrative discrimination improvement
ICAM-1: Soluble intercellular adhesion molecule 1
IL: Interleukin
IMT: Intima-media thickness
IS: Ischemic stroke
LDL-C: Low-density lipoprotein cholesterol
M-CSF: Macrophage colony stimulating factor
MCP-1: Monocyte chemoattractant protein-1
MI: Myocardial infarction
MMP: Metalloproteinase
MPO: Myeloperoxidase
MRI: Magnetic resonance imaging
MR-proADM: Midregional pro-adrenomedullin
MR-proANP: Midregional pro-atrial natriuretic peptide
NF-kB: Nuclear factor-kappa B
NLRP3: Nucleotide-binding leucine-rich repeat-containing pyrin receptor 3
NO: Nitric oxide
NRI: Net reclassification improvement
oxLDL: Oxidative modified LDL-C
OR: Odds ratio
PAI-1: Plasminogen activator inhibitor-1
PAMPs: Pathogen associated molecular patterns
PCT: Procalcitonin
RCT: Randomized controlled trial
ROS: Reactive oxygen species
SD: Standard deviation
SMC: Smooth muscle cell
TIMP: Tissue inhibitors of matrix metalloproteinases
TNFα: Tumor necrosis factor α
TPA: Total plaque area
TRF: Traditional risk factor
US: Ultrasonography
WBC: White blood cells
WHO: The World Health Organization

1 Introduction

1.1 Cardiovascular disease

Cardiovascular disease (CVD) is an umbrella term for a number of pathologies, commonly defined as coronary heart disease (CHD), cerebrovascular disease, peripheral arterial disease, rheumatic and congenital heart disease, and venous thromboembolism.[1] CVD is the most common cause of mortality in developed countries and an important cause of disability leading to major health and economic burdens globally.[2] In 2013, CVD was the most frequent underlying cause of death in the world, accounting for an estimated 17.3 million of the 54 million total deaths (31.5%).[3] Ischemic CVD more specifically refers to diseases where the blood supply and thereby oxygen delivery is insufficient due to an occluded or stenotic artery, potentially leading to tissue damage in the affected organs. Ischemic cardiovascular disease includes coronary artery diseases (myocardial infarction and angina pectoris), ischemic cerebral stroke, transient ischemic attack and peripheral artery disease. In the remaining part of this thesis, CVD refers to myocardial infarction and ischemic stroke.

Myocardial infarction (MI) is myocardial cell death due to prolonged ischemia. The universal definition of MI includes "symptoms suggestive of myocardial ischemia, accompanied by new ST elevation, or new left bundle-branch block, and/or evidence of fresh thrombus by coronary angiography and/or at autopsy," all of which imply a focal arterial occlusion.[4, 5] Myocardial injury is detected when blood levels of sensitive and specific biomarkers, such as cardiac troponin or the MB fraction of creatine kinase (CKMB), are increased. Cardiac troponin I and T are components of the contractile apparatus of myocardial cells and are expressed almost exclusively in the heart, showing high myocardial tissue specificity as well as high clinical sensitivity. To establish the diagnosis of MI, a rise and/or fall in troponin values with at least 1 value above the decision level is required, coupled with a strong pre-test likelihood.[5] Acute or evolving changes in the ST–T waveforms and Q waves of the electrocardiogram (ECG), aid clinicians in timing the event, identifying the infarct-related artery, estimating amount of myocardium at risk and determining therapeutic strategy. The pathophysiological mechanism leading to MI is typically an intraluminal thrombus in one or more coronary arteries causing imbalance between oxygen supply and demand.[5]

The World Health Organization (WHO) introduced in 1970 the definition of stroke that is still in use; "rapidly developing clinical signs of focal (or global) disturbance of cerebral function, lasting more than 24 hours or leading to death, with no apparent cause other than that of vascular origin".[4] Ischemic stroke (IS) is an episode of neurological dysfunction caused by focal cerebral infarction. IS is confirmed by brain imaging (computer tomography (CT) or magnetic resonance imaging (MRI)) or by pathological findings at autopsy.[4] IS accounts for 80-85% of stroke cases, in addition stroke comprises intracerebral hemorrhage (10-15%), and subarachnoid hemorrhage (3-5%). During the last two decades, CT and MRI have become increasingly available in the diagnosis of stroke and can differentiate between stroke types and localize the regions of brain infarction and hemorrhage. Occlusion of cerebral arteries leading to brain infarction can be caused by several mechanisms including atherothrombosis (extra- or intracranial), embolism (cardiogenic typically due to atrial fibrillation or artery-to-artery embolism), primary occlusive disease of the small penetrating arteries, and non-atherosclerotic abnormalities (dissections, vasculitis and coagulopathies). No specific cause can be identified in about 30% of patients ("cryptogenic stroke").

Globally, there were 7.4 million deaths due to ischemic heart disease and 6.7 million stroke deaths in 2015.[3, 6] In Norway, there are approximately 13 000 MIs and 12 000 strokes annually. Men are on average 7-10 years younger than women when they experience their first CVD event.[3] Population based, epidemiologic studies have played an important role in identifying CVD risk factors, i.e., observable characteristics in the preclinical phase associated with increased risk of future CVD events. Several non-modifiable (age, sex and race) and modifiable risk factors have been identified, highlighting opportunities for prevention.[2] Therapeutic and life-style interventions aimed at improving modifiable risk factors such as dyslipidemia, hypertension, diabetes, smoking, and abdominal obesity have been developed and implemented in clinical practice. In addition, new treatment options have evolved, including thrombolytic drugs aimed at dissolving clots and intravascular catheter-based methods for opening stenotic and occluded arteries. Preventive strategies associated with declining incidence and improved treatments with subsequently decreased case fatality, have led to reduced global age-standardized death rates of ischemic heart disease and ischemic stroke by 19.5% and 26.6% respectively since 1990.[7] Still these diseases remain the top two causes of death worldwide, with increasing incidence in many low and middle-income countries.[7] Globally, 80% of CVD deaths take place in low- and middle-income countries,[3] where the availability of health services and

new treatments are limited. The rate of CVD worldwide is predicted to increase due to the global epidemic of obesity and insulin resistance, aging populations and rising prevalence of CVD risk factors in previously low-risk countries.[1] The WHO estimates that 80% of premature heart disease and stroke are preventable and that risk factor improvement can help reduce the growing CVD burden on both individuals and healthcare systems.[1]

1.2 Atherosclerosis

Atherosclerosis is a slowly progressing systemic disease in large and medium sized arteries which represents the underlying cause of the majority of clinical CVD events.[3] The artery wall consists of three layers. The intima is the layer closest to the lumen and consists of endothelial cells (ECs) and the internal basement membrane. The middle layer, tunica media, consists of smooth muscle cells (SMCs) and extracellular matrix. The adventitia is the external layer and mainly consists of loose connective tissue with nerve fibers, small vessels and an external elastic layer. Atherosclerosis is a process where the arterial wall thickens when fatty deposits, inflammation, cells, and scar tissue build up and form atheromas (atherosclerotic plaques) within the sub-intimal layer. In Greek, *athere* means gruel, and *skleros* means hard. Among the first to describe atherosclerosis was Leonardo da Vinci (1452-1519), who stated that "Vessels in the elderly restrict the transit of blood through thickening of the tunics". In 1799, the British physician Caleb Hillier Parry discovered a plaster-like substance within the coronary arteries when performing autopsy on a sheep and he was the first to suggest the correct mechanism of ischemic heart disease.[8] Atheroma rupture was reported for the first time during the autopsy of the Danish artist and sculptor, Bertel Thorvaldsen, who died a sudden cardiac death in the Royal Theatre in Copenhagen in 1844. It was recognized that the vessel wall contained "several atheromatous plaques, one of which quite clearly had ulcerated, pouring the atheromatous mass into the arterial lumen".[8]

As shown in Figure 1, atherosclerosis occurs as an indolent disease progressing throughout adult life. Most individuals with atherosclerosis will never experience clear clinical symptoms related to their disease and subjects who die suddenly because of CVD are commonly unaware of their condition.[9]

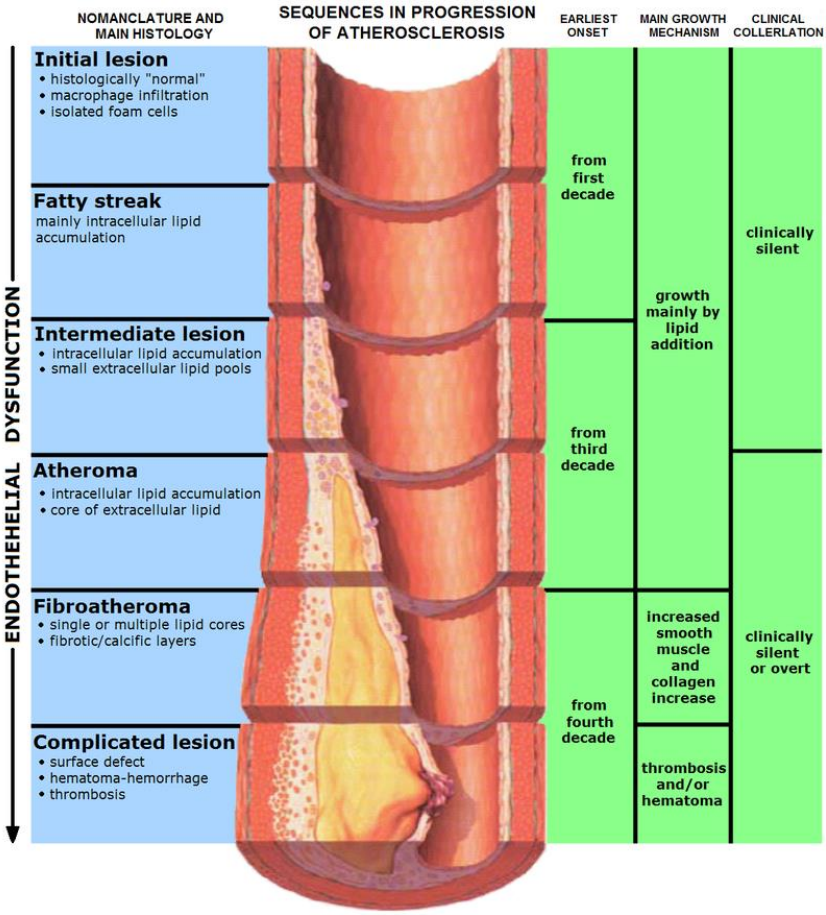

Figure 1 - Development of atherosclerosis throughout life from fatty streak to complicated lesion with potential to cause clinical cardiovascular events. Reproduced in accordance with license CC BY_SA 3.0 (https://creativecommons.org/licenses/by-sa/3.0), via *Wikimedia Commons.*

1.3 Inflammation in atherosclerosis

Celsus described inflammation in the 1st century AD as a localized protective reaction of tissue to irritation, injury or infection. Inflammation is characterized by rubor (redness due to hyperaemia), tumor (swelling, caused by increased permeability of micro-vessels and leakage of proteins to the interstitial space), calor (heat, associated with increased blood flow and metabolic activity), dolor (pain, due to changes in the perivasculature and associated nerve endings) and loss of function.[10, 11] By the end of the 18th century, Rudolf Virchow argued that an inflammatory process with reactive fibrosis induced by proliferating connective tissue cells within the intima caused development of atherosclerotic plaques. He suggested that mechanical forces represented an irritative initiating stimulus and that atherosclerosis was part of a repair mechanism.[12] Virchow's hypothesis gave basis for the popular "response to injury" hypothesis of Russel Ross (1929-1999). Ross postulated that the "lesions of atherosclerosis arise as a result of

focal injury to arterial endothelium, followed by adherence, aggregation and release of platelets".[8] Atherosclerosis proceeds from intima-media thickening to fatty streaks, intermediate lesions and raised plaques to complicated plaques prone for rupture with ability to cause clinical events through thromboembolism. Inflammation is now acknowledged to play an important role at all stages of the disease.

The innate immune response is a rapid response to tissue injury, which detects a broad number of patterns that are commonly found in pathogens, but are foreign to mammals; so-called pathogen associated molecular patterns (PAMPs). Macrophages express a set of pattern recognition receptors including scavenger receptors and toll-like receptors, whose ligands include PAMPs such as lipopolysaccharides on the surface of pathogens, but also low-density lipoprotein cholesterol (LDL-C) modified by oxidation and glycation. Ligation of scavenger receptors can lead to endocytosis and lysosomal degradation of bound ligands. On the other hand, ligation of toll-like receptors results in activation of the transcription factor nuclear factor-kappa B (NF-kB) and mitogen-activated protein kinase pathways, increasing phagocytosis, production of reactive oxygen species and release of cytokines that amplify the inflammatory response.[11, 13]

The adaptive immune response is a slow and more focused defence mechanism depending on the recognition of specific molecular structures and generation of a large number antigen receptors i.e., T-cell receptors and immunoglobulins. When T-cells recognize foreign antigens presented to them, they initiate responses that target precisely that antigen, including direct attack against the specific antigen by cytotoxic T-cells, stimulation of antibody production by B-cells and induction of local inflammatory responses. T-cells differentiate into T-helper cells (T_H1 and T_H2). T_H1 cells produce a number of cytokines (including gamma interferon) coordinating crosstalk with the innate immune system, stimulating macrophages to increase production of mediators including reactive oxygen species (ROS) and pro-inflammatory cytokines. T_H2 cells stimulate maturation of B-cells into anti-body producing plasma cells and may also mute the inflammatory response through production of anti-inflammatory cytokines such as interleukin (IL)-10.[11, 13]

In chronic diseases, the innate and adaptive immune systems interact and approach epithelial cells and mesenchymal cells. Selective and sequential migration of blood cells into tissues and interaction between these blood-based cells with resident tissue cells lead to extracellular matrix remodelling, cellular proliferation and death as well as neoangiogenesis within the affected

organ. A persistent stimulus may preclude resolution of the inflammatory response leading to a chronic inflammatory condition such as atherosclerosis.[11] Pathophysiological processes involved in the development of atherosclerosis are described below and illustrated in Figure 2.

1.3.1 **Mechanisms of atherosclerosis initiation**

Atherosclerosis occurs as focal lesions located within the intima at specific susceptible sites in the arterial tree. Typical sites are branch points, the outer wall of bifurcations, the inner wall of curvatures and cardiac valves, associated with variations in shear stress and flow disturbances. In their normal state, vascular ECs resist contact with leucocytes, maintain a non-thrombotic interface, and regulate vessel permeability and contractility.[14] The initial step in atherosclerosis involves EC activation. Low shear stress associated with non-laminar flow reduces nitric oxide (NO)-dependent athero-protection and leads to increased uptake and permeability of apolipoprotein-B100 (ApoB100) containing LDL-C. High levels of LDL-C cause augmented transcytosis at lesion-susceptible areas. Plasma derived LDL-C is then trapped within the subintimal space and becomes oxidative modified (oxLDL).[15] When exposed to activating stimuli such as changes in plasma homeostasis including hypercholesterolemia, hyperglycaemia, hypertension, microbial constitutes or pro-inflammatory cytokines, ECs shift to a secretory phenotype. This leads to proliferation of the extracellular matrix and development of a hyperplastic multilayered basal lamina,[14] and to expression of vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule 1 (ICAM-1) and members of the selectin family which binds circulating white blood cells (WBC) including monocytes, T-lymphocytes and neutrophils.[11] After adhesion to the ECs, monocytes undergo directed migration into the artery wall, mediated by chemokines such as monocyte chemoattractant protein-1 (MCP-1). Procalcitonin (PCT), a marker of sepsis and pro-hormone of calcitonin, is suggested to act as a chemoattractant during monocyte adhesion and migration. PCT is produced in response to various stimuli, such as lipopolysaccharides or pro-inflammatory cytokines (IL-1β and IL-6).[16]

In the intima, mediators such as macrophage colony stimulating factor (M-CSF) promotes proliferation of recruited monocytes and differentiation into macrophages.[11] These macrophages over-express scavenger receptors and engulf modified lipoprotein particles through endocytosis. Cholesterol esters then accumulate in cytoplasmic droplets in the macrophages, transforming them to lipid-loaded macrophage derived foam cells, which are characteristic for fatty streaks.[14] Cholesterol can crystallize and activate a multimolecular signaling complex known as nucleotide-binding leucine-rich repeat-containing pyrin receptor 3 (NLRP3) inflammasome in

the cytosol.[17, 18] Activation of the NLRP3 inflammasome results in caspase-1 mediated processing of the precursors of inflammatory cytokines IL-1β and IL-18 to their active forms, which subsequently leads to release of IL-6 and amplification of the inflammatory cascade.[17, 18]

1.3.2 **Mechanisms of atherosclerosis progression**

Once present in the arterial wall, cells of the innate immune system produce ROS, cytokines and pro-coagulants that amplify and sustain the inflammatory response. Both ECs and SMCs respond to these signals and are activated to propagate the inflammation by generating a spectrum of mediators (IL-1α, IL-1β, IL-6, IL-18, tumor necrosis factor α (TNFα), M-CSF, MCP-1, ICAM-1 and pro-coagulant tissue factor).[11] Locally expressed cytokines (IL-2 and IL-18) induce a T_H1 dominated response. T_H1-cytokines promote development and progression of disease, whereas T_H2 and T-regulatory cytokines exert anti-atherogenic activities.[14] T_H1 cells secrete inflammatory cytokines, which induce monocyte polarization towards classical activated macrophages (M1), which in turn produce pro-inflammatory cytokines, metalloproteinases (MMPs) and tissue factor. Neopterin is a marker of monocyte activation, and mirrors elevated inflammatory states and vascular oxidative stress. SMCs located in the intima and medial layer of the vessel switch from a contractile to a synthetic phenotype which migrates and proliferates rapidly, synthesizes collagen and expresses increased number of receptors involved in lipid uptake leading to SMC-derived foam cells. MMPs (especially MMP-2 and MMP-9) promote SMC migration from the media to the intima, contributing to fibrous cap formation. OxLDL may also induce trans-differentiation of SMC toward an osteoblastic-like phenotype through the expression of S100 calcium binding proteins.[19] This process represents a key feature in atheroma calcification.[19] Advanced atherosclerotic plaques contain macrophages, SMC- and macrophage-derived foam cells, extracellular lipid droplets and calcified cores. Collagen rich, fibrous plaques are encapsulated by a robust SMC-rich fibrous tissue cap, have smaller lipid cores, less inflammation, more calcification and are considered stable. Plaques that are characterized by large lipid cores, inflammatory cells and thin caps are vulnerable and rupture prone (Figure 2).

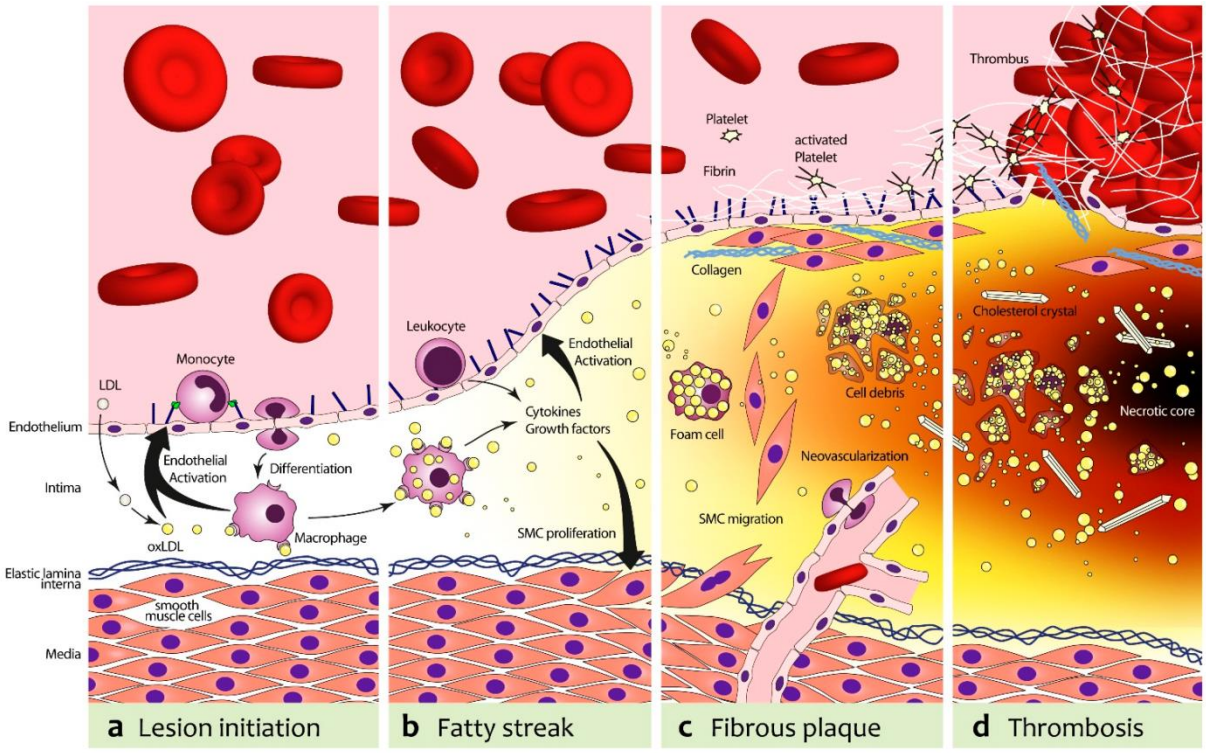

Figure 2 - Pathogenesis of atherosclerosis. (a) In the first stage, LDL-C is deposited in the endothelium and undergoes oxidative modification, resulting in oxidized LDL-C (oxLDL). OxLDL stimulates endothelial cells to express adhesion molecules (VCAM-1, P-Selectin) and various chemokines (MCP-1, IL-8). This leads to recruitment of monocytes, which transmigrate into the intima and differentiate to pro-atherogenic macrophages; (b) Macrophages harvest residual oxLDL via their scavenger receptors and add to the endothelial activation and, subsequently, leukocyte recruitment with the secretion of tumor necrosis factor α (TNFα) and IL-6; (c) The increasing plaque volume promotes neovascularization. Proliferating smooth muscle cells (SMCs) stabilize the nascent fibrous plaque. With deposition of fibrin and activated platelets on the dysfunctional endothelium that expresses tissue factor, a pro-thrombotic milieu is formed; (d) Foam cells can undergo apoptosis and release cell debris and lipids, which will result in the formation of a necrotic core. In addition, proteases secreted from foam cells can destabilize the plaque. This can lead to plaque rupture, in which case extracellular matrix molecules (e.g., collagens, elastin and tissue factor) catalyze thrombotic events. *(Reproduced with permission in accordance to Creative Commons Attribution License 4.0 from Steinl DC, Kaufmann BA. Ultrasound Imaging for Risk Assessment in Atherosclerosis. International Journal of Molecular Sciences*. 2015; 16(5):9749-976. Figure legend is modified)

1.3.3 **Mechanisms of acute thromboembolic complications**

Chronic stable and asymptomatic atherosclerosis does not usually progress to chronic flow-limiting lesions. Thromboembolic complications most commonly result from fibrous cap rupture or superficial erosion of the endothelial monolayer of the atherosclerotic plaque. This initiates local thrombus formation and may cause occlusion at the site of plaque rupture or clots that dislodge from the surface of atherosclerotic lesions and occlude more distal arteries (thromboembolism).[20] Thinning of the fibrous cap, excess of inflammatory cytokines and proteases (inducing digestion of extracellular matrix), decreased collagen synthesis, accumulation of cell debris within the necrotic core and neovascularization are mechanisms that

may result in plaque rupture.[14] Plaque enlargement leads to intraplaque hypoxia, which triggers local neovascularization. The presence of neovessels within atherosclerotic lesions does not only promote plaque growth, but also contributes to its vulnerability.[21] As the atheroma increases in size, neovessels may leak causing intra-plaque hemorrhage and induction of additional ROS formation, inflammation and proteolytic degradation related to angiogenetic factors.

Inflammation in the intima is associated with decreased synthesis and increased breakdown of collagen, preventing repair and stability of the fibrous cap. Gamma interferon produced by T_H1 cells in the atheroma inhibits the production of new collagen by SMCs. Cleavage and degradation of interstitial collagen are dependent of collagenases mostly belonging to the family of MMPs. Active collagenases are not present in normal arteries, but are produced by ECs, SMCs and macrophages in atherosclerotic plaques. MMP-9 is a potent matrix-degrading enzyme and may be involved in arterial remodelling including compensatory artery enlargement at plaque sites and in aneurysm formation. Ubiquitous tissue inhibitors of matrix metalloproteinases (TIMPs) regulate the actions of MMPs and are also present in plaques.[20]

Death of SMCs, macrophages and other types of vascular cells are found in advanced lesions and lead to decreased lesion cellularity, weakening of the fibrous cap, necrotic core formation and lesion instability. Pyroptosis is a pro-inflammatory form of cell death, uniquely dependent on caspase-1 and suspected to play an important role in atherosclerosis.[22] In pyroptosis, the dying cells undergo loss of plasma membrane integrity and DNA fragmentation and release their cytoplasmic content into the extracellular space.[22] Dying cells thus release growth factors, pro-inflammatory cytokines, proteases and intracellular lipid into the extracellular spaces which in turn initiate inflammation, promote plaque disruption and arterial thrombosis.[23] Ruptured plaques are also characterized by defective efferocytosis, i.e. inadequate phagocytic clearance of dead cells.[15]

Polymorph nuclear cells may play a role in plaque destabilization and rupture through release of ROS and pro-inflammatory mediators in the blood and on the endothelial surface. Myeloperoxidase (MPO) is released by activated granulocytes during the respiratory burst and suspected to be involved in plaque rupture.[20] MPO binds to extracellular matrix and converts chloride ions plus hydrogen peroxide to hypochlorus acid, a potent oxidant and chlorinating species. Hypochlorus acid provokes programmed cell death of ECs, linking oxidative stress caused by inflammation to fibrous cap disruption.[20]

Fracture of the cap exposes blood to pro-coagulants in the lipid core and triggers thrombosis. Pathological studies indicate that plaque disruption often occurs subclinically.[24] The interaction between plaque and blood determines the consequences of plaque disruption and hence the composition of the blood is crucial.[20] Platelets activate upon contact with subendothelial extracellular matrix, and aggregate to form a thrombus.[14] Tissue factor is expressed in macrophages upon signals from inflammatory mediators. When exposed to blood, tissue factor activates the coagulation cascade, which generates thrombin and subsequent conversion of fibrinogen to fibrin resulting in blood clotting. Tissue factor is synthesized in the adventitia of normal blood vessels, where it functions to maintain haemostasis after vascular trauma. Tissue factor is not present in the intima of normal arteries, but is found in the lipid-rich cores of atherosclerotic plaques.[25] Blood levels of fibrinogen and the endogenous fibrinolysis inhibitor plasminogen activator inhibitor-1 (PAI-1) regulate coagulation and fibrinolysis. D-dimer is a fibrin degradation product. Levels of these substances may determine formation and stability of a thrombus. Inflammatory signalling alters the synthesis of acute phase reactants such as fibrinogen and CRP in the liver. In this regard, inflammation is involved in both regulating the stability of the plaque and in determining the consequences of plaque rupture; microscopic subclinical mural thrombus or occlusive arterial thrombus with clinical manifestation.[20]

1.4 Traditional risk factors, chronic inflammation and atherosclerosis

Epidemiological data show consistent associations between traditional risk factors (TRFs) and increased levels of inflammatory markers such as IL-6, TNFα and CRP.[26, 27] In the body, free radicals are continuously formed because of oxidative chemical reactions**.** Experimental and clinical studies have demonstrated that TRFs such as hypercholesterolemia, hypertension, diabetes, and smoking are associated with an increased production of ROS.[28] Superoxide dismutases (SODs), including Cu/Zn SOD, represent the major antioxidant defence systems against ROS in vivo. High dose or inadequate removal of ROS results in oxidative stress.[29] ROS have been implicated in key processes of atherosclerosis including oxidative modification of LDL-C, EC activation and regulation of pro-inflammatory cytokines.[30]

High density lipoprotein cholesterol (HDL-C) is inversely correlated to CVD and plaque progression.[31] Cholesterol cannot be degraded within the vessel wall but may be removed by HDL-C containing apolipoprotein-A1 (ApoA1) lipoproteins and transported to the liver for degradation. In addition, HDL-C exerts anti-inflammatory properties. Activation of innate immune response results in reduction of plasma HDL-C levels and remodeling of HDL-C, which

becomes enriched with pro-inflammatory mediators and thus dysfunctional, disturbing its ability to transport cholesterol.[13]

Chronic activation of the renin-angiotensin-system (RAS) may result in constantly enhanced blood pressure and volume overload of the vasculature, causing pathological mechanical vascular wall stress, enhancing the vascular production of ROS and pro-inflammatory cytokines.[30] Vasoactive peptides or their more stable precursors, such as midregional pro-adrenomedullin (MR-proADM), midregional pro-atrial natriuretic peptide (MR-proANP), B-type natriuretic peptide (BNP), copeptin, the C-terminal part of the arginine vasopressin prohormone (CT-proAVP), reflect vascular function and neuro-humoral activity and also play a role in hypertension. Vascular tone and plasma volume is effectively controlled by the active form of MR-proADM and the natriuretic peptides, MR-proANP and BNP. The antidiuretic hypothalamic hormone vasopressin regulates osmotic homeostasis through water retention in the kidneys and acts directly on vascular SMCs. Adrenomedullin has vasodilating effects and is produced by ECs and SMCs. MR-proADM expression is induced by shear stress, ischemia, hypoxia and pro-inflammatory factors such as IL-1β and raised levels are found in hypertension.[32]

Levels of inflammatory markers in blood have shown ability to predict CVD independent of TRFs.[26, 27] Evidence that suggests inflammation as a driver of atherosclerosis is supported by the fact that conditions of chronic inflammatory states, such as rheumatoid arthritis, inflammatory bowel disease, chronic renal failure and obesity, are associated with accelerated atherosclerosis and higher incidence of CVD. Adipose tissue is not only a fat depot, but also an endocrine organ. Macrophages accumulate in visceral adipose tissue, act as scavengers for apoptotic adipocytes and express pro-inflammatory proteins, such as TNFα, IL-1 and IL-6. These cytokines stimulate hepatic inflammation inducing a chronic systemic inflammatory response.[33] Transplanted visceral adipose tissue from obese mice into atherosclerosis-prone Apo-E deficient mice has shown ability to increase atherosclerosis in the recipient animals, suggesting that inflamed adipose tissue exert pro-atherogenic effects.[34] Adiponectin is a protein hormone secreted by adipocytes that modulates a number of metabolic processes, including glucose regulation and fatty acid oxidation and is inversely correlated with body mass and insulin resistance. Adiponectin exerts beneficial effects on endothelial vasorelaxation, supresses generation of ROS and leads to down-regulation of adhesion molecules and pro-inflammatory cytokines. On the other hand, leptin has been related to vascular disorders in human cohorts. Leptin is a hormone predominantly made by adipose cells and involved in regulation of energy homeostasis. Leptin

concentrations are often high in obese subjects. Leptin is associated with EC proliferation, angiogenesis, ROS generation, expression of tissue factor and adhesion molecules.[33]

In patients with chronic kidney disease, accelerated atherosclerosis has been observed. Reasons for this may be increased prevalence of TRFs, such as hypertension, hypercholesterolemia and diabetes. A chronic inflammatory state, calcium phosphate metabolism disturbances, oxidative stress, fluid overload and disturbances in the coagulation system related to kidney disease represent other possible links. Cystatin C and creatinine are reliable markers of renal function. In addition, Cystatin C has emerged as a novel marker of CVD and has been related to inflammation and atherosclerosis.[35]

1.5 C-reactive protein (CRP)

A wide array of inflammatory biomarkers has been studied in relation to cardiovascular disease. C-reactive protein (CRP) is the most extensively studied marker. Properties such as relative stability in frozen samples, long plasma half-life (19h) and ease of testing with standardized assays have facilitated its use.[36] The term "high sensitive CRP" or "hs-CRP" is often used and refers to CRP measured by high-sensitivity assays with lower detection limits of approximately 0.03 mg/L. In comparison, the assays which are regularly used in the clinical setting of diagnosing infection are less sensitive with typical detection limits of 5-8 mg/L.

In the 1990s, studies revealed that increased CRP values were associated with future coronary events. Since then, CRP has shown ability to predict CVD in more than 40 large epidemiological studies.[37] Increase in relative risk estimates for CVD ranges from 1.45 to approximately 2-fold, when comparing the highest with the lowest CRP tertile.[38, 39] This is comparable to the effect of TRFs such as blood cholesterol and blood pressure.[39] A meta-analysis comprising individual participant records from 54 long-term prospective studies[27] showed 1.37 (95% confidence interval (CI) 1.27, 1.48) relative risk increase for CHD and 1.27 (95% CI 1.15, 1.40) for IS per standard deviation (SD) increase in log-transformed CRP after adjustment for TRFs. In most studies, the magnitude of CRP's association with CVD was smaller in women than in men. CRP concentrations are dependent on genetic polymorphisms and show heterogeneity between racial groups and sexes.[40] In addition, raised levels are associated with the presence of TRFs, such as BMI, metabolic syndrome, diabetes mellitus, hypertension, smoking and age. CRP is also related to alcohol consumption, contraceptive drug use, physical exercise, periodontal disease, environmental pollution and chronic inflammatory conditions.[27, 40] Under normal conditions, in

the absence of infections, the intra-individual variability in CRP measured by high sensitivity assays on a year-to year basis corresponds to that of systolic blood pressure and cholesterol.[36] The American Heart Association recommended CRP cut-off points of low CVD risk (<1.0 mg/L), average CVD risk (1.0 to 3.0 mg/L), and high CVD risk (>3.0 mg/L), corresponding to approximate tertiles of CRP in the adult population.[38]

Treatment with statin therapy reduces both LDL–C and CRP levels and leads to reduction in CVD events.[41] A potential role of CRP in the guidance of statin therapy has been proposed. Statin-induced CRP lowering is suggested to derive from both lipoprotein-mediated effects, and from pleiotropic effects of statins related to direct anti-inflammatory actions.[41, 42] In animal models, statins showed ability to limit inflammation, increase collagen content, reduce tissue factor expression and CRP levels in plaques.[43] JUPITER (Justification for the Use of Statin in Prevention: An Intervention Trial Evaluating Rosuvastatin) randomized 17 802 individuals of low to intermediate CVD risk with LDL-C <3.4 mmol/L and CRP >2 mg/L to 20 mg rosuvastatin daily or placebo.[41] The lowest number of CVD events was seen in those treated with rosuvastatin who achieved low levels of both LDL and CRP. However, as a control group with low CRP levels at baseline was missing, the trial could not conclude whether CRP reduction was responsible for the observed benefits. A meta-analysis including 82 000 participants compared clinical outcomes of LDL-C levels in 10 statin trials versus nine non-statin trials. This study questions whether pleiotropic and anti-inflammatory effects of statins contributes to CVD risk reduction beyond LDL-lowering.[42] The REVERSAL (Reversing atherosclerosis with aggressive lipid lowering) trial showed that aggressive lipid lowering with 80 mg compared to 40 mg pravastatin achieved greater reductions in both CRP and LDL-C levels, and was associated with reduced rate of progression of coronary atherosclerosis.[44] The evidence that reducing CRP levels prevents CVD is so far inconclusive.

CRP belongs to the pentraxin family of plasma proteins and circulates in the blood as a pentamer of identical subunits.[36] It is produced in the liver as a response to acute infections, trauma and inflammation and its synthesis is controlled by several cytokines, IL-6 being the most potent driver.[10] CRP binds to phosphocholine residues in bacterial cell membranes, thereby playing an important role in the innate immune response by facilitating the recognition and clearance of bacteria. CRP also binds phosphocholine residues in apoptotic eukaryotic cells, ox-LDL and several mammalian proteins. Aggregated or ligand-bound CRP activates the complement cascade.[10] CRP mRNA is detectable in the walls of diseased blood vessels, which indicates that

CRP is produced locally and not just deposited from blood.[45, 46] Macrophages and SMCs within plaques also produce CRP.[47] Exposure of cultured vascular endothelial cells to CRP inhibits nitric oxide synthase expression, impairing vasoreactivity, and leads to up-regulation of ICAM-1 and VCAM-1, facilitating monocyte adhesion and transmigration.[46] A pro-thrombotic role of CRP has also been suggested. CRP may play an important role in regulating the function of platelets, the extrinsic coagulation system and the fibrinolytic system, thus enhancing the thrombotic response to vascular injury.[10] However, the mechanistic way in which CRP links to CVD is not clearly understood. Whether CRP plays a causal role in atherosclerosis and its complications or is merely a clinical marker of inflammation and cardiovascular risk is continually debated. Plasma CRP levels are weakly correlated to atherosclerosis in humans[48] and CRP's ability to prospectively predict plaque formation and progression has been sparsely studied.

1.6 Atherosclerosis imaging

Since 1958, angiography has been considered the gold standard in the assessment of atherosclerosis. This technique requires percutaneous placement of an access needle with catheters over guide wires and contrast dye is injected into the artery of interest.[8] However, angiography depicts only the contrast-filled lumen, and does not provide information about the vessel wall itself. Along with advances in imaging technology, the ability to detect and quantify subclinical atherosclerosis at different stages and in different vascular beds is continually being improved.[3]

Ultrasonography (US), magnetic resonance imaging (MRI) and computer tomography (CT) are now the most widely applied imaging modalities for studying the vessel wall. The use of multi-slice CT angiography and MRI permit accurate evaluation of lumen diameter, plaque size and composition. However, radiation and nephrotoxic iodine-based contrast agents are drawbacks of CT, and MRI is a time-consuming and expensive examination with frequent contraindications and poor availability, limiting the use of these modalities in large population-based studies.

US is used for visualization of carotid and peripheral arteries located at a depth in tissue which can be reached with ultrasound. Coronary artery imaging is challenging because high temporal resolution is needed to eliminate cardiac motion, and a high spatial resolution is needed to adequately visualize small coronary arteries.[8] Coronary artery calcium (CAC) score by CT, shows equivalence with the total coronary artery atherosclerosis load and is based on axial slices

limited to the cardiac region with quantification of calcium identified as areas of hyper-attenuation. In this setting, CT is performed without the use of intravenous contrast and at low radiation doses and this technique has been applied in population studies.[3]

Two-dimensional B-mode US imaging is a well-acknowledged method for evaluation of atherosclerotic disease in the carotid arteries. It is used to assess degree of stenosis with blood-velocity profiles, carotid intima media thickness (IMT), the presence of plaque and plaque characteristics. US is non-invasive, reliable and reproducible.[49] It is a low cost, low risk and accessible imaging modality that is well tolerated by patients and suitable for population studies and repeated measurements. An estimated 20% of ischemic strokes are caused by carotid atherosclerotic disease.[49] A strong association between the extent of carotid atherosclerosis and coronary atherosclerosis as well as atherosclerosis elsewhere in the arterial tree has been confirmed.[50] Plaques in the carotid artery may therefore serve as a measure of atherosclerotic burden in the individual. The main disadvantages of two-dimensional B-mode US imaging is that it is dependent on the examiners skills and image quality, resulting in observer variability.

Invasive catheter-based intravascular ultrasonography provides more detailed information on plaque morphology, and size and depicts the arterial lumen. Contrast-enhanced US with micro bubble contrast depicts wall irregularities, ulcerations and intraplaque contrast enhancement suggestive of neovascularization.[49] FDG-PET and SPECT represents promising imaging modalities for detection of plaque inflammation.

1.6.1 **Ultrasound assessed atherosclerosis and association with CVD**

Different ultrasonographic measures are used to assess different aspects of the atherosclerotic process; degree of stenosis, intima-media thickness (IMT), presence or absence of atherosclerotic plaques, plaque number, plaque size (thickness, area or volume), surface irregularity, texture and echogenicity.[51]

The degree of luminal stenosis has been serving as the primary criterion for risk stratification of patients and treatment decision-making. Patients who have experienced a recent ischemic stroke, TIA or amaurosis fugax and have extracranial internal carotid artery disease may profit from surgical carotid endarterectomy when internal carotid artery luminal stenosis is >50%.[52] However, stenosis severity is a poor predictor of fatal and non-fatal stroke in asymptomatic individuals for whom the annual risk is suggested to be ~2% with >60% stenosis, advocating the

need for further risk stratification and other preventive strategies.[49] Measurement of stenosis is also limited by the phenomenon of compensatory vessel enlargement. The artery accommodates to the plaque and stenosis is considered a late stadium of atherosclerosis, likely resulting from plaque rupture with scarring.[20]

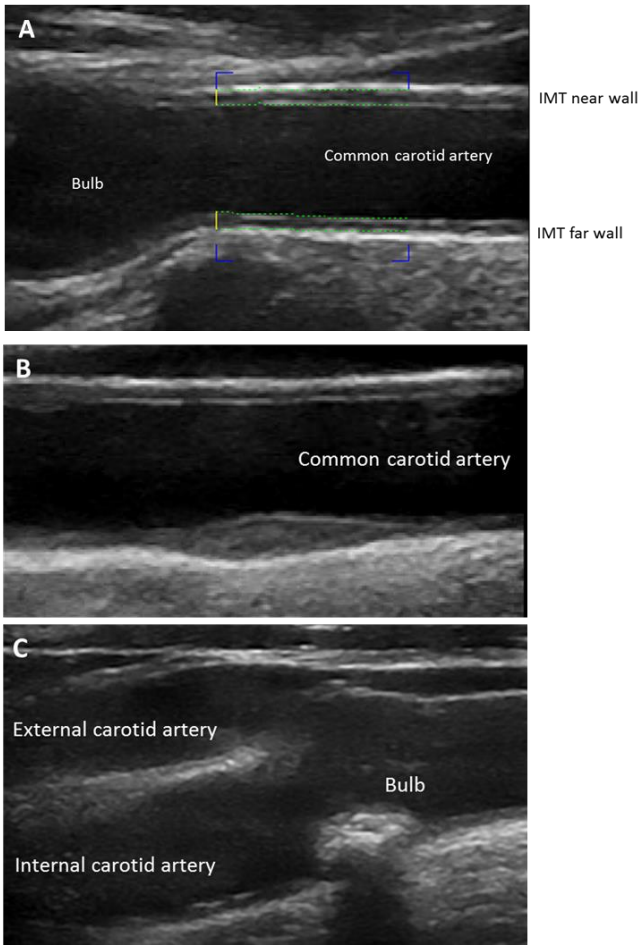

Figure 3 - Ultrasonographic measures of carotid atherosclerosis. (A) Intima media thickness (IMT) in the near and far walls of the common catorid artery.(B) Plaque of low echogenicity in the far wall of the common carotid artery. (C) Plaque of high echogenicity in the far wall of the carotid bulb.

IMT is the marker of subclinical atherosclerosis, which has been most commonly assessed in population studies. As depicted in Figure 3, IMT represents the thickness of two layers (the intima and media) of the vessel wall. Carotid intima-media thickening is thought to be an early manifestation of atherosclerosis, because thickening precedes the development of atherosclerotic plaque. However, epidemiological studies have been incoherent with regard to which part of the artery they measured (common carotid, internal carotid, or bulb) and whether plaques were

included in the measurements. IMT is most often assessed in the common carotid artery (cIMT), a site where atherosclerotic plaques rarely develop. cIMT probably largely represents medial SMC hypertrophy related to hypertension, rather than subintimal changes indicative of atherosclerosis.[53] Assessment of cIMT has been widely used to predict cardiovascular risk but may not be useful for risk stratification in a general population.[54] In addition, it is not feasible to measure progression of IMT within individuals over time, and in large cohorts IMT progression did not predict events.[55]

Quantification of plaque burden by assessment of plaque prevalence (Figure 3) and total plaque area (TPA) in carotid arteries is superior to IMT, as it is a stronger predictor of cardiovascular events.[56, 57] The presence of plaques increases the risk of stroke by 1.5-fold,[58] whereas being in the highest TPA quartile was associated with 1.7-fold increased risk of stroke compared to no plaque.[56] Also, being in the highest TPA tertile was associated with a 1.7-fold increased risk of MI compared to no plaque.[59] In addition, plaque progression can be reliably evaluated within individuals within months.[60] Progression of carotid atherosclerosis is related to higher risk of vascular events compared to atherosclerosis that remain stable or regress over time.[53, 60, 61] Measurement of plaque volume by 3D ultrasound may be even more sensitive to changes than TPA, for instance when evaluating the effect of therapy.[62]

Vulnerable, rupture-prone plaques tend to have large lipid cores (cross-sectional plaque area of at least 25%), thin fibrous caps (<200µm thick) or intraplaque hemorrhage and thus appear echolucent with irregular surface and ulcerations detected by colour-Doppler ultrasound[49] (Figure 3). Grey-scale median (GSM) is an objective computerized measurement of echogenicity.[49] However, evidence regarding the value of assessing plaque echogenicity in CVD prediction is diverging [63-65]. In spite of major advances in imaging technology with potential to identify vulnerable plaque characteristics, this has not led to improved ability for risk prediction.[24] Still, the complex exchange of cellular, molecular and biomechanical factors indicative of symptomatic plaque disruption and its sequelae cannot be accurately foreseen by any of the available imaging techniques.[9] Studies with repeated assessments have shown that plaque morphology may change over a few months gaining or losing vulnerable characteristics, presumably secondary to subclinical rupture and healing.[20, 24] In this way, atherosclerosis is a systemic condition which remains unpredictable concerning which particular lesion may cause a clinical event. Thus, some argue in favour of a greater focus on the atherosclerotic disease burden, rather than on the features of individual plaques, and advocate that detecting a state of

vulnerability represented by widespread atherosclerosis and inflammation may be more important that detecting individual vulnerable sites.[24]

1.7 Risk stratification and novel therapeutic targets in CVD prevention

The Framingham Heart Study was initiated in 1947 in Massachusetts to study CVD events in a stable population, and in 1960 the concept of risk factors was introduced. Risk factors are observable in the preclinical phase and have also been defined as factors that are "associated with a disease by virtue of its participation in the causal pathway leading to the disease".[38] Risk-multiplying effects were acknowledged when several risk factors were present at the same time, and led to the development of 10-year absolute cardiovascular risk equations. The first was the Framingham Risk Score in 1998.[66] TRFs are incorporated in these risk equations, which calculate an individual's risk of experiencing a CVD event within the next 10 years. Issues regarding applicability of the Framingham Risk Score to other populations have led to the development of various risk calculators, most of them include variations of the original TRFs age, sex, hypercholesterolemia, hypertension, and smoking, which account for most of the risk in ischemic CVD. Such risk assessment equations are used as guiding tools for preventive strategies in the primary prevention setting. Some CVD prevention strategies are beneficial nearly for all and generally recommended, e.g., healthy diet, exercise and smoking cessation. Others are associated with considerable costs and risks for adverse effects, e.g., preventive medications such as aspirin, antihypertensive and lipid lowering drugs, and are reserved for use in persons for whom the benefits of interventions are expected to be large enough to outweigh the costs and risks.[66] Subjects who score high on risk calculators, usually >20% risk of CVD in the next 10 years, are candidates for more intensive risk reduction interventions, including blood pressure and lipid lowering medications (statins) in addition to lifestyle interventions. Nonetheless, approximately 1/3 of individuals who subsequently experience CVD events are erroneously classified to be at low risk by TRFs, and CVD events also occur in subjects treated with prophylactic medications.[67] Therefore, a wide array of blood biomarkers and imaging of subclinical atherosclerosis are being investigated for detection of subclinical disease, refinement of risk assessment and guidance in preventive strategies.

In 1998, the National Institutes of Health Biomarkers Definitions Working Group defined a biomarker as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention." An ideal novel biomarker for CVD assessment should demonstrate quantitative

differences in subjects with and without disease. For a biomarker to be capable of discriminating individuals at risk independent of TRFs, it would require a robust association with CVD events in prospective studies and absence of collinearity with TRFs.[36] Methods for evaluation of novel risk markers have evolved during the last decades. Initially the focus was on detecting an association between the novel marker and CVD events after multivariable adjustment for TRFs. Subsequently, focus shifted to the use of C-statistic i.e., area under the receiver operator characteristic curve (AUC), as a metric of improved discrimination between subjects who did and did not develop CVD.[66] To significantly improve C-statistic or AUC the odds ratio between high and low risk categories needs to be more than 7, which has been deemed hard to achieve.[36] A biomarker is considered valuable if it has ability to change clinical management in health and cost-efficient ways. In the setting of CVD, this implies ability to correctly reclassify subjects who remain event-free into lower risk categories, and those who will suffer events into higher-risk categories supporting a more aggressive treatment approach. Hence, statistical methods have been developed to assess whether adding information from a novel marker to standard risk assessment with TRFs improves reclassification across a treatment decision threshold.[68]

Many of the investigated serological markers, such as CRP, are unspecific markers of inflammation which may be upregulated due to different biological processes. The studied markers are often correlated with each other and with TRFs. On the other hand, atherosclerosis is common and increases by age. The prevalence of plaque in the carotid arteries in a general population rises from <3 % in the age-group 25-35 years, to 50-60 % in men aged 55-70 years and 40-50 % in women aged 55-70 years.[69, 70] Even higher prevalence of up to 80 % has been reported in Icelandic and Finnish populations.[71] This implies that less than 10% of the population who test positive for atherosclerosis will experience a near-term CVD event.[67] For both CRP and atherosclerosis imaging measures, the associations with CVD are partly explained by strong correlations with TRFs, and conflicting findings regarding added incremental value in risk prediction exist. The assessment of multiple markers simultaneously may increase sensitivity and specificity for detection of unstable atherosclerosis. Identification of reliable imaging and serological markers of disease activity may thus be essential to single out vulnerable patients and improve the cost-effectiveness of screening for carotid atherosclerosis in the primary prevention setting.

In addition to a role in risk assessment, linking novel serological markers to different stages of atherosclerosis and clinical CVD outcomes may provide insights to the pathophysiological

mechanisms involved in disease progression. The knowledge gathered from epidemiological studies, in addition to experimental, genetic and gene expression studies represents valuable contributions in the search for novel therapeutic targets in CVD prevention. The attributable vascular risk associated with inflammation is substantial and targeted anti-inflammatory therapies in animal models have shown promise, but it remains unknown whether inhibition of inflammatory pathways in humans will lower vascular event rates.[72] It is also uncertain whether the risk of serious adverse events, such as infection and cancer, might outweigh a potential effect on CVD prevention in humans.

2 Aims of the thesis

The objectives of this thesis was

1. - To assess cross-sectional associations between CRP and carotid plaque presence and plaque burden measured as total plaque area (TPA).
   - To explore whether CRP predicts novel plaque formation and plaque progression, independent of traditional risk factors. (Paper I)

2. - To investigate the associations between CRP and carotid atherosclerosis, alone and in combination, with incident IS and MI.
   - To assess whether CRP mediates the risk of IS and MI in subjects with subclinical carotid atherosclerosis.
   - To assess whether CRP and carotid atherosclerosis, alone and in combination, add incremental value beyond that obtained from traditional risk factors in risk prediction for IS and MI. (Paper II)

3. - To assess the association between 28 circulating protein biomarkers measured at baseline and formation and progression of carotid plaque at 6-years follow-up. (Paper III)

3 Subjects and methods

3.1 Study population and ethics

The Tromsø Study is a longitudinal population-based multipurpose cohort study carried out in the municipality of Tromsø, Norway. A total of seven cross-sectional health surveys, with high attendance rates, have been conducted (Tromsø 1-7) with 6-7 years intervals in the period 1974-2017. CVD was initially the main focus of the study, but other research areas have been added throughout the years**.** "The aim has been to include large, representative samples of the population of Tromsø, with invitation of whole birth cohorts and random samples".[73] Overall participation rates were high, ranging from 79% in the 5th survey to 66% in the 6th survey.[73] Tromsø 4-7 included a second visit with a more extensive examination for some of the participants. Subjects eligible for the second visit were identified before they were invited to the first visit at each survey, and 76%, 85% and 64% of the eligible attended the second visit in the 4th, 5th and 6th surveys, respectively.[73] If they attended the first visit, they were invited to the second visit 2-4 weeks later. Ultrasound examination of the right carotid artery was performed for the first time at the second visit of Tromsø 4 (1994/1995) and repeated in Tromsø 5 (2001/2002), Tromsø 6 (2007/2008) and Tromsø 7 (2016/2017). The papers included in this thesis are all based on prospective follow-up studies on subjects who attended the second visit of the 4th, 5th and/or 6th Tromsø surveys and had reliable carotid ultrasound measures on plaque presence and total plaque area (Figure 4). In the 4th survey, all inhabitants aged 55-74 years and 5-10% samples in other 5-year age groups (25-54 and 75-85 years) were offered an ultrasound examination of the right carotid artery. All participants who were invited to the second visit in Tromsø 4 and who were still alive and resided in Tromsø, were invited to follow-up ultrasound examinations in the 5th (2001/2002) and 6th (2007/2008) surveys. In addition, all individuals aged 50-62 or 75-84 and a 20% random sample of subjects aged 63-74 were invited to the second visit of Tromsø 6. The number of participants who attended the ultrasound examinations were 6727, 5454 and 7084 in the 4th, 5th and 6th survey, respectively. All subjects who participated in Tromsø 4-6 were given information brochures (Appendix 1) and were asked to give written consent to medical research prior to the examinations. Participants were free to withdraw their consent at any time. Participants without valid written consent to medical research (n=71) were excluded. The study was approved by the Regional Committee for Medical Health and Research Ethics and the Norwegian Data Inspectorate. Dates of emigration were obtained from the

Population Registry of Norway. Inclusion criteria differed in the three papers included in this thesis and are displayed in the flowchart below.

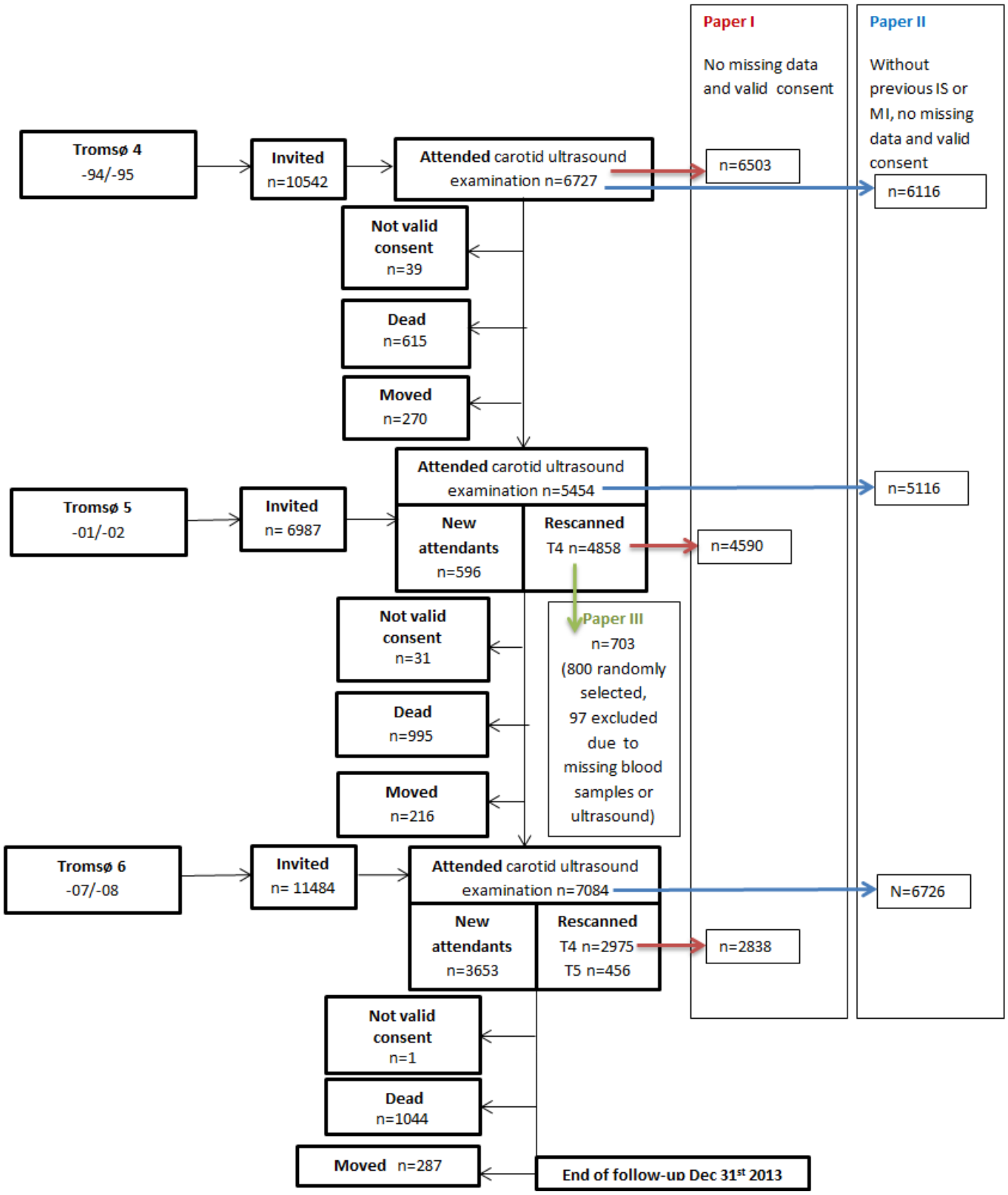

Figure 4. Flowchart of the study population.

Participants - Paper I

The study participants were recruited from the 4th survey of the Tromsø Study.[73] Eligible were all who participated in the carotid ultrasound examination in the 4th survey (1994/1995; baseline) and had CRP measurements, complete information on all relevant TRFs and outcomes (plaque presenece and TPA) assessed at baseline (n=6503). Of these, 4730 and 2917 were rescanned in the 5th and 6th survey, respectively, of whom 4590 participants from the 5th and 2838 participants from the 6th survey had valid information on all covariate and outcome measures, and these were included in the analyses. The maximal follow-up time was 13 years. The participants attended on average 2.2 surveys, and 2595 subjects had complete covariate and outcome information assessed at all three surveys. Of the 6503 participants included in the study, 1530 attendants died and 455 moved out of the municipality during the follow-up period (1994/2008).

Participants - Paper II

Eligible for this study were participants who attended one or more carotid ultrasound examinations in the 4th, 5th and 6th surveys. Participants without valid written consent (n=71), participants with known pre-baseline history of IS (n=121) or MI (n=527), and participants who did not have complete information on CRP, ultrasound measurements and relevant TRFs (n=467) in at least one of the attended surveys were excluded. Thus, our population consisted of 10 109 unique individuals, of whom 4932 attended once, 2505 twice and 2672 attended three surveys (Figure 5). Subjects were followed from the date of enrollment until December 31, 2013. During follow-up 671 and 1079 participants experienced first time IS and MI, respectively, 2249 participants died and 721 moved from the municipality.

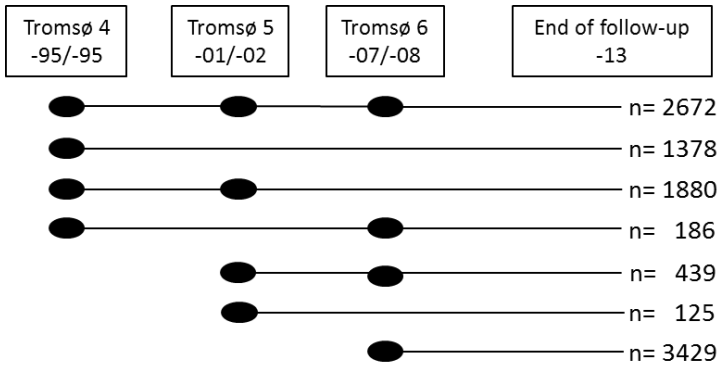

Figure 5 Overview of study inclusion. Dots indicate participation at the survey, and lines indicate observation periods. A total of 10 109 unique individuals were included in the study, of whom 4932 attended once, 2505 attended twice and 2672 three surveys.

Participants - Paper III

The study design was a nested case-control study with 703 participants who participated both in the 4th and 5th surveys of the Tromsø Study. Four groups were randomly selected from the ultrasound cohort on the basis of carotid ultrasound findings at follow-up. **Group 1**: Study participants who had no plaque at baseline nor at follow-up (n=126); **Group 2**: Study participants without plaque at baseline and novel plaque detected at follow-up (n=187); **Group 3**: Study participants with prevalent plaque at baseline and stable plaque (no increase in total plaque area) at follow-up (n=194); **Group 4:** Study participants with prevalent plaque at baseline and progression of plaque (increase in total plaque area) detected at follow-up (n=196). There were originally 200 subjects in each group and they were matched on age and sex. Because of missing blood samples the total number of subjects in the study was 703, the groups remained balanced with regards to age and sex, but the number of participants was not equal in the four groups.

3.2 Cardiovascular risk factors

Information on TRFs was collected by physical examination (blood pressure and body mass index (BMI)), non-fasting blood samples (total cholesterol, HDL-C, glycosylated hemoglobin (HbA1c)) and self-administered questionnaires (prevalent diabetes, current smoking, former MI and stroke, and use of antihypertensive, lipid lowering and diabetic medication) (Appendix 2). Diabetes was defined as self-reported diabetes, daily use of oral diabetic medication or insulin, or HbA1c levels >6.5%. CVD was defined as previous MI or stroke. Further details are presented in the papers.

In Paper I and II, information on TRFs obtained at all surveys (4th, 5th, and 6th) were applied in analyses. We used time-varying covariates, meaning that subjects who attended multiple surveys had their exposure variables and TRFs updated at each survey. In Paper III, we used baseline measurements of TRFs.

3.3 Carotid ultrasound examination

In the 4th and 5th survey, B-mode ultrasonography was performed with an Acuson Xp10 128, ART-upgraded duplex scanner equipped with a 7.5 MHz linear-array transducer. In the 6th survey, a GE Vivid 7 scanner with a linear 12-MHz transducer was used. Different sonographers did the baseline and follow-up scanning, but followed standardized examination techniques, measurements and reading procedures (Appendix 3). All sonographers completed a 2-month pre-

study training protocol. The far and near walls of the right common carotid artery, the bifurcation (bulb) and the internal carotid artery (six locations) were scanned for the presence of plaques. A plaque was defined as a localized thickening of the vessel wall of more than 50% compared with the adjacent intima-media thickness. For each plaque, a still image was recorded with the transducer parallel to the vessel wall and perpendicular to the point of maximum plaque thickness. Each plaque was manually outlined and total plaque area (TPA) was calculated as the sum of all plaque areas in mm^2.[74]

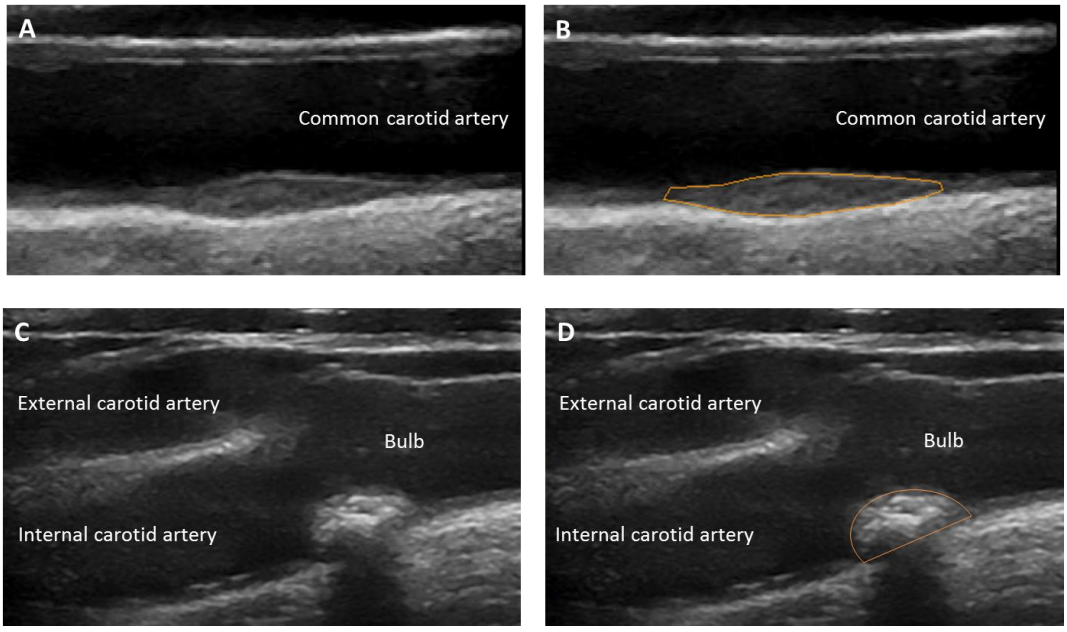

Figure 6 – Measurements of plaque area in the carotid artery. (A) Plaque of low echogenicity in the far wall of the common carotid artery. (B) Outlining of the plaque area which measures 20.8 mm^2. (C) Plaque of high echogenicity in the far wall of the carotid bulb. (D) Outlining of the plaque area which measures 19.3 mm^2.

3.4 Blood biomarkers

CRP was analyzed with a particle-enhanced immunoturbidimetric assay on a Modular P (4^{th} and 6^{th} surveys) or Hitachi 917 (5^{th} survey) autoanalyzer (Roche Hitachi, Mannheim, Germany), with reagents from Roche Diagnostics (Mannheim, Germany). Samples from the 4th survey were analyzed in thawed aliquots after 12 years of storage at −70 °C, while samples from the 5^{th} and 6^{th} surveys were analyzed at time of the surveys. The lower detection limit of the high-sensitivity CRP assay was 0.03 mg /L, and measurements of CRP lower than 0.03 mg/L were set at this value. The analytical coefficient of variation for CRP levels between 0.1 mg/L and 20 mg/L was < 4 %. CRP was measured by these methods in Papers I and II.

In Paper III, a panel of 28 novel biomarkers that previously had shown promising results on the association with CVD were selected and analyzed in blood obtained at baseline. The selected biomarkers have proposed links to atherosclerosis through different pathophysiological mechanisms; inflammatory markers (CRP, fibrinogen, white blood cells (WBC), monocyte count, neopterin, IL-6, IL-18, ICAM-1, VCAM-1, caspase-1, MMP-9, TIMP-1, D-dimer, PCT, protein S-100); markers of oxidative stress (MPO, Cu/Zn SOD); metabolic markers (adiponectin, leptin, ApoA1, ApoB100, ApoB100/ApoA1 ratio); markers of hemodynamic stress (BNP, CT-proAVP, MR-proADM, MR-proANP); and markers of renal function (creatinine, cystatin-C). The study blood samples underwent no more than three freeze/thaw cycles from time of receipt to protein data production. All samples were kept at 4º C between sample dilutions, and were otherwise stored at -70º C until assay production. Fibrinogen, creatinine, and WBC were measured at the Department of Clinical Chemistry, University Hospital of North Norway, Tromsø. All other biochemical analyses were performed at the Mainz Biomarker Laboratory (details in Paper III). According to manufacturers, all inter- and intra-assay coefficients of variation were below 10%, except inter-assay coefficients for adiponectin, IL-18 and PCT which ranged between 10 and 20%.

3.5 Ascertainment of clinical endpoints

Based on data from hospital records, autopsy records and death certificates, an end-point committee of trained physicians validated hospitalized and out-of-hospital events of incident IS and MI. By national unique 11-digit identification numbers, the Tromsø Study participant list was linked to national and local diagnosis registries including the National Causes of Death Registry, the Population Registry of Norway, the discharge diagnosis registry (outpatient diagnoses included) at the University Hospital of North Norway (UNN). UNN is the only hospital in the municipality of Tromsø, the nearest hospital is located approximately 250 km away by road (148 km by air). Fatal events that occurred outside of hospital were identified through linkage to the national Causes of Death Registry at Statistics Norway, and death certificates, autopsy reports, and information from additional sources, such as records from nursing homes, general practitioners, and ambulance services, were used for validation. Discharge letters from hospitalizations in other hospitals were also collected when appropriate. To identify all possible first-ever MI and IS cases, we used a wide search strategy that included the International Classification of Diseases (ICD) 9 codes 410-414, 430-438 and 798-799 from 1994–1998 and thereafter ICD 10 codes I20-I25, I60-I69, R96, R98, and R99. IS was defined as

rapidly developing clinical signs of focal or global disturbance of cerebral function, with symptoms lasting ≥24 hours or leading to death with no apparent cause other than vascular origin, when CT, MRI or autopsy had ruled out intracerebral or subarachnoid hemorrhage. Modified WHO MONICA/MORGAM[75] criteria for MI were used, and included clinical symptoms and signs, findings in electrocardiogram, values of cardiac biomarkers and autopsy reports if applicable. At the University Hospital, biomarkers used included creatine kinase (CK) and its MB fraction (CK-MB) throughout the study period, and troponin from 2000. Biomarker levels were generally recorded three times during the first 3 days following admission or MI onset. When circumstances suggestive of invalid biomarker values were present, the significance of biomarker results were downregulated. Cases meeting diagnostic criteria for definite or probable fatal or non-fatal first-ever MI were classified as MI. Silent MIs as defined by ECG only were not included as cases because of difficulties in determining the exact date of the event.[76]

3.6 Statistical analyses

SAS statistical software package SAS 9.4 (SAS Institute, Cary, NC, USA) was used for all data analyses. Baseline characteristics were presented as percentages, means with SDs, medians with interquartile ranges or geometric means for non-normally distributed variables. When assessed as continuous variables, TPA was square root transformed (sqrtTPA) and CRP log transformed to approximate normal distribution and improve regression model fit. Associations were investigated in age and sex adjusted models and subsequently in models adjusted for traditional risk factors (multivariable-adjusted). Sex-stratified associations were assessed in Paper I and II, but not in Paper III due to lack of statistical power. A two-sided level p-value <0.05 was considered as significant in all analyses, except when examining interactions where p-value <0.2 was considered statistically significant. More detailed descriptions of the statistical methods are found in the papers, whereas main points are highlighted below.

Paper I

Presence of plaque (yes/no) and total plaque area (TPA) in mm^2 were the outcome (dependent) variables and CRP was the exposure (independent) variable. Cross-sectional and prospective associations were examined.

When plaque was treated as a dichotomous variable, generalized estimating equations with a logit link function were applied, and correlated observations within individuals were adjusted for

by an exchangeable correlation structure. In this structure, the correlations between subsequent measurements on the same individual are assumed to be the same, irrespective of the length of the time interval.[77] Odds ratio (OR) of plaque presence compared to no plaque was estimated for a defined change in CRP (1 SD increase or higher risk categories compared to CRP <1 mg/L). When examining the cross-sectional associations between CRP and TPA (continuous variable), we used linear mixed models. Correlated observations within individuals were adjusted for by adding a random intercept to the model, allowing intercepts to differ between subjects, but estimated regression coefficients for the covariates are the same for all subjects.[77] Linear mixed models calculates the β-coefficient, which represents the estimated change in TPA associated with a defined change in CRP.

A second linear mixed model was set up to simultaneously assess the cross-sectional and prospective relationship between CRP and TPA.[78] The cross-sectional component analyzed the association between baseline CRP and estimated TPA at baseline, whereas the prospective component analyzed the association between baseline CRP and TPA progression rate (CRP x time) during the observation period.[79] These models were fit with random intercepts and slopes, allowing both for baseline TPA and progression of TPA over time (slope) to differ between individuals. The normality assumption for linear mixed models was confirmed by graphical inspection of the residuals.

Paper II

First-ever IS and MI were the outcome variables. The exposure variables were CRP in predefined risk categories (CRP <1, CRP 1-3 and CRP >3 mg/L) and categories of TPA (no plaque, below and above the median TPA). Cox proportional hazard regression models with time-varying covariates and age as time scale[80] were used to assess the association between CRP and TPA alone and in combination (CRP+TPA) with risk of IS and MI. Follow-up time and risk estimates were calculated separately for IS and MI. By assigning new observation periods with updated values of risk factors at the time of subsequent study attendance, we utilized individual person data from repeated surveys, thereby taking into account changes in exposure status during follow-up. Due to differences in event censoring, the 10 109 participants contributed with 17 668 observation periods for IS and 17 454 observation periods for MI.

For each exposure variable, we calculated incidence rates and hazard ratios (HRs) with 95% CIs for IS and MI using the low-risk groups as reference (CRP <1 mg/L and no plaque). HR is the

person's instantaneous risk of experiencing the disease of interest, at any time-point in one group (exposed) compared to another group (unexposed). The impact of CRP on the relationship between TPA and the two outcomes was assessed by calculating the percentage change of HR in the different TPA categories when CRP (log-transformed) was added to age- and sex-adjusted models. Multiplicative interactions between CRP and TPA were assessed. To investigate synergistic effects of atherosclerosis and CRP on the risk of IS and MI, we calculated incidence rates and HRs for the other eight constellations of TPA and CRP, and these were compared to the no-plaque group with CRP <1 mg/L. Additive interaction and synergism was evaluated using the Rothman synergy index[81] to determine whether the joint effects of CRP and atherosclerosis on the risk of IS and MI exceeded the sum of effects from each factor alone in age- and sex-adjusted models. A synergy index greater than 1.0 suggests that the effect of the joint exposures of two risk factors is greater than the sum of the separate effects.

Finally, the added value by TPA and CRP in risk prediction was evaluated by comparing the discrimination power of a model based on the Framingham risk factors with models that additionally included TPA alone, CRP alone, and TPA and CRP together. We calculated Harrell's C-index[82], relative integrative discrimination improvement (IDI) and net reclassification improvement (NRI).

For all Cox proportional hazard regression models, the proportional hazard assumption was verified by visual inspection of log–log survival plots.

Paper III

Plaque group at follow-up was the outcome variable, and the 28 biomarkers measured in blood obtained at baseline were exposure variables. We used general linear models to assess differences in biomarker levels across plaque groups. False discovery rates (FDR) were calculated to adjust for multiple comparisons.[83] For each biomarker that significantly differed between groups, multinomial logistic regression models were used to assess the association between baseline biomarker level and plaque group, adjusted for age and sex and further adjusted for TRFs. The no-plaque group was defined as reference category. Odds ratios (OR) for outcome were reported per 1 SD change in continuous variables or for presence vs. absence of binary variables. All significant biomarkers in univariable models and TRFs were candidates for a final multivariable analysis using a backward selection procedure with a retention p-value of 0.05.

We also performed analyses to evaluate the composite measure of the aggregate number of biomarkers in the highest third with respect to plaque progression.[84] We considered the biomarkers which were significantly associated with plaque progression after adjustment for TRFs, and used a logistic regression model to estimate OR for being in the plaque progression group versus the no-plaque group according to number of biomarkers in the upper tertile.[85-87]

3.6.1 **Missing data**

In Paper I, observations with complete data on outcome, exposure and adjusting variables were included in the analyses. In Paper II, missing data were handled by carrying forward values from previous surveys, when applicable. In Paper III, missing data were assumed to be missing at random and handled by multiple imputation by chained equations in SAS, using the FCS command to impute 20 data sets.[88, 89] This method handles different types of variables (continuous, binary and categorical). The imputed values are drawn from the posterior predictive distribution of the missing data, conditional on the observed data. Rubin's rule was used to combine the results for the imputed data sets. The combined estimate is the mean of the individual regression coefficients from each of the 20 data sets. The total variance is determined by the within-imputation variance and the between imputation variance.[89]

4 Main results

4.1 Paper I

In cross-sectional analyses, we confirmed an association between CRP and carotid plaque prevalence as well as TPA in both sexes. After adjustment for TRFs, the cross-sectional associations were most prominent in men; CRP was associated with TPA in men only at baseline, but in both sexes when considering all surveys. However, the magnitude of the association remained larger in men. For women, there was a significant higher plaque prevalence when CRP was >3 compared to CRP < 1 mg/L (OR 1.20, 95 % CI 1.04, 1.39). For men, this association was weaker (OR 1.15, 95 % CI 0.99, 1.34). When treated as a continuous variable, CRP was associated with plaque prevalence in men only.

For men, baseline CRP >3 mg/L vs. <1 mg/L was associated with TPA progression (p=0.03). However, in multivariable-adjusted models, baseline CRP did not predict TPA-progression in either sex. In men who were plaque-free at baseline, the risk of novel plaque formation increased significantly with baseline level of CRP. The risk for plaque at end of follow-up was 44% higher in men with baseline CRP >3 mg/L compared to men with baseline CRP <1 mg/L (OR 1.44, CI 1.08, 1.92). However, this association was attenuated to non-significant upon adjustment for TRFs. There was no association between baseline CRP and novel plaque formation in women.

4.2 Paper II

Serum CRP levels and carotid atherosclerosis were individually associated with increased risk of IS and MI independent of TRFs. CRP level >3 mg/L vs. <1 mg/L was associated with increased risk of IS (HR 1.84, 95% CI 1.49, 2.26) and MI (HR 1.46, 95% CI 1.23, 1.73) in multivariable-adjusted models. There was no significant interaction with sex for either outcome. Both TPA below and TPA above the median were associated with higher risk of IS and MI compared to no plaque. For IS, HRs (95 % CIs) were 1.33 (1.08, 1.65) and 1.65 (1.36, 2.01), referring to TPA below and above median, respectively. The corresponding HRs (95 % CIs) for MI were 1.31 (1.11, 1.55) and 1.64 (1.41, 1.92). For MI, but not for IS, there was a significant interaction between plaque category and sex (p=0.02), suggesting a stronger association between TPA and risk of MI in women than in men.

TPA showed a weak correlation to CRP with Spearman correlation coefficient of 0.13 ($p < 0.001$), and risk estimates for subjects with atherosclerosis were only slightly attenuated by adding CRP to the models (1.7– 8.6%). For both outcomes, the joint presence of TPA > median and CRP >3 mg/L were associated with the highest incidence rates. However, a synergistic effect was evident for IS only, with a synergy index of 1.72 (95% CI 1.06, 2.81). There were no significant multiplicative interactions between CRP and TPA categories for either outcome.

TPA alone and the combination of CRP and TPA achieved a significant improvement in risk prediction beyond Framingham risk factors, with most prominent effects in the group classified at intermediate risk by Framingham risk factors. For IS, the highest categorical NRI was seen when including both variables (CRP+TPA) as continuous variables, 6.6% ($p=0.007$) for the population and 21.6% ($p<0.001$) for the intermediate risk group. For MI, the highest overall NRI of 5.0% ($p=0.01$) for the population and 12.0% ($p=0.02$) for the intermediate risk group were seen when both variables were included as categorical variables.

4.3 Paper III

The crude baseline level of 12 biomarkers differed significantly between the four plaque groups (no plaque, novel plaque, stable plaque and plaque progression). These markers were CRP, fibrinogen, WBC, neopterin, D-dimer, IL-6, caspase-1, ICAM-1, ApoA1, ApoB100, ApoB100/ApoA1 ratio and MPO. Adjustment for multiple comparisons revealed FDR < 0.05 for seven biomarkers (fibrinogen, WBC, IL-6, caspase-1, ICAM-1, MPO and ApoB100/ApoA1 ratio). The mean baseline levels of these biomarkers were, except for two, highest in the plaque progression group and lowest in the no-plaque group. The exceptions were neopterin and ApoA1. The highest baseline level of neopterin was observed in the no-plaque group. The highest level for ApoA1 was observed in the novel plaque group.

Age- and sex-adjusted levels of fibrinogen, ApoB100, ApoB100/ApoA1 ratio, WBC, CRP, MPO, D-dimer, caspase-1, and IL-6 were significantly associated with plaque progression. In addition, an increase in caspase-1 increased the corresponding odds of novel plaque formation, while higher neopterin level decreased the odds for novel plaque formation. The associations between MPO, caspase-1 and IL-6 and plaque progression and between neopterin and novel plaque formation remained significant after adjustment for TRFs. When subjects with former CVD were excluded, IL-6 and neopterin remained the only significant biomarkers for plaque progression and novel plaque formation with ORs (95% CIs) 1.36 (1.05, 1.77) and 0.73 (0.57,

0.94), respectively. In the final regression analysis, which included TRFs and the 12 significant biomarkers from the univariable models, IL-6 remained a significant predictor of plaque progression using a backward selection procedure.

A multimarker model suggested that OR of plaque progression increased with increasing number of biomarkers in the upper tertile (considering IL-6, caspase-1, and MPO). After adjustment for TRFs, individuals with two biomarkers in the upper tertile had a 2.2-fold higher odds, and individuals with three biomarkers in the upper tertile a 4.4-fold higher odds of plaque progression at follow-up, compared to subjects with none of the selected biomarkers in the upper tertile.

5 Discussion

5.1 Methodological considerations

5.1.1 Study design

According to the epidemiologist Kenneth Rothman "the objective of an epidemiological study is to obtain a valid and precise estimate of the frequency of a disease or of the effect of an exposure on the occurrence of a disease in the source population of the study".[90] Cohort studies are observational studies where a group of people (cohort) is defined and investigated. The group consists of individuals who are at risk of developing a specific disease or health outcome. All individuals in the cohort will be observed for a period of time in order to measure the frequency of disease-occurrence (incidence) among those exposed to the suspected causal agent, compared to those not exposed.[91]

Cross-sectional studies, in which exposure and outcome are assessed at the same point in time, provide important information on associations. However, association is not causation and cross-sectional studies are affected by the antecedent-consequent bias, similar to the chicken and egg question (i.e., "which came first?"). In order to use findings from epidemiological studies in primary prevention and other interventions that aim at modifying the probability of the outcome of interest, it is essential to establish whether or not an association is causal.[92] Inferring causation is the most challenging problem in epidemiology. The Bradford Hill criteria from 1965 comprise aspects of a statistical association (strength of association, consistency, specificity, temporality, biological gradient, plausibility, coherence, experiment, and analogy) and when present, strengthens the inference that the statistical association is also causal. The overarching questions that these criteria seek to address are whether confounding and bias are reasonable alternative explanations for the observed statistical association.[93] The most important criterium is the rule of temporality, the cause has to precede the effect. In longitudinal studies, exposure is assessed at a certain point in time and the population under study is followed for a period of time with prospective ascertainment of the outcome of interest. Although longitudinal studies are not able to prove a causal relationship, the fact that exposure is assessed before outcome may support the Bradford Hill criteria of temporality and strengthen the possibility of a causal association. Determining true causality requires, however, further corroboration from experimental trials.

In medicine, the randomized controlled trial (RCT) is the gold standard for establishing causality. Randomization should ensure that the population groups remain close to similar in every aspect, except for the exposure (or intervention) under study. However, it may be impossible or unethical to randomize participants to potentially harmful exposures in order to study their effect on outcomes. This limits the utility of RCT's in studying risk factors for disease. Another approach is genetic epidemiology with application of Mendelian randomization design, which can provide unbiased estimates of causal exposure-disease associations. The basic idea is to study associations between the inherited genetic polymorphisms, known to affect the presence or level of the exposure of interest (the phenotype) and its association to the outcome under study. This method requires a strong and specific association between the genetic trait and exposure of interest and absence of linkage disequilibrium, i.e. the genetic trait is linked with other genetic factors that influence the risk of outcome.[92]

The papers in this thesis are all based on the Tromsø Study, which is a population based longitudinal cohort study with repeated health surveys. The main strengths of the Tromsø Study are the large sample size, the prospective design, and the repeated and standardized assessments of cardiovascular risk factors, blood samples, carotid ultrasound and CVD events in a general population. The nested case-control study design, which was applied in Paper III, combines some of the features and advantages of both cohort and case-control designs. The case groups consist of a representative sample of individuals with the outcome of interest (in this case subjects with novel plaque, stable plaque and progression of plaque) occurring in the specified follow up period of six years. The control group (subjects with no plaque at follow-up examination) was selected from individuals at risk at the same time as each case was defined. This design is less prone to selection and information bias compared to traditional case-based case-control studies. This is because cases and controls are selected from the same source cohort and exposures are assessed before the disease occurs unlike in a traditional cohort study.[92] A properly executed case-control study nested in a cohort is valid if the corresponding analysis of the full cohort is valid.[94] This study design is efficient for addressing research questions when additional information that was not obtained or measured for the whole cohort is needed. In our study, blood samples were collected at baseline and stored in freezers. The serum samples for cases and controls could thus be analyzed at reduced costs.[92]

In population studies, providing information relevant to the general population and allowing generalization beyond the study population itself, requires both internal and external validity.

Internal validity is a prerequisite of external validity. Accuracy, the degree to which a measurement or estimate represent the true value of the attribute being measured, is essential in epidemiology. Threats to the accuracy of epidemiological studies are random errors (lack of precision) and systematic errors (bias).[90, 92]

5.1.2 **Internal validity**

Internal validity refers to whether the inferences drawn from the sample to the population under study are valid.[90] Internal validity may be threatened by three types of error; selection bias, information bias, and confounding.

5.1.2.1 **Selection bias**

Selection bias is systematic error in the recruitment or retention of study subjects. Selection bias is present when individuals have different probabilities of being included in the study sample according to relevant study characteristics. This results in the study participants being different from non-participants in regard to the exposure and outcome of interest. The validity of studies to document incidence or prevalence of disease or exposure in the source population relies on a sample of study participants that represents the actual population. The estimated association between exposure and outcome may also be biased if participation is influenced by exposure or the disease under study.[90, 92] Whenever possible, study subjects should be chosen from a defined reference population. In the Tromsø Study, the invitation of total birth cohorts and random samples from other age groups based on information from the official population registry of inhabitants of Tromsø, ensures the invitation of a representative study population. Selection bias may still be a problem if participation rates are low. In Tromsø 4, the participation rate was high, 77% of those invited to the first visit, and 76% of those eligible to the second visit participated. In Tromsø 5, the rate was even higher; 79% of those invited to the first visit and 85% of those eligible to the second visit participated. The participation rate was somewhat lower in the 6th survey, where 66% and 64% of those eligible attended the first and second visit, respectively. The main targeted age group was 40-80 years. For the youngest and the oldest age groups and for men, the participation rate tended to be lower.[73] The participation rate was also lower among those who had not participated in previous surveys of The Tromsø Study. The educational level was higher among participants than in the general Tromsø population. Responders tended to be non-smokers and married compared to non-responders.[73] Recently, another Norwegian cohort study (HUNT) compared participants to non-participants and concluded that non-participants

had lower socioeconomic status, higher mortality and higher prevalence of several chronic diseases. Non-participants had less healthy lifestyle in terms of tobacco smoking and physical activity, and poorer general health.[95] The Tromsø Study is comparable to HUNT. Legal restrictions have precluded analyses of mortality and morbidity among non-attenders. However, a mortality follow-up study of persons invited to CVD surveys in 5 areas of Norway found that age-adjusted all-cause mortality rate was 3.7 times higher in non-attending women and 2.2 times higher in non-attending men, compared with attendees.[96]

Selection bias may also occur in a cohort study if there are differential losses to follow-up, meaning that individuals who are lost to follow-up have different probabilities of the outcome of interest than those who remain in the cohort. This can occur when losses to follow-up are related to morbidity and mortality from causes other than the outcome of interest (competing risk of death), refusal or migration.[92] Lower mortality rates have previously been documented in participants who attended multiple surveys, compared to those who attended only Tromsø 4.[73] When considering clinical endpoints such as IS and MI, the loss to follow-up is negligible due to usage of the unique personal identity number to search in official health registries. When evaluating changes in carotid TPA, which requires repeated ultrasound measurements, the statistical power to detect associations with the exposure under investigation may be diminished if subjects with the most severe atherosclerosis are lost to follow-up. Survival bias, with higher representation during follow-up of attendees with a more favorable risk profile, compared to diseased persons of the same birth cohort, may represent a source of selection bias. Differences in baseline characteristics between subjects who were lost to follow-up compared to subjects who attended follow-up examinations, were assessed in Paper I and confirmed that subjects with the most unfavorable levels of TRFs and atherosclerosis at baseline, were more likely to drop out from follow-up examinations. In Paper I, the use of linear mixed models and generalized estimating equations enable the inclusion of information from participants who only attended one survey and ability to update information on CRP, TRFs and carotid plaque on subsequent study attendance for assessment of cross-sectional associations. The method of updating CRP, plaque status and TRFs on subsequent study attendance was also applied in Paper II. However, when assessing the prospective associations in Paper I and III, attendance in two or more surveys was required. In this situation a biased relative risk or rate ratio estimate will only ensue if losses to follow-up are biased according both to outcome and exposure (differential).[92] High rates of non-participation may indeed introduce bias in prevalence rates, but there is little evidence

supporting biased estimates of association due to non-participation.[97, 98] Hence, we do not suspect that the associations between circulating biomarkers and atherosclerosis are different in the participants who were lost to follow up than for those who remained in the study.

There were attempts to improve participation rates and thereby reduce selection bias by sending invitation-reminders, having easily available study centers (e.g., in a shopping center) and promoting the health benefits of participation on the individual and community level.

5.1.2.2 **Information bias and misclassification**

Information bias results from "imperfect definitions of study variables or flawed data collection procedures".[92] It follows, that a significant proportion of the study participants may be misclassified, i.e. placed in an incorrect exposure, covariate or outcome category. There are two types of misclassification bias; non-differential (independent of other study variables) and differential (dependent on other study variables). Non-differential misclassification occurs when the degree of misclassification of exposure is independent of case-control status or vice versa. The effect of non-differential misclassification is usually attenuation of the effect estimate. Differential misclassification occurs when the rate of misclassification differs between the groups being compared. The error in the effect estimate resulting from differential misclassification is difficult to predict. Misclassification of covariates (potential confounders) may also affect the efficiency of adjustment for confounding effects. Non-differential misclassification of a confounder tends to bias the association estimate toward the null hypothesis.[92] Sources of error resulting in misclassification may be random (lack of precision, reliability) or systematic (lack of validity, bias). Reliability (reproducibility) refers to the extent to which the results obtained by a test are replicated if the test is repeated. Reliability is reflected through the confidence interval and depends on study size and study efficiency. Validity refers to the method's ability to measure what it is intended to measure, i.e. to distinguish between those who have a disease (or other characteristics) and those who do not. High reliability is a prerequisite for high validity.[90, 92] In cohort studies, the tests that are used may not be the best available tests with regard to sensitivity and specificity. A large amount of healthy individuals is to be tested. Therefore, the test should not be too time-consuming, expensive or invasive, but still have acceptable test-performance characteristics. The validity of the tests used should be examined through validation studies where the test-performance is compared to the "gold-standard" .[92]

Traditional risk factors

Exposure identification bias may affect cohort studies when there are technical or other sorts of bias in the baseline measurements. Since exposure and adjusting variables are assessed before the outcome of interest occurs, such errors tend to be non-differential with regard to disease status. Blood pressure and BMI were assessed in the Tromsø Study. Weight and height were measured, and BMI was calculated using standardized methods. Blood pressure was recorded by specially trained personnel with an automatic device. Three readings were recorded with one-minute intervals. The average of the final two readings was used in the analyses in order to reduce misclassification due to random measurement error.

In case-control studies, more serious exposure identification bias may occur when exposure is assessed after disease status is ascertained. The outcome may influence the reporting of cases and controls differentially (recall bias), and result in differential misclassification. When a well-defined cohort, such as the Tromsø Study, is available, a nested case control study allows the evaluation of certain hypothesis free of recall bias. However, in cohort studies recall bias may be present at the outset of the study when categorization of individuals by level of exposure relies on recalled information from distant or recent past.[92] Information about smoking status, use of lipid lowering and antihypertensive medication and history of former diseases, such as diabetes, stroke, MI and hypertension, was obtained through self-administered questionnaires at baseline. This type of information is prone to respondent bias and recall bias, which may result in misclassification. When people feel stigmatized by a condition or habit, they are more likely to give misleading answers (respondent bias). Smoking may be a sensitive topic leading to misclassification of smoking status. 2.1% of the "never-smokers" in Tromsø 6 had reported >10 pack-years in one of the previous surveys.[99] However, validation studies on self-reported smoking have shown that the information given in questionnaires is in general accurate.[100] We used current smoking as a measure of smoking, as data on previous smoking habits, including calculation of pack years, is more prone to recall bias.

The sensitivity of self-reported diabetes has been found to be moderate to good in previous studies.[101] To increase sensitivity, diabetes was defined as self-reported diabetes, regular use of insulin or oral antidiabetics or HbA1c >6.5%. As the study participants were unaware of any hypotheses about the relation between the variables under study, the misclassifications of self-reported former disease status are likely to be non-differential.

Blood samples were for practical reasons assessed non-fasting. Regarding total cholesterol and HDL cholesterol, fasting or eating before blood collection does not have a marked effect on measurements. Non-fasting triglycerides are problematic because of large variation in pre-and postprandial levels, and triglycerides were therefore not included in analyses.

Blood biomarkers

The exposure variables of main interest in our study were biomarkers assessed in blood samples. Performance characteristics of biomarker immunoassays should be known and acceptable. The coefficient of variation (CV) is a measure of the analytic random variation or imprecision of a test. It is the standard deviation expressed as a percentage of the mean value of two sets of paired observations. It is calculated for each pair of observations and then averaged over all pairs.[92] When each sample is measured duplicate the degree to which the duplicate results differ may be assessed by calculating the intra-assay CVs. When many samples are tested, it is often necessary that blood samples are run on multiple assay plates. The inter-assay CV is an expression of plate-to-plate consistency. Inter-assay CVs of less than 15% are generally acceptable. Intra-assay CVs should be less than 10%. Simulation studies have demonstrated that the chance of finding a more than 1.5 fold difference in two measurements of the same sample when the coefficient of variation is <10% has a probability of <0.001.[102] CVs should be reported for concentrations that reflect the range of results found in the specimens. According to manufacturers, all inter- and intra-assay coefficients of variation were below 10%, except inter-assay coefficients for Adiponectin, IL-18 and PCT, which ranged between 10-20%. The analytical coefficient of variation for CRP levels between 0.1 mg /L and 20 mg/L was <4%. Although the coefficient of variation was small, it may have led to random errors in marker measurements and attenuation of risk estimates.

If the stability of biomarkers is affected by freezing, thawing, or storage, bias may be introduced by the use of frozen blood samples. CRP stability in frozen samples is previously reported to be acceptable with high correlations between CRP values obtained before and after storage.[103] In Paper III, the 28 markers were analyzed only once in frozen blood samples obtained at baseline. Pro-inflammatory cytokines are short acting and prone to fluctuations causing substantial within-person variation. Using only baseline values tend to underestimate the real association between biomarkers and outcome due to the regression dilution effect. It is advised to collect samples at several points in time and use the average of all values as this tends to prevent non-differential misclassification.[92] In Tromsø 6, CRP was assessed both at the first and the second visit, and

6707 subjects had duplicate measurements, of whom 6425 had CRP <10 mg/L at both visits. For these subjects, the Spearman correlation coefficient between visits was 0.75, intraclass correlation coefficient was 0.75 (95% CI 0.74, 0.76), and the intra-individual CV was 39.0% which is comparable to other studies[104] and to within-person variability for total cholesterol and systolic blood pressure.[27] Considering CRP risk categories (<1mg/L, 1-3 mg/L and>3 mg/L) 33.3% of participants changed risk category between the two measurements. 52.4 % of participants who had CRP >3 mg/L at the first visit were still in this category upon the subsequent measurement. Others have found that 40% of patients with chronic coronary artery disease changed risk category between two consecutive measurements of CRP.[105] It has been suggested that discrepancies concerning the incremental value of CRP in CVD risk prediction may arise from the substantial day to day variability of CRP blood levels, which may cause misclassification of subjects from low to moderate or high risk.[105]

Ultrasound measurements

Carotid ultrasound assessment of plaque presence and total plaque area were exposure variables (Paper II) and outcome variables (Papers I + III). The reliability of the ultrasound assessment of plaque detection and plaque area measurement have been addressed in previous studies for all surveys and found to be acceptable. Details about the inter- and intra-observer reproducibility and inter-equipment variability have been published previously.[31, 74, 106] At each survey, reproducibility was assessed by inviting a sample of the participants to a second ultrasound examination within three weeks from the first scan. On each occasion, two or three sonographers examined each subject. The sonographers had no knowledge of each other's results or results from previous assessments. There were 107 paired observations in the 4th survey, 83 in the 5th and 71 in the 6th. Reproducibility of plaque area measurements was assessed in combined data from Tromsø 4th and 5th,[74] and separately in the 6th survey.[51]

Between- and within-sonographer agreement on plaque occurrence in Tromsø 4 was substantial with Kappa (κ) values (95% CI) of 0.72 (0.60, 0.84) and 0.76 (0.63, 0.89), respectively, indicating substantial agreement.[106] Reproducibility of plaque detection did not differ significantly between the sonographers. The inter-observer agreement was 0.67 (0.58, 0.76) and the intra-observer agreement 0.80 (0.70, 0.91) in the 5th survey[74], and 0.53 (0.40, 0.66) and 0.63 (0.44, 0.82) respectively in the 6th survey.[51]

Inter-observer and intra-observer variability of pairwise plaque area measurements are shown in Table 1. If the mean arithmetic difference is not equal to zero, this indicates systematic measurement errors (bias) between or within sonographers. The mean absolute difference represents the typical magnitude of this bias.[74] The arithmetic differences between paired observations were plotted against their average to examine whether differences were constant over the range of measurements (Bland Altman plots), and no systematic errors were detected.[51] In the case of normally distributed differences, 95% of the differences will be found within a range of ±1.96 SDs of the mean arithmetic difference (limits of agreement).

Table 1. Inter-observer and intra-observer variability of pairwise plaque area (mm^2) 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 (-0.5, 0.6)	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. Reproduced with permission from M. Herder.[51]

The use of different ultrasonography equipment in the 4th + 5th and the 6th surveys, and non-standardized uptake angles is likely to have increased the measurement error between surveys. The inter-equipment variability between GE Vivid 7 and Acuson XP10 was tested in 79 subjects, of whom 38 had ≥1 plaques. All subjects were examined with Acuson XP10 first. To minimize the influence of sonographer and reader variability, all examinations were performed by the same sonographer. Readings of TPA were done by another person, blinded to the identity of the participants. For TPA, the mean absolute difference was 6.5 mm^2 and the mean arithmetic difference 2.4 mm^2 (95% CI, −0.5, 5.4), indicating no systematic differences between machines. The coefficient of variation was 26.4% and the correlation coefficient 0.89. In the analyses, we used square root-transformed TPA values, for which the mean arithmetic difference was 0.2

(95% CI, −0.06, 0.50), the mean absolute difference 0.68, the coefficient of variation 13.2%, and limits of agreement ±1.7.[31]

In Paper I, the impact of imprecision in measurements on plaque progression is partly diminished by the large sample size. In Paper II, we defined three categories of plaque; TPA below and above the median, while subjects with no plaque constituted the reference category. We aimed to reduce the effect of measurement errors related to change of equipment and sonographers by defining TPA medians separately at each survey for men and women. In order to decrease the impact of imprecision in the plaque measurements on our estimates in Paper III, we used the mean absolute difference between two observers as a measure of the typical magnitude of the measurement error in observations. Accordingly, stable plaque was defined as change in TPA of less than ±2.9 mm^2 (Table 1).[74] To reduce the risk of misclassification in the plaque progression group, we included subjects with the largest TPA progression. Thus, median increase of TPA between the 4^{th} and 5^{th} surveys was 38.6 mm^2 (range 29.8, 124.5) in the plaque progression group, and 0.30 mm^2 (range -2.86, 2.87) in the stable plaque group. Other choice of cutoffs would probably have affected the risk estimates. The ideal situation would have been to assess the associations between circulating biomarkers and change in TPA in all participants of both Tromsø 4 and 5, but this was not possible due to limited resources.

A limitation of our study is that our ultrasound protocol only included examination of the right carotid artery, whereas plaques in the left carotid artery were not acknowledged. Previous studies have reported on the symmetry in distribution and composition of carotid plaques.[107, 108] Total wall volumes of the left and right carotid arteries were found to correlate with concordance correlation coefficient of 0.71.[108] Although most individuals had bilateral carotid disease, unilateral plaque was more often found to be located in the left carotid artery, and left-sided plaques were thicker than plaques on the contralateral side.[107]

In the conduct of the study, the reproducibility of the ultrasound measures could possibly have been improved by the use of standardized uptake angles, more intensive training, and use of fewer sonographers. However, the latter was hard to achieve due to large examination volumes and long time span of the cohort study.

Clinical endpoint ascertainment

To ensure accurate classification of endpoints IS and MI in Paper III, several steps were taken (details outlined in section 3.5). The loss to follow-up was negligible due to usage of the unique personal identity number to search official health registries. One single hospital provides all hospital care in the region, which facilitates the completeness of our outcome registries. Nonetheless, some IS and MI remained undoubtedly unidentified. Case identification was retrospective, and some non-hospitalized, non-fatal cases may not have been identified. Reasons for this are typically sparse or atypical symptomatology or old age leading to non-referral and non-detection. Improvements in radiological imaging and implementation of CT and MRI modalities may have increased sensitivity in detection of small ischemic lesions during the course of the study. Our definition of IS was based on clinical symptoms and exclusion of hemorrhage. However, improved treatment options leading to more rigorous case seeking behavior among clinicians with lower threshold for referral and increased public awareness, may have led to higher detection rates at the end compared to the beginning of the study. Regarding MI, the biomarkers used for diagnoses changed during the course of the study and from year 2000 troponins, which are more sensitive, were included and enabled the detection of smaller amounts of cardiac necrosis.[76] Women tend to have more atypical symptoms and a higher prevalence of unrecognized silent MIs.[109] An increased awareness of heart disease in general, and in women particularly, in recent years may have led to a higher detection rate at the end of the study.[110] The outcome identification biases described above are suspected to be non-differential and may have attenuated the association estimates.

5.1.2.3 **Confounding and interaction**

A confounding variable (confounder) is a factor, which distorts the true association between exposure and outcome, as it may influence both the magnitude and direction of the association. The confounding variable is associated with both the outcome and the exposure, but not affected by either the exposure or outcome. It accounts for some of the observed association between the exposure and outcome.[92] The TRFs, i.e. age, smoking, total cholesterol, HDL-C, systolic blood pressure, diabetes, BMI, and use of antihypertensive drugs and lipid lowering drugs, were considered potential confounders as previous research have indicated an association with CVD, atherosclerotic disease,[31, 41, 111, 112] and inflammatory markers.[26, 27] In our papers, levels of these TRFs varied across CRP categories (Paper I), plaque categories (Paper III) and in subjects with incident MI or IS compared to event-free participants (Paper II), supporting their role as potential

confounders. A confounder can be statistically adjusted for by including it in a multivariable analysis together with the exposure variable under study. The idea behind adjustment, is to use a statistical model to estimate what the association between exposure and outcome would be at a constant level of the suspected confounding variables. Whether a TRF acts as a confounder may be evaluated by different strategies. The "significance test of the covariate" strategy relies on the confounder being revealed by the significance level of each TRF's respective regression coefficient in multivariate analyses.[92] However, this method may be inaccurate because the p-value of the covariate is solely a reflection of the association between the confounder and the outcome. More commonly used is the "change-in-estimate" strategy, in which confounders are defined as variables that alter the unadjusted exposure-outcome effect by a certain percentage. A cut-off of 10% is regularly cited in the literature.[90] This strategy has been claimed to be more accurate as it accounts for both covariate-outcome and covariate-exposure association. In Paper I, we evaluated the impact each TRF had on the association between CRP and carotid atherosclerosis, by singly including each TRF in the age-adjusted models and observing the change in regression coefficients. In Paper II, we evaluated CRP as a confounder in the association between carotid TPA and CVD events (IS and MI).

In general, a potential confounder should not be an intermediate step in the causal pathway between the suspected risk factor and the outcome. It is considered inappropriate to adjust for an intermediate cause or a mechanistic link. Exceptions to this rule occur when the investigator intentionally explores alternative mechanisms that could explain the association between the exposure and outcome of interest.[92] If CRP and atherosclerosis represent intermediate steps in the pathway between TRFs and clinical CVD events, it would not be suitable to adjust for CRP or atherosclerosis when examining the association between smoking and MI. On the other hand, if smoking influences both CRP level and risk for MI, smoking should be adjusted for when examining the association between CRP and MI. Independent of their status as potential confounders, adjustment for TRFs is grounded when the research question is: "Do novel biomarkers add incremental value to prediction of plaque formation and progression beyond information obtained from TRFs?" Nevertheless, if a residual association between inflammatory markers and atherosclerosis progression exists after adjustment for TRFs, this does not necessarily mean that a true association or a causal pathway exists. Residual confounding may explain the association. Controlling for imperfectly measured blood pressure or an incorrectly categorized smoking variable may lead to incomplete adjustment and residual confounding. In

addition, unknown confounders that have not been accounted for may be present (e.g., other inflammatory markers) and some known potential confounders may not have been included in the analyses due to missing information (e.g., periodontitis, previous infections) or uncertainty related to these data (e.g., alcohol consumption and physical activity). In addition, any observed observation may occur merely by chance.

Whether to adjust for pre-baseline CVD was considered for Paper I and III. The risk of CVD is greater in individuals with a history of previous CVD, and plaques in the carotid artery serves as a surrogate endpoint of CVD. Over-adjustment may occur when adjustment is unintentionally carried out for a variable that is in the causal pathway between the exposure and outcome, or so strongly related to either the exposure or the outcome that the true relationship is distorted. Over-adjustment may occur when different variables representing overlapping constructs are simultaneously adjusted for, and their collinearity would cause the corresponding regression coefficients to be meaningless. Diastolic blood pressure was highly inter-correlated (Pearsons r=0.77) with systolic blood pressure and therefore not included in analyses due to issues of multicollinearity. Over-adjustment can obscure a true effect or create an apparent effect when none exists.[92] Instead of adjusting for CVD, we assessed the effect of inflammatory markers on plaque development separately in subjects without former CVD in sensitivity analyses (Paper III). This implicates that the effects of biomarkers are assessed in a primary prevention setting.

The population under study may be stratified according to certain risk factors to examine interaction (effect modification), i.e., whether the exposure variable has varying effects at different levels of another variable. By stratifying according to sex, we could examine whether the effect of CRP on plaque development or CVD events was different in men and women (Paper I+II). In Paper II, we examined whether CRP predicted IS and MI differently in the presence and absence of plaque. If interaction is present, crude risk estimates differ between strata. We also assessed the interaction by adding an interaction term to the model (CRP x TPA). In comparison with stratified analysis, assessing interaction by the use of interaction-terms in multivariable-adjusted models, increase the statistical efficiency and also allows for evaluation of interaction between continuous variables.[92]

Several studies indicate that the joint presence of several novel biomarkers increase the predictive value in assessment of cardiovascular risk ("multimarker approach").[85] A combination of non-invasive tests have been shown to improve their prognostic accuracy compared to the use

of single tests alone.[113] This strategy is already in use when examining the presence of multiple TRFs.[92] The within-individual variability in level of inflammatory markers may at least partially be rectified by combining more than one inflammatory index for prediction. In this regard, additive interaction is of interest. It is difficult to evaluate additive interaction in regression analyses, but stratified analyses can be done to evaluate this. In Paper II, we assessed the additive interaction of TPA and CRP on risk of IS and MI. In Paper III, we assessed whether simultaneously raised levels of multiple circulating biomarkers, which were individually associated with plaque progression, altered the OR for being in the plaque progression group.

5.1.3 **Temporal changes in variables**

In cohorts with long follow-up, temporary fluctuations in modifiable risk factors (CRP, TPA and TRFs) over time may result in underestimation of the true association between exposure and outcome (regression dilution bias).[114] An approach to minimize the impact of such bias is to perform analyses with time-varying covariates, and such methods were applied in Papers I and II. In Paper II, the first time-point that a participant could enter the study was in 1994, and they were followed for a maximum of 19 years until end of follow-up December 31, 2013. In the Tromsø Study, information on how risk factors change within an individual during the course of the study is often available due to multiple measurements on the same individual at different surveys. Therefore, we chose to update risk factor information in analyses. For instance, the prevalence of smokers decreased dramatically during the course of the study. In a time-fixed model, using only information from the time of study entrance, those who stopped smoking during follow-up would be misclassified as smokers during the remaining follow-up. If these subjects due to smoking cessation in fact had a lower risk of MI, the association between smoking and MI would be diluted.[115]

In Paper III, we used risk factors measured at baseline as exposure variables because the aim was to explore the predictive ability of these markers. Repeated biomarker samplings could have enabled monitoring the change in biomarker level over time in relation to outcome. A more frequent assessment of risk factors in general may have reduced measurement errors and reflected true exposure levels over time.[51]

When modeling associations of change in a continuous variable, such as TPA, which has been measured on several occasions, the phenomenon of regression to the mean should be considered. Extreme values at one measurement point will tend to reverse towards a less extreme value at

subsequent measurements. This variation may be caused by random measurement error or random fluctuations in a subject, and represents an alternative explanation for change-scores in non-randomized studies such as cohort studies. Within an individual, extreme values are likely to be followed by less extreme values, closer to the subjects' true mean. Within groups, regression to the mean is important to recognize especially, when comparisons are done in groups that are categorized on the basis of initial values.[116] Many authors recommend adjustment for baseline values in all prospective studies of change to avoid the effect of any random differences in the initial levels across the groups that are being compared.[116] However, inclusion of the measured baseline as a covariate can also result in biased estimates, exacerbated by measurement error.[79] In Paper I, measured baseline TPA was not included when modeling TPA progression. In the linear mixed model, the cross-sectional and prospective associations between exposures and TPA were jointly modeled. Baseline TPA was accounted for by the cross-sectional term, estimating the baseline TPA using both fixed and random effects. Adjusting for an estimated baseline, allows us to control for cross-sectional confounding without inducing bias.[79]

5.1.4 **Missing data**

Missing data occurs in nearly all epidemiological studies. There are several reasons for this, including inadequate response to questionnaires, equipment failure, and loss or errors in laboratory handling of samples. Three categories of missing mechanisms have been proposed; i) missing completely at random, where the probability of missingness is unrelated to both observed and unobserved data, ii) missing at random, where the probability of missingness is conditional on the observed data, and iii) missing not at random, where the probability of missing depends on unobserved data.[89]

In Paper I, we used complete case analyses, losing 224 individuals (3.3%) of the original Tromsø 4 population due to missing values in one or more variables. Complete case analyses assume that data is missing completely at random. As the percentage of individuals with missing data was small, we believe that their exclusion does not influence the results substantially. In Paper II, we carried forward observations when applicable. This allowed us to include observations from subjects who had missing data for one or more variables in subsequent surveys if they had observations for these variables in a previous survey.

In Paper III, there were up to 11% of participants who had missing information for one or more biomarkers. As these missing values were assumed to be mostly related to equipment failure,

loss or errors in laboratory handling of samples, we did not suspect this to bias the study sample. The subjects with missing values were similar to subjects with complete values on all observations with regard to relevant exposure and outcome measures. However, we performed multiple imputation to gain more power in the statistical analyses. In the regression model, where all biomarkers which significantly differed across plaque groups were included together, the percentage of missing values was greatest (15%). Thus, we assumed data to be missing at random, and used observed data to impute 20 data sets.

5.1.5 **Statistical considerations**

We aimed to utilize all acquired longitudinal data, including repeated measurements on the same individuals by updating values of exposure, outcome and confounding variables in the case of subsequent study attendance. However, when data are collected multiple times from the same individual, these observations are not independent of each other and this must be accounted for in the analyses.[77] In this regard, generalized estimating equations and linear mixed models are applicable statistical techniques. (Paper I)

In Paper II, we chose to use age as time scale in Cox models with time varying covariates. Time-on-study as time scale may introduce bias if the covariates included are associated with age and especially in the setting of time-varying covariates.[80] When age is used as time-scale, the risk of outcome is compared between subjects at the same age, instead of the same follow-up time, ensuring a more effective adjustment for age.[80]

Sensitivity analyses may be used to obtain a range of "corrected estimates" under different assumptions about the levels of misclassification.[92] CRP rises in cases of acute infections and inflammation. In Paper I, we aimed to ensure that our results were not confounded by former history of CVD and temporary acute inflammation. Therefore, we repeated the analyses with exclusion of subjects with self-reported former CVD (n=545) and observations of CRP >10 mg/L (n=668). Prospective analyses with TPA as outcome measure were also repeated including only subjects with prevalent plaque at baseline. To evaluate associations between CRP and TPA progression in subjects where change could truly be evaluated, analyses were also rerun only on subjects who attended all three surveys.

In Paper II, sensitivity analyses were performed by regular Cox models with time-fixed covariates, using values of exposure and confounder information at time of study entrance, and each individual contributing data only once.

In Paper III, analyses were carried out for the whole study sample, and separately for subjects without former history of CVD to assess the predictive value of biomarkers in a primary prevention setting. In addition, complete case analyses were compared to results from imputed data sets.

With a retention p-value of 0.05, there is a 5% probability of making type I errors (detecting false positive associations). When the number of statistical tests performed simultaneously is increased, the chance of type I error will increase. Various methods are used to correct hypothesis-testing procedures under these circumstances. The popular Bonferroni correction is based on the concept of familywise error rate, which is the probability of making one or more type I errors in all the hypotheses tests conducted. The retention p-value is down-regulated accordingly for each conducted hypothesis test. For example, if 1000 proteins were to be tested, we would test each protein at a significance level of 0.00005. This is a conservative method with the cost of a loss in statistical power, which may lead to missed findings. False-discovery rate (FDR) methods, which control the proportion of events reported as significant that are actually false positives, is probably a more appropriate method to correct for multiple testing. While the Bonferroni false positive rate of 0.05 means that 5% of all results will be truly negative, the FDR value of 0.05 means that 5% of declared positive results are truly negative. If many p-values fall into the range where the null hypothesis of no association should be rejected, the FDR is much less conservative.[83] It thus adjusts for the actual p-value distribution of the data, and balances type II (cases for which the null hypotheses is false, but our decision rule does not yield a significant result) vs. type I error. The risk of type II error increases with the number of variables included in the regression models, as the degrees of freedom, and thus statistical power decreases.[92]

Novel risk markers should be evaluated, not only on their individual predictive abilities, but also on the predictive value added beyond established predictors. Difference in the area under the receiver operating characteristic curve (AUC) is a common method to compare two models. AUC summarizes how well the model separates subjects who did and did not experience an event. It quantifies a tradeoff between the benefit of a model (true positive or sensitivity) vs. its

costs (false positive or 1-specificity). AUC is calculated by comparing the estimated probability of all possible pairs in a dataset between individuals experiencing an event and those not experiencing an event. If the individual experiencing an event has a higher predicted probability, that pair would be labeled 'concordant' and assigned a value of 1. Conversely, if the individual experiencing an event has a lower probability, the pair would be labeled 'discordant' and assigned a value of 0. AUC or C-statistic will be the average of all pairs and ranges from 0.5 (no discrimination) to 1 (perfect discrimination).[68] Measures of discrimination such as the C-statistic, are not able to detect small improvements in model performance, if a marker is added to a model that already includes important predictors. NRI quantifies to which extent a model which includes the new predictor, improves the classification in clinically meaningful predefined risk categories for participants with and without the outcome, compared to the baseline model with established predictors.[68] NRI is the sum of NRI for cases plus NRI for non-cases. NRI for cases is the percentage of cases correctly classified upwards minus the percentage of cases erroneously classified downward by the new model, compared to the baseline model. NRI for non-cases is the percentage of non-cases correctly classified downwards minus the percentage of non-cases erroneously classified upwards by the new model.[68] Definitions of these risk categories are however arbitrary and differs across studies complicating comparisons. To circumvent this problem, the category-free continuous NRI or IDI may be evaluated. IDI does not require predefined risk thresholds. IDI represents the estimated improvement in the average sensitivity, minus estimated decrease in average specificity summarized over all possible thresholds of the model with the added predictor, compared to the baseline model. The absolute IDI depends on the event rate observed in a given data, whereas the relative IDI is a percentage which may be compared across studies.[68]

5.2 External validity

External validity refers to the ability to generalize results from our study to other populations. Ensuring internal validity is necessary for external validity. Random errors have less impact in large samples. Misclassification of exposure variables in longitudinal studies is usually not a substantial problem, since they will be non-differential and will in general underestimate the true association.[92] The Norwegian Population Registry was the source for the invitations to the Tromsø Study. The age and sex distribution and risk factor levels in the Tromsø Study is not substantially different from the Norwegian population, and comparable to other Western populations. However, the subjects invited to the second visit, were on average older. The

Tromsø population consists mainly of Caucasians and extrapolation to populations of other ethnicities may be limited. Hence, our findings should be applicable to similar middle-aged European populations.

5.3 Ethical considerations

In the information brochure given to individuals upon invitation, the dual aim of the Tromsø Study is presented (Appendix 1). On the individual level, the aim is to identify individuals who either suffer from CVD or are at high risk of developing CVD without knowing it. Identification of these individuals is important in order to initiate appropriate preventive treatment strategies. On the public research level, the aim is to gain new knowledge about the occurrence of diseases (cardiovascular, cancer etc.), the risk factors for diseases and how these diseases can be prevented.

Chapter 8 in The Norwegian Health Research Act deals with transparency and the right of access to research. It states that "research participants have the right of access to person- identifiable and pseudonym personal health data about themselves. The data, that access is granted to, must be presented in a way that is adapted to the capabilities and the needs of the individual".[117] If consented to, information about certain selected individual results in the Tromsø Study were passed on to the general practitioner for further follow-up. These results included blood pressure, height, weight, HDL-C, and total cholesterol. Lifestyle cohort research studies are "non-interventional". The sample has been randomly selected to be representative for the population under study. However, with the feedback of individual findings, the sample may receive a more aggressive preventive approach with respect to lifestyle and medications because of being part of the study, more than what is expected for the general population. This may introduce bias and jeopardize the validity of the study. Observer bias (interviewer bias) is introduced if the interviewer treats cases and controls differently, and this could result in differential misclassification. This problem has been addressed in the Tromsø Study in general, by having trained test personnel that are not directly involved in the research and therefore not biased by scientific hypotheses in their measurements. Standard protocols and standard informational procedures contribute to minimizing errors. The test personnel were masked to the ultrasound findings in previous surveys. However, when study participants were informed about the presence of plaques in the their carotid artery, this might have increased their motivation for lifestyle changes and influenced the initiation of preventive treatments,[118, 119] introducing biased estimates of the association between baseline level of exposure variables and TPA progression.

In longitudinal studies, researchers must balance the crucial need to maintain a representative sample population with the responsibility to offer health advice. Also, the feedback of results may raise unnecessary health concerns among the participants. When considered to be ethically tenable, bias related to influencing on the cohort's health development may partly be prevented by restricting the feedback of results from the surveys.

5.4 Discussion of main results

5.4.1 C-reactive protein in atherosclerosis - A risk marker but not a causal factor?

In Paper I, we report cross-sectional associations between CRP and prevalent plaque and between CRP and TPA, which were stronger in men than in women and independent of TRFs. In prospective analyses, age-adjusted baseline CRP predicted TPA progression and novel plaque formation in men, but not in women. When adjusted for TRFs, baseline CRP did not predict novel plaque formation nor TPA progression in neither men nor women.

The role of CRP in atherosclerosis has been debated continuously during the last decades. Is it a causal factor or an epiphenomenon to the atherosclerotic process? CRP has shown ability to predict CVD in a meta-analysis comprising individual participant records from 54 long-term prospective studies.[27] In small case-control trials, CRP was associated with the presence of carotid artery stenosis.[120, 121] CRP was also cross-sectionally associated with IMT in a meta-analysis of individual participant data from 20 prospective cohort studies (PROG-IMT) involving 49 097 participants free of pre-existing CVD.[122] Our research group has previously reported a cross-sectional relationship between CRP and TPA in men,[123] but not all studies have confirmed an independent cross-sectional association between CRP and carotid plaque.[124, 125] The strength and consistency of cross-sectional associations differed somewhat between the statistical models applied in our study. While CRP levels have shown a dose–response relationship to CVD risk independently of TRFs in prospective studies, the data on associations with extent of carotid atherosclerosis are inconclusive.[38] Schulze Horn et al.[126] found an association between CRP and IMT in 3092 middle-aged participants. However, CRP was associated with IMT to the same degree as to more advanced stages of atherosclerosis, indicating that CRP may identify vascular risk patients, but may not be suited to monitor progression of the disease.[126]

In our study, the association between CRP and carotid atherosclerosis was weaker in women than in men. The association between baseline CRP and TPA in women was attenuated to non-significant upon adjustment for TRFs. Except for a cross-sectional study on the Framingham offspring,[127] most other studies support our findings and report a stronger association between subclinical carotid atherosclerosis and CRP in men.[123, 128-130] On average, women experience their first CVD event (MI or stroke) 7-10 years later in life than men, and a protective effect of their natural estrogen status prior to menopause on vascular inflammation and atherosclerosis has

been suggested.[131, 132] Previous work from our research group and others, have also documented a lower prevalence of carotid plaque in women than in men at the same age.[31, 133] The male-to-female ratio in plaque prevalence peaks at age 45-49 years and then declines steadily.[69] A sex difference in plaque morphology has also been reported with men having a higher proportion of echolucent plaques than women throughout life.[69, 134] Echolucent plaques are associated with higher intraplaque inflammation. Calcification and inflammation may represent distinct processes within the atherosclerotic plaque, and calcification is associated with stable asymptomatic carotid disease.[135] Anti-inflammatory effects of female sex-hormones may shift the atherosclerotic process in females towards a less inflammatory, more calcifying and slower progressive development, which in turn may explain the less prominent association between CRP and plaque in women.

A few other population-based studies have reported on the prospective association between CRP and subclinical atherosclerosis.[122, 125, 136, 137] Some have found baseline CRP to predict progressive atherosclerotic disease defined as increase in plaque score and progression of stenosis.[136, 138, 139] In a study of 486 subjects, of whom 72% were 65 years or older, CRP was an independent predictor of new carotid plaques within three years.[125] The Austrian Stroke Prevention Study demonstrated a significant relationship between baseline CRP and baseline carotid atherosclerosis, as well as progression of atherosclerosis during the observational period of 6 years.[137] However, CRP did not predict progression of IMT in the PROG-IMT meta-analysis.[122] Our study did not confirm the temporality criterion of a causal relationship, as high levels of CRP did not independently predict plaque formation and progression. Differences in time span, exposure level, study design, analytic strategies and publication bias may have threatened the consistency of published results.

Whether CRP reflects a response to TRFs or rise secondarily due to inflammatory processes within the atherosclerotic plaque is not clear. CRP is linked to abdominal obesity, insulin resistance, diabetes mellitus, hypercholesterolemia, and cigarette smoking.[27, 67, 140] Abdominal adipocytes produce inflammatory cytokines, including IL-6, which is a potent messenger for CRP secretion in the liver.[140] Results from the Multi-Ethnic Study of Atherosclerosis indicated that in the absence of obesity, CRP was not associated with coronary calcium and only weakly associated with IMT, whereas obesity was related to both imaging outcomes,[141] suggesting a

complex interplay between metabolic disorders, inflammation and serum lipids in atheroma formation.

Serum levels of CRP have been associated with vulnerable plaque features detected by MRI[142], but not plaque inflammation assessed by FDG-PET[143, 144] or immune pathological analysis.[145] In addition, results regarding its associations to unstable plaque features such as echogenicity are diverging.[120, 123] Although CRP is associated with prevalent atherosclerosis beyond TRFs, these associations are weak and inconsistent. Other circulating inflammatory markers may better reflect the inflammatory process within the plaque, and thereby show higher sensitivity and specificity for the detection and monitoring of inflammatory atherosclerotic disease.[21, 37, 146]

Evidence drawn from experimental manipulation, particularly RCTs in which disease risk declines following an intervention or cessation of exposure, is the strongest support for causal inference. Data suggest that cardiovascular preventive medication, such as lipid lowering agents, angiotensin converting enzyme inhibitors, angiotensin receptor blockers, antidiabetic agents, anti-inflammatory and antiplatelet agents and beta-adrenoreceptor antagonists, lower serum levels of CRP.[147] Treatment with statins reduces both low-density lipoprotein cholesterol and CRP levels. Reduction of CRP by statins is proposed to contribute to additional CVD risk reduction benefit beyond that obtained from cholesterol lowering.[41] However, in the Heart Protection Study of 20 536 participants randomized to simvastatin 40 mg vs. placebo, baseline CRP levels did not predict benefit of therapy, and there was clear evidence of benefit in subjects with both low LDL and low CRP at baseline.[148] In the Anglo-Scandinavian Cardiac Outcomes Trial (ASCOT), CRP did not predict outcome in relation to statin treatment.[149] Mendelian randomization studies both in the Copenhagen study,[150] and in a combined study of 194 418 participants, including 46 557 patients with prevalent or incident coronary heart disease,[151] concluded that CRP gene variants associated with increased CRP levels did not lead to increased risk of ischemic atherosclerotic disease. When using anti-sense oligonucleotide targeted to CRP production and pharmaceutical grade CRP infusions, no upstream effects on systemic inflammation were observed in direct response to alterations in CRP production.[146] In addition, Mechanistic studies in mouse models did not find evidence of a causal role of CRP in atherosclerotic development.[152, 153]

All in all, our results and the current body of evidence do not support a causal role for CRP in the initiation and progression of atherosclerosis and suggest that CRP may link to CVD by other

mechanisms. Thus, our findings indicate that CRP may be considered as a risk marker, a potential tool to identify subjects with prevalent atherosclerosis, but questions its role as a therapeutic target in haltering progressive atherosclerotic disease.

5.4.2 **Joint effect of carotid plaque and CRP in determination of CVD risk.**

In Paper II, we found that both elevated serum levels of CRP >3 mg/L and carotid total plaque were individually associated with increased risk of IS and MI. For plaque area, the RR estimates in our study were stronger for women than men, concordant with previous findings from the Tromsø Study suggesting that assessment of carotid plaque may be a more important tool for risk stratification of women than men.[154] CRP is evidently linked to increased risk of CVD, yet the underlying mechanisms behind these associations are not fully understood. As discussed in Paper I, it has been suggested that inflammatory active and rupture-prone plaques may themselves be a source of CRP.[140] However, as CRP only minimally attenuated the risk in individuals with prevalent plaque, our results suggest that CRP and prevalent plaque do not represent the same underlying risk factor, i.e. unstable atherosclerotic plaques, in relation to clinical outcomes.

Novel biomarkers that may improve the identification of subjects at risk and guide preventive treatment are long awaited. Assessment of carotid plaque has been proposed as a risk modifier in subjects classified to be at intermediate risk by TRFs.[155] NRIs added by plaque-measures in CVD risk prediction have previously been reported by the Multi-Ethnic Study of Atherosclerosis (MESA), Atherosclerosis Risk in Communities Study (ARIC) and Three City Study, ranging from 7.7-13.1% for the whole population and approximately 20% for the intermediate risk groups[156-159] concurring with our findings. Some of these studies included IMT in addition to plaque measures and the studies differed somewhat in plaque assessment methods as well as definition of outcomes. Plaque in the carotid artery may be considered an end organ manifestation of genetic and environmental risk factors that serves as a proxy of generalized atherosclerosis, calling for more aggressive risk factor management. Whether carotid ultrasound assessment leads to treatment decisions, which improves outcomes and justifies the cost-effectiveness of screening is not clarified. The most recent European Guidelines on CVD prevention recommend that atherosclerotic plaque detection by carotid artery scanning may be considered as a risk modifier in CVD risk assessment for individuals with calculated CVD risks around the decisional thresholds for medical intervention based on the major TRFs. However, it is suggested that less than 10% of the population who test positive for atherosclerosis will experience a near-term event.[67] Identification of reliable imaging and serological markers of

disease activity is therefore essential to improve the selection of vulnerable patients and cost-effectiveness of screening with carotid ultrasound in the primary prevention setting.

CRP is the most extensively studied circulating biomarker in relation to CVD. In a large meta-analysis of individual participant data (n= 246 669), addition of CRP to TRFs yielded a modest significant improvement in C-index by 0.0039 ($p < 0.001$), and NRI of 1.52%.[160] For subjects classified to be at intermediate risk by TRFs, incorporation of CRP in the risk assessment model resulted in 5.2% being reclassified to a higher risk category, thus eligible for statin therapy. Adhering to current CVD prevention guidelines, this could potentially prevent one additional CVD event in 10 years from 400-500 subjects screened.[160] Controversy about the usefulness and prognostic value of CRP in CVD prediction still remains.[161, 162]

Few studies have explored whether CRPs ability to predict CVD is dependent on the presence of atherosclerosis.[48, 163-165] Experimental studies have indicated that CRP may initiate mechanisms involved in plaque rupture and thrombus formation.[166, 167] Transgenic mice that express human CRP demonstrate accelerated thrombosis after arterial injury, compared to non-transgenic control mice,[168] and administration of CRP to human beings activates the blood coagulation system.[169] These observations suggest that CRP increases the risk of CVD by activating the blood coagulation system, rather than by promoting atherosclerotic plaque progression. Thus, an interaction between higher serum levels of CRP and inflammatory active plaques may increase the risk of thromboembolic complications, and explain the attributable risk of CRP in CVD.[170] The joint presence of elevated CRP and plaques >TPA median was associated with the highest risks of both IS and MI, but synergistic effects were evident for IS only. This concurs with results from the Cardiovascular Health Study, where Cao and colleagues simultaneously measured carotid intima-media thickness, plaque characteristics, and CRP, and found that all three parameters independently predicted 12-year incidence of CVD events and mortality in 5888 elderly participants.[163] Elevated CRP was a particularly useful predictor in the presence of subclinical atherosclerosis with a 72% increase in risk for CVD and 52% increase in total mortality. By contrast, CRP did not add predictive power in the absence of carotid atherosclerosis. Cumulative event rates suggested a possible additive interaction for composite CVD and all-cause mortality with an excess risk attributable to the interaction of CRP and subclinical atherosclerosis of 54% for CVD death and 79% for all-cause mortality. Additive effects of CRP and extent of coronary artery disease on risk for future MI in angina patients have also been reported.[48] Contradictory, in the ARIC population soluble biomarkers, including CRP,

were associated with CVD events with a similar magnitude in the presence and absence of atherosclerosis, and the researchers concluded that the presence of atherosclerosis assessed by IMT and plaque did not influence the association between biomarkers and CVD.[164] However, additive interaction of these measures was not assessed in that study.[164] The significance of CRP as a risk marker may in addition differ according to plaque subtype and vulnerability, non-calcified plaques with necrotic cores is suggested to have higher levels of inflammation.[171] Park et al.[165] concluded that elevated CRP is a predictor of adverse cardiovascular events in asymptomatic self-referred middle-aged Korean patients with non-calcified coronary plaques, but not in patients with calcified or mixed plaques on coronary CT scan. In that population, the highest event rate was found in patients with non-calcified plaques and hsCRP >3 mg/L.[165] Regression dilution bias due to intra-individual changes in CRP and plaque status may play a role in prospective studies with long follow-up and may have led to bias towards the null. To our knowledge, no previous studies have assessed these additive effects using time-varying exposure variables.

As Mendelian randomization studies and animal studies have not supported a causal role of CRP in CVD, it may be more likely that CRP is a non-specific marker of inflammation that rises secondarily to up-stream processes more, directly linked to the pathogenesis of CVD.[146] However, a limitation of Mendelian randomization studies is that the power to detect meaningful gene–environment interaction is low.[172] To our knowledge, it has not been tested whether gene polymorphisms associated with increased serum levels of CRP may have different effects in determining CVD events in the presence and absence of atherosclerosis.

CRP is closely correlated with diabetes mellitus, hypercholesterolemia, and cigarette smoking.[27, 67, 140] These are all conditions that lead to a pro-thrombotic state.[67] CRP has been found to inhibit release of plasminogen activator inhibitor (PAI-1) from vascular ECs,[173, 174] and induce tissue factor expression by monocytes[175] and SMCs in vitro,[176] thereby shifting the fibrinolytic balance to promote intravascular fibrin formation. CRP is mainly found as a circulating pentamer in the circulation. When CRP binds to one of its ligands, for instance in a denaturizing oxidative environment, it dissociates in a non-reversible manner to non-soluble monomers (mCRP). Recent research suggests mCRP to be an effector; a potential regulator of signaling pathways associated with thrombosis, angiogenesis and inflammation, whereas pentameric CRP acts as a facilitator.[177] Thus, further knowledge about the binding ligands which lead to dissociation and subsequent induction of local inflammation, may unravel new promising therapeutic targets.

Simultaneous assessment of carotid atherosclerosis and CRP led to minimal, but significant improvements in risk prediction judged by C-index and categorical NRI. TRFs have well-known limitations for accurate assessment of individual cardiovascular risk.[67, 178] Thus, our results suggest that the combined assessment of subclinical atherosclerosis and CRP may improve CVD risk stratification.

5.4.3 **Interleukin-6 is a predictor of plaque progression**

In Paper III, we reported IL-6 as an independent predictor of plaque progression. MPO and caspase-1 were independent predictors of plaque progression, but these effects disappeared when excluding subjects with former CVD, suggesting an association to more advanced stages of atherosclerosis. Neopterin was found to be protective of novel plaque formation (OR 0.73, 95% CI 0.57, 0.93).

IL-6 is a master pro-inflammatory cytokine. It is produced by different cell types, including activated monocytes, macrophages, endothelial cells, adipocytes and T_H2-cells, upon induction by vasoactive peptides, ROS and other cytokines. IL-6 amplifies the inflammatory cascade by stimulating hepatic synthesis of acute phase reactants, such as CRP and fibrinogen and is also a pro-coagulant cytokine.[179] IL-6 has a variety of other functions, including activation of endothelial cells, activation of the hypothalamic-pituitary-adrenal axis, oxidation of lipoproteins and promotion of lymphocyte proliferation and differentiation.[30] IL-6 has shown ability to predict cardiovascular events in more than 25 prospective epidemiological cohort studies. According to a meta-analysis performed by the Emerging Risk Factors Collaboration, 1 SD increase in log transformed IL-6 yielded a 25% increased risk of future CVD events.[26] IL-6 was associated to IMT in a meta-analysis of 14 832 participants[180] and to severity of coronary artery calcium score in another study.[181] IL-6 has also been associated with progression of carotid artery stenosis[182] and IMT[183] in high risk populations. Compared to CRP, evidence more uniformly suggests a causal role of IL-6 in atherosclerosis. In murine experiments, exogenous administrated IL-6 enhanced the development of fatty streaks,[184] and lifetime IL-6 deficiency was associated with enhanced atherosclerotic plaque formation.[185] Mendelian randomization studies also suggest that IL-6 signaling pathways play a causal role in CVD. In two meta-analyses of polymorphism in the IL-6 signaling pathways, individuals with a variant in the IL-6 receptor that impairs IL-6 signaling had lower levels of CRP as well as a decreased risk for coronary heart disease.[186] Drawbacks when considering IL-6 as risk marker are issues related to assay-stability, short half-life, circadian and post-prandial variation, and the fact that no

clinically approved assay for IL-6 exists.[146] The picture is further complicated by the fact that IL-6 has shown ability to exert both pro- or anti-atherogenic effects depending on the environmental circumstances and whether it acts through the classic membrane IL-6 receptor or trans-signaling through the soluble receptor. Selective interference with the IL-6 trans-signaling represents a promising strategy to overcome the adverse effects observed under the treatment with anti-IL-6 receptor antibodies.[30]

There is a growing interest in caspase-1 and its effects in pyroptosis and activation of IL-1β concerning atherosclerosis and plaque destabilization. Pyroptosis is a pro-inflammatory form of cell death, uniquely dependent on caspase-1 and suspected to play an important role in plaque destabilization. Plaque cholesterol can activate the multimolecular signaling complex NLRP3 inflammasome.[17] Activation of the NLRP3 inflammasome results in caspase-1-mediated production of IL-1β and ultimately IL-6, which amplify the inflammatory cascade. This finding offers a mechanistic link between hypercholesterolemia and vascular inflammation.[18] To our knowledge, the association between caspase-1 and progressive atherosclerotic disease is not previously documented. Expression of NLRP3, caspase-1, IL-1β, and IL-18 mRNA was significantly increased in carotid artery plaque tissues obtained during endarterectomy surgery compared to normal arteries from transplant donors.[17] IL-1β secretion appeared to be an important pathway in carotid plaque tissue in a larger study of gene expression in carotid atherosclerosis.[135] Linking caspase-1 and IL-1β activation to plaque progression is especially relevant concerning the recently published Canakinumab Anti-inflammatory Thrombosis Outcomes Study (CANTOS).[187] In CANTOS, canakinumab, a monoclonal antibody against IL-1β, was tested for secondary prophylaxis in 10 061 post myocardial infarction patients with elevated levels of CRP. Results suggested a significant 15% lower rate of recurrent cardiovascular events in the group using canakinumab at a dose of 150 mg every 3 months compared to placebo at 48 months. These results thus encourage further research on the detection and testing of anti-inflammatory therapies targeted to the central inflammatory signaling (IL-1 – TNFα – IL-6) pathway for CVD prevention.[187]

MPO is an enzyme secreted by activated macrophages, and is linked to both oxidative stress and inflammation. MPO may reduce the bioavailability of NO, resulting in endothelial dysfunction, in particular endothelium dependent vasorelaxation.[188] MPO is involved in the oxidation process of LDL, promoting foam cell formation in the vascular wall.[189] Finally, MPO may play a role in plaque destabilization by activating metalloproteinases, thereby weakening the fibrous cap[188] and

may thus be involved at all stages of atherosclerosis from initiation to plaque rupture.[190] MPO has been associated to subclinical atherosclerosis,[191] stenosis progression[188], plaque inflammation[143], and increased risk of CVD events, and may show additive effect to subclinical atherosclerosis in CVD risk determination.[191]

Our results regarding neopterin contradict the findings from other studies.[192, 193] However, a recent paper support anti-inflammatory and anti-atherosclerotic properties of neopterin by in vitro and in vivo experiments. The authors suggest that neopterin increases in circulating blood in patients with coronary artery disease to counter inflammation and atherosclerosis.[194]

As many of the markers are inter-correlated and probably reflects aspects of the same biological processes, the simultaneous assessment of multiple markers may increase sensitivity and specificity of unstable atherosclerosis. Our results suggest that IL-6, caspase-1 and MPO should be considered promising candidates in future studies.

6 Conclusions and implications for future research

Imaging of subclinical atherosclerosis and circulating biomarkers of inflammation provide promising strategies for improving our ability to identify individuals at increased risk of CVD, and to guide and evaluate interventions.

Our findings did not support a causal role of CRP in the formation and progression of atherosclerosis, but suggested CRP to be a marker of prevalent atherosclerosis. The joint presence of carotid atherosclerosis and CRP was associated with the highest risk of both IS and MI, suggesting that the combined assessment of these measures may improve clinical risk prediction.

The novelty of CRP as a risk marker of CVD is limited. However, the mechanistic way by which CRP relates to CVD is still not fully understood. In addition to animal studies, gene expression studies and Mendelian randomization studies, our approach studying the associations between potential biomarkers and different stages of disease development (subclinical and clinical) is useful for improving our understanding of mechanistic links.

When deciding to use certain biomarkers in CVD risk assessment, it is important that such markers influence on treatment decisions which subsequently lead to reductions in the risk for clinical events and improve quality of life for patients. Regarding CRP and plaque assessment in the carotid arteries, future research should aim to document the cost-effectiveness of screening. If therapies, which lead to plaque regression and lower levels of inflammatory markers, indeed decreases the risk of CVD events, this will further support the use of these biomarkers as surrogate endpoints and in individual monitoring of treatment effects. This may again benefit studies aimed at evaluating the effect of new preventive treatments, compared to large, protracted and costly studies based on reducing CVD events.

Future research should also aim to establish a molecular signature for unstable atherosclerosis that improves CVD risk prediction at the individual level. IL-6, MPO and caspase-1 represent promising markers in this regard. As many of the inflammatory biomarkers are inter-correlated and may exert both pro-inflammatory and anti-inflammatory effects, statistical methods which may elucidate complex patterns and co-variances between multiple markers is warranted.

Still a great amount of clinical CVD events cannot be prevented by available drug therapies, including statins.[195] The ultimate test of the inflammatory hypothesis in atherosclerosis relies on anti-inflammatory targeted drug trials. So far, two large randomized controlled trials of post myocardial infarction patients have followed this approach, the CANTOS trial[187] and the ongoing Cardiovascular Inflammation Reduction Trial (CIRT).[72] Better knowledge of the cellular and molecular mechanisms involved in atherosclerosis holds promise to unravel new risk markers and therapeutic targets for CVD in the future.

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Errata

Paper I

Typing error in the abstract: β = 0.0.029 (CI, 0.003-0.056) should be β = 0.029 (95% CI, 0.003-0.056).

In Supplemental Table 1; For Tromsø 6; n should be =2838, not 2828.

Paper III

In the "Materials and Methods" section *2.1. Subjects*: it was erroneously stated that the 5th survey was performed in 2000/2001. It should read 2001/2002. Almost all examinations were performed in 2001, whereas a few were done in January 2002.

An error had also occurred in the methods section on page 3, second paragraph (*2.4. Ultrasonography*). The paragraph is now printed correctly below.

Progression of plaque was defined as an increase in TPA above the mean absolute difference (2.9 mm^2) between 2 independent measurements performed by 2 independent sonographers, as a measure of the typical magnitude of the measurement error. Stable plaque size was defined as change in TPA of less than ±2.9 mm^2. To reduce risk of misclassification in the plaque progression group, we included subjects with the largest TPA progression.

An erratum will be submitted to *Atherosclerosis* regarding the errors in Paper III.

Paper I

Paper II

Paper III

Appendix I

Letter of Invitation to The Tromsø Study

4th, 5th and 6th surveys

You are invited to the large health survey in the municipality of Tromsø 1994 - 95

We will reach everyone

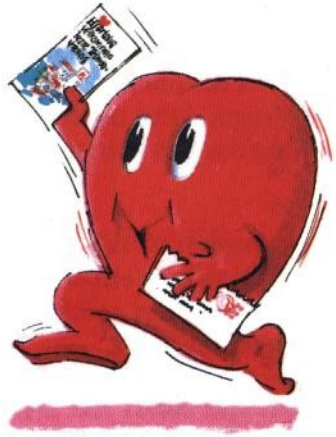
We will start in the outskirts of the municipality. Here, the examination will take place in schools and other premises – see the information in the invitation accompanying this letter.
From late October 1994 until summer 1995, the examination will take place in Mellomveien 50 (the Elisabeth centre; the old maternity hospital). We prefer that you attend at the location specified in the invitation letter.

Why did you receive this offer?

Because we offer this examination to everyone born 1969 or earlier.

What is the purpose?

The survey is first and foremost aimed at cardiovascular diseases, but is also important to gather new knowledge about other serious chronic diseases (amongst them cancer).
This time we will also study musculo-skeletal pain conditions, for instance fibromyalgia. Therefore, some people will be invited to a separate examination in the fall of 1995.
Large cardiovascular surveys were carried out in Tromsø in 1974, 1979-80, and 1986-87. The attendance rate was high, and several cases of cardiovascular disease were detected – who are now being treated.
The surveys have also contributed with important knowledge to combat these diseases. The knowledge we gained through the previous surveys, made the University of Tromsø to one of the renowned research centres in the world with regard to cardiovascular diseases. Again, we aim to detect hitherto undiscovered cardiovascular disease. We also hope to reach those at particular high risk, so that they may get the possibility of prevention and other measures to stop the development of disease. Cardiovascular diseases are still one of our largest health problems.



Not only for your own sake ...

The examination not only is important for you personally. It is also important that the results may be used in medical research, for instance by using them together with information about disease that occur in the future. Thereby we will learn more about how cardiovascular diseases, cancer, and other population diseases develop, and how they may be prevented. By attending the survey, you are helping to fight these diseases.

The examination includes

- **Measurement of height and weight**
- **Measurement of blood pressure**
- **Blood sample.** In this sample, we will measure the content of lipids (e.g. cholesterol), calcium and a liver enzyme. The result of these measurements will be forwarded to your doctor if you consent. The result of other analyses will be used for medical research only. The blood sample will be frozen to make it possible to perform other blood analyses in order to study disease development. Before such analyses are performed, the study will be presented to the Regional Ethical Committee of North Norway.

ECG is a test that registers the heart activity. We will use a simplified version, and the results will be used for research purposes only.

- **Questionnaire**
- **Special examination.** Everybody born between 1920 –1939 and a sample of the others, will be offered a more extensive examination for free. The content of the examination varies somewhat, but will provide a better examination of the heart, the aortic artery, atherosclerosis, and the tendency to osteoporosis. You will get an appointment for the examination when you attend.

Questionnaire

This you will find on the reverse side of the invitation letter. Please fill in the questionnaire beforehand and bring it to the examination site. If some questions are difficult to answer, you may get some help when you attend.

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 the Regional Ethical Committee of North Norway. For the information to be used in medical research, you have to consent. Your consent is also necessary if your doctor shall have the results of the analyses (and which you will be mailed the results of) and of your answers to the questionnaire enclosed with this letter. When attending, we therefore ask you to give your consent that:

- a letter with your results is sent to your family doctor, and will be stored in your medical record
- that your blood sample may be used for medical research. The purpose of such research is to learn about causes of diseases.
- that your results may be used for medical research, by linking that information with other health- and disease registries (for instance cancer registry and causes of death registry) and with information form the previous health surveys in Tromso. Before the information is used for analyses, your name and personal identification number will be removed. Even if you give your consent now, you may withdraw your consent later.

Invitation 4th Survey, 1st visit

Follow-up examination

Some of those who are examined may later be referred to their own doctor for a more thorough control. If you are in need of treatment, you will be offered such treatment.

What does it cost?

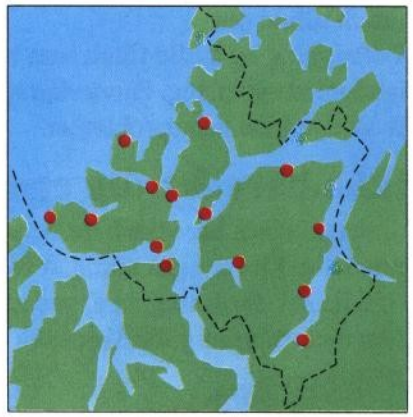
A small fee is necessary for this examination. It is very modest compared to the actual cost. You will find the amount in the letter you have received now. The special examination is free of charge. If you will need an examination by your own doctor or at the Regional hospital, you will have to pay the ordinary fee.

Clothing

Because of the blood pressure measuring, we ask you to wear clothes that are sleeveless or with short sleeves that are not tight. It is not necessary to take the clothes off.

Places that will be visited by the health survey

- Kaldfjord
- Tromvik
- Lakselvbukt
- Sjursnes
- Breivikeidet
- Fagernes
- Skittenelv
- Ersfjordbotn
- Straumsbukta
- Brensholmen
- Vikran
- Trondjord
- Sjøtun
- Tromsø sentrum



Welcome!

Sincerely

- The municipality health service
- The Faculty of medicine, University of Tromsø

Statens helseundersøkelser

(The National Health Screening Service)

Invitation 4th Survey, 1st visit

Repro & Grise A/S 360 95 955

(Heartily welcome, dear Tromsø inhabitant)

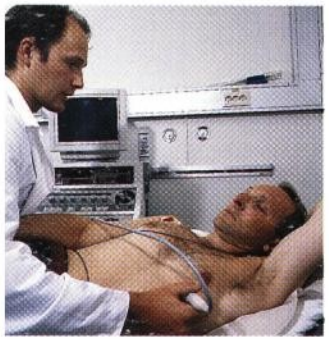
Hjertelig velkommen, kjære Tromsø-væring

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

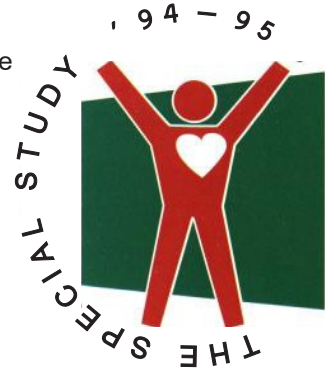
The urine samples are used to indicate kidney function through measuring the amount of protein and creatinine substances. The result is most accurate if urine from the separate days are examined.

✓ 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

Invitation 4th Survey, 2nd visit

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

Invitation 4th Survey, 2nd visit

SPESIALUNDERSØKELSEN '94 - 95

You are invited to the special study in Tromsø

LUNDBLAD GRAFISK AS, TROMSØ

Welcome to the fifth round of the Tromsø Study!

-a collaboration between:

Department of Community Medicine,
University of Tromsø
tlf: 77 64 48 16 (kl. 9 - 11) Tromsous@ism.uit.no

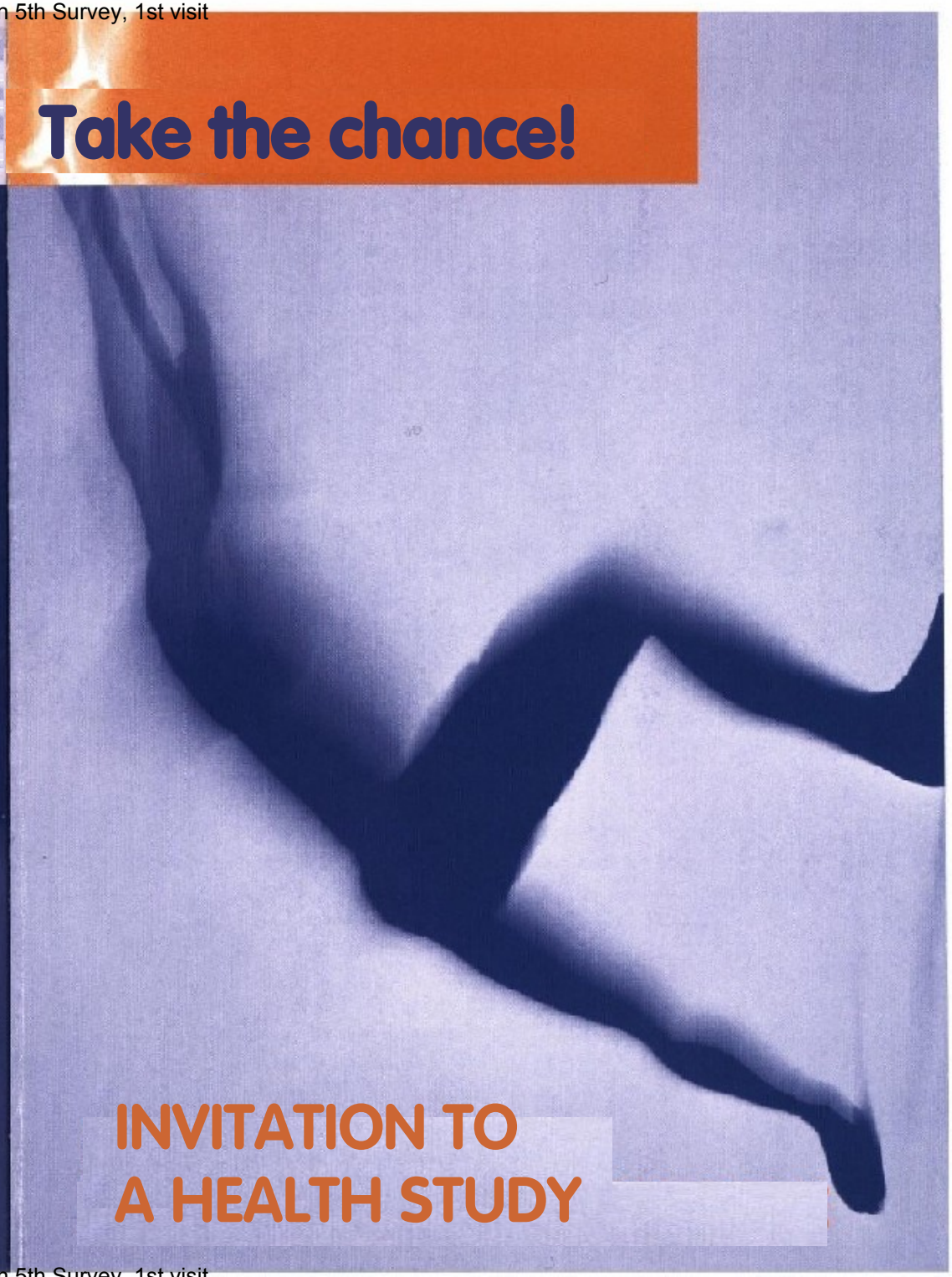
National Health Screening Service
tlf: 22 24 21 00 (kl. 9 - 15) post@shus.no

You will find more information about the health survey on the homepage of the National Health Screening Service

www.shus.no

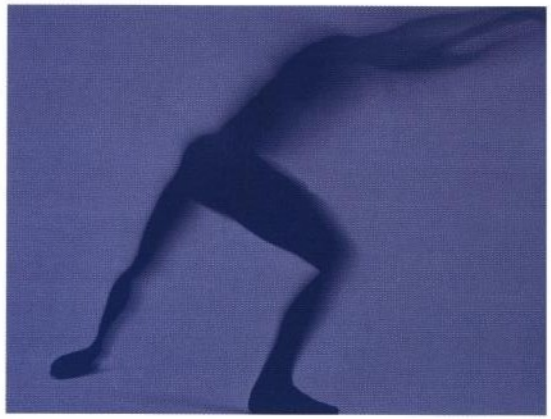
ISBN 82-91210-51-4

Take the chance!



INVITATION TO A HEALTH STUDY

Would you like to participate in the fifth survey of the Tromsø study?



Why a new round of the Tromsø study?

Large health studies were conducted in Tromsø in 1974, 1979-80, 1986-87, and 1994-95. These surveys have given us important knowledge concerning cardiovascular epidemiology and other serious diseases, such as cancer.

The main purpose of another Tromsø study is to monitor any changes in the health of the population since last survey. We will analyze the information we have about a person, both personal data and results from analysis of frozen blood, and see if there are relationships to diseases that occur. This way we learn more on how cardiovascular diseases, cancer and other major diseases develop and how they can be prevented.

Why are we asking you to participate?

We ask everyone who participated in the Special Study in the Tromsø study in 1994-95 and a selection of others older than 29 years.

Where are you going to meet?

The survey will for the vast majority take place in Elizabeth Center in Tromsø. For some of the outer places in the municipality, the survey will take place locally. Those concerned are notified in this letter.

On the front page of the questionnaire that you receive with this letter are the opening hours for the health survey and when you have to attend the survey. If you cannot attend at that time, you are welcome any another time during the opening hours of the survey. There is no need to tell us about this – just show up when we are open.

What does the study include?

The Tromsø study is first and foremost a research project. Through following up as many as possible from the study of 1994-95, we gain valuable information of health and disease in the population of Tromsø.

Participants' general health status will be examined with regard to certain diseases and risk conditions. If you have a high risk of developing cardiovascular diseases you will be notified of this.

On the day of the examination you will be guided through the survey and there will be an opportunity to ask questions. Your height, weight and waist circumference is measured, as well as blood pressure, and a blood sample is taken. Your lung capacity is determined, in addition to simple tests of vision and strength. Tests to determine osteoporosis is are also conducted.
The blood sample may later be analyzed for fatty substances, blood sugar, indicators of infections, diet, hormones, liver- and kidney function, and bone markers.

Everyone who participated in the Special Study in 1994-95 is also offered to take part in another Special Study. This study provides information on the heart and the main arteries in neck and abdomen, and offers a more detailed analysis on tendency of osteoporosis. This survey is also located at the Elisabeth-center in Tromsø. A time will be scheduled for you and information is provided upon arrival.

The Questionnaires

With this letter a questionnaire is attached. We kindly ask you to complete this form at home and bring it on the day of the examination.

If you are unsure of how to answer a question, leave it blank. You will be aided at the examination.

Everyone who participates in the study will be given an additional questionnaire of other factors which might affect your health. The questionnaire is to be completed at home and sent to the National Health Screening Service in the enclosed envelope.

Future analysis of blood

The blood which is frozen will be used for medical research only, in order to find factors influencing disease. In most cases this means that data from people with a disease is compared to data from those without it. The comparison is done on already collected data and the new analysis from the frozen blood.

We might want to analyze parts of the DNA from the frozen blood cells. Because DNA is important for the regulating and development in human being, we need knowledge on DNA to understand why diseases evolve. Analysis of this kind are only conducted after the Data Inspectorate has given a permission and if The Regional Committee for Research Ethics has no objections to the analysis.

We need your consent

When you attend the study, you will be asked to sign a consent form where you agree to the following six points:

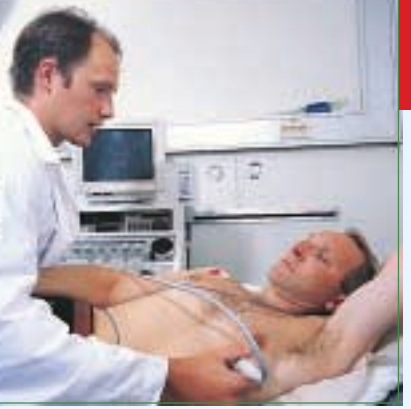
- That we may contact you with recommendations of follow ups, treatment or prevention of disease.
- That we may ask you to participate in similar studies in the future.
- That we may use the results for medical research.
- That the results, after legal approval from the Data Inspectorate, may be linked with information about you in other registries, to be used for research purposes. This might be registries including information on health, pension and disease, and also data on income, education and occupation, in addition to information from previous health studies in Tromsø. Examples of such registries are the Cancer Registry, the Cause of Death registry and population censuses. In these cases your name and social security number are removed when data is analyzed.
- That the blood sample may be stored and used for medical research. All use of this sample will only take place after approval from the Data Inspectorate and if The Regional Committees for Research Ethics has no objections.
- That the blood sample may also be used for analysis of DNA.

Even if you approve to this now, you are entitled to change your opinion later and also ask to have your profile deleted from the registry. You may also decline to consent to one or more of the points above. The Data Inspectorate has given consent to this fifth survey of the Tromsø Study, and the Regional Committee for Research Ethics has no objections. We keep your results confidential and safe. Everyone employed in the Tromsø Study has signed a confidentiality agreement.

When will you receive your results?

About four weeks after you participated in the study you will receive a letter wherein your recorded values for cholesterol, blood pressure and blood sugar are stated. You will also receive more information on the different risk factors.

People who are found to be at particularly high risk of developing cardiovascular diseases and diabetes will be recommended to seek further examination from their own doctor.



YOU ARE INVITED TO THE SPECIAL STUDY

The Special Study

The Special Study uses advanced technology which makes images of blood vessels and the heart, and provides information on fatty tissue content and skeletal structure. The latter requires x-ray equipment, although in very low doses. The three former is done with ultrasound, reflecting it against a small device held to the skin (pictured). These tests do not penetrate the skin and are not painful. The Special Study also involves blood-, urine-, and respiratory tests, as well as registering heart activity (EKG). Moreover, basic memory tests and word recognition tests are conducted, as well as the degree of finger mobility.

Why are we asking you?

We invite everyone who participated in the Special Study in 1994-95 to take part now.

What is the purpose?

Many diseases evolve over a long period of time, 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. We are especially interested in studying the changes since 1994-95 and their implication towards disease developing later. This way we hope to increase knowledge on 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. We then see if the arteries are clogged or if 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 determine risk of osteoporosis and fractures, and if there is a correlation between body fat and disease.

ECG and blood pressure

- ECG is registering heart activity which provides information on heart diseases. An ECG is done by attaching sensors to arms, legs and chest. Blood pressure is checked on both the upper arm and the ankle.

Respiratory test

- Through breathing into a machine, lung function is determined.

Blood sample

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

Further follow up

- If more examinations or treatment are required it will be offered to you.
- Some participants may be asked to take part in later studies for further research.

Invitation 5th Survey, 2nd visit

PRACTICAL INFORMATION

Place and time

The examination will take place in the 2nd floor of the Elizabeth Center - the old maternity hospital (Mellom-veien 50) on the floor below the Tromsø study. The examination takes about 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 48 16*** *or* e-mail: ***Tromsous@ism.uit.no***

Urine sample

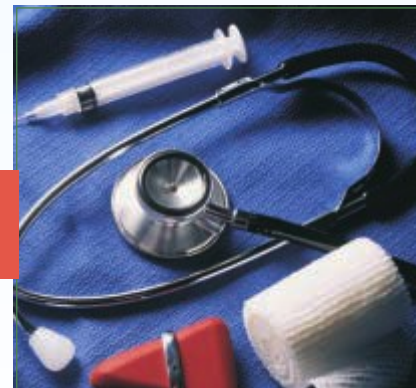
You have been given three urine glasses marked 1, 2 and 3. We wish that you take a morning urine sample in each glass in the last three days before the special study. You have therefore got a glass for every morning. Follow the instructions provided with glasses.

Fall

You are asked to register falls until the Special Study.

Clothing

Because of the blood pressure measuring and sampling of the ECG, , we ask you to wear clothes that are not tight on the arm and the leg. 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. At the examination of bone density, you will not undress yourself, but it is important not to have metal objects in the clothes. such as zipper lock, buttons, loops, or spikes of metal.



Tromsø 6
- en del av Tromsøundersøkelsen

Vil du være med i den 6. Tromsøundersøkelsen?

» viktig forskning
» undersøkelse av egen helse
» forebygging av helseproblemer

Hva er Tromsøundersøkelsen?

Tromsøundersøkelsen er et stort forskningsprosjekt. Opplysninger som samles inn skal brukes til å gi oss kunnskap som kan bedre menneskers helse.

Den første Tromsøundersøkelsen ble gjennomført allerede i 1974, og dette er den sjette i rekken. Et viktig mål med undersøkelsen er å få kunnskap om hvorfor noen blir syke mens andre beholder god helse gjennom livet.

Visste du at ..?

Den som deltar på Tromsøundersøkelsen får også en enkel undersøkelse av sin egen helse.

Hva forskes det på i Tromsøundersøkelsen?

Tromsøundersøkelsen gjennomføres først og fremst for å kunne øke kunnskapen om de store folkehelseproblemene og forhold som påvirker disse, blant annet:

- Hjerte- og karsykdommer
- Lungesykdommer (f.eks. KOLS)
- Diabetes
- Stoffskiftesykdommer
- Kreftsykdommer
- Psykiske plager
- Demens
- Muskel- og skjelettplager

Undersøkelsen vil også bli benyttet til forskning om bruk og effekter av legemidler, trivsel, livskvalitet, livsstil, døgnrytme, smerter, sosial ulikhet, fysisk aktivitet, kosthold, bruk av helsetjenester og alternativ behandling. Det vil også bli undersøkt om miljøgifter kan påvises i blodet og om disse innvirker på helsa.

Videre vil det bli gjort forskning på kvinnesykdommer, sykdommer i fordøyelsesorganer, allergi, nyrer og urinveier, nervesystemet, sanseorganer og hud. Det vil også bli forsket på arbeidsuførhet som følge av disse sykdommene eller tilstandene. En del av prosjektene vil spesielt undersøke samspillet mellom arv, miljø, sykdom og helse. Til slike prosjekter vil det bli hentet ut DNA (arvestoff) fra blodprøvene.

Det er allerede planlagt mange forskningsprosjekter som skal benytte data fra Tromsøundersøkelsen. Du vil finne en liste over disse på vår internettside:

http://www.tromso6.no

Vil du delta?

Ved å delta på Tromsøundersøkelsen er du med på å bidra til forskning om hvordan sykdom kan forebygges og behandles, hva som fremmer god helse, og hva som er årsak til helseproblemer.

Hvorfor spør vi deg?

Alle som møtte til spesialundersøkelsene i Tromsøundersøkelsen i 1994 og 2001, og et tilfeldig uttrukket utvalg av personer som er over 30 år og som er innbyggere i Tromsø kommune, blir spurt om å delta.

Alle er viktige!

Hver deltaker er like viktig, enten du er ung eller gammel, frisk eller syk. Det har vært stort frammøte til de tidligere Tromsøundersøkelsene. Godt oppmøte er viktig for gode forskningsresultater. Det er en styrke for forskningen at de som har vært med i tidligere Tromsøundersøkelser møter fram på nytt.

Frivillig

Det er frivillig å delta. Det vil ikke få noen konsekvenser for deg dersom du ikke deltar eller velger å trekke deg fra undersøkelsen på et senere tidspunkt. Du må ikke gi noen begrunnelse dersom du ønsker å trekke deg fra undersøkelsen.

Visste du at ..?

Du kan delta på Tromsøundersøkelsen selv om det er deler av undersøkelsen du ikke ønsker å være med på.

Din helse

Cirka fire uker etter undersøkelsen vil du få et brev med resultatene fra målinger av kolesterol og blodtrykk. Dersom det er nødvendig, vil du bli anbefalt å ta kontakt med din fastlege. Det blir ikke gitt rutinemessig tilbakemelding om resultater av andre blodprøver eller målinger.

Dersom resultatet av prøvene viser at det er nødvendig med oppfølging av lege eller henvisning til spesialist, vil du bli orientert om det. Ved behov for henvisning til spesialist, vi vil sørge for at slik henvisning blir sendt.

Du kan reservere deg mot å få vite resultatene av prøvene dine. Men hvis et prøveresultat er slik at det er nødvendig med rask legebehandling, vil du uansett bli kontaktet.

Tromsøundersøkelsen er gratis. Trenger du videre undersøkelse / oppfølging av fastlegen eller i spesialisthelsetjenesten, betaler du vanlig egenandel.

Slik foregår undersøkelsen

Sammen med dette informasjonsskrivet ligger det et ark med praktiske opplysninger og beskjed om hvor og når du kan møte fram. Her står også åpningstidene for undersøkelsen. Hvis du vil delta og den foreslåtte tiden ikke passer, kan du komme en annen dag. Du trenger ikke melde fra om dette på forhånd.

Unngå før undersøkelsen

For at resultatene skal bli mest mulig korrekt, er det en fordel om du avstår fra alkohol og smertestillende medisiner 12 timer før undersøkelsen.

Påkledning

Vekt og høyde, liv- og hoftevidde måles med lett påkledning, men uten sko. For at det skal gå raskt å måle blodtrykk, er det en fordel om du har plagg som ikke strammer over armen og benet. Ha gjerne et kortermet plagg innerst.

Spørreskjema

Sammen med denne brosjyren har du fått et spørreskjema som du skal fylle ut og ta med til undersøkelsen. Hvis du er i tvil om hvordan du skal svare på et eller flere av spørsmålene, lar du det stå åpent. Personalet på undersøkelsen hjelper deg da med utfyllingen om du ønsker det.

Utfylte svar i spørreskjema er like viktig for forskningen som resultater fra blodprøver og undersøkelser.

Regelmessig bruk av legemidler

Ved frammøte til undersøkelsen vil du bli intervjuet om hva slags legemidler du har brukt regelmessig de siste fire ukene, og om noen av de legemidlene du har brukt siste 24 timer. Navn på legemidler du bruker fast kan besvares i skjemaet på forhånd. Ta gjerne med deg legemidlene du bruker ved frammøte til undersøkelsen.

Undersøkelser

Når du møter fram, vil kvalifisert helsepersonell veilede deg gjennom undersøkelsen og svare på spørsmål. Du vil bli intervjuet og få utlevert et nytt spørreskjema med en frankert svarkonvolutt. Spørreskjemaet kan også besvares mens du er tilstede på undersøkelsen, og du vil kunne få hjelp underveis. Hver enkelt undersøkelse varer bare noen minutter. Totalt vil undersøkelsen vare cirka en time.

De måler høyde, vekt, hoftevidde og livvidde, de måler blodtrykket og tar blodprøve av deg. I tillegg vil følgende undersøkelser bli gjort:

- Beintetthetsmåling (måling av beinmasse) i den ene armen med svake røntgenstråler. Målingene brukes til å undersøke risiko for beinskjørhet og brudd.
- Bakterieprøve fra nese og hals fra om lag halvparten av deltagerne, for å se etter gule stafylokokker, en bakterie som normalt finnes på hud og slimhinner hos mennesker, men som i enkelte tilfeller kan forårsake alvorlige infeksjoner. Prøven gjøres med fuktet vattpensel.
- Smerteølsomhet som måler hvordan kroppen reagerer på smerte. Du blir bedt om å holde hånden i isvann i opptil 1 minutt. Underveis registreres blodtrykk og du angir hvor mye smerte du kjenner. Du kan ta hånden ut av vannet før tiden er ute hvis det blir for ubehagelig.
- Hårprøve. Vi vil be om å få noen hårstrå for å undersøke forekomsten av spormetaller som kvikksølv.
- Fysisk aktivitet og kosthold. Vi planlegger at utvalgte deltakere vil bli bedt om å registrere fysisk aktivitet (aktivitetsmålere som skritttellere og lignende) og kosthold i en periode.

Blodprøver

Blodet fordeles på fem glass, men til sammen utgjør det ikke mer enn 45 milliliter, som er mindre enn en tidel av det en blodgiver gir. For de aller fleste vil det være tilstrekkelig med ett stikk. Disse analysene blir gjort:

- Måling av kolesterol og andre fettstoffer, blodsukker, blodlegemer, stoffskifteprøver, hormoner, markører for betennelsesreaksjoner, allergi, mage- og tarmfunksjon, lever- og nyrefunksjon samt muskel- og beinmarkører.
- DNA (arvestoff) vil bli lagret til bruk i forskningsprosjekter som er omtalt i denne brosjyren og som kartlegger sammenhengen mellom arv og miljø, sykdom og helse. DNA vil ikke bli brukt til andre formål enn forskning.
- Miljøgifter, blant annet sporstoffer, spormetaller og organiske stoffer. Forekomsten i blodet skal sammenlignes med tilsvarende målinger i andre befolkninger. Forskere vil studere om miljøgifter kan påvirke helsa vår.

Spesialundersøkelsen

Når første del av Tromsøundersøkelsen er gjennomført, kan du bli forespurt om å delta i en eller flere deler av Spesialundersøkelsen noen uker senere. Over halvparten vil bli spurt om dette. Hele Spesialundersøkelsen vil vare cirka en time, og

varigheten vil være avhengig av hvor mange deler du blir spurt om å være med på. Ved oppmøte til Spesialundersøkelsen vil det bli tatt ny blodprøve som skal brukes til samme formål som beskrevet for første del av undersøkelsen. Deler av blodprøven blir frosset ned for senere bruk i forskning som er beskrevet i denne brosjyren.

Hvilke undersøkelser gjøres i Spesialundersøkelsen?

- Ultralyd av blodårene (arteriene) på halsen. Undersøkelsen gjøres for å se etter forkalkninger og innsnevringer av årene. Undersøkelsen kartlegger også blodforsyningen til hjernen.
- Ultralyd av hjertet gjøres for å undersøke hjertets form og funksjon.
- Måling av beintetthet i rygg/hofte og kroppens fettmengde. Målingene brukes til å undersøke risiko for beinskjørhet og brudd, og for studier om sammenhengen mellom kroppsfett, beinmasse og brudd.
- Fotografering av øyebunn. Fotografiet vil vise tilstanden for blodkarene i øyet som også sier noe om blodkarene i kroppen. Ved øyestasjonen tas fotografi av øyebunnen din. Deltagerne får en øyedråpe i hvert øye en tid før fotografering for at pupillene skal utvide seg. Dette kan svi noe og synet kan forbigående bli noe uklart. Effekten går gradvis over, og etter en time er den borte. I tillegg vil det gjøres en enkel synstest som du vil få svar på umiddelbart.
- Tester av hukommelse gjøres ved hjelp av enkle spørsmål og omfatter også evne til gjenkjenning av ord og grad av fingerbevegelighet.
- EKG og blodtrykk. EKG er en registrering av hjerterytmen som også kan gi informasjon om hjertesykdom. Ved registrering festes ledninger til kroppen. Blodtrykket måles både på overarmen og ved ankelen.
- Pusteprøve. Dette er en enkel undersøkelse av lungefunksjonen. Du skal puste så hardt du klarer gjennom et munnstykke. Hvor mye luft som blåses ut pr. sekund, er et mål på lungefunksjonen din.
- Ny bakterieprøve fra nese og hals. Prøven utføres på samme måte som i første del av undersøkelsen.
- Urinprøve. Du vil bli bedt om å avlevere urinprøver fra de tre siste dagene før spesialundersøkelsen. Du gis alt nødvendig utstyr. Urinen blir lagret til bruk i forskning som er beskrevet i denne brosjyren.

For å sikre høy kvalitet på forskningsdata ønsker vi å undersøke et lite utvalg som møter til undersøkelsen to ganger med circa en ukes mellomrom. De som er aktuelle vil bli forespurt om dette ved frammøte.

Nye prosjekter

Noen deltakere vil i ettertid bli spurt om å delta i videre undersøkelser. Hvis dette gjelder deg, vil du få en forespørsel i posten. Du er ikke forpliktet til å delta selv om du har deltatt i andre deler av Tromsøundersøkelsen. Omtale av alle delprosjektene finner du på nettsiden vår:

http://www.tromso6.no

Forsikring og finansiering

Deltakere i Tromsøundersøkelsen er forsikret gjennom Norsk Pasientskadeerstatning.

Tromsøundersøkelsen er finansiert av Universitetet i Tromsø, Helse Nord HF samt ulike forskningsfond.

Etikk, personvern og sikkerhet

Du kan være trygg på at informasjon som gis til Tromsøundersøkelsen vil bli behandlet med respekt for personvern og privatliv, og i samsvar med lover og forskrifter. Alle medarbeidere som jobber med undersøkelsen har taushetsplikt. Opplysningene som samles inn vil bare bli brukt til godkjente forskningsformål.

Alle opplysninger om deltakere vil bli lagret på datamaskin. Navn og personnummer blir fjernet og erstattet med en kode. Kodenøkkelen oppbevares separat og kun noen få, autoriserte medarbeidere har tilgang til denne.

Den enkelte forsker får ikke tilgang til opplysninger som gjør det mulig å identifisere enkeltpersoner. Hver enkelt deltaker har en rett til å vite hvilke opplysninger som er lagret om en selv.

For alle prosjekter kreves det at prosjektlederen tilhører en kompetent forskningsinstitusjon.

Tromsøundersøkelsen har konsesjon fra Datatilsynet og er godkjent av Regional komité for medisinsk forskningsetikk, Nord-Norge.

Sammenstilling med andre registre

Opplysninger om deg fra den sjette Tromsøundersøkelsen kan bli knyttet sammen med opplysninger fra tidligere Tromsøundersøkelser. For enkelte prosjekter kan det være aktuelt å sammenstille opplysninger om deg med opplysninger fra barn, søsken, foreldre og besteforeldre hvis disse har deltatt i Tromsøundersøkelsen.

For spesielle forskningsprosjekter kan det være aktuelt å sammenstille informasjon fra Tromsøundersøkelsen med nasjonale helseregistre som Reseptregisteret, Medisinsk fødselsregistrer, Kreftregisteret, Norsk pasientregister og Dødsårsaksregisteret, og andre nasjonale registre over sykdommer som det forskes på i Tromsøundersøkelsen.

I tillegg kan det være aktuelt å innhente helseopplysninger fra primær- og spesialisthelsetjenesten til bruk i forskning på sykdommer og helseproblemer som er nevnt i denne brosjyren, for eksempel hjerte-karsykdom, diabetes og beinbrudd. I slike tilfeller innhentes nytt samtykke, eller annen type godkjenning (dispensasjon fra taushetsplikten).

Informasjon fra Tromsøundersøkelsen kan også bli sammenstilt med registre ved Statistisk sentralbyrå, for eksempel om miljø, befolkning, utdanning, inntekt, offentlige ytelser, yrkesdeltakelse og andre forhold som kan ha betydning for helsa.

Slike sammenstillinger krever noen ganger forhåndsgodkjenning av offentlige instanser, for eksempel Regional komité for medisinsk forskningsetikk, Datatilsynet eller NAV.

Bruk av innsamlede data i framtiden

Data fra Tromsøundersøkelsen vil kun bli brukt til forskning og vil ikke kunne brukes til andre formål.

Opplysninger og prøver som du gir, blir oppbevart på ubestemt tid til bruk i forskning til formål som nevnt i denne brosjyren. I noen tilfeller kan det bli aktuelt å gjøre analyser av blodprøver ved forskningsinstitusjoner i utlandet. Hvis dette gjøres, vil det skje i en slik form at våre utenlandske samarbeidspartnere ikke kan knytte prøvene opp mot deg som person.

Hva som er aktuelle problemstillinger i medisinsk forskning forandrer seg hele tiden. I framtiden kan data bli brukt i forskningsprosjekter som i dag ikke er planlagt, forutsatt at det er i samsvar med gjeldende lover og forskrifter. For alle slike nye prosjekter kreves det at prosjektet er godkjent av Regional komité for medisinsk forskningsetikk og Datatilsynet.

Tromsøundersøkelsen informerer om nye forskningsprosjekter på: http://www.tromso6.no
Her kan du også lese om forskningsresultatene fra Tromsøundersøkelsen. Forskningsresultater vil ellers bli publisert i internasjonale og nasjonale tidsskrifter, på faglige konferanser og møter. Det vil ikke være mulig å identifisere enkeltpersoner når forskningsresultatene offentliggjøres.

Samtykke

Hvis du vil delta i den sjette Tromsøundersøkelsen, må du gi skriftlig samtykke til dette. Personalet på Tromsøundersøkelsen vil kunne gi mer informasjon om undersøkelsen, og kan svare deg dersom du har spørsmål i forbindelse med samtykket.

Det er viktig å vite at selv om du sier ja til dette nå, kan du senere ombestemme deg. Du kan når som helst etter undersøkelsen trekke ditt samtykke tilbake. Allerede innsamlede data blir lagret videre, men kan ikke lenger knyttes til deg som person, og dine data vil ikke bli brukt i nye forskningsprosjekter. Du kan be om at blodprøven din blir ødelagt.

Hvis du vil trekke tilbake ditt samtykke, henvend deg til:

Tromsøundersøkelsen, Inst. for samfunnsmedisin
Universitetet i Tromsø
9037 Tromsø
telefon: 77 64 48 16
telefaks: 77 64 48 31
e-post: tromsous@ism.uit.no
internett: www.tromso6.no

Hvis vi i framtiden ønsker å forske på nye spørsmål som ikke er beskrevet i denne brosjyren, kan det bli nødvendig å be deg om et nytt samtykke.

Vil du delta?

Følgende tekst er en kopi av dokumentet du blir bedt om å signere når du møter fram til undersøkelsen:

Samtykke til bruk av helseopplysninger i forskning - den 6. Tromsøundersøkelsen

I brosjyren jeg har fått tilsendt, har jeg lest om undersøkelsens innhold og formål, og jeg har hatt mulighet til å stille spørsmål. Jeg samtykker herved i å delta i undersøkelsen [dato/signatur].

Tromsø-
undersøkelsen

Tromsøundersøkelsen
Institutt for samfunnsmedisin, Universitetet i Tromsø
9037 TROMSØ
telefon: 77 64 48 16
telefaks: 77 64 48 31
epost: tromsous@ism.uit.no
internett: www.tromso6.no

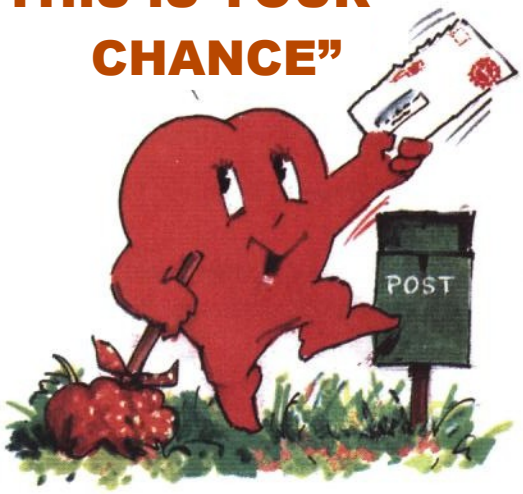
Appendix II

Questionnaires in The Tromsø Study

4th, 5th and 6th surveys

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

Tromsø Health Survey
for the over 70s

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. Finally, the survey should give knowledge about the older part of the population. We would therefore like you to answer the questions below.

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 1year?

.....24 -28

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

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

- Very good .....29 ❑ 1
- Good ..... ❑ 2
- Difficult ..... ❑ 3
- Very difficult ..... ❑ 4

How old were your parents when they died?

- Mother .....30 _____Years
- Father .....32 _____Years

HOME

Who do you live with?
Tick once for each item and give the number.

	Yes	No	Number
Spouse/partner 34	❑	❑	
Other people over 18 years 35	❑	❑	_____
People under 18 years 38	❑	❑	_____

What type of house do you live in?

- Villa/ detached house .....41 ❑ 1
- Farm ..... ❑ 2
- Flat/apartment ..... ❑ 3
- Terraced /semi-detached house ..... ❑ 4
- Other ..... ❑ 5

How long have you lived in your present home? .....42 _____years

	Yes	No
Is your home adapted to your needs? 44	❑	❑
If "No", do you have problems with:		
Living space 45	❑	❑
Variable temperature, too cold/too warm 46	❑	❑
Stairs 47	❑	❑
Toilet 48	❑	❑
Bath/shower 49	❑	❑
Maintenance 50	❑	❑
Other (please specify) 51	❑	❑

Would you like to move into a retirement home? ...52 ❑ ❑

PREVIOUS WORK AND FINANCIAL SITUATION

How will you describe the type of work you had for the last 5-10 years before you retired?

- Mostly sedentary work? .....53 ❑ 1
  (e.g. office work, mounting)
- Work that requires a lot of walking? ..... ❑ 2
  (e.g. shop assistant, housewife, teaching)
- Work that requires a lot of walking and lifting? ..... ❑ 3
  (e.g. postman, nurse, construction)
- Heavy manual work ..... ❑ 4
  (e.g. forestry, heavy farm-work, heavy construction)

Did you do any of the following jobs (full-time or part-time)?
Tick one box only for each item.

	Yes	No
Driver 54	❑	❑
Farmer 55	❑	❑
Fisherman 56	❑	❑

How old were you when you retired? .....57 _____Years

What kind of pension do you have?

- Basic state pension .....59 ❑
- An additional pension .....60 ❑

How is your current financial situation?

- Very good .....61 ❑ 1
- Good ..... ❑ 2
- Difficult ..... ❑ 3
- Very difficult ..... ❑ 4

HEALTH AND ILLNESS

Has your state of health changed in the last year?

- Yes, it has got worse .....62 ❑ 1
- No, unchanged ..... ❑ 2
- Yes, it has got better ..... ❑ 3

How do you feel your health is now compared to others of your age?

- Much worse .....63 ❑ 1
- A little worse ..... ❑ 2
- About the same ..... ❑ 3
- A little better ..... ❑ 4
- Much better ..... ❑ 5

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 64	❑	❑	_____
Wrist /forearm fracture 67	❑	❑	_____
Whiplash 70	❑	❑	_____
Injury requiring hospital admission 73	❑	❑	_____
Gastric ulcer 76	❑	❑	_____
Duodenal ulcer 79	❑	❑	_____
Gastric/duodenal ulcer surgery 82	❑	❑	_____
Neck surgery 85	❑	❑	_____

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

	Yes	No
Cancer 88	❑	❑
Epilepsy	❑	❑
Migraine	❑	❑
Parkinson's disease	❑	❑
Chronic bronchitis	❑	❑
Psoriasis 93	❑	❑
Osteoporosis	❑	❑
Fibromyalgia/fibrositis/chronic pain syndrome	❑	❑
Psychological problems for which you have sought help	❑	❑
Thyroid disease	❑	❑
Liver disease 98	❑	❑
Recurrent urinary incontinence	❑	❑
Glaucoma	❑	❑
Cataract	❑	❑
Arthrosis (osteoarthritis)	❑	❑
Rheumatoid arthritis 103	❑	❑
Kidney stones	❑	❑
Appendectomy	❑	❑
Allergy and hypersensitivity		
Atopic eczema (e.g. childhood eczema)	❑	❑
Hand eczema	❑	❑
Hey fever 108	❑	❑
Food allergy	❑	❑
Other hypersensitivity (not allergy)	❑	❑

How many times have you had a common cold, influenza (flu), diarrhoea/vomiting or similar in the last 6 months? 111 _____ times

	Yes	No
Have you had this in the last 14 days? 113	❑	❑

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 114	❑	❑	❑	❑	❑	❑
Heart attack before age 60 120	❑	❑	❑	❑	❑	❑
Cancer 126	❑	❑	❑	❑	❑	❑
Hypertension 132	❑	❑	❑	❑	❑	❑
Asthma 138	❑	❑	❑	❑	❑	❑
Osteoporosis 144	❑	❑	❑	❑	❑	❑
Arthrosis (osteoarthritis) 150	❑	❑	❑	❑	❑	❑
Psychological problems 156	❑	❑	❑	❑	❑	❑
Dementia 162	❑	❑	❑	❑	❑	❑
Diabetes 168	❑	❑	❑	❑	❑	❑
- age when they got diabetes 174	__	__	__	__	__	

SYMPTOMS

	Yes	No
Do you cough about daily for some periods of the year? 184	❑	❑
If "Yes":		
Is your cough productive? 185	❑	❑
Have you had this kind of cough for as long as 3 months in each of the last two years? 186	❑	❑
Have you had episodes with wheezing in your chest?187	❑	❑
If "Yes", has this occurred:		
Tick one box only for each item.		
At night 188	❑	❑
In connection with respiratory infections	❑	❑
In connection with physical exertion	❑	❑
In connection with very cold weather 191	❑	❑
Have you noticed sudden changes in your pulse or heart rhythm in the last year? 192	❑	❑
Have you lost weight in the last year? 193	❑	❑
If "Yes":		
How many kilograms? 194	_____ kg	

How often do you suffer from sleeplessness?

- Never, or just a few times a year .....196 ❑ 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 .....197 ❑ 1
- Especially during the polar night ..... ❑ 2
- Especially during the midnight sun season ..... ❑ 3
- Especially in spring and autumn ..... ❑ 4

	Yes	No
Do you usually take a nap during the day? 198	❑	❑
Do you feel that you usually get enough sleep?	❑	❑

Do you suffer from:	No	A little	A lot
Dizziness 200	❑	❑	❑
Poor memory	❑	❑	❑
Lack of energy	❑	❑	❑
Constipation 203	❑	❑	❑

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

- Not at all ..... 204 ☐
- Only a little ..... ☐
- Some ..... ☐
- Very much ..... ☐

BODILY FUNCTIONS

Can you manage the following everyday activities on your own without help from others?

	Yes	With some help	No
Walking indoors on one level 205	☐	☐	☐
Walking up/down stairs	☐	☐	☐
Walking outdoors	☐	☐	☐
Walking approx. 500 metres	☐	☐	☐
Going to the toilet	☐	☐	☐
Washing yourself 210	☐	☐	☐
Taking a bath/shower	☐	☐	☐
Dressing and undressing	☐	☐	☐
Getting in and out of bed	☐	☐	☐
Eating	☐	☐	☐
Cooking 215	☐	☐	☐
Doing light housework *(e.g. washing up)*	☐	☐	☐
Doing heavier housework *(e.g. cleaning floor)* ..	☐	☐	☐
Go shopping	☐	☐	☐
Take the bus	☐	☐	☐

	Yes	With difficulty	No
Can you hear normal speech (if necessary with hearing aid)? 220	☐	☐	☐
Can you read (if necessary with glasses)? 221	☐	☐	☐

Are you dependent on any of the following aids? ?

	Yes	No
Walking stick 222	☐	☐
Crutches	☐	☐
Walking frame/zimmer frame	☐	☐
Wheelchair	☐	☐
Hearing aid	☐	☐
Safety alarm device..... 227	☐	☐

USE OF HEALTH SERVICES

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

	Number of times the past year
To a general practitioner (GP)/emergency GP 228	
To a psychologist or psychiatrist	
To an other medical specialist (not at a hospital)	
To a hospital out-patient clinic 234	
Admitted to a hospital	
To a physiotherapist	
To a chiropractor 240	
To a acupuncturist	
To a dentist	
To a chiropodist 246	
To an alternative practitioner (homoeopath, foot zone therapist, etc.)	
To a healer, faith healer, clairvoyant	

Do you have home aid?	Yes	No
Private 252	☐	☐
Municipal	☐	☐

Do you receive home nursing care? ..... ☐ ☐

Are you pleased with the health care and home assistance services in the municipality?

	Yes	No	Don't know
Assigned family GP 255	☐	☐	☐
Home nursing care	☐	☐	☐
Home assistance services	☐	☐	☐

Do you feel confident that you will receive health care and home assistance services if you need it?

- Confident ..... 258 ☐ 1
- Not confident ..... ☐ 2
- Very unsure ..... ☐ 3
- Don't know ..... ☐ 4

MEDICATION AND DIETARY SUPPLEMENTS

Have you for any length of time in the last 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 ..... 259 _____ months
- Sleeping pills ..... _____ months
- Tranquillizers ..... _____ months
- Antidepressants ..... 265 _____ months
- Allergy drugs ..... _____ months
- Asthma drugs ..... _____ months
- Heart medicines (not blood pressure) ..... 271 _____ months
- Insulin ..... _____ months
- Diabetes tablets ..... _____ months
- Drugs for hypothyroidism (Thyroxine) ..... 277 _____ months
- Cortisone tablets ..... _____ months
- Remedies for constipation ..... _____ months

Dietary supplements:

- Iron tablets ..... 283 _____ months
- Vitamin D supplements ..... _____ months
- Other vitamin supplements ..... _____ months
- Calcium tablets or bone meal ..... 289 _____ months
- Cod liver oil or fish oil capsules ..... _____ months

FAMILY AND FRIENDS

	Yes	No
Do you have close relatives who can give you help and support when you need it? 293	☐	☐
If "Yes", who can give you help?		
Spouse/partner 294	☐	
Children	☐	
Others	☐	

How many good friends do you have whom you can talk confidentially with and who give you help when you need it? ..... 297 ____ good friends
Do not count people you live with, but do include other relatives!

	Yes	No
Do you feel you have enough good friends?..... 299	☐	☐

Do you feel that you belong to a community (group of people) who can depend on each other and who feel committed to each other (e.g. a political party, religious group, relatives, neighbours, work place, or organisation)?

- Strong sense of belonging ..... 300 ☐ 1
- Some sense of belonging ..... ☐ 2
- Not sure ..... ☐ 3
- Little or no sense of belonging ..... ☐ 4

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 ..... 301 ☐ 1
- 1-2 times a month ..... ☐ 2
- Approximately once a week ..... ☐ 3
- More than once a week ..... ☐ 4

FOOD HABITS

	Number
How many meals a day do you normally eat (dinner and bread meals)? 302	_____
How many times a week do you eat warm dinner? 304	_____

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 type is most similar to:	☐ 306	☐	☐	☐	☐ 310

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

- Butter ..... 311 ☐
- Hard margarine ..... ☐
- Soft margarine ..... ☐
- Butter/margarine blend ..... ☐
- Oils ..... 315 ☐

How much (in number of glasses, cups, potatoes or slices) do you usually eat/drink daily the following foodstuffs?

Tick one box for each foodstuff.

	None	Less than 1	1-2	3 or more
Milk of all types (glasses) 316	☐	☐	☐	☐
Orange juice (glasses)	☐	☐	☐	☐
Potatoes	☐	☐	☐	☐
Slices of bread in total (incl. crispbread)	☐	☐	☐	☐
Slices of bread with				
– fish (e.g. mackerel in tomato sauce)	☐	☐	☐	☐
– cheese *(e.g. Gouda/Norvegia)*	☐	☐	☐	☐
– smoked cod caviare 322	☐ 1	☐ 2	☐ 3	☐ 4

How many times per week do you normally eat the following foodstuffs?

Tick for all foodstuffs listed.

	Never	Less than 1	1	2 or more
Yoghurt 323	☐	☐	☐	☐
Boiled or fried egg	☐	☐	☐	☐
Breakfast cereal/oatmeal, etc.	☐	☐	☐	☐
Dinner with				
– unprocessed meat	☐	☐	☐	☐
– fatty fish (e.g. salmon/red-fish)	☐	☐	☐	☐
– lean fish (e.g. cod) 328	☐	☐	☐	☐
– vegetables (fresh or cooked)	☐	☐	☐	☐
Carrots (fresh or cooked)	☐	☐	☐	☐
Cauliflower/cabbage/broccoli	☐	☐	☐	☐
Apples/pears	☐	☐	☐	☐
Oranges, mandarins, etc. 333	☐ 1	☐ 2	☐ 3	☐ 4

WELL BEING

How content do you generally feel with growing old?

- Good ..... 334 ☐ 1
- Quite good ..... ☐ 2
- Up and down ..... ☐ 3
- Bad ..... ☐ 4

What is your view of the future?

- Bright ..... 335 ☐ 1
- Not too bad ..... ☐ 2
- Quite worried ..... ☐ 3
- Dark ..... ☐ 4

TO BE ANSWERED BY WOMEN ONLY

MENSTRUATION

How old were you when you started menstruating? ..... 336 _____ years

How old were you when you stopped menstruating? ..... 338 _____ years

PREGNANCY

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

If you have given birth, fill in for each child the year of birth and approximately how many months you breastfed the child. If you have given birth to more than 6 children, note their birth year and number of months you breastfed at the space provided below for comments.

Child	Year of birth:	Number of months breastfed:
1	342 _____	_____
2	346 _____	_____
3	_____	_____
4	_____	_____
5	358 _____	_____
6	_____	_____

	Yes	No
Have you during pregnancy had high blood pressure and/or proteinuria? 366	☐	☐

If "Yes", during which pregnancy?	Pregnancy First	Later
High blood pressure 367	☐	☐
Proteinuria 369	☐	☐

ESTROGEN

Do you use, or have you ever used estrogen:

	Now	Previously	Never
Tablets or patches 371	☐	☐	☐
Cream or suppositories 372	☐	☐	☐

If you use estrogen, what brand do you currently use?

..... 373

Your comments:

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

Tromsø Health Survey

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: day/month/year

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

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

E

Health survey

Personal invitation

Do not write here:

E13 (Municipality) (County) (Country) E15 (Mark)

E1. YOUR OWN HEALTH

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

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

Do you have, or have you had?:

	Yes	No	Age first time
Asthma	☐	☐	
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	☐	☐	

Do you get pain or discomfort in the chest when:

	Yes	No
Walking up hills, stairs, or walking fast on level ground?	☐	☐

If you get such pain, do you usually:

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

	Yes	No
If you stop, does the pain disappear within 10 minutes?	☐	☐
Can such pain occur even if you are at rest?	☐	☐

E2. ILLNESS IN THE FAMILY

Have one or more of your parents or siblings had:

	Yes	No	Don't know
A heart attack (heart wounds) or angina pectoris (heart cramp)	☐	☐	☐

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	☐	☐	☐	☐	☐	☐

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
☐					

E3. COMPLAINTS

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

	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

E4. TEETH, MUSCLE AND SKELETON

How many teeth have you lost/extracted? *Number of teeth*
(disregard milk-teeth and wisdom teeth)

Have you been bothered by pain and/or stiffness in muscles and joints during the last 4 weeks?

	No complaint	Little complaint	Severe complaint
Neck / shoulders	☐	☐	☐
Arms, hands	☐	☐	☐
Upper part of the back	☐	☐	☐
Lumbar regions	☐	☐	☐
Hips, legs, feet	☐	☐	☐
Other places	☐	☐	☐

Have you ever had:

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

Have you fallen down during the last year? *(Tick once only)*

No ☐ 1 | Yes, 1-2 times ☐ 2 | Yes, more than 2 times ☐ 3

E5. EXERCISE AND PHYSICAL ACTIVITY

How has your physical activity been during this last year?
Think of a weekly average for the year.
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

E6. BODY WEIGHT

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

E7. EDUCATION

How many years of education have you completed? *Number of years* ☐☐

(include all the years you have attended school or studied)

E8. FOOD AND BEVERAGES

How often do you usually eat these foods?
(Tick once for each 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	☐	☐	☐	☐	☐	☐
Fat fish (e.g. salmon, trout, mackerel, herring)	☐	☐	☐	☐	☐	☐
	1	2	3	4	5	6

Do you use dietary supplements:

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

How much of the following do you usually drink?
(Tick once for each 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	☐	☐	☐	☐	☐
Soft drink, mineral water	☐	☐	☐	☐	☐
	1	2	3	4	5

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 type of coffee	☐☐
Tea	☐☐

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	About 1 time a week ☐ 6	2-3 times a week ☐ 7	4-7 times a week ☐ 8

To those who have consumed the last year:

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

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

E9. SMOKING

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

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

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

If you have NEVER smoked daily;
Go to question E11 (BODILY FUNCTIONS AND SAFETY)

If you smoke daily now, do you smoke:

	Yes	No
Cigarettes?.....	☐	☐
Cigars/cigarillos?	☐	☐
A pipe?.....	☐	☐

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

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* ☐☐

E10. BODILY FUNCTIONS AND SAFETY

Would you feel safe by walking alone in the evening in the area where you live?

Yes	A little unsafe	Very unsafe
☐ 1 2 3	☐	☐

When it comes to mobility, sight and hearing, can you:
(Tick once for each line)

	Without problems	With some problems	With great problems	No
Take a 5 minute walk in fairly high pace?	☐	☐	☐	☐
Read ordinary text in newspaper, if necessary with glasses?	☐	☐	☐	☐
Hear what is said in a normal conversation?	☐	☐	☐	☐
	1	2	3	4

Do you because of chronic health problems have difficulties with: *(Tick once for each line)*

	No difficulties	Some difficulties	Great difficulties
Move around in your home?	☐	☐	☐
Get out of your home by yourself?	☐	☐	☐
Participate in organization or other leisure time activities?	☐	☐	☐
Use public transport?	☐	☐	☐
Perform necessary daily shopping?	☐	☐	☐

E11. USE OF HEALTH SERVICES

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
A general practitioner (GP)	☐	☐	☐
Specialist *(private or out-patient clinic)*	☐	☐	☐
Emergency GP *(private or public)*	☐	☐	☐
Hospital admission	☐	☐	☐
Home nursing care	☐	☐	☐
Physiotherapist	☐	☐	☐
Chiropractor	☐	☐	☐
Municipal home care	☐	☐	☐
Dentist	☐	☐	☐
Alternative practitioner	☐	☐	☐

Are you confident that you will receive health care and home assistance if you need it?

YES ☐ 1 NO ☐ 2 Don't know ☐ 3

E12. FAMILY AND FRIENDS

Do you live: At home? ☐ 1 In an institution/shared apartment? ☐ 2

Do you live with:

	YES	NO
Spouse/ partner?	☐	☐
Other people?	☐	☐

How many good friends do you have?
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 your children and other relatives.....

Number of friends ☐☐

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

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

How many associations, sport clubs, groups, religious communities, or similar do you take part in?
(write 0 if none)

Number ☐☐

E13. CHILDHOOD/YOUTH AND AFFILIATION

How long altogether have you lived in the county? ☐☐ years

How long altogether have you lived in the municipality? ☐☐ years

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: _____

Have you moved during the last five years?

No ☐ 1 Yes, once ☐ 2 Yes, more than once ☐ 3

E14. USE OF MEDICINES

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

Do you use?
(Tick once for each line)

	Now	previously, but not now	Never used
Blood pressure lowering drugs	☐	☐	☐
Cholesterol-lowering drugs	☐	☐	☐
Drugs for osteoporosis	☐	☐	☐
Insulin	☐	☐	☐
Tablets for diabetes	☐	☐	☐

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

State the name of the medicines you are using now and the reason you are taking the medicines (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	How long have you used the medicine: One year or more
		☐	☐
		☐	☐
		☐	☐
		☐	☐
		☐	☐
		☐	☐
		☐	☐

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

E15. THE REST OF THE FORM IS TO BE ANSWERED BY WOMEN ONLY

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

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

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

Do you use, or have you ever used estrogen? *Total number of years*

	Never	Previously	Now	
Tablets or patches	☐	☐	☐	☐☐
Cream or suppositories	☐	☐	☐	☐☐

If you use estrogen, which brand you use now?

_____

Have you ever used contraceptives pills? ..... Yes ☐ No ☐

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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?

IE 050000 - 1042-1 - 24.000 - Beyer Hecos 02.01

Tromsø-undersøkelsen

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

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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
Heart attack	☐	☐	
Angina pectoris	☐	☐	
Stroke/brain hemorrhage	☐	☐	
Atrial fibrillation	☐	☐	
High blood pressure	☐	☐	
Osteoporosis	☐	☐	
Asthma	☐	☐	
Chronic bronchitis/Emphysyma/COPD	☐	☐	
Diabetes mellitus	☐	☐	
Psychological problems *(for which you have sought help)*	☐	☐	
Low metabolism	☐	☐	
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 different situations. Have you experienced some of them in the last week (including today)?** (Tick once for each complaint)

	No complaint	Little complaint	Pretty much	Very much
Sudden fear without reason	☐	☐	☐	☐
You felt afraid or worried	☐	☐	☐	☐
Faintness or dizziness	☐	☐	☐	☐
You felt tense or upset	☐	☐	☐	☐
Easily blamed yourself	☐	☐	☐	☐
Sleeping problems	☐	☐	☐	☐
Depressed, sad	☐	☐	☐	☐
You felt useless, worthless	☐	☐	☐	☐
Feeling that life is a struggle	☐	☐	☐	☐
Feeling of hopelessness with regard to the future	☐	☐	☐	☐

USE OF HEALTH SERVICES

7 **Have you during the past year 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 medical practitioner *(homeopath, acupuncturist, foot zone therapist, herbal medical 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 MEDICINE

10 **Do you take, or have you taken some of the following medications?** (Tick once for each line)

	Never used	Now	Earlier	Age first time
Drugs for high blood pressure	☐	☐	☐	
Lipid lowering drugs	☐	☐	☐	
Drugs for heart disease	☐	☐	☐	
Diuretics	☐	☐	☐	
Medications for osteoporosis	☐	☐	☐	
Insulin	☐	☐	☐	
Tablets for diabetes	☐	☐	☐	
Drugs for metabolism Thyroxine/levaxin	☐	☐	☐	

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

	Not used 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 names of all medications -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 the space is not enough for all medications, use an additional paper of your own.

When attending the survey centre 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/cohabitant	☐	☐	
Other persons older than 18 years	☐	☐	
Persons younger than 18 years	☐	☐	

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

	Parents	Children	Siblings
Myocardial infarction	☐	☐	☐
Myocardial infarction before 60 years	☐	☐	☐
Angina pectoris	☐	☐	☐
Stroke/brain haemorrhage	☐	☐	☐
Osteoporosis	☐	☐	☐
Stomach/duodenal ulcer	☐	☐	☐
Asthma	☐	☐	☐
Diabetes mellitus	☐	☐	☐
Dementia	☐	☐	☐
Psychological problems	☐	☐	☐
Drugs/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. sports 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 one)

☐ Primary, 1-2 years secondary school
☐ Vocational school
☐ High secondary school (A-level)
☐ College/university less than 4 years
☐ College/university 4 years or more

19 **What is your main occupation/activity?** (Tick one)

☐ 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 (are in a sick leave)
- ☐ Rehabilitation benefit
- ☐ Full disability pension
- ☐ Partial disability pension
- ☐ Unemployment benefits
- ☐ Transition benefit for single parents
- ☐ Social welfare benefits

21 **What was the households total taxable income last year?** Include income from work, social 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 outdoors 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 you activity varies much, for example between summer and winter, then give an average. The question refers only to the last year.** (Tick the one that fits best)

- ☐ Reading, watching TV, or other sedentary activity.
- ☐ Walking, cycling, or other forms of exercise at least 4 hours a week *(here including walking or cycling to place of work, Sunday-walking, 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 more infrequently
- ☐ 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 stopped?**

Number of years ☐☐

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

Number of cigarettes ☐☐

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

Number of 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 fruits 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 per week do you eat hot dinner?**

Number ☐☐

41 **How often do you usually eat these products?**
(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/meatloaf/meatballs)*	☐	☐	☐	☐	☐
Fruits, vegetables, berries	☐	☐	☐	☐	☐
Lean fish	☐	☐	☐	☐	☐
Fat fish *(e.g. salmon, trout, mackerel, herring, halibut, redfish)*	☐	☐	☐	☐	☐

42 **How much do you normally 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 supplements?**

	Daily	Sometimes	No
Cod liver oil or fish oil capsules	☐	☐	☐
Omega 3 capsules *(fish oil, seal oil)*	☐	☐	☐
Vitamins and/or mineral supplements	☐	☐	☐

QUESTONS FOR WOMEN

46 **Are you currently pregnant?**

☐ 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 **During pregnancy, have you had high blood pressure?**

☐ Yes ☐ No

50 **If yes, which pregnancy?**

☐ The first ☐ Second or later

51 **During pregnancy, have you had proteinuria?**

☐ Yes ☐ No

52 **If yes, 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 IUD or similar ☐ Yes ☐ No

Hormone treatment for menopausal problems ☐ Yes ☐ No

When attending the survey centre you will get a questionnaire about menstruation and possible use of hormones. Write down on a paper the names of all the hormones you have used and bring the paper with you. You will also be asked whether your menstruation have ceased and possibly when and why.

Appendix III

Ultrasound protocol in The Tromsø Study

4th, 5th and 6th surveys

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. **Scoring of plaque-echogenicity.** 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

Procedure for measurements of 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. For optimization of uptakes, adjust gain by turning knob marked ***2D***.
9. Plaque detection: Plaques are defined as an atherosclerotic lesion in the intima with focal protrusion into the lumen of the artery 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 (bulb)
    Near wall of bifurcation (bulb)
    Far wall of internal carotid artery
    Near wall of internal carotid artery

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

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

Plaque images should be used for detection of plaque thickness, plaque area and plaque echogenicity (GSM). As a main rule, one representative image from each location should be used for both size and echogenicity 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 images of the same plaque. Label plaque to show localization and purpose of measurement, eg: PLAQUE_CCA_FAR_WALL AREA for area measurement, PLAQUE_CCA_FAR_WALL ECHO for echogenicity measurements. If there are no plaques in in any part of the artery, capture one representative image of the artery and label as NO_PLAQUES.

11. Continue with R-triggered uptakes of the intima-media thickness in the distal part of common carotid artery (far wall and near wall) and in the bifurcation (far wall). It is important to depict each segment of the artery (CCA, bulb) so that the ultrasound beam is perpendicular on the longitudinal axis of the artery. Furthermore, IMT should be measured in a full diameter of the artery. Ideally, the artery should be depicted horizontally on the screen with visualization of the typical “double line” contour of the intima media complex in both near and far wall.

    Start with CCA. 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 images. Select Freeze and choose the three most representative images, which should be at least 10 images apart and save. Each image 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 (divergence of parallel walls). It is important to place the **+** as precisely as possible. To end ECG trigging, select ***ECG TRIG*** once more (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 images not good enough for measuring 1 cm, but is of sufficient quality in a shorter segment, this segment 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 the 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 images should be labeled IMT_CCA_MISSING and/or IMT_BULB_MISSING.

12. Participants who fulfill one of the following criteria should be referred to the Department of Neurology's outpatient clinic:
    a. Plaque in the CCA, bifurcation or ICA with s possible or definite maximum thickness of ≥50% of the original lumen diameter (stenosis).
    b. Possible or definite occlusion of the CCA, bifurcation or ICA.
    c. Technical difficulties which arises any doubt as to whether the above mentioned criteria are fulfilled.

    The participant should be informed about the 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. The referral will for most persons act as a safety precaution, ensuring that preventive measures can be installed.

    Save 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 deliver to mail administrators at the end of the day.

13. 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 images (***Save all***), select images for saving (***Select***) or not to save images (***None***). Normally select ***Save All***, or ***Select*** if there are images that can be deleted.
14. Clean probe with soft tissue paper after examination.
15. Next participant is registered by selecting ***New exam***.
16. At the end of the day: Turn off VIVID 7. Clean keyboard and probe with moist tissue paper. Dry off with tissue paper.