Inga Thorsen Vengen

Inflammation and atherosclerosis

Risk associations in the HUNT surveys

Thesis for the degree of Philosophiae Doctor

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Norwegian University of Science and Technology
Faculty of Medicine
Department of Laboratory Medicine, Children's and Women's Health

NTNU – Trondheim
Norwegian University of
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Inflammasjon og aterosklerose
Risikofaktorer i HUNT-undersøkelsene

Iskemisk hjertesykdom og hjerteinfarkt forårsakes av aterosklerose («åreforkalkning») og påfølgende forsnevring i blodårene til hjertet (koronararteriene), og er den vanligste dødsårsaken i Norge. Aterosklerose er en kronisk betennelsesprosess (inflammasjon), og inflammasjonsceller som *monocytter* og *nøytrofile granulocytter* er involvert. Andre deler av immunforsvaret er også aktivert, deriblant *komplementsystemet*. Røyking, ugunstig kosthold og fysisk inaktivitet er livsstilsfaktorer som kan bidra til utvikling av høyt blodtrykk, høyt kolesterol, overvekt, og diabetes, og dermed til økt risiko for iskemisk hjertesykdom. Videre er arvelig belastning en viktig risikofaktor.

Avhandlingen er bygget på følgende hypoteser: 1) diabetes kan forverre ateroskleroseutviklingen ved å øke inflammasjonen og 2) genetisk variasjon (polymorfismer) i immunforsvaret kan påvirke risikoen for aterosklerose. Målet for avhandlingen var å belyse sammenhenger mellom ulike aktører i inflammasjonsprosessen og iskemisk hjertesykdom. Avhandlingen består av tre artikler, basert på data fra den første og den andre helseundersøkelsen i Nord-Trøndelag, HUNT1 (1984-86) og HUNT2 (1995-97).

Artikkel I og II: 200 personer med nyoppdaget diabetes og 198 matchede kontroller ble fulgt i opptil 20 år etter HUNT1. Dødsfall grunnet iskemisk hjertesykdom ble registrert. Vi målte fire ulike inflammasjonsmarkører: *neopterin* fra aktiverte monocytter, *C-reaktivt protein* (CRP), en generell markør (Artikkel I), og *laktoferrin* og *myeloperoxidase* fra nøytrofile granulocytter (Artikkel II). Vi undersøkte hvordan risiko for død av iskemisk hjertesykdom var relatert til konsentrasjonen av de forskjellige markørene. De i diabetesgruppen som hadde neopterinkonsentrasjoner i det høyeste området, hadde dobbelt så høy risiko for å dø av iskemisk hjertesykdom som de som hadde lavest neopterinkonsentrasjoner. Det samme gjaldt for CRP. De som hadde høy konsentrasjon av laktoferrin hadde også over to ganger økt risiko for å dø av iskemisk hjertesykdom. Dette gjaldt ikke for myeloperoxidase. Det var ingen tilsvarende sammenhenger i kontrollgruppen.

Artikkel III: Alle som deltok i HUNT2 (n = 57133) ble fulgt opp for første-gangs hjerteinfarkt til og med 2008. De 370 yngste infarktpasientene, samt 370 matchede kontroller ble inkludert. Vi undersøkte polymorfismer i genene for *mannose-bindende lektin* (MBL) og *fikoliner*, som aktiverer komplementsystemet. Genetisk variasjon i MBL-genet som resulterer i manglende eller dysfunksjonelt MBL var assosiert med doblet risiko for hjerteinfarkt.

Studiene var ikke designet for å finne årsakssammenhenger, men bidrar til økt forståelse i ateroskleroseprosessen, slik at nye hypoteser kan genereres.

Kandidat: Inga Thorsen Vengen
Institutt: Institutt for laboratoriemedisin, barne- og kvinnesykdommer
Veileder: Vibeke Videm
Biveileder: Rune Wiseth
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Overnevnte avhandling er funnet verdig til å forsvares offentlig for graden PhD i klinisk medisin. Disputas finner sted i Auditoriet i Laboratoriesenteret, fredag 15. juni 2012 kl 12.15.

If I can stop one heart from breaking
I shall not live in vain
If I can ease one life the aching
Or cool one pain
Or help one fainting robin
Unto his nest again
I shall not live in vain

Emily Dickinson

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Acknowledgements

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I would like to thank *Nina Sandberg* and *Oddrun Storrø* for excellent assistance and good times in the lab. I would also like to thank my colleagues in our small research group: *Kristine, Erik, Kristin, Yunita* and *Tone.*

I want to thank my parents, brother and friends for being there and for supporting me, I could not have made it without you. My family has always stimulated me to expand my horizons, and supported my decisions unconditionally, and I am very grateful for that. Last, I would like to thank *Stig*, my best friend and husband, for his loving support and ability to always make me smile.

Trondheim, February 2012
Inga Thorsen Vengen

List of papers

This thesis is based on the three following papers, and they will be referred to by their Roman numbers.

I Vengen IT, Dale AC, Wiseth R, Midthjell K, Videm V. **Neopterin predicts the risk for fatal ischemic heart disease in type 2 diabetes mellitus. Long-term follow-up of the HUNT 1 study.** *Atherosclerosis* 2009;207:239-44.

II Vengen IT, Dale AC, Wiseth R, Midthjell K, Videm V. **Lactoferrin is a novel predictor of fatal ischemic heart disease in diabetes mellitus type 2: Long-term follow-up of the HUNT 1 study.** *Atherosclerosis* 2010;212(2):614-20.

III Vengen IT, Madsen HO, Garred P, Platou C, Vatten L, Videm V. **Mannose-binding lectin deficiency is associated with myocardial infarction: the HUNT2 Study in Norway**. (Submitted)

Abbreviations

AGE	Advanced glycation end products
apoB	Apolipoprotein B
BMI	Body mass index
CI	Confidence interval
CRP	C-reactive protein
CVD	Cardiovascular disease
ER stress	Endoplasmatic reticulum stress
FCN1, FCN2, FCN3	The genes coding for ficolins
GWAS	Genome-wide association study
HDL cholesterol	High-density lipoprotein cholesterol
HR	Hazard ratio
HUNT	The Nord-Trøndelag Health Study
ICAM-1	Intercellular adhesion molecule 1
LDL cholesterol	Low-density lipoprotein cholesterol
MBL	Mannose-binding lectin
MBL2	The gene coding for mannose-binding lectin
MI	Myocardial infarction
NF-κB	Nuclear factor-κB
OR	Odds ratio
PCR	Polymerase chain reaction
SNP	Single nucleotide polymorphism
TLR	Toll-like receptor
VCAM-1	Vascular cell adhesion molecule 1
VLA-4	Very late antigen 4
VLDL cholesterol	Very low-density lipoprotein cholesterol
WHR	Waist-hip ratio
WHO	World Health Organization

Summary

Ischemic heart disease is the leading cause of death in Norway and is caused by coronary atherosclerosis. The atherosclerotic process is characterized by accumulation of lipids and inflammatory cells in the vessel wall. Inflammation is central in the pathophysiology, but the roles of the different components of the immune system are not fully understood. Both lifestyle and genetic predisposition are risk factors for development of atherosclerosis, however risk estimation is unsatisfactory. Inflammatory mediators are therefore studied to better understand the process and to improve clinical risk prediction.

This thesis consists of three studies, using data and biomaterial from the population-based HUNT surveys.

Type 2 diabetes is a lifestyle-related disease characterized by insulin resistance, hyperinsulinemia, hyperglycemia and activation of inflammation. The inherent inflammation may accelerate atherosclerotic development. *We therefore investigated the predictive value of inflammatory biomarkers on fatal ischemic heart disease, in a group of 200 newly diagnosed diabetes patients and 198 controls who were followed for up to 20 years (Study I &II).*

Polymorphisms in inflammatory genes may be related to the risk of atherosclerosis. Persons who experience a myocardial infarction at a young age are more likely to have a genetic predisposition in addition to the conventional risk factors. Certain polymorphisms in the gene coding for mannose-binding lectin (*MBL2*) cause deficiency of the protein. Mannose-binding lectin activates the lectin pathway of the complement cascade, and *MBL2* genotypes as well as variation in protein concentration have been linked to atherosclerosis in different risk populations. Ficolins also activate the lectin pathway. *We therefore performed a candidate gene study of functional polymorphisms in MBL2 and ficolin genes in a group of young patients with myocardial infarctions and controls.*

We found that a marker of monocyte activation, neopterin, and a well-known marker of general inflammation, C-reactive protein, were significant predictors of fatal ischemic heart disease in the group of newly diagnosed diabetes patients (Study I). We also found that lactoferrin, but not myeloperoxidase (markers from neutrophil granulocytes) was a predictor of

death from ischemic heart disease in the same study group (Study II). These associations were not present in the control group.

Lastly, we found that polymorphisms corresponding to mannose-binding lectin deficiency were associated with doubling of the risk for myocardial infarction.

This thesis consists of three studies, which contribute with small pixels to a large and complicated picture. They were not designed to reveal causal relationships, but they may lend support to former hypotheses, or generate new ones.

Clarifications

The literature in the field of atherosclerosis and inflammation is extensive. In the Introduction, I have used papers and reviews published up to January 2012 to give a current status of the research related to the general background. However, in the sections where I present the different inflammatory mediators, I have used the literature that was available before our studies were planned and published. In the Discussion, our findings are discussed in relation to recent publications in the field.

1 Introduction

1.1 Cardiovascular disease

1.1.1 Definition

According to World Health Organization (WHO), cardiovascular diseases comprise of the diseases shown in Table 1.1. The group can be divided into those caused by atherosclerosis (**bold**) and a cluster of other diseases (*italics*). In this thesis, "CVD" or "atherosclerotic CVD" will be used denoting ischemic heart disease, cerebrovascular disease and diseases of the aorta and arteries. The term "all-cause CVD" will be used when referring to the total group of diseases in section *1.1.2*.

Cardiovascular diseases
• **Ischemic heart disease**
• **Cerebrovascular disease**
• **Diseases of the aorta and arteries (including hypertension and peripheral vascular disease)**
• *Rheumatic heart disease*
• *Congenital heart disease*
• *Cardiomyopathies*
• *Cardiac arrhythmias*

Table 1.1 Definition of cardiovascular diseases

1.1.2 Mortality

All-cause CVD is the leading cause of mortality and disability in the world (Figure 1.1) [1]. According to WHO, causes of mortality are grouped as follows: communicable, maternal and perinatal and nutritional conditions, injuries and non-communicable diseases. All-cause CVD is one of the non-communicable diseases, together with diabetes, some types of cancer and chronic respiratory diseases. In Figure 1.1 the magnitude of total cardiovascular mortality is shown in green.

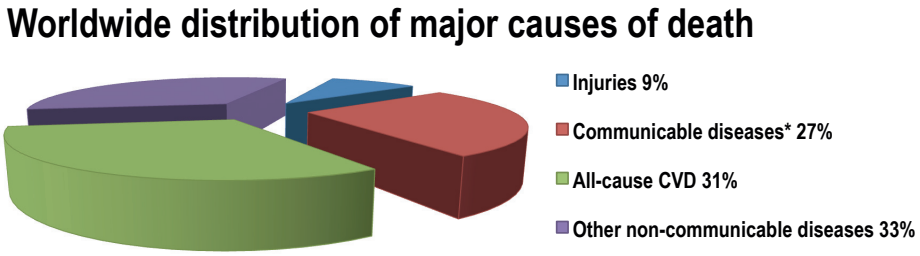

Figure 1.1 All-cause CVD is the leading cause of death worldwide. *Communicable, maternal, perinatal and nutritional conditions. Modified from WHO.

In 2008 17.3 million people died from all-cause CVDs, and the prognosis shows that by 2030, 23.6 million people will die from these diseases every year [2]. For the past two decades, CVD mortality rates have been declining in high-income countries like Norway [3, 4]. However, in low- and middle-income countries, the rates are inclining alarmingly [1]. Despite the decline, CVDs caused 37% of all deaths in Norway in 2010, with the subgroup ischemic heart disease as the leading cause [4]. Worldwide, ischemic heart disease is also the main cause of death and healthy years of life lost, as detailed in Figure 1.2 [1]. In this thesis, the focus will be on ischemic heart disease.

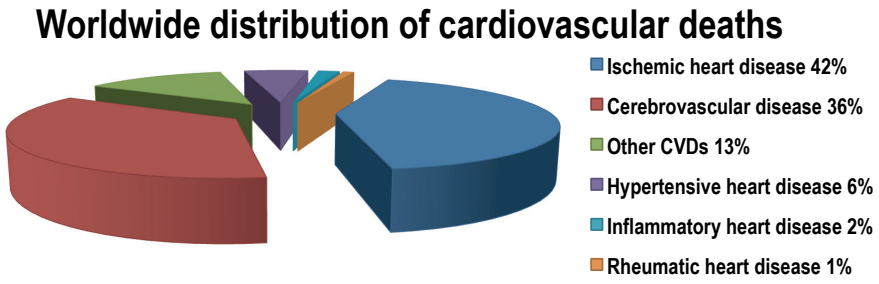

Figure 1.2 Deaths due to myocardial infarction, stroke and other types of cardiovascular diseases worldwide. Modified from WHO

1.1.3 Risk factors

The main behavioural risk factors for atherosclerotic CVD are tobacco use, an unhealthy diet and physical inactivity (Figure 1.3). Lifestyle factors may result in conditions like diabetes, obesity, hypertension and high blood lipids, i.e. metabolic risk factors. The increase in CVD mortality in low- and middle-income countries is closely related to the underlying causes as shown in Figure 1.3. On the other hand, the observed decline in CVD mortality in Western countries may be attributed to reductions in risk factors like cholesterol, smoking, systolic blood pressure and physical inactivity. However, opposite trends are now observed for diabetes and obesity. Furthermore, the decrease in CVD mortality may also be a result of improved treatment [5, 6].

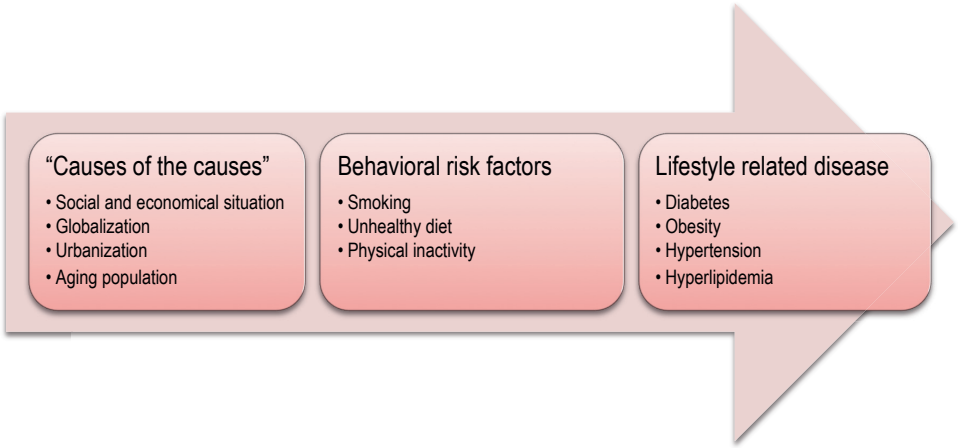

Figure 1.3 Risk factors for atherosclerotic CVD.

Unlike lifestyle and habits, some risk factors are non-modifiable. The incidence of atherosclerotic CVD increases with age, and men are at higher risk than women. This is true until menopause, when the gender difference attenuates and the incidence for women increases abruptly [7]. In addition, studies of familial burden of CVDs and twin studies indicate that genetic susceptibility accounts for 20-60% of the total risk [8-10].

Clinical prediction of atherosclerotic risk is based on combinations of the factors mentioned above: age, gender, anthropometric, metabolic, socioeconomic and lifestyle variables. In Norway, a risk calculator called Norrisk is recommended, which includes age, gender, smoking (yes/no), systolic blood pressure, total cholesterol, family history of premature CVD and glucose tolerance status [11]. However, for many patients, the first sign of ischemic heart disease is admission to a hospital with chest pain, and 15-20% of coronary events occur in patients with no major traditional risk factors [12]. This calls for methods of earlier detection of subclinical disease and better methods for assessing individual risk for cardiovascular events.

1.1.4 Ischemic heart disease

Morphology

Ischemic heart disease is caused by atherosclerosis in the coronary arteries, with narrowing of the lumen and reduced oxygen supply to the myocardium. Briefly, the atherosclerotic process starts with fatty streaks [13] and flow-related intimal thickening at branch points already in youth, and continues by building of atheromas as depictured in Figure 1.4 [14]. The endothelium is activated and attracts inflammatory cells, a process that will be described in section *1.2.* In the early phase, the lumen diameter is maintained because of outward (positive) remodelling [15]. At a point, the endothelium becomes dysfunctional and unable to compensate, and the plaque begins to protrude into the lumen, which gradually decreases blood flow. Plaques covered with a thick fibrous cap are more stable and less likely to rupture than plaques with a thin fibrous cap and a necrotic core. Eventually, a plaque erosion [16, 17] or more often a plaque rupture [18] allows the blood to come into contact with the highly thrombogenic contents of the plaque (i.e. collagen, necrotic cells), which leads to intraluminal clot formation and thrombosis. The result is reduced or obstructed blood flow, and symptoms of ischemia: either unstable angina pectoris or a myocardial infarction (MI).

Clinical presentation

The first three stages in Figure 1.4 are clinically silent, while the atheroma in stage IV may or may not be clinically significant. Even though a plaque does not cause much narrowing of the lumen, it may be vulnerable to rupture. Morbidity and mortality from atherosclerosis is mostly caused by stage V and VI lesions [14]. Occlusion of more than 50% of the diameter impairs coronary blood flow, and substantial decrease in flow is seen at 70% stenosis [19].

Chronic stable angina pectoris is characterized by chest pain occurring during physical activity or emotional stress, with pain episodes lasting for less than 15 minutes, and with pain relief at rest or by the use of sublingual nitroglycerin. The pain is typically retrosternal and radiating to the arm, neck and jaw. The diagnosis is clinical, but exercise electrocardiography (ECG) may reveal myocardial ischemia during high effort activity.

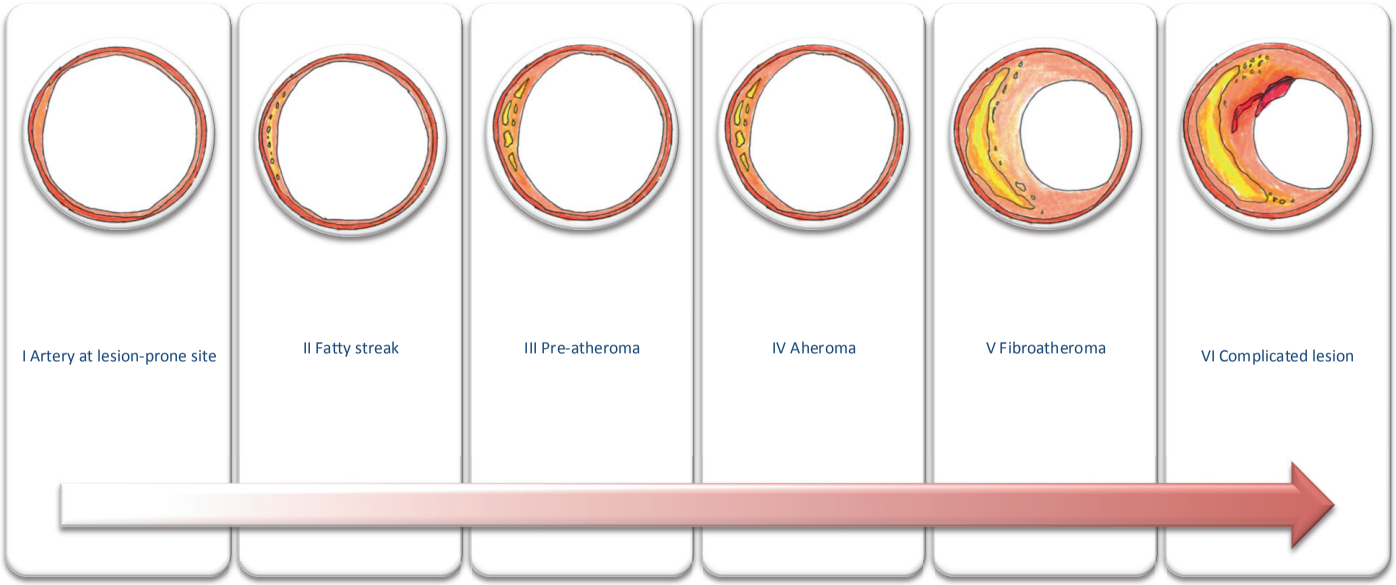

Figure 1.4 The atherosclerotic process starts with endothelial activation and adaptive thickening of the intima in areas with shear stress and endothelial damage (I). Excess blood lipids accumulate in the intima. The endothelium is activated and attracts monocytes, which ingest lipids and become foam cells. The first visible traces are fatty streaks (II). An atheroma builds up (III), with gradual formation of a core of extracellular lipids (IV). Smooth muscle cells invade the intima and produce collagen that creates a fibrous cap (V). Eventually, a surface defect can cause thrombosis and/or haemorrhage and lead to an ischemic event (VI).

Unstable angina or acute MI is caused by plaque disruption [20], Figure 1.5. A superficial rupture may cause transient or repetitive occlusion and give unstable angina. MI is the result of a thrombotic occlusion due to deeper ulceration of the plaque, where the lipid core, collagen and tissue factor are exposed. The symptoms are strong, radiating pain, shortness of breath, nausea, vomiting and sweating, and the diagnosis is based on the symptoms, myocardial ischemia on ECG and elevated cardiac enzymes in the blood [21, 22].

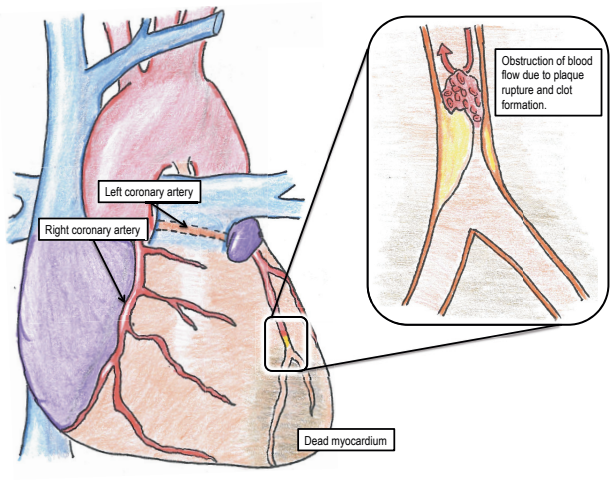

Figure 1.5 Myocardial infarction due to plaque rupture.

1.2 Atherosclerosis – a chronic inflammatory disease

Inflammation is regarded the central pathogenetic process of atherosclerosis. The main function of inflammation is to eliminate any pathogenic insult and remove damaged tissue in order to restore tissue homeostasis. A possible role of inflammation in atherosclerosis was already recognised in the mid 19th century, when Virchow and von Rokitansky discovered inflammatory cells in atherosclerotic plaques [23, 24]. However, they did not agree whether inflammation was responsible for (Virchow) or a consequence of (von Rokitansky) atherosclerosis. Others believed infections to cause the disease, a theory that is not entirely abandoned [25]. In the beginning of the 20th century, Anchikov and co-workers demonstrated the crucial role of cholesterol in the process [26], and for many years atherosclerosis was viewed merely a passive lipid deposition disease. The concept was replaced by a theory of "response-to injury", where endothelial damage led to platelet adhesion and subsequent smooth muscle cell proliferation [27]. However, through the last three decades, the understanding of the pathogenesis of atherosclerosis has changed remarkably, and inflammation has gained a key position in our understanding of this very complex process [28, 29].

1.2.1 Inflammatory mediators and biomarkers in risk prediction

Considering that inflammation plays a pivotal part in the pathology of atherosclerosis, biomarkers of inflammation have emerged as potential risk predictors of atherosclerotic CVD. The ultimate goal for clinical use of any biomarker is to improve patient care by earlier detection of subclinical disease, diagnosis of acute or chronic syndromes, more precise risk stratification, appropriate selection of therapy, or monitoring disease progression / response to therapy [30]. Ideally, such biomarkers would indicate disease aetiology and reflect causal pathways. However, in an intricate process like inflammation, it may be naïve to seek single markers fulfilling these criteria. In practice, both causal and by-standing markers, alone or in combination, may be useful.

Several principles for evaluation of novel cardiovascular biomarkers have been suggested. The first phases of evaluation are exploratory, where the association between the marker and outcome is established, in basal and epidemiological studies. *The studies in the present thesis are at this level*. Next, the question of whether the marker adds new predictive information and its clinical utility is evaluated. The area under the receiver-operator characteristic curve (summarized by the C statistic), and risk reclassification are two approaches used for evaluation of clinical value. Finally, whether use of the marker helps improve clinical outcome and the cost-effectiveness are assessed [30-32]. Causality of biomarkers can be assessed without bias by the use of Mendelian randomization studies. In these studies, genetically determined concentrations of a marker are linked to the risk of an event, and it is viewed a "natural" randomized controlled trial [33].

1.2.2 C-reactive protein

C-reactive protein (CRP) is a marker of general inflammation that has received massive attention as a possible predictor of CVD [34-44].

CRP is an acute-phase protein, which is produced in the liver and adipose tissue [45] in response to interleukin-6 from macrophages, T-cells and adipocytes [46]. It increases 10,000-fold in response to severe infections or extensive tissue damage [47]. In between such spikes, the concentration is fairly stable within the individual. High-sensitivity assays are available for detection of CRP

concentrations below the normal range, i.e. less than 5 mg/L. In prediction of cardiovascular events, the following intervals of CRP concentrations are suggested for risk classification: lower than 1 mg/L: low risk, 1-3 mg/L: intermediate risk, and higher than 3 mg/L: high risk.

There are two main topics in the present debate about this marker: 1) CRP and causality in atherosclerosis and 2) the role of CRP in clinical decision-making. First, on one hand there are biological studies supporting CRP in the pathogenesis of plaque development [48]. On the other hand, Mendelian randomisation studies have rejected increased CRP as a cause of atherosclerosis [41, 42]. Second, classical risk factors are used to evaluate individual cardiovascular risk, e.g. using the Framingham model. Measurement of CRP is suggested in persons who are classified as being at intermediate risk (10-year risk of 10-20%) [37, 49]. Some argue that this will lead to reclassification in as much as 30% of these persons [50], whereas others point out the relatively small improvement in risk prediction compared to established risk factors [38].

While the usefulness of CRP as a target of therapy is investigated, there is a constant search for more specific inflammatory markers of atherosclerosis. A possible approach to increase the understanding of the process, and maybe also identify new biomarkers, is to test hypotheses based on knowledge of the pathophysiology, which is further described in the following sections.

1.2.3 Lipids

Atherosclerosis is often initiated at sites of disturbed laminar flow, such as bifurcations [51]. In the earliest stages, apolipoprotein B (apoB)-containing lipoproteins accumulate in the intima [52] (Figure 1.6). ApoB-lipoproteins are secreted as very low-density lipoproteins (VLDL) from the liver or as chylomicrons from the intestines. VLDLs are transformed to the atherogenic low-density lipoprotein (LDL) and chylomicrons are converted by lipolysis to remnant lipoproteins, which are also atherogenic. High-density lipoproteins (HDL), containing apolipoprotein A, on the other hand, contribute to reverse cholesterol transport, and are anti-atherogenic [53, 54]. HDL, LDL and total cholesterol are firmly established as participants in the atherosclerotic process and are used in clinical risk assessment. Moreover, treatment with cholesterol-

lowering drugs (i.e. statins), have substantially decreased the risk for CVD [55]. Triglycerides, another component of circulating lipids, are on the other hand probably not directly involved in the pathogenesis [56].

1.2.4 Monocytes

The endothelium overlying the lipoproteins is activated and secretes chemoattractants, which attract circulating inflammatory cells. This is a feature of *endothelial dysfunction*, which denotes a pro-inflammatory and pro-thrombotic state with impaired vasodilation [57]. The first cells that are recruited are monocytes [58, 59].

Monocytes are derived from the common myeloid progenitor cell in the bone marrow and are important phagocytes in the early defence line in the innate immune system. In atherosclerosis, they are recruited continuously, from the initial stages of plaque formation to advanced lesions, and the accumulation is proportional with lesion size [60]. The monocytes are tethered to the endothelium and roll over endothelial cells through interaction between adhesion molecules, as depicted in Figure 1.6. Selectins like P-selectin, integrins like very late antigen-4 (VLA-4) and CD11/CD18, and molecules like vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1) mediate this process. P-selectin on endothelial cells and its ligand on monocytes initiate rolling and activation of integrins. Activated VLA-4 on the monocytes interacts with VCAM-1 and leads to firm adhesion, which is followed by diapedesis, i.e. subendothelial entrance of the monocytes [58]. Driven by factors like macrophage colony stimulating factor, monocytes in the intima develop into macrophages or cells with dendritic features.

Macrophages in the atherosclerotic lesions ingest apoB-lipoproteins via scavenger receptors [61] and probably also through other mechanisms. The resulting lipid-loaded macrophages are termed foam cells (Figure 1.6). The cholesteryl esters of the lipoproteins are hydrolysed to free cholesterol and fatty acids [62]. Ingestion of lipoproteins stimulates inflammatory signalling because of cholesterol enrichment of the plasma membrane [63]. The retained lipoproteins may be oxidized or enzymatically modified, which enhances the inflammatory response to the accumulation. Stimulation of macrophages through toll-like

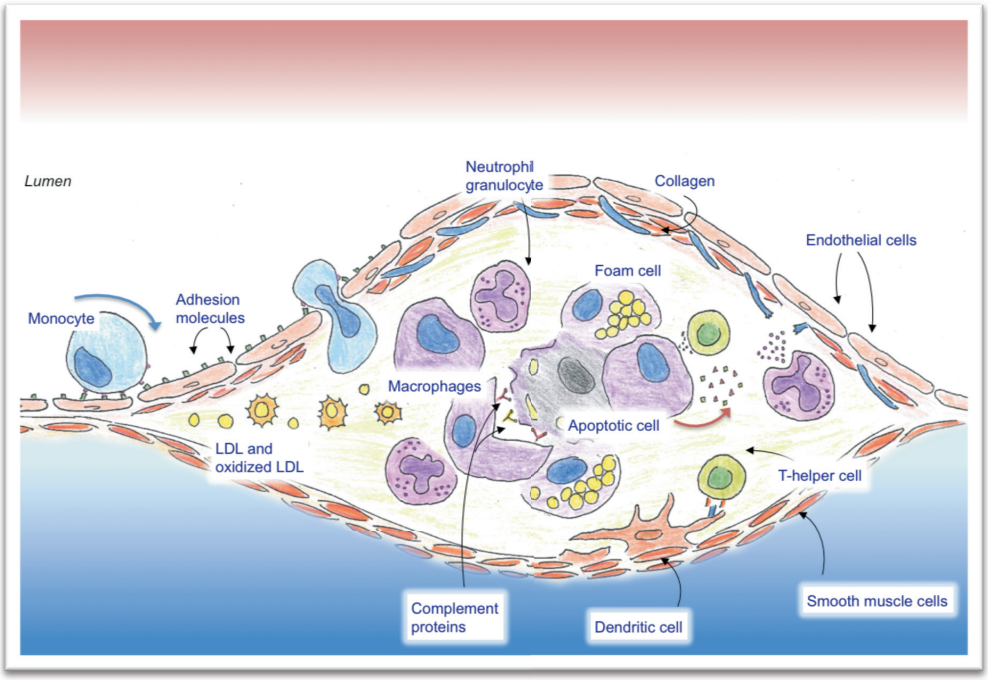

Figure 1.6 Inflammatory cells in the atherosclerotic plaque.
Lipoproteins accumulate in the intima and monocytes are attracted. The monocytes develop into macrophages, which ingest LDL and oxidized LDL and become foam cells. T-helper cells interact with dendritic cells and activate macrophages. Smooth muscle cells proliferate and produce collagen. Neutrophil granulocytes are activated and release substances that can lead to formation of vulnerable plaques. The complement system is involved in the clearing of apoptotic cells. Inefficient removal of dying cells may lead to the formation of a necrotic core.

receptors (TLRs) and other cell surface receptors leads to activation of the pro-inflammatory transcription factor nuclear factor κ-B (NF-κB), and subsequent production of various cytokines [64]. TLRs are pattern recognition receptors that recognize molecules on microbes as well as other danger signals, and activate the immune system for defence, and may play a central role in the pathogenesis of atherosclerosis [64]. NF-κB is a regulator of the immune response, and is activated through various receptors in response to bacterial or viral antigens, free radicals, oxidized LDL, ultraviolet irradiation, cytokines or other cellular stresses [65]. Oxidized LDL may upregulate the expression of TLRs on macrophages, which may lead to a sustained inflammatory response in atherosclerotic plaques [66].

Macrophages in the lesion actively promote development of vulnerable plaques, which are characterized by thinning of the fibrous cap and formation of a necrotic core. They release proteases that degrade collagen, they inhibit collagen production and promote apoptosis in smooth muscle cells [59]. Formation of a necrotic core is a result of apoptosis of macrophages and defective phagocytic clearance (efferocytosis) [67]. Several factors may contribute to apoptosis, and prolonged endoplasmatic reticulum (ER) stress is an emerging concept [68]. An effective efferocytosis is characterized by 1) clearing of the cells before membrane damage lead to leakage of toxic intracellular material, 2) induction of anti-inflammatory response (e.g. interleukin-10 and tumour necrosis factor-β) in the efferocytes and 3) survival of the efferocytes. The precise causes of defective efferocytosis in advanced lesions are not known, but may be related to oxidative stress caused by the engulfed apoptotic cells.

Neopterin

Neopterin is released from activated macrophages upon stimulation with interferon-γ from T-helper cells [69]. It is the oxidized product of 7,8-dihydroneopterin, a by-product of the guanosine pathway. [70]. Interferon-γ possesses many pro-atherosclerotic properties [71], and could thus be an attractive candidate in risk prediction. However, due to its short half-life in circulation, measurement is not feasible. Moreover, neopterin in itself induces a pro-atherothrombotic phenotype in coronary endothelial cells [72]. Neopterin release is closely correlated to macrophage release of hydrogen peroxide [73] and reflects the amount of oxidative stress in the process . It also promotes apoptosis in smooth muscle cells and promotes plaque growth [74]. Therefore, as neopterin reflects interferon-γ [75] and macrophage activity, and furthermore may be an effector molecule in the atherosclerotic process, it has emerged as a promising marker of coronary disease.

Neopterin has been recognized as a marker of disease activity in atherosclerosis, i.e. it is increased in patients with acute coronary syndromes versus chronic stable angina [76-80]. It is associated with stenosis complexity [81, 82] and plaque instability [83], and it is a predictor of major cardiovascular events in patients with chronic stable angina [84, 85] and MI [86-89]. Whether it is linked to the

extent of atherosclerosis is under debate [90-92]. Altogether, these studies indicate that neopterin is closely linked to plaque vulnerability and a potential marker of future cardiovascular events.

1.2.5 Neutrophil granulocytes

In contrast to monocytes, neutrophil granulocytes have received little attention in the pathogenesis of atherosclerosis until recently [93, 94]. However, the neutrophil count is known as an independent predictor of cardiovascular events, both in the general population [95-100] and in persons with atherosclerosis [101-108].

The lack of attention may be due to the low frequency of neutrophils detected in atherosclerotic plaques. Poor detection methods may partly explain this. Additionally, neutrophils are relatively short-lived as they undergo rapid apoptosis and phagocytic clearance, especially compared to macrophages, dendritic cells and T-lymphocytes. Nevertheless, neutrophils in inflamed tissue live twice as long as unchallenged neutrophils [109-111], and neutrophils from unstable coronary plaques have demonstrated increased telomerase activity compared to blood neutrophils, indicating a prolonged life span [112].

Improved staining techniques have allowed more precise detection of neutrophils in atherosclerotic lesions [113]. Studies of pathophysiology in mice and men (and monkeys) show that neutrophils are recruited continuously through all stages of the atherosclerotic development [113-122]. Depletion of neutrophils in mice reduced plaque size [121] and the number of monocytes and macrophages in the arterial wall [117], indicating an important role in the initiation of atherosclerosis, possibly by recruitment and activation of macrophages [123]. Furthermore, the neutrophils seem to be central in the development of vulnerable plaques [82, 124] (Figure 1.6), especially considering that they are particularly numerous in rupture-prone [122] and culprit lesions [115].

Neutrophil granulocytes are phagocytes formed in the bone marrow, which are massively released to circulation in response to infection and inflammation. In steady state the circulating count is low. However, increased numbers are reported in response to hyperlipidaemia [117].

The neutrophil granulocytes exert their pro-inflammatory actions through interaction with platelets, macrophages, dendritic cells and other

lymphocytes [94]. Neutrophil function depends on controlled hierarchic mobilization of cytoplasmic granules and secretory vesicles, which contain antimicrobial proteins, proteases, components of the respiratory burst oxidase, membrane-bound receptors for endothelial adhesion molecules, extracellular matrix proteins, and soluble mediators of inflammation [93].

There are three different granule subsets, primary, secondary and tertiary. The secretory vesicles contain receptors that mediate firm adhesion to endothelium and degranulate most easily. Then follows exocytosis of gelatinase from tertiary granules, which helps degradation of collagen in the basement membrane. Subsequently, there is degranulation of secondary, or specific granules, identified for example by lactoferrin. Lastly, the primary or azurophilic, granules are mobilized, which contain myeloperoxidase and other proteases [125]. Fusion of primary and secondary granules with the phagosome facilitates oxygen-dependent and –independent bactericidal activity. Intracellular granule proteins are released in abundance from activated neutrophils, through degranulation and leakage during phagosome formation and cell death. The granule proteins can contribute to tissue damage and thus formation of vulnerable plaques [122], and are therefore of interest as biomarkers.

Myeloperoxidase

Serum concentrations of myeloperoxidase have been associated with future cardiovascular events, both in previously healthy persons [126] and in persons with acute coronary disease [127-131]. Furthermore, myeloperoxidase is present in atherosclerotic plaques [118, 121, 132].

Myeloperoxidase is a powerful oxidative agent and there are several mechanisms through which myeloperoxidase may contribute to atherosclerosis. Myeloperoxidase can induce reactive oxygen radicals (ROS) that oxidize LDL and may thus accelerate foam cell formation [133, 134]. Myeloperoxidase may also induce impaired function of HDL and thus impaired reverse cholesterol transport [135, 136]. Myeloperoxidase may activate proteases that contribute to weakening of the fibrous cap and formation of vulnerable plaques [137, 138]

Lactoferrin

On the other hand, neutrophils may also exert anti-inflammatory effects, as they contain mediators that act to limit inflammation and maintain homeostasis [139]. Lactoferrin, located in the secondary granules, is an iron-binding glycoprotein of the transferrin family, and is upregulated in inflammatory responses [140-142]. It is also produced in exocrine secretions (breast milk, tears, saliva, etc.) and it is released by mucosal epithelium, but the amount in circulation mainly originates from neutrophils [143]. Lactoferrin has immune-modulatory properties and exerts anti-microbial and anti-inflammatory effects [144].

Lactoferrin is less studied in the context of atherosclerosis, but one study by our group demonstrated higher concentrations in patients with significant coronary artery stenosis compared to those without stenosis [145]. Furthermore, it was increased in other inflammatory diseases like systemic lupus erythematosus and rheumatoid arthritis [146].

Under normal circumstances however, many of lactoferrin's effects may protect against atherosclerosis. It inhibits both uptake of cholesterol in macrophages [147, 148] and upregulation of adhesion molecules on endothelial cells [149]. In rodents it has been shown that bovine lactoferrin improves the lipid profile [150]. It also affects the production of various cytokines and may thereby influence in the regulation of immune responses [151-154].

1.2.6 The adaptive immune response

T-lymphocytes are recruited to atherosclerotic plaques in the same manner as monocytes and neutrophils [155]. Dendritic cells in lymph nodes, which have ingested antigens like apoB lipoproteins, activate the T-cells. They are then recruited to the plaque, and are reactivated by local macrophages and dendritic cells [155].

CD4+ T lymphocytes with defective cell surface expression of CD28, an important receptor for co-stimulation (CD4+CD28^{null} T-cells), are a subset of long-lived cytotoxic T cells normally not found in healthy individuals. However, in some inflammatory diseases [156], acute coronary syndromes [157, 158] and recurrent plaque instability [159] the CD4+CD28^{null} T-cell count is increased. These cells undergo clonal expansion and release large amounts of interferon-γ, which

activates monocytes and leads to production of neopterin [158, 160, 161] (Figure 1.6). Clonal expansion has been observed in atherosclerotic plaques, supporting the concept of an antigen-specific response. Both exogenous antigens (e.g. microbial proteins) and autoantigens (e.g. oxidized LDL or heat shock proteins) may induce the clonal expansion.

B-cells and mast cells are only occasionally found in atherosclerotic lesions. B-cells may have protective effects, while mast cells produce pro-inflammatory mediators. Dendritic cells may both induce tolerance but also activate the adaptive immune response within the plaque [155].

1.2.7 The complement system

The complement system is another part of the innate immune system that may have a dual effect on the progression of atherosclerosis. It consists of small proteins mainly found in the circulation. When activated, complement activation products lead to opsonisation of pathogens for phagocytosis, pro-inflammatory effects and formation of the membrane attack complex, which may cause cell lysis. The complement system also bridges innate and adaptive immune responses, and it is necessary for removal of immune complexes, apoptotic cells and debris [162, 163].

Activation of the complement system goes through three stages; 1) initiation, 2) amplification by assembly of C3 and C5 convertases and 3) the effector functions carried out by anaphylatoxins, opsonins and the terminal complement complex (Figure 1.7). The cascade is activated through the classical, the lectin or the alternative pathway, or by thrombin from the coagulation cascade. The pathways share the same downstream events: the cleaving and activation of C3 and C5.

The classical pathway is activated by pentraxins, like CRP, or antibodies bound to their target antigens. The alternative pathway is constantly activated by spontaneous hydrolysis, but is limited by C3b interaction with self cells. However, if C3b binds to a foreign surface, the C3 convertase is stabilized, and the activation escalates. Mannose-binding lectin (MBL) and ficolins activate the lectin pathway through binding of mannose-binding lectin associated serine proteases to carbohydrates on microbial surfaces [164].

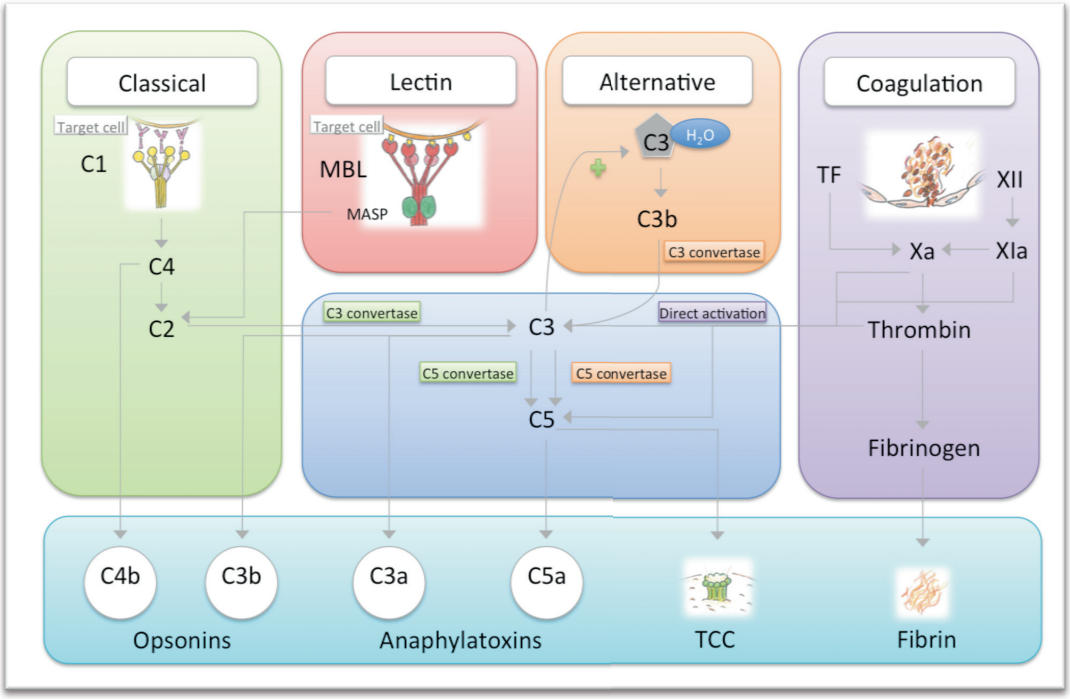

Figure 1.7 Activation of the complement cascade. Modified with permission from the publisher (Speidl, 2010)

Complement activation is involved in the atherosclerotic process, from the formation of fatty streaks to plaque rupture [165-167]. Within the plaque, the classical pathway may be activated by autoantibodies against LDL or heat shock proteins [155], or through interaction with platelets or endothelial cells [168]. Furthermore, interaction with CRP may modify activation of the classical pathway, either by increased activity or by facilitating LDL removal [169]. The alternative pathway may be activated by enzymatically changed LDL [170], apoptotic cells and cell debris [171], free cholesterol [172] or P-selectin [168, 173]. Activation of the lectin pathway may be linked to the presence of *Chlamydia pneumoniae* in the atherosclerotic plaque [174] or through interaction with dying cells [175, 176] (Figure 1.6).

Studies in knockout mice have shown increased atherosclerotic lesions when the classical [177] and lectin [178] pathways are inhibited, indicating the importance of apoptotic cell removal mediated by opsonins. On the other hand, a

pro-atherogenic role of the alternative pathway has been suggested, as knockout mice lacking factor B (an important activator of the alternative pathway) had decreased aortic root plaque size [179]. These findings underline the dual role of complement activation in atherosclerosis.

MBL deficiency

MBL deficiency, i.e. lack of functional MBL, was first identified as a humoral defect of phagocytosis, related to the complement system in children with recurrent infections [180-182]. Later, MBL was recognized as an activator of the complement cascade [183], and the lectin pathway was distinguished from the classical and alternative pathway [184, 185]. Eventually, the children's disease was linked to lack of MBL [186]. Six functional single nucleotide polymorphisms (SNPs) were found in *MBL2*. The inferred haplotypes correspond to three levels of functional MBL concentration in serum: *normal*, *intermediate* and *deficient* [187].

MBL deficiency has been investigated in many clinical conditions [188], and in 1998 Madsen et al found an association with severe atherosclerosis in patients going through coronary surgery [189]. After this initial report, others have found both increased and decreased risk of atherosclerosis and coronary artery disease associated with *MBL2* genotypes and serum concentrations. On one hand, normal or high concentrations of MBL/normal *MBL2* genotype have been associated with increased risk of atherosclerosis [190-196]. On the other hand, the majority of studies reports associations of MBL deficiency/low genotype and increased risk [189, 191, 197-205].

Ficolin-1, Ficolin-2 and Ficolin-3 also activate the lectin pathway. Both MBL and ficolins recognize foreign molecular patterns of microorganisms, and they bind to dying cells, which leads to opsonisation and removal by phagocytes [188, 206]. Different genetic variants are found in the ficolin genes (*FCN1, FCN2* and *FCN3*), which affect their stability, binding capacity or concentration [207], but less is known about their role in heath and disease.

1.2.8 Altered inflammation and the risk of CVD

Conditions where the inflammatory processes are altered may affect the risk of CVD. At this point, the background of the thesis diverges into lifestyle-related

disease on one hand, and genetic predisposition on the other. In the following sections (*1.3* and *1.4)*, the specific background for the different studies will be presented, and the rationale for inclusion of the investigated inflammatory mediators will be presented.

1.3 Inflammatory diseases and risk of atherosclerosis

Specific background for study I and II

Persons with autoimmune diseases like rheumatoid arthritis, other systemic connective tissue diseases [208, 209] or diabetes are at an increased risk for developing premature ischemic heart disease. In patients with rheumatoid arthritis the prevalence of CVDs is similar to that in persons with type 2 diabetes of corresponding duration [210, 211]. Diabetes and rheumatoid arthritis share many of the conventional risk factors for CVDs, but these do not entirely explain the increased risk [210]. The two diseases also have another feature in common; i.e. chronic inflammation [208, 212], and the inherent inflammation may accelerate the atherosclerotic process. In this thesis, the lifestyle-related disease type 2 diabetes will be in focus.

1.3.1 Type 2 diabetes

The prevalence of type 2 diabetes is increasing worldwide, and the estimated annual increase for persons older than 30 years is 1.4% in Norway [213]. This development is closely related to lifestyle risk factors, as already shown in Figure 1.3. Diabetes is an important risk factor for CVD and the risk of MI in a diabetes patient without CVD is similar to that of a non-diabetic patient who has previously had an MI [214]. Data from Norwegian population studies show that 50% of persons with diabetes die from ischemic heart disease [215].

Type 2 diabetes is seldom diagnosed based on the classical symptoms of hyperglycemia: thirst, polydipsia and weight loss. The disease is more often discovered through blood sugar measurements at the presentation of other conditions, like fatigue, infections, hypertension or heart disease. The awareness of type 2 diabetes has also increased, and persons with anthropometric and lifestyle risk factors are more frequently screened.

The co-occurrence of abdominal obesity, hyperglycemia, dyslipidemia and hypertension is known as the metabolic syndrome [216, 217], and is a risk factor for both CVD and diabetes. The combination of little physical activity [218], obesity [219] and ageing [220], can result in insulin resistance (Figure 1.8). The insulin-producing β-cells of the pancreas respond by increasing the production, resulting in hyperinsulinemia, until they cannot compensate any longer. β-cell dysfunction may be caused by a genetic predisposition [221] and overnutrition. Glucotoxisity, lipotoxisity, oxidative stress and ER stress can result in β-cell failure and is closely linked to inflammation [212, 222, 223] (Figure 1.8). The consequence is hyperglycemia and overt type 2 diabetes mellitus.

Hyperinsulinemia contributes to an altered metabolism of proteins, lipids (enhanced formation of VLDL and triglycerides, and hence LDL) and carbohydrates, and development of hypertension [224]. A high concentration of insulin is known to be atherogenic in itself: it enhances cholesterol transport into smooth muscle cells, formation of lipid in smooth muscle cells, proliferation of smooth muscle cells, increases the formation of lipid plaques, and augments collagen synthesis in the vascular wall [224].

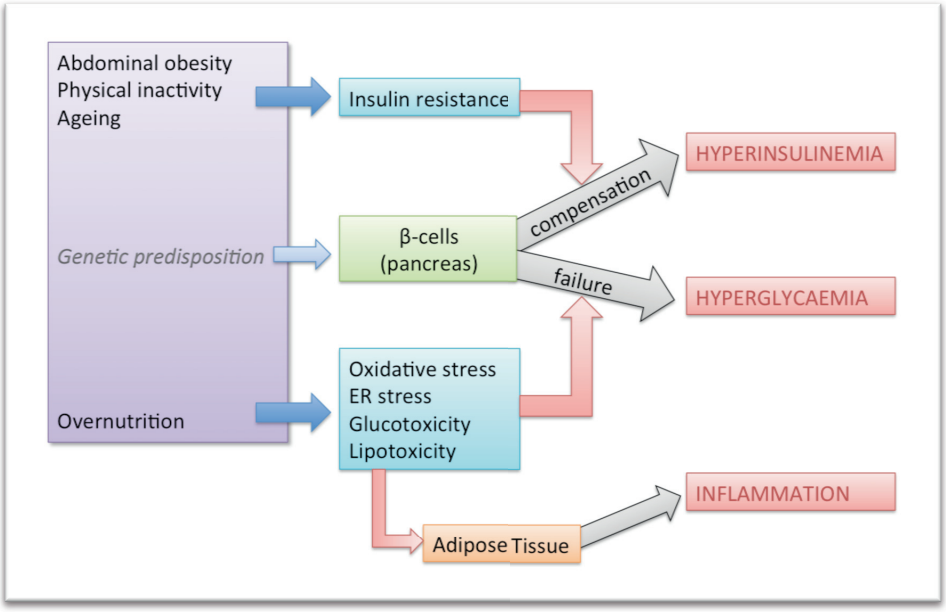

Figure 1.8 Simplified overview of mechanisms of hyperinsulinemia, hyperglycemia and inflammation in type 2 diabetes

In many studies, diabetes is treated as a confounder. However, as the pathophysiology of atherosclerosis may be altered/exaggerated and the diabetes population increases, it can be argued that diabetes patients should be investigated separately. In this thesis, one main focus has been to investigate the predictive value of different inflammatory biomarkers on fatal ischemic heart disease and comparing diabetes patients to controls.

Diabetes and general inflammation (Study I)

In addition to being a metabolic disorder, diabetes is regarded an inflammatory disease [212]. The cellular stresses caused by overnutrition can induce inflammation, or are exacerbated by inflammation (Figure 1.8). Hypoxia in expanding adipose tissue may initiate accumulation of macrophages [225]. Moreover, macrophages are recruited to pancreatic islets, probably in response to hyperglycemia and free fatty acids that cause metabolic stress and induce an inflammatory response in the β-cells [212]. Different inflammatory pathways may be activated through the cellular stresses, mostly in leukocytes in the liver and adipose tissue. Free fatty acids and advanced glycation end products (AGEs) may activate TLR4 and receptors for advanced glycation end products. This may lead to activation of the transcription factor NF-κB, which induces production of pro-inflammatory cytokines that can promote insulin resistance and also have a positive feedback effect [212, 222, 223].

Certain biomarkers have been shown to predict development of type 2 diabetes [226], among them is CRP [227, 228]. Increased concentrations of CRP are also associated with cardiovascular events in type 2 diabetes [229, 230], although its predictive value was partly explained by other risk factors in one study [231].

Based on the dominant role CRP has obtained in cardiovascular risk prediction and diabetes, *CRP* was included in our analyses. We performed an evaluation of CRP in risk prediction of fatal IHD in a diabetes population, and comparison with other, perhaps more specific markers.

Diabetes and macrophage activation (Study I)

Individuals with type 2 diabetes and coronary artery disease have more vulnerable plaques, with large necrotic cores and inflammatory cell infiltrates [232,

[233]. Increased apoptosis of macrophages due to ER stress and defective efferocytosis may be exacerbated by insulin resistance and hyperinsulinemia [234]. In addition, long-term hyperglycemia leads to production of AGEs, which bind to receptors on macrophages and smooth muscle cells and may contribute to increased inflammation, apoptosis and thinning of the fibrous cap [232].

The crucial position of macrophages in the development of plaques and plaque rupture, along with the influence of insulin resistance, led to inclusion of *neopterin* in our analyses. We studied the predictive value of this relatively specific marker of plaque vulnerability and macrophage activation on fatal ischemic heart disease in a group of diabetes patients compared to controls.

Diabetes and neutrophil dysfunction (Study II)

White blood cell count is associated with the development of diabetes [235, 236]. Persons with type 2 diabetes are at an increased risk of infections, partly due to defect neutrophil granulocytes. The neutrophils display impaired chemotaxis, microbicidal activity and phagocytosis [237-239], and excessive release of pro-inflammatory cytokines [240]. Both hyperlipidemia [241] and hyperglycemia [242-244] may 'prime' neutrophils, causing increased inflammation [245]. 'Priming' means that the neutrophil is moved from a resting to an intermediate state, where the cell is prone to become activated [246].

Moreover, other studies show that neutrophils are associated with the risk of atherosclerosis, including in type 2 diabetes. In one study, neutrophil/lymphocyte ratio was associated with ischemic cardiovascular disease in diabetes [247]. High glucose concentrations increased endothelial-neutrophil cell adhesion, which may contribute to the atherosclerotic process [248]. Furthermore, in a study of unstable coronary plaques, there were higher amounts of neutrophils in plaques from diabetes patients [119]. Markers of neutrophil activation are thus potential candidates in risk prediction.

In patients with endothelial dysfunction, stable angina and acute coronary syndromes [130, 249, 250], myeloperoxidase was associated with diabetes. Furthermore, increased myeloperoxidase concentrations have been linked to increased lipid values [251]. Regarding the potentially positive effects of lactoferrin, these may be attenuated in diabetes, as lactoferrin may be inhibited by glycation

[252, 253]. Low lactoferrin concentrations in diabetes may also reflect neutrophil dysfunction [254], which could influence the atherosclerotic process. As neutrophil function is impaired in diabetes, and the neutrophils are important in the pathophysiology of atherosclerosis, both myeloperoxidase and lactoferrin were included in our analyses. Myeloperoxidase and lactoferrin were selected because of their diverse functions and as they represent the two most important granule subsets.

In summary, four different markers of inflammation were included for evaluation of predictive ability of fatal ischemic heart disease in a group of diabetes patients compared to controls. In study I, CRP, a general marker of low-grade inflammation, and neopterin, a specific marker of monocyte activation, were compared. In study II, two markers from neutrophil granulocytes were included, i.e. myeloperoxidase and lactoferrin.

1.4 Genetic predisposition for CVD

Specific background for study III

Genetic predisposition represents another aspect of the individual risk for CVD. Familial clustering of premature coronary artery disease is an independent risk factor of CVD, and is considered as evidence for a significant genetic burden. In persons who experience an early cardiovascular event, the genetic influence may be more significant and less dependent on non-genetic risk factors than at older age [8, 9]. A few Mendelian disorders (i.e. with a monogenetic cause) have been discovered, such as familial hypercholesterolemia, where there is a mutation in the genes coding for the LDL receptor or apoB. However, most cases of CVD are multifactorial, i.e. result from several genes, each with a small effect, working alone or in combination with modifying genes or environmental factors.

In the search for genes related to different diseases, the main approaches have been family linkage studies and association studies. Family linkage studies detect genetic markers that follow a disease or trait in family members. The genetic markers indicate a chromosomal region, in which the genes associated with the disease are located. This approach has been successful in discovering diseases with a monogenetic causes, but less so in complex diseases like CVD.

Association studies have been performed on candidate genes, which are identified by linkage studies, or based on knowledge of the pathological process. The relevant genes can then be investigated in case-control studies. However, very few of the findings have been replicated in other studies.

Over the last few years, another approach has become possible, namely the genome-wide association studies (GWAS). The Human Genome Project [255], the SNP Consortium [256] and the HapMap project [257] have collectively provided a catalogue of ~10 million common DNA variants, primarily SNPs. Based on knowledge of these SNPs and their linkage disequilibrium (non-random association of alleles at different loci), approximately 1 million SNPs are selected as 'tag' SNPs. GWAS allow an hypothesis-free approach: the genome of cases and controls is 'scanned' with micro-array chips that detect the 'tag' SNPs. Specific GWA analyses can then identify associations between haplotypes and the phenotype of interest, using a threshold of significance at $p < 5x10^{-8}$ due to multiple testing. The rationale behind the GWAS is the "common disease, common variants"-hypothesis: a limited number of genetic variants with high frequency (>5%) contribute to common diseases. GWAS studies in CVD have resulted in the discovery of several common variants with moderate effects [258, 259]. For the time being, however, the approach is unable to detect rare SNPs that may have substantial impact.

1.4.1 Variations in inflammatory genes and the risk of atherosclerosis

Considering that inflammation is essential in the pathogenesis of atherosclerosis, alterations in genes coding for inflammatory proteins could influence the development of the disease. Thus, genetic variations in several inflammatory pathways and proteins have been investigated, for example IL-6, tumor necrosis factor α and TLR4 [260-263].

Polymorphisms related to activation of the complement system have received attention as potential candidate genes, as already mentioned. *MBL2* has several polymorphic sites, closely linked to alterations in protein function and concentration. Most of the findings on MBL deficiency and atherosclerosis were done in high-risk populations, e.g. with high prevalence of CVD [197], systemic lupus erythematosus [201, 203], rheumatoid arthritis [191] or diabetes [202], and less is

known about the risk in young and healthy individuals. Even if little is known about the ficolins compared to MBL in health and disease [264], they can also be considered potential candidates, as they activate the lectin pathway.

As activation of the complement system through the lectin pathway possibly protects against atherosclerosis [178], we hypothesized that polymorphisms in *MBL2* causing MBL deficiency are related to the development of MI. Further, variations in the ficolin genes may be associated with atherosclerosis as well. These associations were investigated in a case-control study of young and middle-aged MI patients.

2 Hypotheses and aims

We hypothesized that 1) type 2 diabetes accelerates the atherosclerotic process by increasing inflammation, and that 2) markers of inflammation are related to atherosclerosis and can be used in risk prediction of ischemic heart disease. This led to the aims of study I and II.

We also hypothesized that 3) persons who experience MI at an early age are likely to have genetic risk factors in addition to conventional risk factors, 4) polymorphisms in inflammatory genes increase the risk of MI and specifically 5) polymorphisms in genes of proteins that activate the lectin pathway of the complement cascade lead to increased risk of MI. This led to the aims of study III.

The specific aims were to

Study I: Evaluate serum concentrations of CRP (marker of low-grade inflammation) and neopterin (marker of monocyte activation) as predictors of fatal ischemic heart disease in persons with newly diagnosed diabetes compared with controls.

Study II: Evaluate serum concentrations of lactoferrin and myeloperoxidase (markers of neutrophil granulocytes) as predictors of fatal ischemic heart disease in persons with newly diagnosed diabetes compared with controls.

Study III: Determine the influence of functional polymorphisms in *MBL2* and genes of the ficolins on risk of MI in young and middle aged individuals.

3 Populations and methods

3.1 HUNT

The county of Nord-Trøndelag is located in the middle of Norway (Figure 3.1) and has a stable population of about 131 000 (2010). The population is fairly representative for Norway as a whole. The inhabitants are served by two local hospitals in Levanger and Namsos, and one central university hospital, St. Olav's Hospital in Trondheim. The Nord-Trøndelag health studies (HUNT studies) are the most comprehensive health surveys in Norway. They were carried out in 1984-86 (HUNT1), 1995-97 (HUNT2) and in 2006-08 (HUNT3). The data included in this thesis are based on HUNT1 and HUNT2.

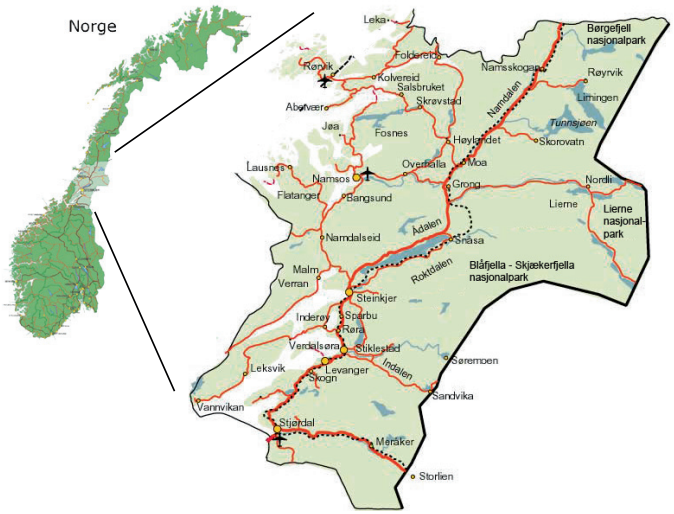

Figure 3.1 The county of Nord-Trøndelag. Reprinted with permission from North Trøndelag County Council.

3.2 HUNT1 (Study I & II)

All inhabitants over 20 years in the county were invited, and 74 599 (88.1%) participated. They received a questionnaire (Q1-H1, Appendix) by mail together with an invitation letter. A second questionnaire (Q2-H1, Appendix) was handed out at the clinical examination. Briefly, the questionnaires contained questions about previous illness, contact with general physician and hospitalization for the last 12 months, lifestyle and quality of life. Height, weight, blood pressure and pulse were measured, as previously described [265].

A random blood glucose sample was drawn from all participants aged 40 or older, in order to screen for unknown diabetes mellitus type 2. The 1980 WHO definition of diabetes was used [266]. If random blood glucose was ≥ 8 mmol/L, the subjects were invited back within 1-5 days for a fasting blood glucose sample. Fasting blood glucose ≥ 7 mmol/L was diagnosed as type 2 diabetes. If the fasting blood glucose was < 7 mmol/L, an oral glucose tolerance test was performed. A two-hour post-load value ≥ 11 mmol/L was also classified as diabetes. Through this procedure 428 persons were diagnosed with previously unknown type 2 diabetes (Figure 3.2).

The objectives for the original studies were to study prevalence of diabetes [267], CVD risk factors among newly diagnosed diabetes patients [268] and to evaluate the effect of glycemic control on long-term morbidity and mortality [215, 265, 269, 270]. The individuals in the case group were followed for up to 10 years, but it was not feasible to monitor all 428 newly diagnosed diabetes patients. The inclusion process and reasons for exclusion are shown in Figure 3.2, and is described in detail elsewhere [268]. 205 persons with newly diagnosed diabetes and 205 controls were included in a follow-up study, but because of missing blood samples, the final study groups consisted of 200 diabetes patients and 198 controls. Study I and II of this thesis were conducted in these groups.

The study participants underwent a comprehensive clinical examination and completed questionnaire Q2-H1 if it had not been done previously. A medical history was recorded, and a general physical examination performed. The examination included auscultation, pulse palpation, measurement of blood pressure, electrocardiogram (ECG) recording, reflex testing, measurement of height, weight and waist and hip circumference. In addition, a venous blood sample was drawn and analysed for creatinine, urea (carbamide), total cholesterol, HDL cholesterol, and C-peptide, and serum was stored for future analyses.

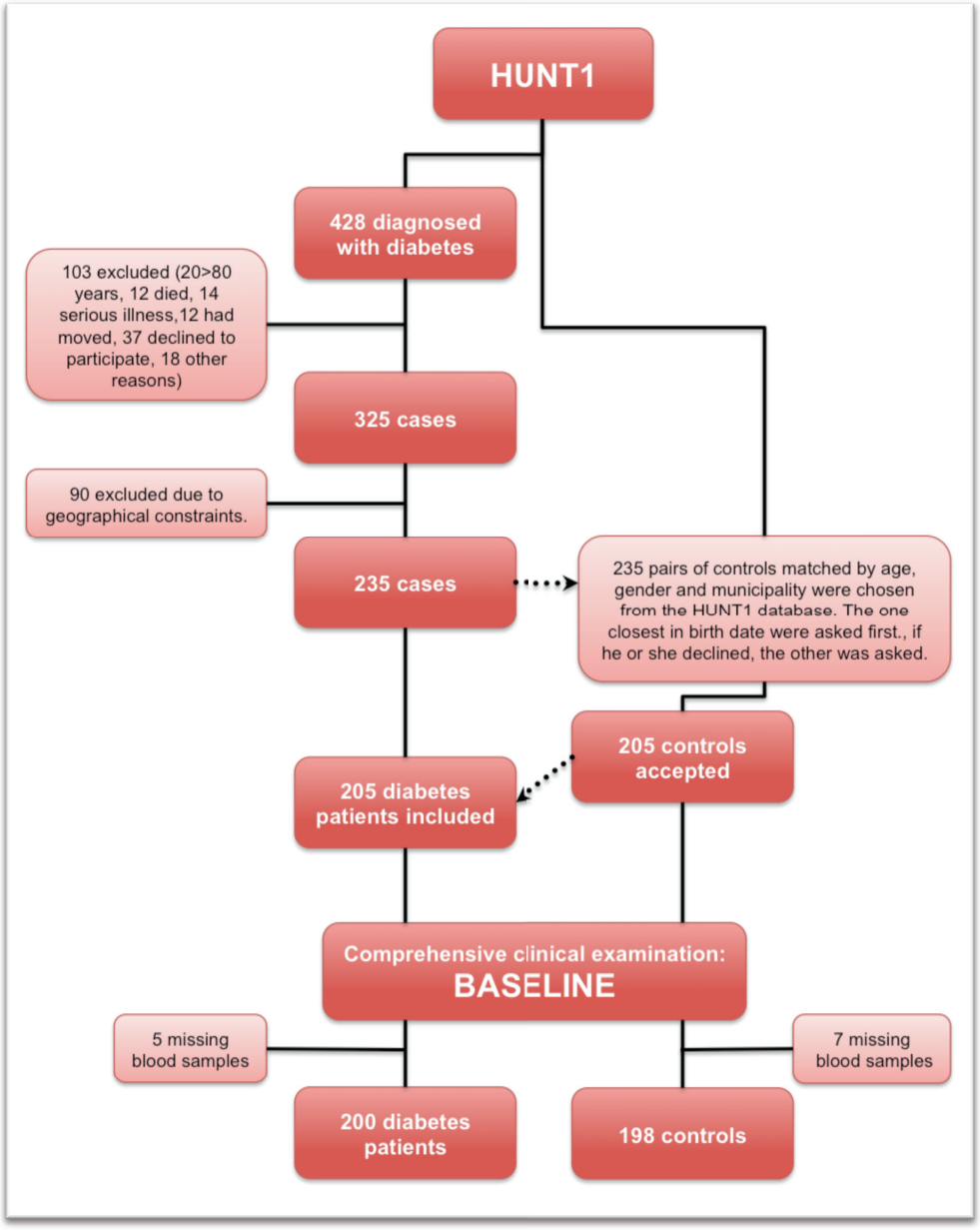

Figure 3.2 – Study I & II, inclusion process. Modified and reprinted with permission from Elsevier.

3.2.1 Mortality

The Norwegian Causes of Death Registry, Statistics Norway, receives all death certificates from Norwegian citizens. The HUNT1 data was linked to the Causes of Death Registry by means of the 11-digit personal ID-number, and causes of death were obtained. Causes of death were classified according to the International Classification of Disease (ICD) system. The number of deaths from ischemic heart disease (ICD-9 code 410-414 and ICD-10 code I20-I25) was obtained. Individual person time at risk was calculated from the day of clinical examination until death from ischemic heart disease, death from other causes or until study termination of the study on 31st of December 2004.

3.2.2. Serum analyses

The serum samples were stored at -40 °C until 2005 when they were moved to -80 °C. They had been thawed two times, once for use in analysis in 1987 and once for aliquotation in 2007. C-reactive protein, neopterin, lactoferrin and myeloperoxidase were analysed in the stored serum. In-house enzyme-linked immune-sorbent assays were used to detect lactoferrin, myeloperoxidase [271, 272] and CRP. For detection of CRP, rabbit anti-human CRP antibody (Sigma, St. Louis, MO, USA) was used to coat microtiter plates. Diluted samples and standards were added, and the secondary antibody was goat anti-human CRP (Sigma). Peroxidase-conjugated anti-goat antibody (Dako Cytomation, Glostrup, Denmark) was added, and o-phenylenediamine 0.15mg/mL with 0.015% H_2O_2 was used as substrate. The reaction was stopped with 2M H2SO4, and optical density was measured at 492 nm (Sunrise microplate reader, Tecan, Männedorf, Switzerland). The assay was calibrated against a commercial assay (Immunlite 2000 High Sensitivity, Diagnostic Products, Los Angeles, California, USA). Neopterin was analysed using a commercial kit, as indicated by the manufacturer (Brahms, Henningsdorf, Germany).

3.3 HUNT2 (Study III)

The second wave of the Nord-Trøndelag health study took place in 1995-97. All inhabitants aged 13 or older were invited. Totally, about 75 000 (70%) participated, and 65 237 were in the adult population [273]. Participants answered questionnaires (Q1-H2, Q2-H2, Appendix), and a venous blood sample was drawn from those 20 years or older, for analysis of total cholesterol, HDL cholesterol, triglycerides, glucose and creatinine. At the clinical examination blood pressure, heart rate, height, weight, hip- and waist circumference were measured.

A case-control study (nested within the HUNT cohort) on genetic predisposition for MI was performed. Of the 65 237 HUNT2 participants, 57 133 individuals met the inclusion criteria for the baseline cohort (Figure 3.3). The inclusion criteria were available DNA and no previous CVD. Reasons for exclusion were: DNA not available (n = 2687), previous MI (n = 850), previous angina pectoris (n = 1850), previous stroke (n = 846) or a combination of the above mentioned (n = 1871).

The baseline cohort was linked to a registry of MIs in Nord-Trøndelag. Based on information from medical records from the only two hospitals in the county (Levanger Hospital and Namsos Hospital), incident MIs were registered. Registration was initiated in 2000, and MIs from the onset of HUNT2 (1995) until 2000 were registered retrospectively. From 2001, the registration has been continuous. MIs in study III were registered in the period from 1995 to the end of 2008. Criteria for MI were 1) specific changes in biomarkers for myocardial damage, 2) specific symptoms according to case history information and 3) specific ECG changes [21]. As shown in Figure 3.3, 1691 individuals from the baseline cohort had been hospitalized with MI during the inclusion period. Based on power calculations (as detailed in *3.6.2*), the 370 youngest MI-patients were chosen as cases, and 370 controls were randomly selected, matched by age (±2 years) and gender. All controls were at risk for MI at the time when their matched case had the MI event.

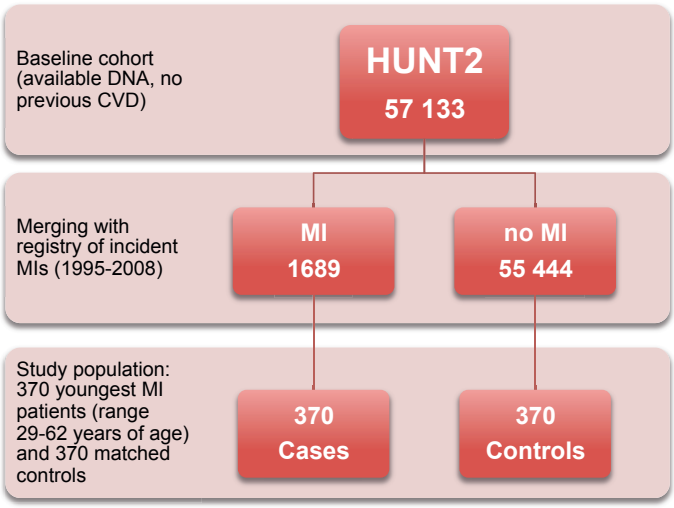

Figure 3.3– Study III, inclusion process. MI: myocardial infarction.

Total cholesterol > 6.2 mmol/L was classified as hypercholesterolemia [274]. The Framingham risk score [275] was calculated based on variables from the HUNT2 database (age, HDL-cholesterol, total cholesterol, systolic blood pressure, antihypertensive treatment, smoking and diabetes). A modified set of criteria was used to classify the metabolic syndrome [217]. The criteria were 1) *central obesity*, (i.e. men: waist circumference ≥ 94 cm; women: waist circumference ≥ 80 cm) plus two of the following four criteria 2a) *low HDL cholesterol* (men < 1.03 mmol/L; women < 1.29 mmol/L), 2b) *hypertension* (systolic blood pressure ≥ 130 mmHg or diastolic blood pressure ≥ 85 mm Hg, or treatment for hypertension), 2c) *fasting plasma glucose ≥ 5.6 mmol/L* or previously diagnosed *type 2 diabetes*, 2d) *fasting triglycerides > 1.7 mmol/L*. MI before the age of 60 in first-degree relatives was considered a positive family history.

3.3.1 Genetic analyses

DNA was extracted from peripheral leukocytes by the HUNT biobank. Two methods were used, one manual (Puregene, Gentra Systems, Minneapolis, MN) and one robotic (Autopure LS, Gentra Systems).

The MBL gene (*MBL2*) was sequenced in 1989 [276, 277]. *MBL2* lies on chromosome 10q11.1–q21 and consists of four exons interrupted by three introns. Exon 1 encodes the signal peptide of the protein. Three point mutations

(*B*, *C* and *D*, wild type is denoted *A*) are found in exon 1 [278-280], which all have an effect on serum concentration of functional MBL. Additionally, three promoter polymorphisms exist: -550 (*H/L* variant), -221 (*X/Y* variant) and the last in position +4 in the 5'-untranslated portion of the gene (*P/Q* variant) [280-282].

In study III, the most significant *MBL2* promoter polymorphism (*X/Y*, rs7096206) and the three exon 1 polymorphisms (*B*; codon 54; rs1800450, *C;* codon 57; rs1800451 and *D;* codon 52; rs5030737) were analysed. To simplify the interpretation, the structural variant alleles are pooled to one *O*-allele. The *O*-alleles are always found on a *Y* background, and this defective genotype is thus denoted *YO*. Combining the promoter variant with the structural alleles gives rise to six haplotypes and three haplotype groups which correspond to serum concentrations of functional MBL as shown in Table 3.1 [187].

MBL2 haplotype groups		Concentration of functional MBL
YA/YA ***YA/XA***	*YA/YA, YA/XA*	Normal
XA/XA ***YA/YO***	*XA/XA, YA/YO*	Intermediate
XA/YO ***YO/YO***	*XA/YO, YO/YO*	Deficient

Table 3.1 MBL haplotype groups and corresponding serum concentrations

Among the ficolins (Table 3.2), we included one common promoter polymorphism in *FCN1* (-542), and two different SNPs in *FCN2*, which are known to cause increased (+6424) and decreased (+6359) binding capacity of N-acetylglucosamine, a microbial carbohydrate [283]. *FCN3* is less polymorphic, but a frame-shift variation (+1637) is known to cause a 50% reduction of serum ficolin-3 in heterozygotes, and total ficolin-3 deficiency in homozygotes [284]. This was also included in spite of a very low frequency. *FCN1* and *FCN2* lie on chromosome 9q34, while *FCN3* lie on chromosome 1p36.11.

	rs number	Position	Frequency*	Rationale
FCN1	rs10120023	-542, promoter	0.31	Common, but unknown function
FCN2	rs17549193	+6359, exon 8, amino acid substitution	0.25	Reduced binding of *N*-acetylglucosamine
FCN2	rs7851696	+6424, exon 8, amino acid substitution	0.1	Increased binding of *N*-acetylglucosamine
FCN3	rs28357092	+1637, exon 5, frame-shift	0.01	Heterozygosity causes 50% serum reduction, homozygosity cases total deficiency.

Table 3.2 Overview of the ficolins genes. * Minor allele frequency Denmark [207]

Genotyping was performed using polymerase chain reactions (PCR) (DNA Engine Gradient Cycler, PTC-200, MJ Research, St. Bruno, Canada) and subsequent pyrosequencing (Pyro sequencer PSQ 96MA (Pyrosequencing AB; Biotage, Uppsala, Sweden))[285]. Four different PCRs were performed (Table 3.3).

About 20 ng DNA was used in amplification of the sequences in question. A 25 µL PCR reaction was performed, using 0.2 mM deoxyribonucleotide triphosphate (dNTP), 0.5 U Taq polymerase, 1.5 mM $MgCl_2$ together with the PCR primers in PCR buffer. One of the primers in each pair was biotinylated. The PCR reaction was performed using the following program: denaturation at 95 °C for 2 minutes, followed by 40 cycles á 94 °C for 30 seconds, 58 °C for 30 seconds, 72 °C for 30 seconds, and a final elongation period for 5 minutes at 72 °C. Evaluation of the PCR products by agarose electrophoresis showed specific bands for the expected molecular weights. The PCR product was further used for pyrosequencing.

The pyrosequencing was performed with a standard protocol. Briefly, streptavidin sepharose beads (GE Healthcare, Uppsala, Sweden) were attached to the biotinylated PCR product. The beads were captured on a filter plate, and the liquid was removed by vacuum filtration (Vacuum Preparation Tool, Biotage AB). The immobilized DNA strands were then washed in 70% ethanol (10 seconds), and subsequently in 0.5M NaOH (10 seconds). Finally, the samples were transferred to a PSQ 96-plate and resuspended in annealing buffer with 40 pmol of the relevant sequencing primer. This mixture was incubated for 2 minutes at 80 °C, and cooled down to room temperature before analysis on the

pyrosequencer. The principle for pyrosequencing is to "sequence by synthesis". The complimentary DNA strand is synthesized one base at the time, and a correct incorporation leads to a visible light signal detected by a camera. The light signals generate a pyrogram, which reveals the sequence [286].

PCR reaction	Gene	Primer	Primer sequence (5´-3´)
PCR 1	*MBL2* exon 1, codon 52, 54 and 57 (*D*, *B*, *C*)	Forward Reverse Sequencing	CCTTCCCTGAGTTTTCTCAC AACAGCCCAACACGTACCTG CGTACCTGGTTCCCCCTTTTCT
PCR 2	*MBL2* promoter -221 (*X/Y*)	Forward Reverse Sequencing	TGGTGTGAGAAAACTCAGGGAAG GCACGGTCCCATTTGTTCTC CTGGAAGACTATAAACATGCTT
PCR 3	*FCN1* -542	Forward Reverse Sequencing	TCCCAAATACTATTTCCATCATATC CTTCAATTTCTCCAGCTGTAACT ATCTTGCACCAGCCC
PCR 3	*FCN3* +1637	Forward Reverse Sequencing	GAGCCAGGGCGCCACCTT CCCCCCTCGGTGTCCATGT CTACCTGAGGGCAGG
PCR 4	*FCN2* +6359, +6424	Forward Reverse Sequencing +6359 Sequencing +6424	TCACATTTCCTCCTGCACAGG TTGACACATGGCAGTTTTTGTAC CACAGGAGATTCCCTGA GATCTTAACACCGGAAATT

Table 3.4 PCR reactions, SNPs and primers

3.4 Shared variables (Study I, II & III)

Having a systolic blood pressure ≥ 140 mmHg, diastolic blood pressure ≥ 90 mmHg or using anti-hypertensive medication was classified as hypertension. Based on self-reporting, the participants were classified as smokers or non-smokers (never/previous), and previous CVD was defined as a having had MI, angina pectoris or stroke.

3.5 Ethics

The study protocols conformed to the Helsinki Declaration, and the studies were approved by the Regional Committee for Medical Research Ethics and the Data Inspectorate Norway. All HUNT participants signed a consent that included participation in morbidity and mortality follow-up studies.

3.6 Statistical analysis

Data are given as means, hazard ratios (HRs) or odds ratios (ORs) with 95% confidence intervals (95% CI), or as numbers with proportions in parenthesis. P-values < 0.05 were considered statistically significant, except for the comparison of haplotype frequencies, section *3.6.2*.

3.6.1 Study I & II

Comparison of continuous variables between diabetes patients and controls were done with the Mann-Whitney U test, due to non-normal distribution of the data. The Chi square test was used to compare categorical variables.

The incidence of fatal ischemic heart disease was plotted in Kaplan-Meyer plots. The biomarkers were divided into tertiles. According to the baseline concentration of the biomarkers, uni- and multivariate Cox regression analyses were used to estimate hazard ratios and 95%CI for death from ischemic heart disease. In multivariate analyses, adjustments were made for age, gender, body mass index (continuous), total cholesterol (continuous), previous cardiovascular disease (yes/no) and hypertension (yes/no). Departures from the proportional hazards assumption were evaluated using graphical procedures (log-log plots).

In study II, validation of the Cox regression analyses was performed. To get robust estimates for calculation of HR and 95 % CI, the coefficients from the models were bootstrapped (400 runs). Bootstrapping also provides a corrected value of Somer's Dxy rank correlation coefficient between predicted log hazards and observed survival time. The value of Dxy varies from -1 to 1, where 0 denotes no correlation, while an absolute value of 1 denotes full correlation. An absolute value over 0.3 indicates a strong relationship. The corrected Dxy adjust for overfitting and is therefore more accurate than the uncorrected.

Data in study I and II were analysed with SPSS statistical software, version 15.0 for Windows (SPSS Inc., Chicago, Illinois, USA). The internal validation in study II was performed with the Design package (version 2.3-0) in the R statistical environment (version 2.6.1) [287].

3.6.2 Study III

A power calculation was performed based on an expected frequency of 0.08 of the *MBL2* combined low expressing haplotype (*YO/YO+XA/YO*) in Caucasians. To detect an odds ratio (OR) of 2.0, assuming a power of 80 % and a 5% significance level, 320 persons were required in each group. 370 persons were included in each group, in order to account for possible variations in the genotype distribution in small datasets. The study population was too small for analyses stratified by gender.

Because the cases and controls were individually matched, paired tests were used. Due to non-normal distribution, Wilcoxon's signed rank test was used to compare continuous and ordinal variables. McNemar's test was used to compare the number of discordant pairs. The Chi square test was used to compare allele frequencies. Deviation from the Hardy-Weinberg equilibrium was calculated using the Chi square test.

Conditional logistic regression was performed to analyse the relationship between MI and the three inferred *MBL2* haplotype groups. Three additional models were developed, where adjustments were made for conventional risk factors of MI, the Framingham risk score and the metabolic syndrome, respectively.

To avoid false positive conclusions, the alpha levels of significance for the comparisons of haplotype frequencies between cases and controls were obtained by permutation testing, using 10,000 permutations. This method is considered the gold standard, and a result is considered significant if the observed p-value is lower than the empirical p-value found under permutation.

Permutation testing was performed using the R package, version 2.14.1[287], and Stata/MP for Mac, version 11.2, (Stata Corp., College Station, Texas) was used for other analyses in study III.

4 Results

4.1 Study I & II

Individuals with diabetes had higher BMI, were more often hypertensive and had a higher frequency of established cardiovascular disease than the control group. There was no difference in smoking status (data not shown).

44 persons with diabetes and 28 persons without diabetes died from ischemic heart disease in the follow-up period. Mean individual person time at risk was 12.6 years (range 0.1 – 19.7 years). Among those who died from ischemic heart disease, 16 persons with diabetes and 8 persons without diabetes had known cardiovascular disease at baseline (p = 0.49). Both total cholesterol and HDL-cholesterol were lower in the diabetes group, but the total cholesterol – HDL-cholesterol ratio were higher compared to individuals without diabetes.

The highest tertile of neopterin and CRP, and the two highest tertiles of lactoferrin were individual predictors of fatal ischemic heart disease in patients with diabetes mellitus (Table 4.1, Figure 4.1). Myeloperoxidase did not predict ischemic heart death in the diabetes patients. None of the biomarkers predicted death from ischemic heart disease in the control group.

		Hazard Ratio	95% Confidence interval
Study I	Neopterin		
	Tertile I	1.0	Reference
	Tertile II	1.39	0.58-3.37
	Tertile III	2.59	1.11-6.01
	CRP		
	Tertile I	1.0	Reference
	Tertile II	1.79	0.74-4.33
	Tertile III	2.45	1.05-5.69
Study II	Lactoferrin		
	Tertile I	1.0	Reference
	Tertile II	2.54	1.00-6.45
	Tertile III	4.06	1.72-9.60
	Myeloperoxidase		
	Tertile I	1.0	Reference
	Tertile II	1.25	0.54-2.90
	Tertile III	1.94	0.85-4.43

Table 4.1 Hazard ratios for the tertiles of the biomarkers in the newly diagnosed diabetes patients. Adjusted for age, gender hypertension, BMI, previous CVD and total cholesterol.

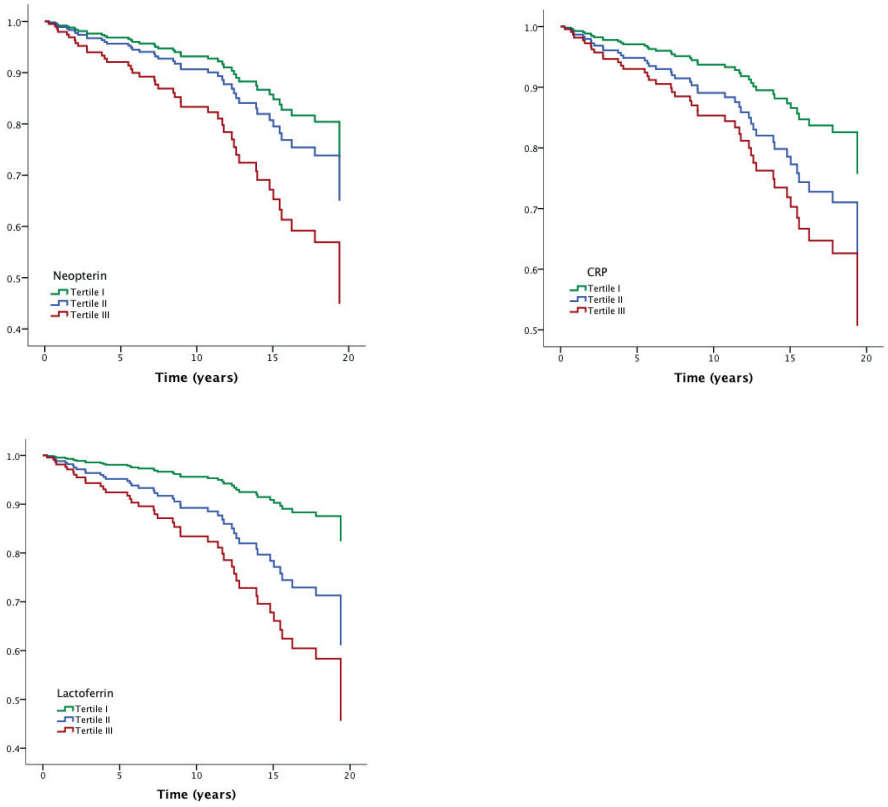

Figure 4.1 Survival curves according to tertiles of the biomarkers at baseline in the diabetes group. Adjusted for age, gender hypertension, BMI, previous CVD and total cholesterol. Modified and reprinted with permission from Elsevier.

When CRP was included in the multiadjusted models with neopterin and lactoferrin, neopterin and the two highest tertiles of lactoferrin remained significant whereas CRP did not (Table 4.2).

Multiadjusted models				
Study I	Neopterin	1.0	**CRP**	1.0
		1.48 (0.61-3.62)		1.85 (0.75-4.55)
		2.39 (1.01-5.69)		2.12 (0.89-5.05)
Study II	Lactoferrin	1.0	**CRP**	1.0
		2.67 (1.03-6.93)		2.07 (0.84-5.10)
		3.97 (1.60-9.90)		1.88 (0.78-4.54)

Table 4.2 Hazard ratios for the tertiles of neopterin and lactoferrin in the newly diagnosed diabetes patients. Adjusted for age, gender, hypertension, BMI, previous CVD, total cholesterol and CRP.

For lactoferrin, the changes in HR after bootstrapping were small (data not shown), and it did not alter the significance of the variables. Somer's Dxy for the model including lactoferrin in the persons with diabetes was -0.55 before correction and -0.47 after correction by bootstrapping.

CRP concentrations were higher in the diabetes group compared to the control group at baseline ($p < 0.0001$), whereas neopterin did not differ between the two groups. In the diabetes group, neopterin was significantly higher among those with preciously known CVD than in those without ($p = 0.001$). There were no differences in lactoferrin or myeloperoxidase concentrations between the groups at baseline, or between those with and without previous CVD.

4.2 Study III

The MI cases suffered from more cardiovascular risk factors at baseline: they had higher BMI, waist-hip ratio, and Framingham risk score, and more unfavourable lipid values. Hypertension, diabetes, smoking, metabolic syndrome and a family history of cardiovascular diseases were more frequent in the case group.

The *MBL2* genotypes were pooled into three haplotype groups as shown in Table 4.3. There were more individuals with *MBL2* haplotypes causing MBL deficiency in the case group compared to the controls. There were no significant differences in the ficolin genotypes between the groups.

	***MBL2* genotypes**		***MBL2* haplotypes**		**Serum concentrations of functional MBL**
	Cases	Controls	Cases	Controls	
YA/YA	112 (30 %)	117 (32 %)			
YA/XA	86 (23 %)	100 (27 %)	198 (54 %)	217 (59 %)	Normal
XA/XA	18 (5 %)	12 (3 %)			
YA/YO	91 (25 %)	108 (29 %)	109 (29 %)	120 (32 %)	Intermediate
XA/YO	43(12 %)	24 (7 %)			
YO/YO	20 (5 %)	9 (2 %)	63 (17 %)	33 (9 %)	Deficient
p-value		**0.025**		**0.005**	
Alpha level by permutation		**0.028**		**0.023**	

Table 4.3. Haplotype frequencies for *MBL2*.

Conditional logistic regression showed that *MBL2* haplotypes causing MBL deficiency were positively associated with MI. The odds for being MBL deficient was twice as high in the case group compared to controls, OR 2.04 (1.29 – 3.24), p = 0.003. There were minor changes in ORs when adjustments were made for conventional cardiovascular risk factors (hypertension, hypercholesterolemia, BMI, smoking and diabetes) (data not shown). Due to missing data on family history in 131 case-control pairs (71 cases and 66 controls, p=0.64), this was not included in the analysis.

5 Discussion

Several aspects of inflammation and ischemic heart disease were examined in this thesis.

5.1 Inflammatory biomarkers as predictors of fatal ischemic heart disease – Study I & II

5.1.1 Risk prediction in newly diagnosed diabetes patients

CRP, neopterin and lactoferrin were independent predictors of fatal ischemic heart disease in a group with newly diagnosed diabetes, but not in the control group. These three biomarkers represent different aspects of the inflammatory response.

When CRP was added to multivariate Cox regression analyses including neopterin and lactoferrin in turn, both of the cellular markers remained significant, whereas CRP did not. Neopterin and lactoferrin represent cells involved in plaque formation and may thus be more specific than CRP, which more generally reflects inflammation.

Neopterin has emerged as a promising marker of plaque instability [288-293]. Our results support this in diabetes patients, a group known to present with rupture-prone plaques. Moreover, we found that neopterin was increased in persons with previous CVD compared to those without in the diabetes group, which indicates that neopterin is related to active atherosclerotic disease among these patients. Few other studies have focused on neopterin, diabetes and atherosclerosis. In a Swedish study, neopterin was clearly related to severity of critical limb ischemia in diabetes patients, and it was increased in persons with critical limb ischemia compared to controls [294]. In contrast to CRP, neopterin was also an independent predictor of mortality in multivariate logistic regression among the same patients, but the association was lost when all inflammatory markers were included in the analysis [295].

In a recent study CD4+CD28^{null} T-cells were more abundant in diabetes patients than controls [296]. Furthermore, there was a strong association with first cardiovascular event in the diabetes patients. This was not the case for CRP. The

number of CD4+CD28^{null} T-cells was correlated with glycemic control and poor outcome after an acute cardiovascular event [296]. As these T-cells are active producers of interferon-γ, which stimulate macrophages to neopterin production [160], this finding could indicate one possible mechanism for increased neopterin and plaque vulnerability in diabetes. The mechanism behind the clonal expansion is not known, but it may reflect a chronic response to exogenous antigens or autoantigens [161, 297]. Altogether, neopterin may be especially promising for risk prediction of atherosclerotic events in diabetes patients.

CRP was included in our analyses both for evaluation of predictive ability of fatal ischemic heart disease, and for comparison with the other markers. The finding that the association of CRP and fatal ischemic heart disease was weakened by inclusion of neopterin or lactoferrin in our study indicates a limited potential of CRP for prediction of fatal ischemic heart disease in diabetes. This is supported by other studies: Bruno et al [298] found that CRP was associated with short-time mortality in a population-based study on diabetes patients. However, the usefulness of CRP for 5-year mortality prediction was limited. Moreover, another study of patients with acute coronary disease demonstrated that in diabetes patients, CRP was not associated with 1-year events, contrary to patients without diabetes [299].

CRP was increased at baseline in the diabetes group compared to the controls, consistent with the findings of others [227, 228]. This may reflect a diffuse low-grade inflammation already present in persons with diabetes, due to hyperglycemia and subsequent increase in IL-6 [300], or increased adipose tissue. Furthermore, CRP was not associated with established CVD among the diabetes patients, in consistence with the findings of Bowden et al. [301]. The increased basal CRP may contribute to attenuating the predictive ability of CRP of cardiovascular events in this patient group.

In theory, *lactoferrin*, with its anti-inflammatory properties [142, 144] could provide protection against atherosclerosis. Orally administrated lactoferrin was associated with decreasing adipose tissue in obesity [302] and lowering of blood glucose [303]. Lactoferrin enhances the effects of insulin, which may contribute to

its anti-inflammatory effects [304-306]. In obese subjects and persons with altered glucose tolerance, high lactoferrin concentration was associated with endothelial function, and low lactoferrin concentration was associated with an unfavourable lipid profile [307]. Moreover, lactoferrin improves post-prandial lipid profile in severely obese subjects [308]. Lactoferrin also inhibits macrophage uptake of cholesterol [147, 148] and upregulation of adhesion molecules and pro-inflammatory cytokines in endothelial cells [149, 309]. These studies indicate that lactoferrin could possibly protect against atherosclerosis.

Among the newly diagnosed diabetes patients in our study, however, the two highest tertiles of lactoferrin were predictors of fatal ischemic heart disease. In diabetes, the anti-inflammatory effects of serum lactoferrin may be impaired due to glycation [253]. Another explanation may be that the increased concentration of lactoferrin acts as a surrogate marker for neutrophil count and activity [310] associated with coronary atherosclerosis. This also applies for the previous findings from our group [145], where lactoferrin was associated with the presence of significant coronary artery stenosis, as well as for other inflammatory diseases like rheumatoid arthritis [146, 311].

Furthermore, lactoferrin may worsen atherosclerosis by inhibiting apoptosis of neutrophil granulocytes [311, 312]. At inflammatory sites, neutrophils have to live long enough to fight the pathogen, but an inappropriate delay of apoptosis may lead to tissue damage [313]. Neutrophil apoptosis induces anti-inflammatory mechanisms in macrophages in order to resolve the process [314], and a prolonged neutrophil life span therefore indicates both increased inflammation and deprived anti-inflammatory activity in macrophages, which may accelerate the atherosclerotic development.

Delayed neutrophil apoptosis is seen in unstable angina patients [315, 316], compared to patients with stable angina. This was especially pronounced in full blood experiments compared to isolated cells, indicating that soluble factors or other blood components may activate survival signals [316]. In diabetes, neutrophils may be primed and activated due to hyperglycemia, AGEs [242-244] or hyperlipidemia [241]. Priming of neutrophil granulocytes can also lead to excessive release of lactoferrin at the site of inflammation, and as lactoferrin inhibits neutrophil apoptosis, this may contribute to a prolonged and chronic

inflammation [311, 312]. Moreover, primed neutrophils also enhance the synthesis of neopterin, which may contribute to development of unstable plaques and increase the risk of fatal ischemic heart disease [289].

Some studies show that lactoferrin activates macrophages and induce release of pro-inflammatory cytokines [151, 317]. However, this is controversial, as lactoferrin may act as a scavenger of LPS [144].

Myeloperoxidase was the only biomarker not associated with fatal ischemic heart disease among the diabetes patients. This is supported by a study of diabetes patients with stable angina: Myeloperoxidase could not predict hemodynamically significant coronary artery disease in this group [318]. Although lactoferrin and myeloperoxidase both stem from neutrophil granulocytes, the specific granules containing lactoferrin are more readily mobilized than the azurophilic granules, which contain myeloperoxidase [319].

5.1.2 Risk prediction in the control group

None of the biomarkers could predict fatal ischemic heart disease in the control group. There are two apparent explanations. First, this might be a type 2 error (false negative result) due to insufficient power. There were only 28 persons who died from ischemic heart disease in the control group, which could be the reason for the lack of findings.

Alternatively, the lack of associations in the control group may be because the markers really are unable to predict death from ischemic heart disease in this group. The nature of the atherosclerotic disease among these individuals may be different from that in the diabetes group, and markers of macrophage activity (neopterin) and neutrophil degranulation (lactoferrin and myeloperoxidase) may not emerge in this population. Our results underline the differences between persons with and without diabetes, and support separate analyses for individuals with diabetes.

Regarding CRP, the results in the control group do not lend support to either of the two issues presented in section *1.2*.2 (1) CRP and causality in atherosclerosis and 2) CRP's role in clinical decision-making). This may be related to lack of power, but our findings are supported by others. Further

genetic studies in recent years have refuted a causal role of CRP in atherosclerosis [320, 321]. Moreover, a large meta-analysis concluded that the association between CRP and coronary heart disease was considerably weakened by adjustment for conventional risk factors [322]. In order to improve risk prediction, a marker has to provide information beyond the classical risk factors, and thus have a sufficiently low correlation with those. The observation that CRP predicts cardiovascular events may be explained by other factors than causality. The increase in CRP could possibly mark other risk factors (confounding), or be caused by subclinical atherosclerosis (reverse causation). Altogether, the current evidence indicates that CRP is not an ideal tool for risk prediction of CVD [323]. Our data confirms this conclusion in diabetes patients, where neopterin and lactoferrin emerged as stronger markers.

5.1.3 Methodological considerations (Study I & II)

Some of the main strengths of HUNT1 are that it is population-based and provides a long observation time, in our case up to 20 years. In general, the participation rate was high (88%) [265], compared to other national and international population studies [324-326]. However, non-participants may represent a selection problem. The non-participation study after HUNT1 showed no health-related selection in the young age groups [265]. However, in older age groups non-participants had more health problems than participants. This may have influenced the representativity of our study. This may also have influenced the total number of newly diagnosed diabetes patients and hence the number of participants in the study, as the diabetes patients presented with poorer general health, even though they were unaware of their diabetes.

As demonstrated in Figure 3.2, 193 of the newly diagnosed diabetes patients were not included in the follow-up, due to several reasons. Those who were excluded due to age or illness, or died before start of the follow-up period, constitute a possible selection bias. Moving, declining to participate and other reasons may also be related to poor health and thereby contribute to a skewed selection. However, the study was mainly designed to follow the newly diagnosed diabetes patients with annual HbA1c measurements, and practical

considerations were prioritized. This could result in an increased risk of false negative conclusions.

In HUNT1, serum samples were only drawn from the diabetes patients and their controls. The samples were stored for > 20 years, which may have reduced the quality. However, all samples were handled identically and the concentrations were compared within groups. Furthermore, the concentrations correspond to those found in fresh samples, but the reported values are not directly comparable. Nevertheless, blood samples for measurements of leukocyte-related molecules should preferably be collected in EDTA tubes, as coagulation activates the complement system and the leukocytes.

Because of some missing information on smoking, adjustments could not be made in the main analyses. However, smoking was not a predictor and it did not influence the biomarkers in the Cox regression models among those with valid information. Statins are known to lower inflammatory parameters, but statins were not in regular use in Norway when HUNT1 was initiated. The limitations of the HUNT1 study would contribute to an increased risk of type 2 errors. Our results are therefore robust.

The Cause of Death Registry, Statistics Norway, receives death certificates for all Norwegian citizens. As very few of the certificates are based on autopsy, there is a risk of misclassification.

5.2 Study III

5.2.1 MBL deficiency and risk of MI

We found that *MBL2* polymorphisms corresponding to MBL deficiency were associated with a doubling of the risk of MI. This finding corroborates previous findings regarding the association between MBL and atherosclerosis [189, 191, 197, 203, 204], and confirms our hypothesis. However, most of the previous results were found in high-risk populations. Recently, Siezenga et al, showed that low *MBL2* genotype, but not MBL serum concentrations were associated with cardiovascular events in a group of diabetes patients [327]. The present results from a group of relatively young and healthy individuals are partly confirmatory, and indicate that the general population may also be at risk.

Although the MI patients were below 62 years of age, they presented with several cardiovascular risk factors. Nevertheless, the association of variant *MBL2* haplotypes corresponding to MBL deficiency and MI was independent of conventional risk factors, indicating that MBL deficiency acts through other mechanisms. Our results lend support to the hypothesis that lack of functional MBL leads to defect efferocytosis and thereby increased atherosclerosis [328].

Nevertheless, others have found an increased risk of CVD in persons with normal MBL [190, 193, 195, 196]. The duality of MBL in the pathogenesis of atherosclerosis was demonstrated in a study of persons with rheumatoid arthritis by Troelsen et al [191], where both high and low MBL concentrations were linked to increased intima-media thickness of the carotid artery. This shows that both high and low MBL activity can be harmful. Furthermore, in some studies the relative cardio-protective effects of MBL may be blurred, as activation of the lectin pathway seems to play an important role in ischemia-reperfusion injury [329, 330]. Altogether, our findings support a protective role of MBL in atherosclerosis, but underline the complexity of the disease.

5.2.2 Ficolins

There were no significant differences in ficolin polymorphisms between cases and controls. The ficolins were included for an exploratory purpose, as they activate the lectin pathway of the complement system. Further studies are needed to explore the functions of ficolins and their potential clinical correlates.

5.2.3 Genetic predisposition for MI

The present study was not designed to test predictive ability. However, the clinical usefulness of genetic variations in risk prediction is an important topic to address. The apparent advantages of genetics are that they allow early risk prediction as they are fixed from birth. They are not necessarily affected by other illness, even though interactions may occur, and it is relatively easy to obtain a reliable result. However, the effect sizes found in studies so far are small, and even though genetic variations are associated with incident cases, they fail to improve risk reclassification and C-statistics.

A simple and free tool in clinical risk assessment is the question about family history of cardiovascular events. This information covers all genetic variants, and has been found to increase risk prediction [331]. However, family history is not always available or reliable, and it predicts the same risk for all members of the immediate family. Furthermore, compared to family history, a genetic test may provide early information, even before the parents are affected. Unfortunately, some data on a significant family history (MI before 60 years in a first-degree relative), were missing in our study group, and could thus not be included in the conditional logistic regression models. Such an analysis was desirable, as there were almost twice as many with a positive family history in the case group compared to the controls.

5.2.4 Methodological considerations (Study III)

The study group was based on the HUNT2 cohort. An important strength is that this cohort is fairly representative for Norway as a whole, with little ethnic diversity (about 3 % non-Caucasians), and thus suitable for genetic studies. There was a decline in participation from HUNT1 to HUNT2, and as mentioned, non-participators represent a selection problem. The participation rate was lowest among the young and elderly, but the main reason for not attending among those aged 20-44 years was not health related [273]. Among those aged 40-69 years, the participation rate was fairly high, at 77-81%.

The total decline in participation may be explained by several factors: 1) the screening was more comprehensive, 2) the population seeks regular health checks with their general practitioner and 3) less interest in public health in general [273]. We may have lost some 'young and healthy' individuals that did not participate due to 'lack of time' or 'were not interested', that later had an MI. However, although there may be a selection bias, we think that our study group provides fairly good estimates of *MBL2* haplotype frequencies and association with MI.

Another strength of the study is the method of case finding. The registration was independent of HUNT2 with well-defined criteria for MI, and based on patient charts. Furthermore, our results were significant after

correction for multiple comparisons by permutation testing, strengthening the probability of a causal relationship.

There are few limitations to pyrosequencing, especially when performed on suitable polymorphic regions like the ones in *MBL2* [285]. However, the light response following incorporation of more than five nucleotides is non-linear, and may play a role in the assessment of homopolymeric regions like the one in *FCN3*. All samples with uncertain results were therefore re-sequenced manually.

We chose the candidate gene approach for several reasons. First, the inferred haplotypes of *MBL2* have well-known functional consequences. Second, there are plausible pathogenic mechanisms for how the variants may accelerate the development of atherosclerosis [178]. Third, there are adequate methods and it was feasible to manage the samples. And finally: We and others have previously found a link of MBL deficiency and ischemic heart disease [189, 190, 197, 199, 202] and atherosclerosis [200, 203] in high-risk groups. Our findings were partly confirmatory, and therefore carry less risk of being false positive.

GWAS are efficient and have identified hundreds of new loci associated with different diseases [258, 259]. However, these loci are considered the low-hanging fruit, with small effects, and many of them lie in DNA regions with unknown functions. Furthermore, the frequency of variations like the MBL haplotypes may be too low to appear in GWAS. One has recognized that rare SNPs, which may have significant influence on disease development, are missed in today's GWAS. This has led to deeper sequencing of a large number of people in the 1000 Genome Project and HapMap 3 [332, 333], which may improve the design of GWAS and lead to better understanding of inheritance of complex traits. Meanwhile, different approaches are required, and candidate gene studies are suitable for replication of previous findings in new groups.

5.3 The long and winding road from bench to bedside

Our studies were not designed to test improvement of C-statistics or risk reclassification, but the results provide new insights of possible pathogenetic mechanisms in the development of atherosclerosis. Furthermore, improved understanding of the pathophysiology generates hypotheses, on which new studies can be based.

A combination of circulating markers and/or genetic variants may be necessary in order to successfully improve risk prediction. However, a multi-marker approach should be investigated based on more thorough knowledge of pathology and predictive ability in a large systematic study. The results in this thesis may be of help to guide the selection of markers.

However, factors that are causative are not necessarily good predictors. Two examples are blood pressure and cholesterol [334], which are poor predictors of cardiovascular events. These factors are normally distributed in the population, and many CVD events will happen among those with average measures. Moreover, a marker that is not directly involved in the pathological process can still be a useful predictor. Nevertheless, the search for inflammatory markers to improve risk prediction has so far not been exclusively successful, and thus they have not really challenged the classical risk factors.

In order to be incorporated in individual patient management, a marker or a set of markers must provide precise estimates in risk prediction. This is essential as imprecise prediction can result in over- or undertreatment and confused doctors. Furthermore, it can lead to false reassurance or unnecessary worry in patients and their relations.

Identifying people at risk challenges clinicians, as the border between prevention of future disease and medicalization may be blurred, especially as the diagnostic tools are improved. Risk information may possibly increase the person's feeling of 'control over their life' and thereby quality of life. However, going from feeling healthy to be labelled 'at risk' may lead to insecurity and undermine the sense of integrity and health.

Improved risk prediction is one strategy for prevention of CVD morbidity and mortality. However, evolution has provided humans with an ability to store energy for leaner times and an eager immune system to tackle any foreign intruder. Moreover, genetic variations that are advantageous under some circumstances may be harmful in a state of overnutrition and 21st century lifestyle. This may be a part of the downside of the modern way of life and indicates that in order to really reduce the burden CVDs, we have to deal with behavioural risk factors and the underlying causes.

In these studies, we found associations between certain inflammatory biomarkers and fatal ischemic heart disease in newly diagnosed diabetes patients. We also found that *MBL2* haplotypes causing MBL deficiency were associated with a doubling of the risk for MI before the age of 62 years. There is a long way from associations in population-based studies to clinical usefulness. However, our results shed new light on the pathogenetic mechanisms for the development of atherosclerosis.

6 Conclusions

I Both CRP and neopterin were independent predictors of fatal ischemic heart disease in a group of newly diagnosed diabetes patients, but not in the control group. However, when corrections were made for the opposite marker, neopterin remained significant whereas CRP did not.

II The two highest tertiles of lactoferrin were independent predictors of fatal ischemic heart disease in a group of newly diagnosed diabetes patients, but not in the control group. Myeloperoxidase could neither predict death from ischemic heart disease in diabetes patients nor in controls.

III Functional polymorphisms in *MBL2*, corresponding to MBL deficiency, were associated with a doubling of the risk for MI in young and middle-aged persons. None of the investigated polymorphisms in the ficolins were related to MI.

7 Future perspectives

The studies in this thesis have resulted in new hypotheses that can be addressed in future studies.

It would be of interest to analyse the inflammatory markers and their predictive ability in a large diabetes cohort compared to controls. Prediction of both MI and fatal ischemic heart disease would be relevant. The HUNT studies have the potential for investigating these issues.

A clinically important approach is to assess the effects of anti-inflammatory medication, both in primary/secondary prevention or the acute phase of ischemic heart disease. Both general anti-inflammatory drugs and specific inhibitors of inflammatory mediators are of interest.

The HUNT studies are suited for genetic analyses, and it would be of interest to genotype *MBL2* in all those included in the MI registry and controls, to assess the risk associated with *MBL2* in the population. Furthermore, these results could be linked to mortality data. Serum is also available, and a multi-marker approach combining circulating markers and genetics could be tested in this cohort. Moreover, the cohort is suitable for the new generation GWAS with deeper sequencing analyses.

8 References

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Paper I

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Neopterin predicts the risk for fatal ischemic heart disease in type 2 diabetes mellitus
Long-term follow-up of the HUNT 1 study

Inga Thorsen Vengen[a], Ane Cecilie Dale[b,c], Rune Wiseth[b,c], Kristian Midthjell[d], Vibeke Videm[a,e,*]

[a] Department of Laboratory Medicine, Children's and Women's Health, Norwegian University of Science and Technology, Norway
[b] Department of Circulation and Medical Imaging, Norwegian University of Science and Technology, Norway
[c] Department of Cardiology, Trondheim University Hospital, Trondheim, Norway
[d] Department of Public Health, Norwegian University of Science and Technology, Norway
[e] Department of Immunology and Transfusion Medicine, Trondheim University Hospital, NO-7006 Trondheim, Norway

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ABSTRACT

Aims: Neopterin has emerged as a novel predictor of coronary events. The study aim was to compare the predictive value of neopterin and C-reactive protein (CRP) on long-term risk for fatal ischemic heart disease (IHD) in persons with newly diagnosed diabetes compared to persons without diabetes.
Methods and results: In 1984–1986 a large population study, HUNT 1, was conducted in Norway. During the study, 205 patients were diagnosed with formerly unknown diabetes. A matched control group without diabetes was selected from the HUNT 1 population. Fatal IHD was registered until 2004. Blood samples were drawn at baseline and serum was analysed for neopterin and CRP. Cox regression analysis with correction for age, gender, hypertension, body mass index, established cardiovascular disease and total cholesterol was used to estimate hazard ratios (HR) for fatal IHD. In the diabetes group, neopterin and CRP were independent predictors of fatal IHD, HR 2.59 (1.11–6.01) and 2.45 (1.05–5.69), respectively. Neither CRP nor neopterin were significant predictors of fatal IHD in the control group.
Conclusion: In subjects with diabetes, both neopterin and CRP were independent predictors of fatal IHD, suggesting that these two markers reflect different aspects of the pathogenesis underlying fatal coronary events.

1. Introduction

Diabetes mellitus type 2 is considered both a metabolic and an inflammatory disease [1]. Individuals with impaired glucose metabolism are at increased risk for fatal ischemic heart disease (IHD) [2], and there is firm evidence that inflammation is central in the pathogenesis of atherosclerosis. It has therefore been suggested that diabetes accelerates the atherosclerotic process by increasing inflammation [3], and hyperglycemia is associated with increased markers of inflammation [4]. Obesity is common among diabetes patients, and inflammatory mediators released from visceral and subcutaneous fat are both pro-inflammatory and may lead to decreased insulin sensitivity [5]. Hyperglycemia is also associated with endothelial dysfunction [6].

Several inflammatory markers have been suggested for prediction of risk for future coronary events. C-reactive protein (CRP) measured in high-sensitivity assays (hs-CRP) is most widely studied [7,8]. However, the predictive value of CRP has been disputed and there is a search for stronger predictors among inflammatory markers [9]. Monocytes and macrophages are central in all stages of atherosclerosis, and neopterin is a soluble marker of monocyte activation. Neopterin has been identified as a prognostic factor in acute coronary syndromes [10,11], in survivors of acute myocardial infarction [12], as well as for rapid disease progression in patients with stable angina pectoris [13].

In previous studies assessing the predictive power of different inflammatory markers on future IHD, the presence of diabetes most often has been treated as a confounding factor in the statistical analyses. With the increasing prevalence of subjects with diabetes and their known risk for IHD, it could be argued that the diabetes population should be analysed separately in order to get further insight into differences between subjects with and without diabetes.

The aim of the present study was to evaluate baseline CRP and neopterin as prognostic markers for fatal IHD among subjects with diabetes during long-term follow-up.

* Corresponding author at: Department of Immunology and Transfusion Medicine, Trondheim University Hospital, NO-7006 Trondheim, Norway.
Tel.: +47 725 73321; fax: +47 725 76426.
E-mail address: vibeke.videm@ntnu.no (V. Videm).

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2. Materials and methods

2.1. Study population

During 1984–1986, a large health survey (HUNT 1) was conducted in the county of Nord-Trøndelag in Norway. The county is fairly representative for Norway as a whole with a stable and ethnically homogenous population, with only 3% non-Caucasians.

The HUNT 1 study has been described in detail elsewhere [14,15]. Briefly, 76,885 individuals responded to a questionnaire, and 74,977 (88.1%) attended a clinical examination that included measurements of blood pressure, pulse, height and body weight. Data on smoking habits, exercise and level of education were recorded. In all participants aged 40 years and older, a non-fasting glucose concentration in capillary whole blood was measured. If the glucose concentration in persons without known diabetes was ≥8 mmol/L, fasting glucose was measured within 1–5 days. An oral glucose tolerance test was performed in subjects with fasting blood glucose <7 mmol/L. Persons with fasting glucose ≥7 mmol/L or a 2-h glucose concentration ≥11.0 mmol/L were considered to have diabetes [16].

Through this procedure, a total of 428 persons were diagnosed with previously unknown diabetes. Among these patients, 103 were excluded for various reasons. Due to geographical constraints, it was feasible to follow 235 of these 325 patients. A control group of 205 persons without diabetes matched by age, gender and municipality was selected from the HUNT 1 population, and the 205 corresponding diabetes patients were then included (see Appendix A for further details).

Both groups were invited to a comprehensive baseline clinical examination including a full medical history, ECG, urine samples and measurements of serum creatinine, total cholesterol and HDL-cholesterol. The diabetes patients attended this examination 6–22 months (mean 14) after the baseline survey and the comparison group attended after 12–32 (mean 22) months. A blood sample was drawn from all participants. Frozen serum was stored at −40 °C until 2005 when the samples were moved to −80 °C. Before analysis in the present study the serum had been thawed twice, once for use in analysis in 1987 [17] and once for aliquotation in 2007. 5 patients with newly diagnosed diabetes and 7 control participants were excluded due to missed blood sampling.

Table 1
Background characteristics.

	Newly diagnosed diabetes (n = 200)	Control group (n = 198)
Gender (male/female)	105/95	102/96
Age, years	67 (65–68)	67 (65–68)
BMI, kg/m^2	29.7 (29.0–30.3)	26.2 (25.7–26.7)
Waist-hip ratio	0.92 (0.90–0.93)	0.88 (0.87–0.89)
Random glucose, mmol/L	10.6 (10.0–11.1)	5.2 (5.1–5.4)
Fasting glucose, mmol/L	6.8 (6.5–7.0)	5.0 (4.8–5.1)
Total cholesterol, mmol/L	6.7 (6.5–6.8)	7.3 (7.1–7.5)
HDL-cholesterol, mmol/L	1.24 (1.20–1.29)	1.46 (1.41–1.51)
Total cholesterol–HDL-cholesterol ratio	5.6 (5.4–5.8)	5.2 (5.0–5.5)
Creatinine, μmol/L	86.4 (83.2–89.5)	86.2 (84.4–88.0)
Hypertension[a], number (%)	135 (68%)	98 (49%)
Smoking, number (%)	42 (23%)	35 (21%)
Previous CVD[b], number (%)	44 (22%)	27 (14%)

Continuous variables are given as mean and 95% CI.
[a] Hypertension was defined as blood pressure > 140/90 or the use of antihypertensive medication.
[b] CVD = Cardiovascular disease was defined as prior myocardial infarction, angina pectoris or stroke.

2.2. Study variables

Hypertension was defined as blood pressure ≥140/90 mmHg or as current use of antihypertensive medication. Previous cardiovascular disease (CVD) was defined as having had myocardial infarction, angina pectoris or stroke (self-reported). Body mass index (BMI) was calculated as weight (kg) divided by the squared value of height (m). The ratio of waist to hip circumference was determined. Based on self-reporting, the participants were classified as smokers or non-smokers.

Neopterin was analysed in serum as indicated by the manufacturer, using an enzyme-linked immunosorbent assay (Brahms, Hennigsdorf, Germany). For high-sensitivity detection of CRP, microtiter plates were coated with rabbit anti-human CRP antibody (Sigma, St. Louis, MO, USA). Diluted samples or standards (CRP from human plasma, Sigma) were added. Goat anti-human CRP (Sigma) was used as secondary antibody, and peroxidase-conjugated rabbit anti-goat antibody (Dako Cytomation, Glostrup, Denmark) was employed in the subsequent step. The substrate was o-phenylenediamine 0.15 mg/mL with 0.015% H_2O_2. The reaction was stopped with 2 M H_2SO_4, and the optical density was measured at 492 nm (Sunrise microplate reader, Tecan, Männedorf, Switzerland). The assay was calibrated against a commercial assay (Immunlite 2000 High Sensitivity, Diagnostic Products, Los Angeles, California, USA).

2.3. Follow-up

Information on causes of death was obtained by linking data from our study to the Cause of Death Registry at Statistics Norway, which receives all death certificates of Norwegian citizens. Deaths were classified according to the International Classification of Disease (ICD-9 and ICD-10). Death from IHD was defined by ICD-9: 410–414 and ICD-10: I20-25. We calculated the individual person time at risk from the date of the comprehensive baseline clinical examination until the date of death from IHD, death from other causes or until the end of follow-up on December 31, 2004. The study protocol conformed to the Helsinki declaration, and the study was approved by The Data Inspectorate and recommended by the Regional Committee for Medical Research Ethics.

2.4. Statistical analysis

Baseline characteristics are displayed by proportions or means with 95% confidence intervals (CI), stratified according to diabetes status. Spearman's rank correlation coefficient was used to evaluate the correlation between neopterin and CRP concentrations. Due to non-normal distribution, the data were analysed with Mann Whitney U-test. The Chi-square test was used to compare categorical variables.

Incidences of IHD mortality were plotted in Kaplan–Meier plots. Cox regression analysis was used to estimate hazard ratios (HR) and 95% CI of death from IHD according to the baseline concentrations of the biomarkers. Neopterin and CRP concentrations were divided into tertiles (neopterin <7.9, 7.9–10.5, >10.5 nmol/L, CRP < 1.09, 1.09–2.86, >2.86 mg/L). Departures from the proportional hazards assumption was evaluated using graphical procedures (log–log plots).

All statistical tests were two-sided and p-values below 0.05 were considered statistically significant. Data were analysed with SPSS for Windows (version 15.0 SPSS Inc., Chicago, IL, USA).

3. Results

Background characteristics are shown in Table 1. Subjects with newly diagnosed diabetes had a higher BMI, were more often

Table 2
Concentrations of neopterin and CRP (means and 95% CI), stratified according to previous CVD and diabetes status.

Diabetes	Biomarker	Previous CVD		No previous CVD		*p*-value
		n	Mean (95% CI)	*n*	Mean (95% CI)	
Yes	Neopterin (nmol/L)	44	13.2 (11.1–15.3)	156	9.6 (9.0–10.3)	0.001
	CRP (mg/L)		3.56 (2.49–4.63)		2.98 (2.33–3.62)	0.094
No	Neopterin (nmol/L)	27	10.0 (8.9–11.2)	171	9.7 (9.1–10.3)	0.29
	CRP (mg/L)		1.81 (1.36–2.26)		1.95 (1.69–2.22)	0.71

n = number in each group.

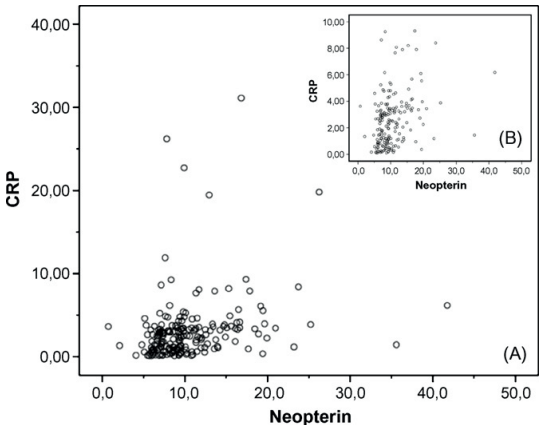

Fig. 1. The correlation of neopterin and CRP concentrations in patients with newly diagnosed diabetes. Panel A: All samples. Panel B: Samples from patients with CRP < 10 mg/L only.

hypertensive, and more often had established cardiovascular disease than the control group. Concentrations both of total and HDL-cholesterol were lower in the diabetes group, resulting in a higher total cholesterol/HDL-cholesterol ratio. Mean individual person time at risk was 12.6 years (range: 0.1–19.7). During follow-up, 44 participants with and 28 participants without diabetes died of IHD. Among the participants who died of IHD, 16 with and 8 without diabetes had established cardiovascular disease at baseline ($p = 0.49$).

At baseline, neopterin was not higher in the diabetes group (10.4 (9.7–11.1) mmol/L) compared to the control group (9.8 (9.2–10.3) mmol/L, $p = 0.65$), as opposed to CRP (3.11 (2.55–3.66) mg/L in the diabetes group and 1.93 (1.70–2.17) mg/L in the control group, $p < 0.0005$). In the group with newly diagnosed diabetes, baseline concentrations of neopterin were significantly higher in the participants with established cardiovascular disease ($p = 0.001$, Table 2). A similar non-significant trend was seen for CRP ($p = 0.094$). In the comparison group, there were no differences in neopterin or CRP concentrations between subjects with or without established cardiovascular disease at baseline. Neopterin and CRP concentrations were significantly correlated ($p < 0.001$), but with a moderate correlation coefficient (diabetes group; Spearman's rho = 0.234, control group; Spearman's rho = 0.353), Fig. 1.

In univariate Cox regression analyses age (continuous) and previous CVD (yes, no) were significant predictors in both groups ($p < 0.0005$). In the diabetes group, neopterin ($p < 0.0005$), gender ($p = 0.047$), diastolic blood pressure (continuous, $p = 0.01$) and creatinine (continuous, $p < 0.0005$) were also associated with fatal IHD. In the control group BMI (continuous, $p = 0.008$) and systolic blood pressure (continuous, $p = 0.005$) were associated with fatal IHD. The multivariate analyses were adjusted for age, gender, hypertension (yes, no), BMI, previous CVD and total cholesterol (continuous). Cre-

Table 3
Hazard ratio for the tertiles for neopterin and CRP in serum from participants with newly diagnosed diabetes and their control group.

	No. of person years	No. of deaths	Death rate per 1000 person years	Hazard ratio[a]	Confidence interval	Hazard ratio[b]	Confidence interval
Newly diagnosed diabetes							
Neopterin[c]							
Tertile I	942.1	10	10.6	1.0	(Reference)	1.0	(Reference)
Tertile II	847.5	12	14.2	1.39	(0.58–3.37)	1.48	(0.61–3.62)
Tertile III	613.9	22	35.8	2.59	(1.11–6.01)	2.39	(1.01–5.69)
CRP[d]							
Tertile I	794.7	8	10.1	1.0	(Reference)	1.0	(Reference)
Tertile II	740.6	14	18.9	1.79	(0.74–4.33)	1.85	(0.75–4.55)
Tertile III	868.2	22	25.3	2.45	(1.05–5.69)	2.12	(0.89–5.05)
Control group							
Neopterin[c]							
Tertile I	959.3	11	11.5	1.0	(Reference)	1.0	(Reference)
Tertile II	927.3	5	5.4	0.43	(0.15–1.26)	0.43	(0.14–1.28)
Tertile III	700.6	12	17.1	1.24	(0.51–2.98)	1.19	(0.49–2.89)
CRP[d]							
Tertile I	1105.5	8	7.2	1.0	(Reference)	1.0	(Reference)
Tertile II	867.4	10	11.5	1.14	(0.43–3.03)	1.28	(0.48–3.37)
Tertile III	628.8	10	15.9	1.66	(0.59–4.65)	1.45	(0.52–4.09)

[a] Adjusted for: age, gender, hypertension, BMI, previous cardiovascular disease and total cholesterol.
[b] Adjusted for: age, gender, hypertension, BMI, previous cardiovascular disease, total cholesterol and opposite biomarker.
[c] Neopterin: Tertile I: <7.9 nmol/L, Tertile II: 7.9–10.5 nmol/L, Tertile III: >10.5 nmol/L.
[d] CRP: Tertile I: <1.09 mg/L, Tertile II: 1.09–2.86 mg/L, Tertile III: > 2.86 mg/L.

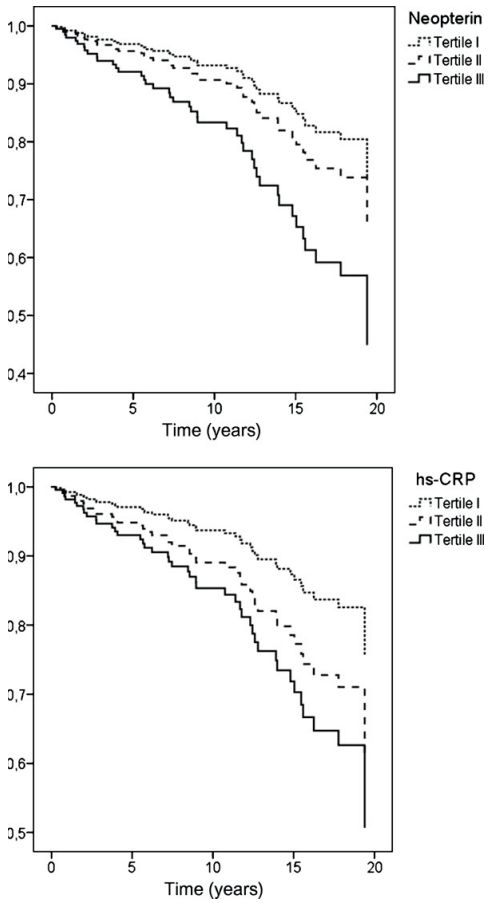

Fig. 2. Neopterin, CRP and death from IHD in patients with newly diagnosed diabetes. Survival curves according to tertile of neopterin (panel A) or CRP (panel B) concentration at baseline. Neopterin: Tertile I: <7.9 nmol/L, Tertile II: 7.9–10.5 nmol/L, Tertile III: >10.5 nmol/L. CRP: Tertile I: <1.09 mg/L, Tertile II: 1.09–2.86 mg/L, Tertile III: >2.86 mg/L. The highest tertile of both markers were significant predictors of fatal IHD; neopterin HR 2.59 (1.11–6.01), $p = 0.027$ and CRP 2.45 (1.05–5.69), $p = 0.038$. The model was adjusted for age, gender, hypertension, body mass index, previous CVD and total cholesterol.

atinine was not included, because it gives only a crude estimate of renal function and the association seen in univariate analysis was lost in multivariate analyses ($p = 0.2$). Some data regarding smoking were missing, but in participants with information on smoking it was not a significant predictor in Cox regression and did not affect the predictive value of the biomarkers.

In Cox regression analysis including either neopterin or CRP, the highest tertile of both markers were significant predictors of fatal IHD in the subjects with diabetes, HR 2.59 (1.11–6.01) and 2.45 (1.05–5.69), respectively (Table 3). There was a change in hazard ratios when the opposite biomarker was included in the analysis; CRP was no longer a significant predictor, CRP (HR 2.12 (0.89–5.05)) whereas neopterin was (HR 2.39 (1.01–5.69)). In the participants without diabetes, none of the biomarkers could predict fatal IHD (Table 3). The survival curves from the Cox regression model for each biomarker in subjects with newly diagnosed diabetes are illustrated in Fig. 2.

4. Discussion

We investigated the predictive value of neopterin and CRP on fatal IHD in 200 patients with newly diagnosed diabetes and a group of 198 matched individuals without diabetes, followed for nearly 20 years. In the group with newly diagnosed diabetes, both neopterin and CRP were predictors of fatal IHD, suggesting that these two markers reflect different aspects of the pathogenesis underlying fatal coronary events. None of the biomarkers could predict fatal IHD in the group without diabetes, possibly due to the limited numbers and small overall risk among the controls. However, this could also reflect that the predictive value of these inflammatory markers for fatal IHD may be stronger in persons with than in persons without diabetes.

Neopterin is released from activated monocytes and macrophages that are involved in the generation of atheromatous plaques from the earliest stages. Interferon-gamma (IFN-γ) from CD4+ T-cells is considered the most important stimulus activating macrophages to synthesize neopterin [18,19]. Neopterin is related to the presence of significant coronary stenosis in stable angina [20], but previous research indicates that neopterin is a marker of plaque activity more than the extent of atherosclerosis [21,22]. Furthermore, by increasing the risk for acute coronary syndromes, plaque activity may be more important for IHD mortality than the extent of atherosclerosis, thus rendering neopterin a strong predictor.

Patients with unstable angina have an increased number of oligoclonal CD4+ T-cells that lack CD28, an important receptor for costimulation upon activation [23]. Such T-cells are active producers of IFN-γ and may thereby stimulate plaque macrophages to increased neopterin release. In turn, neopterin enhances the inflammatory processes in vulnerable plaques. It promotes cellular adhesion molecule and tissue factor expression on endothelial cells, making them pro-atherotrombotic [24].

CRP is produced in hepatocytes, largely as a consequence of gene activation by interleukin-6 (IL-6) [25]. IL-6 is released by many cell types as part of the acute-phase reaction [25], with approximately one third of circulating IL-6 coming from adipose tissue [7]. In contrast to neopterin, CRP is therefore probably a broad marker of inflammation. This does not exclude the possibility that CRP is also directly involved in atheromatosis [26]. However, IL-6 concentrations seem to predict critical coronary stenosis better than does CRP [27]. It has been suggested that CRP is only a moderately strong predictor of future cardiovascular events [28]. Previous work from our group also showed a much stronger correlation between neopterin and coronary artery stenosis than for CRP [20].

In our present study both neopterin and CRP were significant predictors in the multivariate Cox regression models. When corrections were made for the opposite marker, respectively, the HR remained nearly unchanged. This confirms that they represent related, but not similar phenomena, as supported by their weak, albeit significant correlation (Fig. 1). Our findings are in keeping with previous studies in other patient groups. A study comparing neopterin and CRP in patients with lower respiratory tract infections clearly demonstrated that the markers behave differently when responding both to viral and bacterial infections [29]. Furthermore, neopterin, but not CRP, was a predictor of future cardiac events in patients with chronic stable angina [30], in hypertensive patients without obstructive coronary artery disease [31], and during follow-up after acute coronary syndromes [32].

Both biomarkers behaved differently in the participants with newly diagnosed diabetes and the control group. Baseline CRP concentrations were significantly higher in the patients with diabetes. Hyperglycemia is a strong proinflammatory stimulus [4] that also leads to increased IL-6 production [33]. Furthermore, the increased BMI and more abdominal fat in the participants with diabetes could

also contribute to the increased baseline CRP concentrations.In the subjects with newly diagnosed diabetes, the baseline CRP concentrations were unrelated to the presence or absence of CVD. Since only overt conditions (myocardial infarction, angina pectoris or stroke) were considered, undiagnosed presence of low-grade disease may have obscured the data. Alternatively, the stimuli to CRP production related to atherosclerosis may have been much smaller than those caused by diabetes itself. A previous study supports that CRP is not a good predictor of the presence of atherosclerosis in patients with diabetes [34].

Baseline concentrations of neopterin did not differ in subjects with and without newly diagnosed diabetes. In the participants with diabetes, however, neopterin was significantly higher in subjects with previous CVD. An earlier study on critical limb ischemia showed that patients with diabetes had higher concentrations of neopterin than patients without diabetes [35]. These findings may indicate higher atherosclerotic plaque activity in subjects with diabetes, which may in part explain their higher mortality of cardiovascular disease [2].

5. Study limitations

The study population was small and therefore not large enough for stratification by gender, age or whether diabetes was well or poorly regulated. The study also carries a risk of false negative conclusions. Nevertheless, our results suggest that the predictive value of the inflammatory markers studied differ between subjects with and without diabetes.

Use of statins is associated with lower concentration of inflammatory markers [10]. The use of statins was, however, uncommon in Norway during the inclusion period. The diabetes patients and the controls at risk for IHD probably started using statins when these substances were introduced on the market. This would not affect the baseline samples, and the benefit with respect to mortality would rather increase the risk of false negative conclusions.

Other variables related to fatal IHD, such as the left ventricle ejection fraction and coronary artery disease extension by angiography, might influence our findings. However, since HUNT 1 is a population based study, these variables were not available and our results could not be adjusted for them.

The quality of serum stored for up to 20 years may have been reduced. Samples from both groups were handled similarly. Since concentrations of neopterin and CRP were compared between the groups, changes in absolute concentrations due to storage are not supposed to be important. The concentrations correspond to the levels detected in fresh serum samples from similar populations [20].

6. Conclusion

Neopterin was a robust predictor of fatal IHD in patients with newly diagnosed diabetes in our study. Neopterin may be a more plaque-specific marker, whereas CRP reflects non-specific low-grade inflammation common both for atherosclerosis and diabetes. The role of neopterin as a prognostic marker for coronary events warrants further study with particular emphasis on the value of this marker in subjects with diabetes.

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Appendix A

A.1. Study population

In all HUNT 1 participants aged 40 years and older, a non-fasting glucose concentration in capillary whole blood was measured. If the glucose concentration in persons without known diabetes was $\geq$8 mmol/L, fasting glucose was measured within 1–5 days. An oral glucose tolerance test was performed in subjects with fasting blood glucose <7 mmol/L. Persons with fasting glucose $\geq$7 mmol/L or a 2-h glucose concentration $\geq$11.0 mmol/L were considered to have diabetes. Through this procedure, a total of 428 persons were diagnosed with previously unknown diabetes. Among these patients, 103 were excluded; 20 because they were older than 80 years of age, 12 because they died, 14 due to serious illness at baseline, 2 had moved out of the county, 37 declined to participate in follow-up, and 18 persons were not followed up for other reasons; leaving 325 patients with newly confirmed diabetes. Due to geographical constraints, it was feasible to follow 235 of these 325 patients. From the HUNT 1 population 235 pairs of control persons without diabetes, matched to the subjects with newly diagnosed diabetes by age, gender and municipality, were invited to participate. If the first control person declined to participate, the second one was asked, and eventually 205 control persons agreed to participate. The 205 corresponding diabetes patients were then included.

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Lactoferrin is a novel predictor of fatal ischemic heart disease in diabetes mellitus type 2: Long-term follow-up of the HUNT 1 study

Inga Thorsen Vengen[a], Ane Cecilie Dale[b,c], Rune Wiseth[b,c], Kristian Midthjell[d], Vibeke Videm[a,e,*]

[a] Department of Laboratory Medicine, Children's and Women's Health, Norwegian University of Science and Technology, Trondheim, Norway
[b] Department of Circulation and Medical Imaging, Norwegian University of Science and Technology, Trondheim, Norway
[c] Department of Cardiology, Trondheim University Hospital, Trondheim, Norway
[d] Department of Public Health and General Practice, Norwegian University of Science and Technology, Trondheim, Norway
[e] Department of Immunology and Transfusion Medicine, Trondheim University Hospital, Trondheim, Norway

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ABSTRACT

Objectives: The pathogenesis of diabetes and atherosclerosis is linked through inflammation. Neutrophils contribute to atherosclerotic plaque development, and are dysfunctional in diabetes. The aim of this study was to compare the predictive values of two neutrophil degranulation products, myeloperoxidase and lactoferrin, on long-term risk for fatal ischemic heart disease in persons with newly diagnosed diabetes and controls.
Design: Prospective population-based cohort study.
Setting and patients: In 1984–1986, a large population study, HUNT 1, was conducted in Norway. Previously unknown diabetes was diagnosed in 205 persons. A matched control group without diabetes was selected from the HUNT 1.
Main outcome measures: Fatal ischemic heart disease was registered until 2004. Baseline serum was analysed for myeloperoxidase and lactoferrin. Cox regression analysis with adjustments for age, gender, hypertension, body mass index, established cardiovascular disease and total cholesterol was used to estimate hazard ratios for fatal ischemic heart disease.
Results: In the diabetes group (200 subjects), the two highest tertiles of lactoferrin predicted fatal ischemic heart disease, hazard ratio 2.54 (95% CI, 1.00–6.45) and 4.06 (1.72–9.60). Myeloperoxidase did not predict death from ischemic heart disease in subjects with diabetes. In the controls (198 subjects), none of the biomarkers predicted fatal ischemic heart disease.
Conclusion: Increased baseline concentration of lactoferrin strongly predicted the long-term risk for fatal ischemic heart disease in patients with newly diagnosed diabetes. Based on the literature, we hypothesize that the increased concentrations may reflect neutrophil priming caused by hyperglycemia.

1. Introduction

The prevalence of type 2 diabetes is increasing worldwide. Subjects with diabetes have an increased risk for micro- and macrovascular complications, and cardiovascular disease is the most common cause of death in individuals with diabetes [1]. Diabetes is considered both a metabolic and an inflammatory disease. Inflammation also plays an important role in atherosclerosis, and it is hypothesized that diabetes worsens the atherosclerotic plaque activity by promoting inflammation [2]. In previous risk prediction analyses, diabetes has most often been treated as a confounding factor. As the diabetes population is increasing, it could be argued that persons with impaired and normal glucose metabolism should be analysed separately to identify differences between the groups.

Multiple markers of inflammation have been suggested as predictors of future coronary events. High sensitivity C-reactive protein, a marker of low-grade inflammation, has been widely studied, but its predictive value has been disputed [3]. Because additional pro-inflammatory stimuli are present in diabetic patients, it is possible that some inflammatory markers will behave differently in risk prediction analyses in persons with and without diabetes.

Though the role of neutrophils in atherosclerosis remains unclear, the neutrophil count is a marker of ongoing inflammation and has been shown to predict cardiovascular events [4]. In a recent study of postmenopausal women, neutrophil counts predicted future cardiovascular events whereas total leukocyte counts did not [5], supporting a specific role of neutrophils.

* Corresponding author at: Department of Immunology and Transfusion Medicine, St Olavs Hospital, NO-7006 Trondheim, Norway. Tel.: +47 725 73321; fax: +47 725 76426.
E-mail address: vibeke.videm@ntnu.no (V. Videm).

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Myeloperoxidase, a powerful oxidative agent, is mainly located in the primary granules of neutrophil granulocytes with smaller amounts in monocytes/macrophages. Myeloperoxidase catalyzes the formation of reactive oxygen species, promotes atherogenesis and increases the uptake and lowers the removal of cholesterol [6]. Lactoferrin is released from secondary granules of neutrophil granulocytes upon activation. Lactoferrin is an iron-binding glycoprotein of the transferrin family and mainly acts anti-inflammatory. In addition to having anti-microbial properties, it inhibits up-regulation of adhesion molecules on endothelial cells and accumulation of cholesterol in macrophages [7,8]. Thus, it is conceivable that neutrophil degranulation may both enhance and inhibit atherogenesis, depending on the balance between myeloperoxidase, lactoferrin and perhaps other degranulation products.

Patients with diabetes have an impaired neutrophil function, with altered degranulation. There is evidence that neutrophils from diabetes patients are "primed", i.e., they are more prone to becoming activated upon moderate stimulation [9]. This may influence the atherosclerotic process and could contribute to the increased risk of cardiovascular complications in diabetes.

The aim of the present study was to evaluate the two neutrophil degranulation products myeloperoxidase and lactoferrin as prognostic markers during long-term follow-up for fatal ischemic heart disease among subjects with newly diagnosed diabetes, compared to controls.

2. Materials and methods

The first North Trøndelag Health Survey (HUNT 1) was conducted in 1984–1986. The county is fairly representative for Norway as a whole with a stable and ethnically homogenous population, with only 3% non-Caucasians.

The HUNT 1 study has been described in detail elsewhere [10,11]. Briefly, all inhabitants aged 20 years or older (85 100) were invited to participate, and 74 977 (88.1%) attended a clinical examination that also included measurements of blood pressure, height and body weight. Data on smoking habits and other variables (socio-economic data and self-evaluation of present health) were recorded. Non-fasting capillary whole blood glucose was measured in all participants aged 40 years and older. If the glucose concentration in persons without known diabetes was $\geq$8.0 mmol/L, the participants were invited back for an oral glucose tolerance test within 1–5 days. An oral glucose load was given if fasting blood glucose was below 7.0 mmol/L. Persons with fasting glucose $\geq$ 7 mmol/L or a 2-h glucose concentration $\geq$ 11.0 mmol/L were considered to have diabetes, according to WHO 1980 criteria. Of the 2341 subjects who were invited back to further glucose testing, 2315 (99%) met for the follow-up examination.

Through this procedure, a total of 428 persons (18% of the participants with non-fasting glucose $\geq$ 8.0 mmol/L) were diagnosed with diabetes (Fig. 1). Among these patients, 103 were excluded (20 were older than 80 years, 12 had died, 14 had other serious illnesses, 2 had moved, 37 declined to participate, and 18 were excluded for other reasons); leaving 325 patients with newly diagnosed diabetes. Due to geographical constraints, it was feasible to follow 235 of these 325 patients. From the HUNT 1 population 235 pairs of control persons without diabetes, matched to the persons with newly diagnosed diabetes by age, gender and municipality, were invited to participate. If the first control person declined, the second one was asked, and eventually 205 control persons agreed to participate. The 205 corresponding diabetes patients were then included. Both groups were invited to a comprehensive baseline clinical examination including a full medical history and all participants had a non-fasting blood sample drawn. Serum was frozen and stored for future analysis. In our study, 5 patients with newly diagnosed diabetes and 7 control participants were excluded due to missing blood samples. The diabetes patients attended this examination 6–22 months (mean 14) after the HUNT 1 survey and the comparison group attended after 12–32 (mean 22) months. We have previously reported the predictive value of neopterin and C-reactive protein in this cohort [12].

Hypertension was defined as blood pressure $\geq$ 140/90 mm Hg or as current use of anti-hypertensive medication. Body mass index was calculated as weight (kg) divided by the squared value of height (m). Previous cardiovascular disease was defined by self-report as having had myocardial infarction, angina pectoris or stroke. The participants were classified as smokers or non-smokers.

Detection of myeloperoxidase and lactoferrin in the stored serum was done by enzyme immunoassays, as previously described [13,14]. Since previously published concentrations from our laboratory using the same methods have been performed in plasma, the data are not directly comparable. However, pilot experiments showed that parallel measurements in serum and plasma were very strongly correlated (lactoferrin: Pearson's $R = 0.82$, $p < 0.0005$, myeloperoxidase: $R = 0.90$, $p < 0.0005$, unpublished data).

Information on causes of death was obtained by linking data from our study to the Cause of Death Registry at Statistics Norway, which receives all death certificates of Norwegian citizens. Deaths were classified according to the International Classification of Disease (ICD-9 and ICD-10). Death from ischemic heart disease was defined by ICD-9: 410-414 and ICD-10: I20-25. We calculated the individual person time at risk from the date of the comprehensive baseline clinical examination until the date of death from ischemic heart disease, death from other causes or until the end of follow-up on December 31, 2004. The study protocol conformed to the Helsinki declaration and the study was approved by The Data Inspectorate and recommended by the Regional Committee for Medical Research Ethics.

2.1. Statistical analysis

Baseline characteristics are displayed by proportions or means with 95% confidence intervals (CI), stratified according to diabetes status. Due to non-normal distribution of data, continuous data were compared by the Mann–Whitney *U*-test. The Chi square test was used when comparing categorical variables.

Incidences of ischemic heart disease mortality were plotted in Kaplan–Meyer plots. Cox regression analysis was used to estimate hazard ratios and 95% CI of death from ischemic heart disease according to the baseline concentrations of the biomarkers. Variables in the Cox regression analysis were considered significant when the 95% CI for the hazard ratio did not overlap 1. Myeloperoxidase and lactoferrin concentrations were divided into tertiles (myeloperoxidase <569, 569–951 and >951 μg/L, lactoferrin <205, 205–428 and >428 μg/L). In a supplementary analysis, the cut-off concentrations for the tertiles were determined separately for the subjects with diabetes and the controls, respectively. These group-specific tertiles were <592, 592–991 and >991 μg/L for myeloperoxidase and <220, 220–462 and >462 μg/L for lactoferrin in the subjects with diabetes, and <520, 520–892 and >892 μg/L for myeloperoxidase and <190, 190–391 and >391 μg/L for lactoferrin in the control group.

Departures from the proportional hazards assumption were evaluated using graphical procedures (log–log plots). All statistical tests were two-sided and all estimates are reported irrespective of their statistical significance level. *p*-Values below 0.05 were considered statistically significant. Data were analysed with SPSS for Windows (version 15.0 SPSS Inc., Chicago, IL, USA).

Further validation of the Cox regression analyses was performed using the Design package (version 2.3-0 [15]) in the *R* statistical

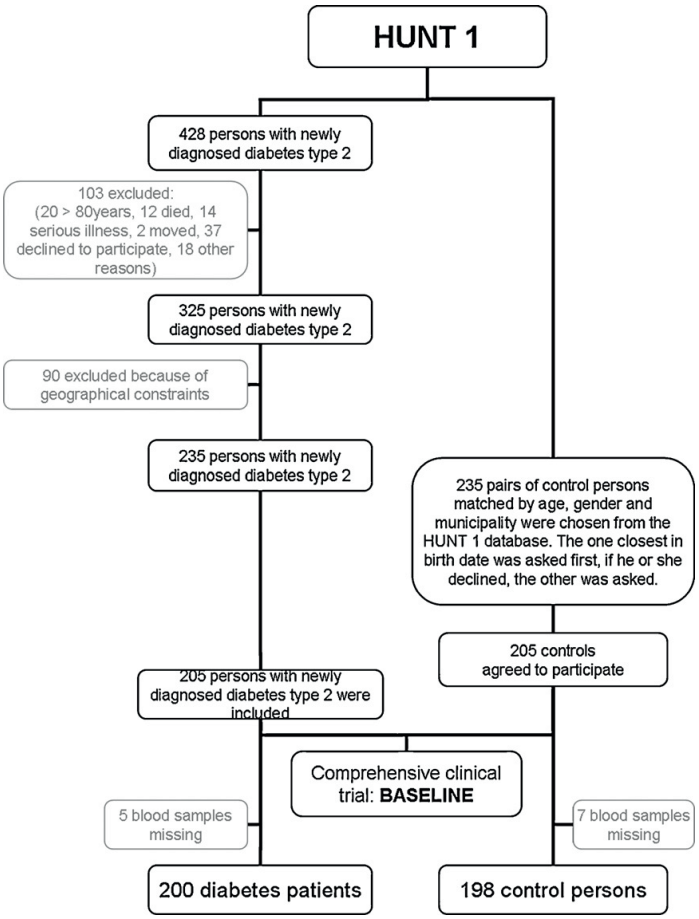

Fig. 1. Inclusion of patients to the study.

environment (version 2.6.1 [16]). The coefficients from the original models were bootstrapped (400 runs) to get robust estimates for calculation of HR and 95% CI. The model including lactoferrin in the subjects with diabetes was internally validated using bootstrapping (400 runs) to achieve a corrected value of Somer's Dxy rank correlation coefficient between predicted log hazards and observed survival time. Dxy provides an estimate of how well the model will discriminate survival in the future. Dxy varies from −1 to +1, where 0 means no correlation and an absolute value of 1 means total correlation. Values with an absolute value above 0.3 are considered to denote a strong relationship. The corrected Dxy penalizes for possible overfitting and is therefore more realistic than the uncorrected coefficient. Since logistic regression modelling was aimed at identifying a potential relationship between lactoferrin concentrations and ischemic heart disease and not to generate an algorithm for clinical risk stratification, prediction accuracy was not calibrated.

3. Results

Subjects with newly diagnosed diabetes had a higher body mass index ($p < 0.0005$), were more often hypertensive ($p < 0.0005$), and more often had established cardiovascular disease ($p < 0.05$) than the control group (Table 1). Concentrations of both total and HDL-cholesterol ($p < 0.0005$) were lower in the diabetes group, but with a higher total cholesterol/HDL-cholesterol ratio ($p < 0.01$). Fasting and non-fasting blood glucose concentrations ($p < 0.0005$) were higher in the group with newly diagnosed diabetes compared to the control group. Triglycerides were only measured in the subjects with newly diagnosed diabetes.

Mean individual person time at risk was 12.6 years (range: 0.1–19.7). During follow-up, 44 participants with and 28 participants without diabetes died of ischemic heart disease. Among the participants who died of ischemic heart disease, 16 with and 8 without diabetes had established cardiovascular disease at baseline ($p = 0.49$).

There were no differences in baseline lactoferrin ($p = 0.14$) and myeloperoxidase ($p = 0.83$) concentrations between the subjects with newly diagnosed diabetes and the controls (Table 1).

In univariate Cox regression analyses age (continuous) and previous cardiovascular disease (yes, no) were significant predictors in both groups ($p < 0.0005$), as previously reported [12]. In the diabetes group, lactoferrin ($p = 0.003$), myeloperoxidase ($p = 0.01$), creatinine (continuous, $p < 0.0005$), diastolic blood pressure (continuous, $p = 0.01$) and gender ($p = 0.047$) were also associated with fatal ischemic heart disease. In the control group, systolic blood pressure (continuous, $p = 0.005$) and body mass index (continuous,

Table 1
Background characteristics.[a].

	Newly diagnosed diabetes (n = 200)	Control group (n = 198)	p-Value
Gender (male/female)	105/95	102/96	0.92
Age, years	67 (65–68)	67 (65–68)	0.95
Body mass index, kg/m^2	29.7 (29.0–30.3)	26.2 (25.7–26.7)	**<0.0005**
Fasting glucose, mmol/L	6.8 (6.5–7.0)	5.0 (4.8–5.1)	**<0.0005**
Non-fasting glucose, mmol/L	10.6 (10.0–11.1)	5.2 (5.1–5.4)	**<0.0005**
Total cholesterol, mmol/L	6.7 (6.5–6.8)	7.3 (7.1–7.5)	**<0.0005**
HDL-cholesterol, mmol/L	1.24 (1.20–1.29)	1.46 (1.41–1.51)	**<0.0005**
Total cholesterol–HDL-cholesterol ratio	5.6 (5.4–5.8)	5.2 (5.0–5.5)	**0.006**
Fasting triglycerides, mmol/L	2.89 (2.56–3.21)	Not measured	–
Hypertension[b], number	135 (68%)	98 (49%)	**<0.0005**
Current smokers[c], number	42 (23%)	35 (21%)	0.56
Previous cardiovascular disease[d], number	44 (22%)	27 (14%)	**0.029**
Lactoferrin, μg/L	473 (405–542)	426 (355–497)	0.14
Myeloperoxidase, μg/L	960 (856–1064)	823 (747–898)	0.83

[a] From Ref. [12], Copyright 2009, with permission from Elsevier.
[b] Hypertension was defined as blood pressure ≥ 140/90 or the use of anti-hypertensive medication.
[c] Data available in 179 persons in the diabetes group and 168 persons in the control group.
[d] Cardiovascular disease was defined as prior myocardial infarction, angina pectoris or stroke.

p = 0.008) were associated with fatal ischemic heart disease. HDL-cholesterol was not significant in either group (p = 0.41 and p = 0.38, respectively), nor were triglycerides in the diabetes group (p = 0.11). The multivariate analyses were adjusted for age, gender, hypertension (yes, no), body mass index, previous cardiovascular disease and total cholesterol (continuous). Creatinine was not included, because it gives only a crude estimate of renal function and the association seen in univariate analysis was lost in multivariate analyses (p = 0.24). Data regarding smoking were missing in a few of the participants, but in those with a complete dataset on smoking it was not a significant predictor in Cox regression and did not affect the predictive value of the biomarkers.

Cox regression analysis in the patients with diabetes showed that subjects in the two highest tertiles of lactoferrin were at significantly higher risk for fatal ischemic heart disease, compared to subjects in the lowest tertile, hazard ratio 2.54 (1.00–6.45) and 4.06 (1.72–9.60) (Table 2). Survival curves are shown in Fig. 2. Myeloperoxidase concentrations did not predict fatal ischemic heart disease. When including myeloperoxidase and lactoferrin in the same analysis, there were only small changes in hazard ratios for lactoferrin: hazard ratio 2.85 (1.06–7.66) and 5.12 (1.72–15.28). In the participants without diabetes, none of the biomarkers could predict fatal ischemic heart disease (Table 2). The changes in HR after bootstrapping were small and the significance of the variables was not altered (Table 2). Somer's Dxy for the model including lactoferrin in the patients with diabetes was −0.55 before correction and −0.47 after correction by bootstrapping.

In the supplementary analysis using group-specific tertiles for lactoferrin and myeloperoxidase, the diabetic subjects in the highest tertile of lactoferrin (hazard ratio 3.41 (1.54–7.57)) as well as the highest tertile for myeloperoxidase (hazard ratio 2.60 (1.11–5.75)) were at significantly higher risk for fatal ischemic heart disease than the subjects in the lowest tertile. When both markers were included simultaneously, the highest tertile of lactoferrin remained

Table 2
Hazard ratios for tertiles of lactoferrin and myeloperoxidase in serum from participants with newly diagnosed diabetes and their control group.

	No. of person years	No. of deaths	Death rate per 1000 person years	Hazard ratio[a]	Confidence interval
Newly diagnosed diabetes					
Lactoferrin[b]					
Tertile I	753.1	8	10.6	1.0	(Reference)
Tertile II	865.6	13	15.0	2.54	(1.00–6.45)
				2.64	*(1.45–4.79)*
Tertile III	784.8	23	29.3	4.06	(1.72–9.60)
				4.40	*(2.55–7.59)*
Myeloperoxidase[b]					
Tertile I	739.7	9	12.2	1.0	(Reference)
Tertile II	879.6	15	17.1	1.25	(0.54–2.90)
				1.31	*(0.53–3.24)*
Tertile III	784.2	20	25.5	1.94	(0.85–4.43)
				2.03	*(0.87–4.72)*
Control group					
Lactoferrin[b]					
Tertile I	955.6	12	12.6	1.0	(Reference)
Tertile II	864.1	9	10.4	0.79	(0.33–1.94)
				0.82	*(0.48–1.42)*
Tertile III	782.0	7	9.0	0.65	(0.25–1.70)
				0.66	*(0.42–1.02)*
Myeloperoxidase[b]					
Tertile I	946.9	10	10.6	1.0	(Reference)
Tertile II	863.1	10	11.6	1.58	(0.63–3.97)
				1.78	*(0.95–3.33)*
Tertile III	791.7	8	10.1	1.39	(0.51–3.76)
				1.47	*(0.62–3.49)*

[a] Adjusted for age, gender, hypertension, body mass index, previous cardiovascular disease and total cholesterol. Bootstrapped estimates are given in italics.
[b] The tertiles for lactoferrin were <205, 205–428 and >428 μg/L, and the tertiles myeloperoxidase were <569, 569–951 and >951 μg/L.

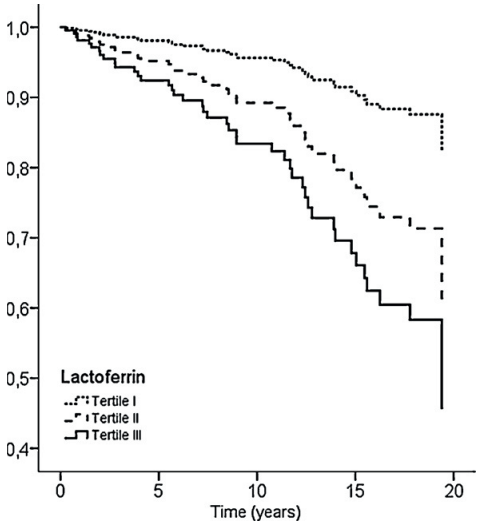

Fig. 2. Lactoferrin and death from ischemic heart disease in patients with newly diagnosed diabetes. Survival curves according to tertile of lactoferrin concentration at baseline. The model was adjusted for age, gender, hypertension, body mass index, previous cardiovascular disease and total cholesterol.

significant (hazard ratio 3.15 (1.10–9.01), whereas the highest tertile of myeloperoxidase did not (hazard ratio (1.17 (0.41–3.39)). In the participants without diabetes, both markers remained unable to predict fatal ischemic heart disease when group-specific tertiles were compared.

4. Discussion

We investigated if baseline concentrations of the neutrophil degranulation products lactoferrin and myeloperoxidase could predict fatal ischemic heart disease in 200 persons with newly diagnosed diabetes and in a group of 198 matched individuals without diabetes when followed for nearly 20 years. Increased concentrations of lactoferrin were associated with increased risk for fatal ischemic heart disease in subjects with newly diagnosed diabetes, whereas none of the biomarkers could predict fatal ischemic heart disease in the control group.

4.1. Choice of markers of neutrophil degranulation

Neutrophils contain several types of granules that are degranulated sequentially during activation. Specific (secondary) and azurophil (primary) granules have distinct functions, and it was considered useful to include one marker for each of these granule types. Lactoferrin and pentraxin 3 are among the constituents of the specific granules, whereas myeloperoxidase, elastase and beta-glucuronidase are found in the azurophil granules. However, pentraxin 3 is released from many cell types, including macrophages, fibroblasts and activated endothelium [17], and thus cannot be considered a marker of neutrophil activation. The same holds true for beta-glucuronidase. Epithelial cells at various mucosal surfaces may also release lactoferrin, but plasma lactoferrin is thought to stem mainly from neutrophils and is significantly correlated with blood neutrophil counts (unpublished data). Myeloperoxidase and neutrophil elastase are also considered relatively neutrophil-specific. Since our lab has extensive experience in quantifying lactoferrin and myeloperoxidase, these markers were preferred.

4.2. Diabetes, neutrophils and atherosclerosis

Most aspects of neutrophil function are impaired in patients with diabetes, including migration to inflammatory sites, phagocytosis, release of lytic proteases, production of reactive oxygen species and apoptosis [18,19]. The role of neutrophils in coronary artery disease is not fully understood. In animal studies, they are the first inflammatory cells appearing in an atherosclerotic plaque [20]. Neutrophils secrete inflammatory mediators and proteases that can cause arterial damage [4]. They can oxidise LDL which in turn recruits macrophages, further contributing to the development of the plaque [21]. Neutrophils are also known to increase the myocardial damage following myocardial infarction. Thus, an altered neutrophil function may influence the atherogenic process in diabetes patients.

Our study demonstrated that an increased baseline concentration of lactoferrin was strongly associated with fatal ischemic heart disease during long-term follow-up of patients with diabetes. The diabetes patients as a group did not have significantly higher baseline concentrations of lactoferrin than the control participants, even if a higher proportion had previous cardiovascular disease (Table 1) and patients with significant coronary stenosis have higher lactoferrin concentrations than patients without [22]. This may be due to the facts that the majority of subjects with diabetes (78%) did not have previous cardiovascular disease and that lactoferrin concentrations overlap between patients with and without significant atherosclerosis [22].

Neutrophil priming in diabetes is induced by hyperglycemia and advanced glycated end-products and is mediated through increased intra-cellular calcium concentrations [23,24]. In diabetes, primed neutrophils contribute to oxidative stress and inflammation, which may lead to endothelial dysfunction [9,25]. Lactoferrin could not predict fatal ischemic heart disease in the control group. This could be due to lack of statistical power. Due to the confidence intervals that widely overlap 1 in the second and third lactoferrin tertiles in the control group, the results are far from significant and the null hypothesis that the hazard ratios are similar in all tertiles cannot be rejected. Therefore, the seemingly falling hazard ratios cannot be considered as representing a trend. Our findings support the hypothesis that increased lactoferrin concentrations reflect neutrophil priming caused by hyperglycemia in persons with diabetes.

An increased baseline concentration of lactoferrin in newly diagnosed diabetes patients may also indicate a more active pro-inflammatory condition and thereby an increased propensity to cardiovascular disease. Alternatively, lactoferrin may act as a surrogate marker of the neutrophil count, which is also related to the level of inflammation. The neutrophil count is a well-known predictor for coronary events [4] and of stenosis complexity [26].

As opposed to most substances released from activated neutrophils, lactoferrin has several anti-inflammatory effects. Lactoferrin acts as a bacteriostatic by binding free iron, which is an essential growth factor for microorganisms. Iron also catalyzes the formation of reactive oxygen species, and as lactoferrin binds iron, this may contribute to its anti-inflammatory properties. Lactoferrin inhibits pro-inflammatory cytokine production in monocytes which may be a feedback mechanism to down-regulate inflammation by preventing neutrophil recruitment and activation [27]. Lactoferrin also contributes to the regulation of immune cells, both by binding of iron and by iron-independent mechanisms [28]. In diabetes, lactoferrin function is inhibited by hyperglycemia and formation of advanced glycated end-products [29]. Thus, the anti-inflammatory effects of lactoferrin may be disturbed in diabetes, which in turn may increase the risk of atherosclerosis.

Under certain circumstances, however, lactoferrin may also act pro-inflammatory by activating macrophages and inducing interleukin-8, tumor necrosis factor-α and nitric oxide production [30]. There are few studies concerning the relationship between lactoferrin and the development of atherosclerosis and ischemic heart disease. One study by Videm et al. demonstrated that increased concentrations of lactoferrin, but not myeloperoxidase, were significantly related to the presence of significant coronary artery stenosis in patients admitted for elective angiography [22]. This finding supports the conclusion of the present study.

Pentraxin 3, which belongs to the same superfamily of acute-phase proteins as C-reactive protein, provides another example of a molecule with diverse effects in relation to cardiovascular disease. Increased concentrations strongly predicted mortality after an acute myocardial infarction [31], but pentraxin 3 showed atheroprotective effects in a knock-out mouse model [17].

4.3. Lactoferrin versus other biomarkers of ischemic heart disease

Myeloperoxidase did not predict death from ischemic heart disease in our study. In the supplementary Cox regression analysis using group-specific cut-off concentrations for myeloperoxidase, subjects with diabetes in the highest tertile of myeloperoxidase concentrations were at significantly higher risk of death from ischemic heart disease. However, this relationship was lost when lactoferrin was also included in the model. This is probably explained by the fact that the two granule proteins are correlated to a certain extent, so that myeloperoxidase was able to "substitute" for lactoferrin when the latter marker was not included. The independent contribution of myeloperoxidase was small, as indicated by the combined model.

Myeloperoxidase is found in atherosclerotic plaques, leads to the formation of reactive oxygen species and exerts several effects on the vasculature [32]. In contrast to lactoferrin, myeloperoxidase has been widely studied as a predictor of coronary events, especially in established coronary artery disease and in the acute phase [33,34]. Since myeloperoxidase and lactoferrin are present in different granules, it is not unreasonable that their behaviour as biomarkers was not equivalent. The specific granules containing lactoferrin are more readily mobilized than the azurophil granules containing myeloperoxidase, which results in easier release of lactoferrin to blood in response to a weaker stimulus. Stimulation of neutrophils results in degranulation of approximately 80% of the total cell content of lactoferrin, as opposed to only 15–20% of total myeloperoxidase [35]. This implies that lactoferrin may have a wider dynamic concentration range than myeloperoxidase, as it is released both when the neutrophils are weakly and strongly stimulated.

Previous work from our group showed that C-reactive protein also was a significant predictor for ischemic heart disease in the study population [12]. After additional adjustment for C-reactive protein in the multivariate model, the two highest tertiles of lactoferrin remained significant and the hazard ratios showed minor changes (hazard ratio 2.67 (1.03–6.93) and hazard ratio 3.97 (1.60–9.90)). Thus, lactoferrin seems to be an independent and strong predictor of fatal ischemic heart disease in patients with diabetes.

4.4. Limitations

Internal validation showed that the logistic regression model including lactoferrin in the patients with diabetes was somewhat overfit, as indicated by a smaller value of Somer's Dxy after bootstrapping. This was not surprising, given the relatively small study group. However, the corrected value of −0.47 still indicates that there will be a strong relationship between predicted log hazards and observed survival time in future studies.

The study population was not large enough for stratification with respect to whether diabetes was well or poorly regulated, or by gender or age. The study carries a risk of false negative conclusions, for example regarding the usefulness of myeloperoxidase as predictor in diabetes patients. It was not designed to prove causal relationships or to investigate pathophysiological mechanisms. Nevertheless, our results suggest that the predictive value of lactoferrin differs between subjects with and without diabetes. Since lactoferrin is a novel marker, substantial work remains to be done before its potential clinical usefulness is clarified. However, the present data indicate that further investigations, e.g. comparison with established risk markers in larger populations, are warranted.

The HUNT 1 study is a population-based study. Variables such as the left ventricular ejection fraction or coronary artery disease extension by angiography, that might influence the risk of fatal ischemic heart disease, were not available and our results could not be adjusted for them.

After storage for up to 20 years, the quality of serum may have been reduced. However, samples from both groups were handled similarly. Since concentrations of myeloperoxidase and lactoferrin were compared between the groups, changes in absolute concentrations due to storage should not be important.

5. Conclusion

Baseline concentration of lactoferrin was a strong predictor of fatal ischemic heart disease in subjects with newly diagnosed diabetes, whereas myeloperoxidase did not predict death from ischemic heart disease in this group. The finding may reflect neutrophil priming and thus over-activation because of hyperglycemia in diabetes. Further studies on the relationship between lactoferrin and cardiovascular disease are warranted.

Competing interests

The authors have no competing interests.

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Paper III

Mannose-binding lectin deficiency is associated with myocardial infarction: the HUNT2 Study in Norway

Inga Thorsen Vengen (MD)[a], Hans O. Madsen (PhD)[b], Peter Garred (MD, PhD)[b], Carl Platou (MD)[c], Lars Vatten (MD, PhD)[d], Vibeke Videm (MD, PhD)[a,e]

[a] Department of Laboratory Medicine, Children´s and Women´s Health, Norwegian University of Science and Technology. [b] Laboratory of Molecular Medicine, Department of Clinical Immunology, Sect. 7631, Rigshospitalet, Faculty of Health Sciences, University of Copenhagen, Denmark. [c] Department of Internal Medicine, Levanger Hospital, Nord-Trøndelag Health Trust, Levanger, Norway. [d] Department of Public Health, Norwegian University of Science and Technology [e] Department of Immunology and Transfusion Medicine, Trondheim University Hospital, Norway.

(*Submitted*)

Abstract

Objectives: Mannose-binding lectin (MBL) and ficolins activate the complement cascade, which is involved in the pathogenesis of atherosclerosis. Functional polymorphisms in the MBL gene (*MBL2*) may be associated with the risk of ischemic heart disease. We therefore studied polymorphisms in *MBL2* and ficolin genes in relation to the risk of myocardial infarction (MI).

Methods and Results: Using the population-based HUNT Study in Norway, 57133 persons were followed up for a first-time MI. Among these, the 370 youngest MI patients were matched by age and gender to 370 controls. Age ranged from 29 to 62 years of age. A younger population was selected because their genetic disposition for cardiovascular disease is likely to be stronger and less dependent on non-genetic risk factors. Polymorphisms in *MBL2* and in the genes of ficolin-1, ficolin-2 and ficolin-3 were genotyped by pyrosequencing and related to the risk of MI, estimated as odds ratios (OR). Variant *MBL2* haplotypes causing MBL deficiency were associated with a two-fold higher risk of MI (OR 2.04, 95%CI 1.29-3.24). Adjustments for conventional cardiovascular risk factors did not substantially influence the association. The ficolins were not associated with MI risk.

Conclusion: In a young to middle aged and relatively healthy Caucasian population, MBL deficiency was associated with a doubling of the risk for MI, independent of conventional risk factors. This supports a protective role of MBL in atherosclerosis.

Introduction

Accumulating evidence suggests that atherosclerosis is an inflammatory disease and that the innate immune system plays a crucial part in the pathophysiology [1]. The complement system is involved at different stages of atherosclerosis, from the early formation of fatty streaks [2] until destabilization of mature plaques. In addition to enhancing the atherosclerotic process, activation of the complement cascade may also have a protective effect by removing cell debris and immune complexes from the atherosclerotic lesions.

The complement system is activated through three possible pathways, denoted as the classic, alternative or lectin pathway. The latter is initiated by mannose-binding lectin (MBL) or by proteins of the ficolin family [3]. During the last decade MBL has received attention as a potential marker of atherosclerosis. The MBL gene (*MBL2*) has several polymorphic sites [4], and the combined genetic profile corresponds to *normal*, *intermediate* or *deficient* serum concentrations of the protein [5]. After the original study by Madsen et al in 1998, where MBL deficiency was associated with increased risk for severe atherosclerosis in relatively young patients [6], polymorphisms in *MBL2* and serum concentrations of the protein have been linked to both increased and reduced risk of atherosclerosis and coronary artery disease in different populations [7-11]. Furthermore, a study in knockout mice demonstrated increased atherosclerotic lesions when the lectin pathway was inhibited [12].

In a pilot study including patients with stable angina pectoris, we found an increased frequency of variant *MBL2* haplotypes corresponding to MBL deficiency in patients with significant coronary artery stenosis compared to patients without significant stenosis (19 % (25 of 131) versus 10% (10 of 103),

p=0.05), (Garred P. and Videm V., unpublished data). Based on the results from that study, we hypothesised that MBL deficiency is related to the development of myocardial infarction (MI) and that variations in the genes coding for MBL and ficolins may be associated with atherosclerosis.

We therefore performed a case-control study, assessing the association of *MBL2* and ficolin genotypes with the risk of a first-time MI at young and middle age, i.e. at an age when the genetic influence may be stronger and less dependent on non-genetic risk factors than at older age.

Methods

This case-control study was generated by linkage of population data from the second wave of the Nord-Trøndelag Health Study (HUNT2) to validated information on incident acute MIs.

HUNT2 was carried out in 1995-1997 as a population-based study and information was collected through comprehensive questionnaires and a clinical examination. All inhabitants 13 years of age and older were invited, and a venous blood sample was drawn from all persons 20 years of age and older. In total, about 75 000 (70 %) of those invited attended the study. The inclusion process is described elsewhere [13].

There are two primary referral hospitals in the county of Nord-Trøndelag (Levanger Hospital and Namsos Hospital). Data on all acute MI hospitalizations from 1995 (corresponding to the commencement of HUNT2) to the end of 2000 were registered retrospectively, whereas from 2001 registration has been done prospectively. MI was diagnosed according to the European Society of

Cardiology/American College of Cardiology consensus guidelines [14]. The criteria were elevated troponin T or troponin I at the same time course with at least one of the following criteria: 1) symptoms consistent with myocardial infarction and/or 2) ECG changes with development of significant Q wave and/or 3) ECG changes consistent with ischemia (ST-segment elevation or depression).

Among participants in HUNT2, the following criteria had to be met to be eligible for the present study: available DNA, and no previous self-reported MI, angina pectoris or stroke. In total, 57 133 individuals met these criteria. We linked these HUNT2 participants to the hospital registrations to ascertain incident cases of MI from baseline at HUNT2 until the end of 2008. During follow-up, 1689 individuals had experienced an MI. Among incident MI patients, the 370 youngest were selected as cases in the study. As controls, we randomly selected 370 participants who were matched to the cases by age (± 2 years) and gender. All controls were at risk of MI at the time when the MI occurred in their respective matched case.

The study protocol conformed to the Helsinki declaration. The study was approved by the Regional Committee for Medical Research Ethics and the Data Inspectorate of Norway. The HUNT2 participants had signed consent to participate in morbidity and mortality follow-up studies.

Clinical information

Measurements of blood pressure, height, weight, waist and hip circumference were done as previously described [13]. Body mass index (BMI) and waist-hip ratio (WHR) were calculated. Concentrations of blood lipids, creatinine and glucose were analysed by standard methods at the Central Laboratory at Levanger

Hospital. Hypertension was defined as systolic blood pressure ≥ 140 mmHg or as diastolic blood pressure ≥ 90 mmHg, or as current use of antihypertensive medication. Information on use of other medications, such as statins or anti-platelet therapy was not available. Hypercholesterolemia was defined as total cholesterol > 6.2 mmol/L. Smoking was classified in three groups: never, former or current smokers. A report of MI before 60 years of age in first-degree relatives was considered as a positive family history. The Framingham risk score [15] was calculated based on the corresponding variables from the HUNT2 database (age, HDL-cholesterol, total cholesterol, systolic blood pressure, antihypertensive treatment, smoking and diabetes). To classify the metabolic syndrome, a modified set of criteria based on The International Diabetes Federation consensus [16] were used. The criteria were 1) *central obesity*, (men: waist circumference ≥ 94 cm; women: waist circumference ≥ 80 cm) plus two of the following four criteria 2a) *low HDL cholesterol* (men < 1.03 mmol/L; women < 1.29 mmol/L), 2b) *hypertension* (systolic blood pressure ≥ 130 mmHg or diastolic blood pressure ≥ 85 mm Hg, or treatment for hypertension), 2c) *fasting plasma glucose ≥ 5.6 mmol/L* or previously diagnosed *type 2 diabetes*, 2d) *fasting triglycerides > 1.7 mmol/L*.

Genotyping

DNA was extracted from peripheral blood leukocytes at the HUNT biobank using a commercial kit (Puregene, Gentra Systems, Minneapolis, MN) or by a robotic method (Autopure LS, Gentra Systems). Genotyping was performed using a combination of polymerase chain reaction (PCR) and pyrosequencing. All of the

single nucleotide polymorphisms (SNPs) are found in the online database http://www.ncbi.nlm.gov/projects/SNP.

Four different SNPs in *MBL2* were investigated (Figure, Panel A). Three of them are in exon 1 and give rise to the structural alleles *B* (codon 54, rs1800450), *C* (codon 57, rs1800451) and *D* (codon 52, rs5030737). Wild type is denoted *A.* The fourth is considered the most important promoter polymorphism: *X/Y* (rs7096206). These SNPs are inherited in haplotypes. To simplify the interpretation, data are presented by pooling the structural alleles *B-D* to one allele denoted *O* [4]. The structural alleles are always found on a *Y* promoter background, thus we used the term *YO* to denote this defective haplotype. Combining the promoter variant with the *A* and *O* alleles results in 6 haplotypes, as shown in Figure, Panel B. These haplotypes were further combined into three haplotype groups: *normal (YA/YA, YA/XA)*, *intermediate (XA/XA, YA/YO)* or *deficient (XA/YO, YO/YO)*, which correspond to serum concentrations of functional MBL [5].

One common polymorphic site in the promoter of the ficolin-1 gene (*FCN1* -542, rs10120023) was genotyped. In the ficolin-2 gene (*FCN2*), two amino acid substituting SNPs in exon 8 were included. They are known to cause increased (*FCN2* +6424, rs7851696) and reduced (*FCN2* +6359, rs17549193) binding capacity of the protein to *N*-acetylglucosamine, respectively [17]. The gene coding for ficolin-3 (*FCN3*) is less polymorphic, but a frame-shift variation in position +1637 (rs28357092) of *FCN3* is known to cause a 50% reduction of serum ficolin-3 in heterozygotes, and total ficolin-3 deficiency in homozygotes [18]. Despite its low allele frequency, this SNP was also included.

Four different PCR reactions were set up: *MBL2* exon 1, *MBL2* promoter, both *FCN2* SNPs, and *FCN1* and *FCN3* in the same reaction. The primers are available in an online supplement (Primers). One primer in each pair was biotinylated. Evaluation of the PCR products by agarose gel electrophoresis showed specific bands of the expected molecular weights. The PCR product was further used for pyrosequencing. We chose the pyrosequencing platform because it has been successfully used for *MBL2* genotyping [19]. Pyrosequencing was performed with a standard protocol on Pyro sequencer PSQ 96MA (Pyrosequencing AB; Biotage, Uppsala, Sweden), using a commercially available kit (PyroMark Gold Q96 Reagents, Qiagen, Germany).

Statistical analyses

Based on an expected frequency of 0.08 of the *MBL2* combined low expressing haplotype (*YO/YO+XA/YO*) in Caucasians, a power calculation was performed. To detect an odds ratio (OR) of 2.0, assuming a power of 80 % and a 5% significance level, 320 persons were required in each group. In order to account for possible variations in the genotype distribution in small datasets, 370 persons were included in each group. The study population was too small for analyses stratified by gender.

McNemar´s test was used to compare numbers of discordant pairs. Due to non-normal distribution of several variables, the Wilcoxon signed rank test was used to evaluate differences in continuous and ordinal variables between pairs. The Chi-square test was used for comparison of allele frequencies. Deviation

from the Hardy-Weinberg equilibrium was calculated by using the chi-square test.

Conditional logistic regression was performed to evaluate associations between the three inferred *MBL2* haplotype groups and risk of MI. Further models were developed, where traditional risk factors (hypertension, hypercholesterolemia, smoking, diabetes and BMI (continuous)), the Framingham risk score or the metabolic syndrome were also included.

All tests were two-sided and the results are presented as means, ORs or HRs (with 95% confidence intervals (CI)). To avoid false positive conclusions, the alpha level of significance for the comparisons of haplotype frequencies between cases and controls were obtained by permutation testing, using 10,000 permutations. By this method, which is considered the gold standard, a result is significant if the observed p-value is lower than the empirical p-value found under permutation. For other tests, p-values below 0.05 were considered statistically significant. Permutation testing was performed using the R package, version 2.14.1 (http://www.r-project.org). All other analyses were performed with Stata/MP for Mac, version 11.2, (Stata Corp., College Station, Texas, USA).

Results

Background characteristics of MI cases and their matched controls are displayed in Table 1. Among cases, baseline measurements of conventional risk factors indicated higher risk of MI in cases than controls: cases had higher BMI, WHR, Framingham risk score and a more unfavourable lipid profile. Furthermore hypertension, diabetes, current smoking, the metabolic syndrome and family history of MI were also more frequent among cases. Creatinine concentrations

were similar in the two groups, and were below 140 μmol/L in all participants, indicating no severe chronic renal failure. Mean age at MI was 53 years (range 29 – 62 years).

There were no significant deviations from the expected Hardy-Weinberg distributions in the control group (for structural alleles, p = 0.39). Frequencies of *MBL2* haplotypes are given in Table 2. There were higher frequencies of variant haplotypes causing MBL deficiency among cases, compared to controls (p=0.025, alpha level by permutations =0.028). Ficolin genotypes are shown in Table 3. There were no significant differences between cases and controls. For *FCN1* -542, however, the number of homozygous individuals was higher in the control group (p=0.07, recessive model). Frequencies of *MBL2* and ficolin alleles are available online (Allele frequencies A and B).

Conditional logistic regression showed that variant *MBL2* haplotypes causing MBL deficiency were positively associated with MI (Table 4). The three haplotype groups, corresponding to functional MBL concentration, were used in the analyses. The odds for MBL deficiency among MI cases was twice as high as in controls (OR=2.04, 95%CI 1.29 – 3.24, p=0.003), and adjustment for conventional cardiovascular risk factors did not substantially influence the association (OR 2.02, 95%CI (1.17-3.47), p=0.012). There was missing data on family history in 131 case-control pairs (71 cases and 66 controls, p=0.64), and information on family history was therefore not included in the analysis.

Discussion

In this population-based case-control study we found that variant *MBL2* haplotypes causing MBL deficiency were associated with a doubling of the risk of MI at middle age (before the age of 62 years). The association was independent of conventional risk factors for MI.

Previous studies support our finding. However, those studies were performed among patients with severe atherosclerosis [6] or other predisposing conditions, such as a high prevalence of coronary artery disease [7] or inflammatory diseases, i.e. systemic lupus erythematosus [11], rheumatoid arthritis [20] or type 2 diabetes mellitus. Our results also suggest that MBL deficiency is a particularly strong risk factor for cardiovascular events among young to middle-aged and apparently healthy individuals.

Although the study population was relatively young, conventional cardiovascular risk factors, including hypertension, hypercholesterolemia, smoking and high BMI were also associated with increased risk of MI. Despite incomplete data, there was also a positive association of family history of MI with MI risk. We chose to study people at middle age, anticipating that underlying causes of an early MI would be more likely to be genetic compared to an older age, when non-genetic causes may dominate. Another reason was our previous finding that *MBL2* was more strongly associated with severe atherosclerosis in the youngest patients going through coronary surgery [6]. At older age, the importance of genetic factors may be difficult to distinguish from the impact of environmental and life style factors and comorbidities.

Previous studies support a cardio-protective role of MBL [21] and activation of the lectin pathway. Rats with MBL deficient macrophages fed on a high-

cholesterol diet were more likely to develop atherosclerotic lesions, which may be explained by reduced removal of apoptotic cells and debris by MBL [12]. In humans, variant *MBL2* alleles may be correlated with increased carotid plaque area [9] and MBL deficient individuals may also have higher postprandial lipid values [22], which in turn may contribute to the development of atherosclerosis [23]. Although the link between infections and atherosclerosis is not verified, a combination of MBL deficiency and infection was related both to the development of coronary artery disease [8] and to reduced flow-mediated vasodilation [24], which is an early marker of endothelial dysfunction. Those results imply plausible mechanisms that may contribute to an increased risk of atherosclerosis in the presence of MBL deficiency.

On the other hand, others have found that high serum MBL-concentrations [10] and wild type *MBL2* may be associated with increased risk of cardiovascular disease. A dual effect of MBL has been suggested, as both high and low serum concentrations of MBL were correlated with increased intima-media thickness of the carotid artery in persons with rheumatoid arthritis [20]. Speidl et al have suggested that activation of the complement cascade by the alternative pathway may be proatherogenic as a result of inflammation, whereas activation through the lectin and classical pathways may have protective effects [25]. It should be noted that MBL and activation of the lectin pathway of complement appears to be central in ischemic reperfusion injury, which may blur the relative cardioprotective effects of MBL in atherosclerosis [26, 27]. Thus, dependent on the local microenvironment in the vessel wall, MBL may be both advantageous and disadvantageous in cardiovascular pathophysiology.

Analysis of haplotypes with known functional consequences and ensuring stringent alpha levels of significance by permutation testing strengthen the probability of a causal relationship, even if the design of our study did not allow direct causal inference. Our results corroborate that genetically determined MBL deficiency is linked to atherosclerosis. However, we cannot exclude that high MBL concentration and an "eager" complement system may also be harmful in the atherosclerotic process under some circumstances.

None of the ficolin polymorphisms were significantly related to MI. One may speculate that being homozygous for *FCN1 -542* yields some protection, but little is known about the effects of this genetic variation, and more research is needed.

This study was not designed to test improvement of risk prediction. However, the results may generate new hypotheses regarding pathophysiology.

Study Limitations

There are some limitations to our study. Serum was not available at the time of the genotyping. However, previous studies have shown that serum concentrations of functional MBL correspond closely to the genotypes [5]. The results were not replicated in a similar cohort, as they were partly confirmatory. Although the population in Norway is assumed to be generally representative for the Caucasian population, we cannot exclude the possibility that MBL may be more important in relation to cardiovascular disease in this population compared to others.

Conclusions

The *MBL2* haplotypes corresponding to functional MBL deficiency were associated with a doubling of the risk for MI in individuals younger than 62 years of age, independent of conventional risk factors. The findings confirm our hypothesis and support a protective role of MBL in atherosclerosis.

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Conflicts of Interest: none declared.

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Table 1. Baseline characteristics

	Cases (n = 370)	**Controls** (n = 370)	p-value
Gender, female / male	88 / 282		---
Age, years	48 (47 – 48)		---
BMI†, kg/m^2	27.4 (27.0 – 27.8)	26.5 (26.1 – 26.9)	0.003
WHR‡	0.89 (0.88 – 0.90)	0.88 (0.87 – 0.89)	0.011
Hypertension	194 (52%)	162 (44%)	0.015
- Systolic blood pressure	140 (139 – 142)	136 (135 – 138)	0.002
- Diastolic blood pressure	85 (84 – 86)	83 (82 – 84)	0.003
Hypercholesterolemia	242 (65%)	146 (39%)	< 0.0005
Diabetes mellitus	13 (4 %)	4 (1 %)	0.049
Total cholesterol, mmol/L	6.8 (6.6 – 6.9)	6.0 (5.9 – 6.2)	< 0.0005
Triglycerides, mmol/L	2.53 (2.35 – 2.70)	2.05 (1.91 – 2.18)	< 0.0005
HDL cholesterol, mmol/L			
- Women	1.37 (1.29 – 1.45)	1.49 (1.40 – 1.58)	0.024
- Men	1.13 (1.08 – 1.17)	1.22 (1.18 – 1.26)	< 0.0005
Smoking			
- Never	68 (19 %)	114 (32%)	
- Former	67 (18 %)	81 (23 %)	
- Current	228 (63 %)	156 (44 %)	< 0.0005
Framingham risk score			
- Women	13.1 (12.0 – 14.2)	9.3 (8.1 – 10.5)	< 0.0005
- Men	13.4 (13.0 – 13.9)	11.6 (11.1 -12.1)	< 0.0005
Metabolic syndrome	37 (10%)	20 (5%)	0.022
Family history§	100 (27%)	54 (15%)	0.001

* Myocardial infarction (MI)
† Body mass index (BMI)
‡ Waist hip ratio (WHR)
§ Myocardial infarction before 60 years in first-degree relatives

Table 2. Haplotype frequencies for *MBL2*

	***MBL2* genotypes**		***MBL2* haplotypes**		***MBL2* recessive model**	
	Cases	Controls	Cases	Controls	Cases	Controls
YA/YA	112 (30 %)	117 (32 %)				
YA/XA	86 (23 %)	100 (27 %)	198 (54 %)	217 (59 %)		
XA/XA	18 (5 %)	12 (3 %)				
YA/YO	91 (25 %)	108 (29 %)	109 (29 %)	120 (32 %)	307 (83 %)	337 (91 %)
XA/YO	43(12 %)	24 (7 %)				
YO/YO	20 (5 %)	9 (2 %)	63 (17 %)	33 (9 %)	63 (17 %)	33 (9 %)
p-value		**0.025**		**0.005**		**0.001**
Alpha level by permutation		0.028		0.023		0.029

Table 3. Genotype frequencies for *FCN1*, *FCN2* and *FCN3*

	Cases	**Controls**	**p-value**
	***FCN1* -542 G/A**		
G/G	148 (40 %)	137 (37 %)	
G/A	177 (48 %)	170 (46 %)	
A/A	45 (12 %)	63 (17 %)	0.19*
	***FCN2* +6359 C/T**		
C/C	181 (49 %)	196 (53 %)	
C/T	157 (42 %)	141 (38 %)	
T/T	32 (9 %)	33 (9 %)	0.46
	***FCN2* +6424 G/T**		
G/G	289 (78 %)	295 (80 %)	
G/T	77 (21 %)	71 (19 %)	
T/T	4 (1 %)	4 (1 %)	0.86
	***FCN3* +1637 C/-** †		
C/C	363 (99 %)	364 (99 %)	
C/-	5 (1 %)	4 (1 %)	
-/-	0 (0 %)	0 (0 %)	0.74

* *FCN1* recessive model: p=0.069

† 4 missing

Table 4. Conditional logistic regression analyses, *MBL2* functional groups

	OR	95 % CI	p-value
Model 1			
YA/YA, YA/XA	1		
XA/XA, YA/YO	1.01	(0.72 – 1.41)	0.96
XA/YO, YO/YO	2.04	(1.29 – 3.24)	0.003
*Model 2**			
YA/YA, YA/XA	1		
XA/XA, YA/YO	1.02	(0.73 – 1.44)	0.89
XA/YO, YO/YO	1.91	(1.19 – 3.08)	0.008
*Model 2 – Adjusted for classical risk factors** †			
YA/YA, YA/XA	1		
XA/XA, YA/YO	1.26	(0.84 – 1.88)	0.27
XA/YO, YO/YO	2.02	(1.17 – 3.47)	0.012
*Model 2 – Adjusted for Framingham risk score** ‡			
YA/YA, YA/XA	1		
XA/XA, YA/YO	1.19	(0.80 – 1.77)	0.39
XA/YO, YO/YO	2.09	(1.22 – 3.59)	0.007
Model 3 – Adjusted for metabolic syndrome §			
YA/YA, YA/XA	1		
XA/XA, YA/YO	1.06	(0.76 – 1.49)	0.73
XA/YO, YO/YO	1.98	(1.25 – 3.16)	0.004

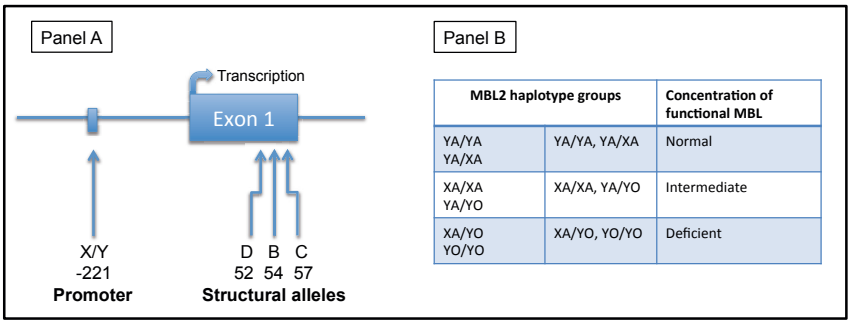
* 26 pairs excluded because one or more missing values.
† Adjusted for classical risk factors: Hypertension (BP > 140/90 or current use of antihypertensive medication), body mass index (kg/m^2, continuous), hypercholesterolemia (total cholesterol > 6.2 mmol/L), diabetes (yes/no) and smoking (never/former/current).
‡ Adjusted for Framingham risk score (age, HDL-cholesterol, total cholesterol, systolic blood pressure, smoking and diabetes)
§ 3 pairs excluded because one or more missing values.

Figure.

Panel A. Simplified figure of the investigated *MBL2* polymorphisms.

Wild type allele is *A.*

Panel B: *MBL2* haplotypes and corresponding concentrations of functional MBL.



Panel B

MBL2 haplotype groups		Concentration of functional MBL
YA/YA YA/XA	YA/YA, YA/XA	Normal
XA/XA YA/YO	XA/XA, YA/YO	Intermediate
XA/YO YO/YO	XA/YO, YO/YO	Deficient

Supplementary Data.

Primers

Gene	**Primer**	**Primer sequence (5´-3´)**
MBL2 exon 1,	Forward	CCTTCCCTGAGTTTTCTCAC
codon 52, 54 and 57	Reverse	AACAGCCCAACACGTACCTG
(*D*, *B*, *C*)	Sequencing	CGTACCTGGTTCCCCCTTTTCT
MBL2 promoter	Forward	TGGTGTGAGAAAACTCAGGGAAG
-221 (*X/Y*)	Reverse	GCACGGTCCCATTTGTTCTC
	Sequencing	CTGGAAGACTATAAACATGCTT
FCN1 -542	Forward	TCCCAAATACTATTTCCATCATATC
	Reverse	CTTCAATTTCTCCAGCTGTAACT
	Sequencing	ATCTTGCACCAGCCC
FCN2 +6359, +6424	Forward	TCACATTTCCTCCTGCACAGG
	Reverse	TTGACACATGGCAGTTTTTGTAC
	Sequencing +6359	CACAGGAGATTCCCTGA
	Sequencing +6424	GATCTTAACACCGGAAATT
FCN3 +1637	Forward	GAGCCAGGGCGCCACCTT
	Reverse	CCCCCCTCGGTGTCCATGT
	Sequencing	CTACCTGAGGGCAGG

Supplementary Data.

Allele frequencies

A. Distribution of *MBL2* alleles

	Cases	**Controls**
SUM *A/A*	**216 (58%)**	**229 (62%)**
A/B	82 (22%)	79 (21%)
A/C	9 (2%)	4 (1%)
A/D	43 (12%)	49 (13%)
SUM *A/O*	**134 (36%)**	**132 (36%)**
B/B	7 (2 %)	2 (0.5%)
B/C	2 (0.5%)	0 (0%)
B/D	7 (2 %)	4 (1 %)
C/C	0 (0%)	0 (0%)
C/D	0 (0%)	1 (0.3%)
D/D	4 (1 %)	2 (0.5%)
SUM *O/O*	**20 (5.4%)**	**9 (2.4%)**
Allele frequency A	566 / 740 (76%)	590 / 740 (80%)
Allele frequency O	174 / 740 (24%)	150 / 740 (20%)
Y/Y	223 (60%)	234 (63%)
X/Y	129 (35%)	124 (34%)
X/X	18 (5%)	12 (3%)
Allele frequency Y	575 / 740 (78%)	592 / 740 (80%)
Allele frequency X	165 / 740 (22%)	148 / 740 (20%)

Supplementary Data.

Allele frequencies

B. Allele frequencies for *FCN1*, *FCN2* and *FCN3*

	Cases	Controls	p-value
FCN1 -542			
G	473/740 (64%)	444/740 (60%)	
A	267/740 (36%)	296/740 (40%)	0.12
FCN2 +6359			
C	519/740 (70%)	533/740 (72%)	
T	221/740 (30%)	207/740 (28%)	0.42
FCN 2 +6424			
G	655/740 (89%)	661/740 (89%)	
T	85/740 (11%)	79/740 (11%)	0.62
FCN3 +1638			
C	731/740 (99%)	732/740 (99%)	
-	5/740 (0.7%)	4/740 (0.5%)	0.74

Appendix
Q1-H1

MELDING OM SKJERMBILDEFOTOGRAFERING OG UNDERSØKELSE AV BLODTRYKK OG BLODSUKKER

Skjermbildefotograferingen kommer nå til ditt distrikt. Denne gangen inngår fotograferingen i en større helseundersøkelse, og vi viser til orienteringen som er gitt i den vedlagte brosjyre.

Tid og sted for frammøte vil du finne nedenfor.

Vennligst fyll ut spørreskjemaet på baksiden og ta det med til undersøkelsen. Ta også med skjermbildebevis, tuberkulinkort eller helsebok om du har.

Det er viktig at du møter fram selv om du nylig har fått kontrollert blodtrykk eller blodsukker, og selv om du er under behandling for høyt blodtrykk eller for sukkersyke.

Med vennlig hilsen

Statens skjermbildefotografering

Postboks 8155 Dep, Oslo 1

Fylkeslegen • Helserådet • Statens Institutt For Folkehelse

Født dato | Personr. | Kommune | Kretsnr.

Møtested | Kjønn | Første bokstav etternavn | Dag og dato | Klokkeslett

H. 14 | V. 18 | SBT_1 21 | DBT_1 24 | PULS 27 | SBT_2 30 | DBT_2 33 | SYKEPL 35

TID 36 | GLUC 39 | GLUC 42 | GLUC 45 | HG 46 | BT 47 | P 48 | Ø.M. 49

A. Hvordan er helsa di for tida?
(Sett kryss i bare *en* rute.)

Dårlig	50	1
Ikke helt god		2
God		3
Svært god		4

B. Har du i løpet av de siste 12 måneder vært hos?

	JA	NEI
Almenpraktiserende lege (distriktslege, privatpraktiserende lege,turnuskandidat)	51	
Bedriftslege	52	
Militærlege	53	
Lege ved sykehus (uten at du var innlagt)	54	
Annen lege	55	

C. Har du vært innlagt i sykehus de siste 5 åra? 56

D. Bruker du, eller har du brukt, medisin for høyt blodtrykk? 57

E. Har du eller har du hatt noen av disse sykdommene?

	JA	NEI
Sukkersyke	58	
Hjerteinfarkt	59	
Angina pectoris (hjertekrampe)	60	
Hjerneslag eller hjerneblødning	61	

F. Har du noen langvarig sykdom, skade eller lidelse av fysisk eller psykisk art som nedsetter dine funksjoner i ditt daglige liv? (Med langvarig menes at det har vart, eller vil vare i minst ett år.) 62

Hvis «JA», vil du si at dine funksjoner er litt, middels eller mye nedsatt?

	LITT	MIDDELS	MYE
Er bevegelseshemmet	63		
Har nedsatt syn	64		
Har nedsatt hørsel	65		
Hemmet pga. kroppslig sykdom	66		
Hemmet pga. psykiske plager	67		

G. Har du noen søsken? (Nålevende eller døde) 68

Hvis «JA», har en eller flere av dem hatt noen av disse sykdommene?

	JA	NEI	VET IKKE
Sukkersyke	69		
Hjerteinfarkt/hjertekrampe	70		
Forhøyet blodtrykk	71		

H. Når du tenker på hvordan du har det for tida, er du stort sett fornøyd med tilværelsen, eller er du stort sett misfornøyd?
(Sett kryss i bare *en* rute.)

Svært fornøyd	72	1
Meget fornøyd		2
Ganske fornøyd		3
Både/og		4
Nokså misfornøyd		5
Meget misfornøyd		6
Svært misfornøyd		7

SE BILDET AV BLODTRYKKSMÅLINGEN I DEN VEDLAGTE BROSJYREN

	JA	NEI	VET IKKE

I. Er blodtrykket ditt målt noen gang før? 73
Hvis «NEI», gå videre til spørsmål M

J. Hvilket år ble blodtrykket målt siste gang?

19 ☐☐ vet ikke 74

Skriv årstallet her (ca.)

K. Hvor ble blodtrykket målt siste gang?
(Sett kryss i bare *en* rute.)

Hos almenpraktiserende lege (distriktslege, privatpraktiserende lege, turnuskandidat	76	1
Hos bedriftslege		2
Hos militærlege		3
På sykehus		4
Hos annen lege		5
Vet ikke		6

L. Hva ble resultatet av målingen?
(Sett kryss i bare *en* rute.)

Jeg skulle begynne med eller fortsette med medisin for høyt blodtrykk	77	1
Jeg skulle komme til kontroll, men skulle *ikke* ta medisin		2
Jeg skulle *ikke* ta medisin og *ikke* komme til kontroll		3

M. Dersom denne helseundersøkelsen viser at du bør undersøkes nærmere: Hvilken almenpraktiserende lege ønsker du da å bli henvist til?

Skriv navnet på legen her

IKKE SKRIV HER

Ingen spesiell lege 78

OM ARBEIDET DITT

N. Er du i arbeid for tida?
(Sett kryss i bare *en* rute.)

Ja, heltidsarbeid (utenom husarbeid)	81	1
Ja, deltidsarbeid (utenom husarbeid)		2
Ja, heltids husarbeid		3
Nei, ikke i arbeid		4

O. Hvis du ikke er i heltids arbeid, er det på grunn av:
(Sett kryss i bare *en* rute.)

Arbeidsløshet, permittering	82	1
Pensjon eller trygd		2
Utdanning eller militærtjeneste		3
Annet		4

HVIS DU ER I ARBEID, VENNLIGST SVAR PÅ DE NESTE TO SPØRSMÅLENE:

P. Er det mye stress og mas på arbeidet ditt?
(Sett kryss i bare *en* rute.)

Nei, ikke i det hele tatt	83	1
Sjelden		2
Ja, en god del		3
Ja, nesten hele tida		4

Q. Kan du sjøl bestemme hvordan arbeidet ditt skal legges opp? (Sett kryss i bare *en* rute)

Nei, ikke i det hele tatt	84	1
I liten grad		2
Ja, stort sett		3
Ja, det bestemmer jeg sjøl		4

Appendix
Q2-H1

Vi takker for frammøtet til undersøkelsen.

Vi vil også be deg være vennlig å fylle ut dette spørreskjemaet. Opplysninger vil bli brukt i et større forskningsarbeid om forhold som har betydning for helsen.

Svar etter beste skjønn. Kryss av for bare en av svar-mulighetene (dersom det ikke står nevnt noe annet). Det utfylte skjema returneres i vedlagte svarkonvolutt. Porto er betalt.

Alle opplysningene er underlagt streng taushetsplikt.

Med hilsen

Statens skjermbildefotografering
Fylkeslegen ● Helserådet ● Statens Institutt For Folkehelse
Institutt for anvendt sosialvitenskapelig forskning/
Institutt for samfunnsforskning

Til etikett

Navn: _____

Adr. : _____

_____

_____

Postnr. Postkontor

F.nr. : _____

MOSJON

Med mosjon mener vi at du f.eks. går tur, går på ski, svømmer eller driver trening/idrett.

Hvor ofte driver du mosjon?
(Ta et gjennomsnitt)

Aldri	12	1
Sjeldnere enn en gang i uka		2
En gang i uka		3
2–3 ganger i uka		4
Omtrent hver dag		5

Dersom du driver slik mosjon så ofte som en eller flere ganger i uka:
Hvor hardt mosjonerer du?
(Ta et gjennomsnitt)

Tar det rolig uten å bli andpusten eller svett	13	1
Tar det så hardt at jeg blir andpusten og svett		2
Tar meg nesten helt ut		3

Hvor lenge holder du på hver gang?
(Ta et gjennomsnitt)

Mindre enn 15 minutter	14	1
16–30 minutter		2
30 minutter–1 time		3
Mer enn 1 time		4

SALT

Hvor ofte bruker du salt kjøtt eller salt fisk/sild til middag?

Aldri, eller sjeldnere enn en gang i måneden	15	1
1–2 ganger i måneden		2
Opptil en gang i uka		3
Opptil to ganger i uka		4
Mer enn to ganger i uka		5

Hvor ofte pleier du å strø ekstra salt på middagsmaten?

Sjelden eller aldri	16	1
Av og til		2
Ofte		3
Alltid eller nesten alltid		4

RØYKEVANER

		JA	NEI
Røyker du daglig for tiden?	17		
Hvis du svarte «JA», røyker du DAGLIG for tiden:		JA	NEI
Sigaretter?	18		
Pipe?	19		
Sigarer (eller serutter/sigarillos)?	20		
Hvis du IKKE røyker SIGARETTER daglig for tiden: Har du røykt SIGARETTER daglig tidligere?	21	JA	NEI

Hvis du svarte «JA», hvor lenge er det siden du sluttet å røyke sigaretter daglig?

Mindre enn 3 måneder	22	1
3 måneder– 1 år		2
1–5 år		3
Mer enn 5 år		4

Hvis du røyker SIGARETTER daglig nå, eller har gjort det tidligere:

Hvor mange sigaretter røyker eller røykte du pr. dag? (Oppgi antall pr. dag medregnet håndrullede) ..... 23 — Antall

Besvares av dem som røyker daglig nå eller har røykt daglig tidligere:
(Gjelder både sigarett-, pipe- og sigar-røykere)

Hvor gammel var du da du begynte å røyke daglig?	25	år
Hvor mange år tilsammen har du røykt daglig?	27	år

ALKOHOLBRUK

Hvor ofte har du drukket alkohol (øl, vin eller brennevin) de SISTE 14 DAGENE?

Jeg har ikke drukket alkohol, men er ikke totalavholdende	29	1
Jeg har drukket 1–4 ganger		2
Jeg har drukket 5–10 ganger		3
Jeg har drukket mer enn 10 ganger		4
Jeg er totalavholdende, drikker aldri alkohol		5

		JA	NEI
Dersom du har drukket alkohol de siste 14 dagene, har det ført til at du noen gang har følt deg beruset?	30		

Har det vært perioder i livet ditt da du har drukket for mye, eller i hvert fall i meste laget?

Nei	31	1
I tvil, kanskje		2
Ja		3

BOSITUASJONEN

Bor du alene eller sammen med andre?
Kryss av for de du bor sammen med. (Her kan du sette flere kryss.)

Bor alene	32	
Ektefelle eller samboer	33	
Foreldre eller svigerforeldre	34	
Andre voksne personer	35	
Barn under 5 år	36	
Barn 6–15 år	37	
Barn over 15 år	38	

Bor du fast i institusjon?
(sykehjem, aldershjem eller liknende) 39

JA | NEI

UTDANNINGEN

Hvilken utdanning har du fullført?
Oppgi bare høyest fullførte utdanning.

7-årig folkeskole eller kortere	40	1
Framhalds- eller fortsettelsesskole		2
9-årig grunnskole		3
Real- eller middelskole, grunnskolens 10. år		4
Ett- eller to-årig videregående skole		5
Artium, økonomisk gymnas eller almenfaglig retning i videregående skoler		6
Høyskole eller universitet, mindre enn 4 år		7
Høyskole eller universitet, 4 år eller mer		8

Har du fullført annen heldags utdanning, og i tilfelle i hvor mange år?

Skriv antall år her .... 41 ____ år

ARBEID

Hvis du er eller har vært i inntektsgivende arbeid, kan du angi hvilken av disse yrkesgruppene ditt yrke faller innenfor? (Hvis du ikke er i arbeid nå, svarer du ut fra det yrket du hadde sist.)

Hvis du har en ektefelle (eller samboer) som er i inntektsgivende arbeid nå, eller har vært det tidligere, angi tilsvarende hvilken yrkesgruppe han/hun tilhører. (Evt. angi om han/hun *ikke* har hatt inntektsgivende arbeid.)

		Deg selv	Ektefellen
Spesialarbeider, ufaglært arbeider	43. 44		
Fagarbeider, håndverker, formann		1	1
Underordnet funksjonær (butikk, kontor, offentlige tjenester)		2	2
Fagfunksjonær (f.eks. sykepleier, tekniker, lærer)		3	3
Overordnet stilling i offentlig eller privat virksomhet		4	4
Gårdbruker eller skogeier		5	5
Fisker		6	6
Selvstendig i akademisk erverv (f.eks. tannlege, advokat)		7	7
Selvstendig næringsdrivende (Industi, transport, handel)		8	8
Har *ikke* hatt inntektsgivende arbeid (f.eks. pga. heltids husarbeid, studier, trygd)		9	9
		0	0

Hvis du er i arbeid (gjelder også heltids husarbeid), **ber vi deg fylle ut de neste spørsmålene:**

Er arbeidet ditt så fysisk anstrengende at du ofte er sliten i kroppen etter en arbeidsdag?

Ja, nesten alltid	45	1
Ganske ofte		2
Ganske sjelden		3
Aldri, eller nesten aldri		4

Krever arbeidet ditt så mye konsentrasjon og oppmerksomhet at du ofte føler deg utslitt etter en arbeidsdag?

Ja, nesten alltid	46	1
Ganske ofte		2
Ganske sjelden		3
Aldri, eller nesten aldri		4

Hvordan trives du alt i alt med arbeidet ditt?

Veldig godt	47	1
Ganske godt		2
Godt		3
Ikke særlig godt		4
Dårlig		5

Hvis du er gårdbruker eller annen selvstendig næringsdrivende, har du noen ansatte som arbeider fast for deg?

Ingen fast ansatte	48	1
1–2 fast ansatte		2
3–10 fast ansatte		3
Mer enn 10 fast ansatte		4

HVORDAN HAR DU DET?

Når du tenker på hvordan du har det for tida, er du stort sett fornøyd med tilværelsen, eller er du stort sett misfornøyd?

Svært fornøyd	49	1
Meget fornøyd		2
Nokså fornøyd		3
Både - og		4
Nokså misfornøyd		5
Meget misfornøyd		6
Svært misfornøyd		7

Føler du deg stort sett sterk og opplagt, eller trett og sliten?

Meget sterk og opplagt	50	1
Sterk og opplagt		2
Ganske sterk og opplagt		3
Både - og		4
Ganske trett og sliten		5
Trett og sliten		6
Svært trett og sliten		7

MEDISIN/PLAGER

Har du vanligvis:

		JA	NEI
Hoste om morgenen?	51	☐	☐
Oppspytt fra brystet om morgenen?	52	☐	☐

Hvor ofte har du brukt smertestillende medisin den siste måneden?

Daglig	53	1
Hver uke, men ikke hver dag		2
Sjeldnere enn hver uke		3
Aldri		4

Hvor ofte har du brukt avslappende/beroligende medisin eller sovemedisin den siste måneden?

Daglig	54	1
Hver uke, men ikke hver dag		2
Sjeldnere enn hver uke		3
Aldri		4

Har du i løpet av siste måned vært plaget av nervøsitet (irritabel, urolig, anspent eller rastløs)**?**

Nesten hele tida	55	1
Ofte		2
Av og til		3
Aldri		4

Har du i løpet av siste måned hatt innsoving- eller søvnproblemer?

Nesten hver natt	56	1
Ofte		2
Av og til		3
Aldri		4

Har du i det store og hele en rolig og god følelse inne i deg?

Nesten hele tida	57	1
Ofte		2
Av og til		3
Aldri		4

VENNER/HJELP

Dersom du ble syk og måtte holde senga i lengre tid, hvor sannsynlig tror du det er at du kunne få nødvendig hjelp og støtte av familie, venner eller naboer?

Svært sannsynlig	58	1
Nokså sannsynlig		2
Usikkert		3
Usannsynlig		4
Helt usannsynlig		5

Hender det ofte at du føler deg ensom?

Meget ofte	59	1
Ofte		2
Av og til		3
Meget sjelden		4
Aldri		5

HVORDAN ER DU?

Har du tendens til å ta dine oppgaver mer alvorlig enn folk flest?

Ja, nettopp slik er jeg	60	1
Ja, stort sett		2
Både - og		3
Nei, stort sett ikke		4
Nei, tvert imot		5

		JA	NEI	VET IKKE
Har du i løpet av det siste året ofte følt at du har presset deg, eller stadig drevet deg selv framover?	61	☐	☐	☐

Føler du deg alltid under tidspress, også når det gjelder daglige gjøremål?

Alltid, eller nesten alltid	62	1
Noen ganger		2
Aldri		3

Er du vanligvis glad eller nedstemt?

Svært nedstemt	63	1
Nedstemt		2
Nokså nedstemt		3
Både - og		4
Nokså glad		5
Glad		6
Svært glad		7

HVA ER VIKTIG?

Synes du det er viktig at man prøver å være fornøyd med det man har?

Dette er særlig viktig	64	1
Dette er viktig		2
Både - og		3
Dette er mindre viktig		4
Dette er overhodet ikke viktig		5

Synes du det er viktig at man kan slå av på kravene?

Dette er særlig viktig	65	1
Dette er viktig		2
Både - og		3
Dette er mindre viktig		4
Dette er overhodet ikke viktig		5

Synes du det er viktig at man alltid er i godt humør?

Dette er særlig viktig	66	1
Dette er viktig		2
Både - og		3
Dette er mindre viktig		4
Dette er overhodet ikke viktig		5

Tusen takk for den hjelp du har gitt oss ved å fylle ut dette skjema.

TILLEGGS-SKJEMA OM BLODTRYKK

På skjemaet du leverte ved helseundersøkelsen, svarte du at du har, eller har brukt, medisin for høyt blodtrykk.

I Nord-Trøndelag har det siden 1980 pågått en undersøkelse om blodtrykksbehandling. Formålet ved undersøkelsen er å gjøre behandlingen bedre. En viktig del av undersøkelsen er å få opplysninger om hvordan du og alle andre med høyt blodtrykk har det, og hvilke erfaringer dere har gjort.

Det er derfor meget viktig at du fyller ut dette skjemaet så nøye som mulig.

Enkelte spørsmål kan være vanskelig å svare på. Prøv likevel å svare etter beste skjønn, og legg vekt på det som er vanlig eller gjennomsnittlig for deg.

Alle opplysninger blir behandlet av oss med streng taushetsplikt.

På forhånd takk!

Når ble det påvist at du hadde høyt blodtrykk første gang? (Skriv årstallet i ruta)

19

Vet ikke ... 67

Hvor ble det påvist?
(Sett kryss i bare *en* av rutene)

Hos almenpraktiserende lege (distriktslege, privatpraktiserende lege, turnuskandidat) ..... 69 — 1
Hos militærlege 2
På sykehus 3
Vet ikke 4

JA | NEI

Bruker du medisin for blodtrykk nå? 70
Hvis «NEI»: Gå til de to siste spm. nederst til venstre.

Hvis «JA»: Når begynte du med medisiner for blodtrykket? (Skriv årstallet i ruta)

19

Vet ikke ... 71

JA | NEI

Bruker du doserings-eske for tabletter? 220

Har du medisinkort som viser hva slags medisin du skal ta? 221

Hender det at du glemmer å ta medisinene?
(Sett kryss i bare *en* av rutene)

Aldri ..... 73 — 1
Sjelden (ca. en gang i mnd.) 2
Oftere 3

Hvor viktig mener du at det er for deg at du tar blodtrykksmedisinen(e) akkurat som foreskrevet?
(Sett kryss i bare *en* av rutene)

Ikke så viktig ..... 74 — 1
Viktig 2
Meget viktig 3

Vet du hva blodtrykket ditt var ved siste kontroll?
(Sett kryss i bare *en* rute)

Nei ..... 75 — 1
Ja 2
Usikker 3

Hvis «JA» eller «USIKKER», skriv hvor mye du tror det var: 76

Ikke skriv her

Skriv her 79

Hvis du har brukt medisin for blodtrykket før, men ikke nå: Når slutta du med medisiner?
(Skriv årstallet i ruta)

19

Vet ikke ... 82

Hvorfor slutta du med medisinene?
(Sett ett eller flere kryss)

Legen bestemte det 84
Jeg fikk plager av medisinene 85
Jeg mente det ikke var nødvendig med medisiner 86
Jeg var redd medisinene var skadelige 87
Annen årsak (skriv hvilken nedenfor) 88

Ikke skriv her

Skriv hvilken årsak det evt. var 89

Har legen gitt deg andre råd i forbindelse med at du har for høyt blodtrykk?
(Sett kryss i bare *en* av rutene)

Nei ..... 91 — 1
Ja 2
Husker ikke 3

Hvis «JA»; Hvilke råd?

92

Ikke skriv her

94

Hvordan opplever du behandlingen for blodtrykket? Gir det deg:
(Sett ett eller flere kryss)

Lettelse, ro, trygghet 96
Anspenthet, engstelse, redsel, uro 97
Dårlig humør, depresjon 98
Ingen spesielle følelser 99

Synes du at det er noen ulemper ved det at du må ha behandling for høyt blodtrykk?

Nei, ingen ulemper 100
Ja .....

Hvis «JA»: Hva synes du er mest plagsomt?
(Sett ett eller flere kryss)

At du må bruke medisiner hver dag 101
At du må gå til legekontroll 102
At du må følge de råd som legen har gitt 103
At du har ubehag av medisinene 104
At du er engstelig for at det er noe alvorlig som feiler deg 105
At du synes det er leit å bli betraktet som «pasient» 106
Annet 107

TILLEGGS-SKJEMA FOR SUKKERSYKE

Du har opplyst at du har sukkersyke. Et viktig mål for helseundersøkelsen er å finne ut hvordan sukkersyke best kan behandles for å gi minst mulig plager.

Alle som har eller har hatt sukkersyke, bes derfor om å svare så godt som mulig på disse spørsmålene om sukkersyke.

Noen har svart på et lignende skjema høsten 1982. Det er likevel av stor betydning at disse fyller ut dette skjemaet.

Alle opplysninger blir behandlet av oss med streng taushetsplikt.

På forhånd takk!

Når ble sukkersyken din oppdaget? ... 19 108
(Skriv årstallet i ruta)

Hvordan ble sukkersyken din oppdaget?

Jeg søkte lege på grunn av symptomer 110 1
Ble oppdaget uten at jeg hadde symptomer (ved legeattest, bedriftskontroll, undersøkelse for annen sykdom i eller utenfor sykehus) 2

Hva slags plager hadde du i tilfelle da sukkersyken ble oppdaget? (kryss evt. i flere ruter).

Ingen plager 111
Unormal tørste 112
Stor vannlating 113
Slapphet 114
Vekttap 115
Underlivskløe 116
Andre plager 117

Hvis «ANDRE PLAGER», skriv hvilke:

_____ 118 Ikke skriv her
_____ 120

JA NEI

Har noen av dine foreldre, søsken eller barn hatt sukkersyke? 122
Hvis «JA», bruker eller brukte noen av disse insulinsprøyter? 123

BEHANDLING

JA NEI

Bruker du insulinsprøyter mot sukkersyken? 124

Hvis «JA», bruker du sprøyter daglig?

Sprøyte en gang daglig 125 1
Sprøyte to eller flere ganger daglig 2

Om du bruker sprøyter, hvor mye insulin tar du tilsammen hver dag?
(Skriv antall ml i ruta – 1 «strek» svarer til 0,1 ml) ..... 126 , ml

Om du bruker sprøyter, hva heter den insulinen du bruker?
(Skriv navnet som står på glasset, begge dersom du bruker to sorter).

_____ 128 Ikke skriv her
_____ 130

JA NEI

Bruker du tabletter mot sukkersyken? 132

Om du bruker tabletter mot sukkersyken, skriv nedenfor hva de heter, antall mg. som står på glasset/pakningen og hvor mange slike tabletter du tar hver dag:
(Skriv om begge sorter dersom du bruker mer enn en type tabletter mot sukkersyke)

133 , 138
_____ 139 Ikke skriv her
Skriv navn på tabletten her — mg. pr. tabl. — antall pr. dag

140 , 145
_____ 146
Skriv navn på tabletten her — mg. pr. tabl. — antall pr. dag

Hvor mange måltider spiser du hver dag? ..... 147 Antall

JA NEI

Føler du at du vet nok om hva slags mat du kan spise? 148

Hvis du skal svare på hva du virkelig spiser, og ikke hva legen din har sagt du bør spise, vil du da si at du: (Kryss av bare i den ruta som kommer nærmest det du virkelig gjør)

Spiser stort sett det samme som de som ikke har sukkersyke 149 1
Spiser hva jeg vil unntatt sukker og søtsaker 2
Bruker på øyemål bestemt mengde brød, potet, melk og frukt 3
Veier/måler bestemt mengde brød, potet, melk og evt. frukt en eller flere dager i uka 4

JA NEI

Kontrollerer du hjemme hvor mye sukker du har i urinen? (Kryss av også om noen hjelper deg eller gjør det for deg) 150

Hva heter den metoden du i tilfelle bruker til å måle sukker i urinen?

_____ 151 Ikke skriv her
Skriv navnet som står på pakningen her

JA NEI

Kontrollerer du noen gang hjemme hvor mye sukker du har i blod (blodsukker)?
(Kryss av også om noen hjelper deg eller gjør det for deg) 152

Hva heter den metoden du i tilfelle bruker til å måle blodsukker?

_____ 153 Ikke skriv her
Skriv navnet på pakningen og navn på evt. apparat du måler med.

Hvis du selv kontrollerer sukker i urin eller blod, hvor ofte gjør du det?
(Kryss av også om noen hjelper deg eller gjør det for deg)

Hver dag 154 1
2-3 dager i uka 2
En dag i uka 3
En dag hver 14. dag 4
En dag i måneden 5
Sjeldnere enn en dag i måneden 6

VENDI

Hvis du selv kontrollerer sukker i urin eller blod: måler du flere ganger om dagen de dagene du gjør det? 155

JA | NEI

Dersom du tar urin- eller blodprøve selv, tar du resultatene med til legen ved kontroll?
(kryss av i den ruta som passer best)

Aldri ..... 156 — 1
Av og til ..... — 2
Oftest ..... — 3
Alltid ..... — 4

JA | NEI

Går du til regelmessig kontroll hos lege for sukkersyken din? 157

Hvis «JA», hvor lenge var det mellom de to siste gangene du var hos legen din til kontroll for sukkersyken?

Antall måneder (skriv i ruta) .... 158 mndr.

Hva slags lege går du til kontroll hos for sukkersyken?
(Sett kryss i bare *en* rute)

Vanlig lege (distriktslege, almenpraktiserende lege, bedriftslege osv.) ..... 160 — 1
Sykehuslege (poliklinikk på sykehus) ..... — 2
Er innlagt i sykehjem eller annen institusjon og får kontroll der ..... — 3
Andre ..... — 4

Ikke skriv her

Hvis «andre», skriv hva slags lege på linja over 161

ANNEN SYKDOM

JA | NEI

Bruker du regelmessig medisin for annet enn sukkersyken? 162

Dersom «JA», skriv hva disse medisinene heter
(Skriv det navnet som står på glasset eller pakningen. Ta med alle sortene du bruker regelmessig. Skriv x bak navnet om du brukte dette også *før* du fikk sukkersyke).

Ikke skriv her

163
166
169
172
175
178
181

JA | NEI

Tror du man er mer utsatt for å få enkelte andre sykdommer dersom man har dårlig kontrollert sukkersyke? 184

Hvis «JA», nevn navnet på 3 slike sykdommer:
(Du behøver ikke å ha hatt disse sykdommene selv).

Ikke skriv her

185
187
189

Har du selv hatt noen vedvarende (kroniske) plager etter at du fikk sukkersyke? 191
(Skriv hva slags sykdom/plager på linjene under).

193
195
197
199
201

UNDERVISNING - STØTTE

JA | NEI

Er du medlem av Norges Landsforbund for Sukkersyke? 203

Har du noen gang deltatt på kurs eller møte om sukkersyke? 204

Får du grunnstønad gjennom trygdekontoret for sukkersyken? 205

Har du søkt om og fått særfradrag i skattelikninga fordi du har sukkersyke? 206

HVORDAN HAR DU DET?

Synes du det er vanskelig å ha sukkersyke?
(kryss av i den ruta som passer best).

Ja, jeg føler det er som en plage hver dag ..... 207 — 1
Ja, jeg tenker ofte på det ..... — 2
Ja, av og til ..... — 3
Nei, sjelden ..... — 4
Nei, jeg tenker nesten aldri på det ..... — 5
Føler meg akkurat som alle som ikke har sukkersyke .. — 6

Dersom du synes det er vanskelig å ha sukkersyke, hva synes du er verst?
(Skriv det du mener på linja nedenfor).

Skriv her

Forteller du til andre at du har sukkersyke?
(kryss av i den ruta som passer best).

Ja, alltid når jeg mener de bør vite det ..... 210 — 1
Ja, men bare om de spør ..... — 2
Nei, helst ikke ..... — 3
Jeg er redd for at andre skal få greie på det ..... — 4

JA | NEI

Har du noen gang hatt for lavt blodsukker?
(«føling», «insulinsjokk») 211

Hvis «JA», hvor mange ganger har du hatt det den siste uka? (Skriv antall ganger i ruta) 212

Hvor mange ganger har du vært innlagt i sykehus de siste 5 årene? (Skriv antall ganger i ruta) 213

Dersom du har ligget i sykehus de siste 5 årene, hva har du ligget der for?
(Skriv på linjene nedenfor)

Ikke skriv her

214
216
218

Appendix
Q1-H2

HELSEUNDERSØKELSEN
I NORD-TRØNDELAG

«JA, nå er det min tur!»

Personlig innbydelse

Spørreskjemaet er en viktig del av Helseundersøkelsen. Her finner du spørsmål om tidligere sykdom og om andre forhold som har betydning for helsa. Vennligst fyll ut skjemaet på forhånd og ta det med til Helseundersøkelsen. Dersom enkelte spørsmål er uklare, lar du dem bare stå ubesvarte til du møter fram, og drøfter dem med personalet som gjennomfører undersøkelsen. Alle svar vil bli behandlet strengt fortrolig.

Flere steder i skjemaet ber vi deg oppgi din alder da eventuell sykdom inntrådte. Hvis du ikke husker nøyaktig hvor gammel du var, skriver du et tall som er nærmest det du antar er korrekt.

Når resultatene fra undersøkelsen foreligger, vil det være enkelte som trenger ny undersøkelse hos egen lege. Dette vil du få beskjed om i det brevet som vi sender deg om dine resultater. Samtidig sender vi melding om resultatene dine til legen din. Det er derfor om å gjøre at du i rubrikken helt til slutt i skjemaet oppgir navnet på den allmennpraktiserende lege, kommunelege eller det helsesenter som du ønsker skal ta hånd om eventuell etterundersøkelse, og som vi skal sende resultatene til.

Med vennlig hilsen

Helsetjenesten i Nord-Trøndelag • Statens helseundersøkelser • Statens Institutt for Folkehelse

DET HANDLER OM HELSA DI

Hvordan er helsa di nå?

Bare ett kryss

Dårlig	12	☐ 1
Ikke helt god		☐ 2
God		☐ 3
Svært god		☐ 4

LUFTVEGSPLAGER

	JA	NEI
Hoster du daglig i perioder av året?		
Hvis JA:		
Er hosten vanligvis ledsaget av oppspytt? 14		
Har du hatt hoste med oppspytt i minst 3 mnd. sammenhengende i hvert av de to siste åra?		
Har du hatt noe anfall med pipende eller tung pust de siste 12 måneder? 16		

	JA	NEI	Alder første gang
Har du eller har du hatt astma? 17			år

	JA	NEI
Har du brukt eller bruker du astmamedisiner? 20		

HJERTE-KARSYKDOMMER, DIABETES

Har du, eller har du hatt:	JA	NEI	Alder første gang
Hjerteinfarkt 21			år
Angina pectoris (hjertekrampe) 24			år
Hjerneslag/hjerneblødning 27			år
Diabetes (sukkersyke) 30			år

Hva ble resultatet siste gang du målte blodtrykket ditt?

Bare ett kryss

Begynne med/fortsette med blodtrykksmedisin	33	☐ 1
Komme til kontroll, men ikke ta blodtrykksmedisin		☐ 2
Ingen kontroll og ingen medisin nødvendig		☐ 3
Har aldri fått målt blodtrykket		☐ 4

Bruker du medisin mot høyt blodtrykk?

Bare ett kryss

Nå	34	☐ 1
Før, men ikke nå		☐ 2
Aldri brukt		☐ 3

	JA	NEI	VET IKKE
Har en eller flere av foreldre eller søsken hatt hjerteinfarkt (sår på hjertet) eller angina pectoris (hjertekrampe)?			

STOFFSKIFTE

Har du noen gang fått påvist:	JA	NEI	Alder første gang
for høyt stoffskifte 36			år
for lavt stoffskifte 39			år
struma 42			år
annen sykdom i skjoldbruskkjertelen			år
Bruker du eller har du brukt noen av disse medisinene:			
Thyroxin 48			år
Neo-Mercazole 51			år
Er du operert i skjoldbruskkjertelen			år
Har du fått radiojodbehandling 57			år

MUSKEL/SKJELETT-PLAGER

	JA	NEI
Har du i løpet av det siste året vært plaget med smerter og/eller stivhet i muskler og ledd som har vart i minst 3 måneder sammenhengende? 60		

Hvis NEI, gå videre til neste side øverst.
Hvis JA, svar på følgende:

Hvor har du hatt disse plagene?	JA	NEI
Nakke 61		
Skuldre (aksler)		
Albuer		
Håndledd, hender		
Bryst/mage 65		
Øvre del av ryggen		
Korsryggen		
Hofter		
Knær		
Ankler, føtter 70		

Hvis du har hatt plager i flere områder i minst 3 mnd. det siste året, setter du ring rundt det ja-krysset hvor plagene har vart lengst

Hvor lenge har plagene vart sammenhengende?

Svar for det området hvor plagene har vart lengst

	Antall mnd.	Antall år
Hvis under 1 år, oppgi antall mnd. 71		
Hvis 1 år eller mer, oppgi antall år. 73		

Har plagene redusert din arbeidsevne det siste året?

Gjelder også hjemmearbeidende. Bare ett kryss

Nei/ubetydelig	I noen grad	I betydelig grad	Vet ikke
☐	☐	☐	☐

	JA	NEI	IKKE I ARBEID
Har du vært sykmeldt pga. disse plagene det siste året? 76			

	JA	NEI
Har plagene ført til redusert aktivitet i fritida?		

Har lege noen gang sagt at du har/har hatt noen av disse sykdommene:

	JA	NEI
Beinskjørhet (osteoporose) ... 78		
Fibromyalgi (fibrositt/kronisk smertesyndrom)		
Leddgikt (reumatoid artritt) ...		
Slitasjegikt (artrose) ...		
Bechterews sykdom ... 82		
Andre langvarige skjelett- eller muskelsykdommer		

Har du noen gang hatt:

	JA	NEI	Alder *siste* gang
Lårhalsbrudd ... 84			år
Brudd i håndledd/underarm ... 87			år
Nakkesleng (whiplash) ... 90			år
Skade som førte til sykehusinnleggelse			år

ANDRE PLAGER

I hvilken grad har du hatt disse plagene i de siste 12 månedene?

	Ikke plaget	Litt plaget	Mye plaget
Kvalme ... 96	☐	☐	☐
Brystbrann/sure oppstøt ...	☐	☐	☐
Diaré ...	☐	☐	☐
Treg mage ...	☐	☐	☐
Hjertebank ...	☐	☐	☐
Åndenød ... 101	☐	☐	☐

ANDRE SYKDOMMER

Har du eller har du noen gang hatt:

	JA	NEI	Alder første gang
Epilepsi ... 102			år
Psykiske plager hvor du har søkt hjelp			år
Kreftsykdom ... 108			år
Annen langvarig sykdom ... 111			

DAGLIGE FUNKSJONER

Har du noen langvarig sykdom, skade eller lidelse av fysisk eller psykisk art som nedsetter dine funksjoner i ditt daglige liv? ... 112

JA	NEI

Langvarig: minst ett år

Hvis JA:

Hvor mye vil du si at dine funksjoner er nedsatt?

	Litt nedsatt	Middels nedsatt	Mye nedsatt
Er bevegelseshemmet ... 113	☐	☐	☐
Har nedsatt syn ...	☐	☐	☐
Har nedsatt hørsel ...	☐	☐	☐
Hemmet pga. kroppslig sykdom.	☐	☐	☐
Hemmet pga. psykiske plager... 117	☐	☐	☐

MENN fortsetter øverst neste spalte

BESVARES BARE AV KVINNER

Hvor mange barn har du født? ... 118
Sett 0 hvis du ikke har født barn

Antall barn

Hvis du har født barn, besvar:

Alder

Hvor gammel var du da du fødte ditt første barn? ... 120 — år

Hvor gammel var du da du fødte ditt siste barn? ... 122 — år
Besvares ikke hvis du har født bare ett barn

Hvor gammel var du da du fikk menstruasjon? ... 124 — år
Sett 0 hvis du ikke noen gang har hatt menstruasjon

Fortsett neste spalte øverst

RØYKING

Røykte noen av de voksne hjemme da du vokste opp? ... 126

JA	NEI

Bor du, eller har du bodd, sammen med noen dagligrøykere etter at du fylte 20 år? ... 127

JA	NEI

Hvor lenge er du vanligvis daglig til stede i røykfylt rom? ... 128
Sett 0 hvis du ikke oppholder deg i røykfylt rom

Antall timer

Røyker du selv?

	JA	NEI
Sigaretter daglig? ... 130		
Sigarer/sigarillos daglig? ...		
Pipe daglig? ... 132		

Aldri røykt daglig ... *(Sett kryss)* ☐

Hvis du har røykt daglig tidligere, hvor lenge er det siden du sluttet? ... 134

Antall år

Hvis du røyker daglig nå eller har røykt tidligere:

Hvor mange sigaretter røyker eller røykte du vanligvis daglig? ... 136

Antall sigaretter

Hvor gammel var du da du begynte å røyke daglig? ... 140

Alder — år

Hvor mange år tilsammen har du røykt daglig? ... 142

Antall år

KAFFE/TE/ALKOHOL

Hvor mange kopper kaffe/te drikker du daglig?
Sett 0 hvis du ikke drikker kaffe/te daglig

	Antall kopper
Kokekaffe ... 144	
Annen kaffe ... 146	
Te ... 148	

Alkohol:
Er du total avholdsmann/-kvinne? ... 150

JA	NEI

Hvor mange ganger i måneden drikker du vanligvis alkohol? ... 151
Regn ikke med lettøl. Sett 0 hvis mindre enn 1 gang i mnd.

Antall ganger

Hvor mange glass øl, vin eller brennevin drikker du vanligvis i løpet av to uker?

Regn ikke med lettøl.
Sett 0 hvis du ikke drikker alkohol 153

Øl	Vin	Brennevin
glass	glass	glass

FYSISK AKTIVITET

I FRITIDA

Hvordan har din fysiske aktivitet i fritida vært det siste året? *Tenk deg et ukentlig gjennomsnitt for året.*

Arbeidsveg regnes som fritid

	Timer pr. uke: Ingen	Under 1	1-2	3 og mer
Lett aktivitet *(ikke svett/andpusten)* ... 159	☐	☐	☐	☐
Hard fysisk aktivitet *(svett/andpusten)* ... 160	☐ 1	☐ 2	☐ 3	☐ 4

UNDER ARBEID
Hvis du er i lønnet eller ulønnet arbeid:

Hvorledes vil du beskrive arbeidet ditt?
Bare ett kryss

For det meste stillesittende arbeid *(f.eks. skrivebordsarbeid, montering)* ... 161 ☐ 1

Arbeid som krever at du går mye *(f.eks. ekspeditørarb., lett industriarb., undervisning)* ... ☐ 2

Arbeid hvor du går og løfter mye *(f.eks. postbud, pleier, bygningsarbeid)* ... ☐ 3

Tungt kroppsarbeid *(f.eks. skogsarbeid, tungt jordbruksarb.,tungt bygningsarb.)* ☐ 4

Bla om!

HVORLEDES FØLER DU DEG?

Har du de siste to ukene følt deg:

	Nei	Litt	En god del	Svært mye
Trygg og rolig? 162	☐	☐	☐	☐
Glad og optimistisk?	☐	☐	☐	☐
Har du følt deg:				
Nervøs og urolig?	☐	☐	☐	☐
Plaget av angst? 165	☐	☐	☐	☐
Irritabel?	☐	☐	☐	☐
Nedfor/deprimert?	☐	☐	☐	☐
Ensom? 168	☐ 1	☐ 2	☐ 3	☐ 4

Her kommer noen flere spørsmål om hvorledes du føler deg. For hvert spørsmål setter du kryss for ett av de fire svarene som best beskriver dine følelser ***den siste uka.*** *Ikke tenk for lenge på svaret - de spontane svarene er best*

Jeg gleder meg fortsatt over ting slik jeg pleide før 169
Avgjort like mye ..... ☐ 1 Bare lite grann ..... ☐ 3
Ikke fullt så mye ..... ☐ 2 Ikke i det hele tatt ..... ☐ 4

Jeg har en urofølelse som om noe forferdelig vil skje 170
Ja, og noe svært ille .... ☐ 1 Litt, bekymrer meg lite . ☐ 3
Ja, ikke så veldig ille ... ☐ 2 Ikke i det hele tatt ..... ☐ 4

Jeg kan le og se det morsomme i situasjoner 171
Like mye nå som før .... ☐ 1 Avgjort ikke som før .... ☐ 3
Ikke like mye nå som før ☐ 2 Ikke i det hele tatt ..... ☐ 4

Jeg har hodet fullt av bekymringer 172
Veldig ofte ..... ☐ 1 Av og til ..... ☐ 3
Ganske ofte ..... ☐ 2 En gang i blant ..... ☐ 4

Jeg er i godt humør 173
Aldri ..... ☐ 1 Ganske ofte ..... ☐ 3
Noen ganger ..... ☐ 2 For det meste ..... ☐ 4

Jeg kan sitte i fred og ro og kjenne meg avslappet 174
Ja, helt klart ..... ☐ 1 Ikke så ofte ..... ☐ 3
Vanligvis ..... ☐ 2 Ikke i det hele tatt ..... ☐ 4

Jeg føler meg som om alt går langsommere 175
Nesten hele tiden ..... ☐ 1 Fra tid til annen ..... ☐ 3
Svært ofte ..... ☐ 2 Ikke i det hele tatt ..... ☐ 4

Jeg føler meg urolig som om jeg har sommerfugler i magen 176
Ikke i det hele tatt ..... ☐ 1 Ganske ofte ..... ☐ 3
Fra tid til annen ..... ☐ 2 Svært ofte ..... ☐ 4

Jeg bryr meg ikke lenger om hvordan jeg ser ut 177
Ja, har sluttet å bry meg ☐ 1 Kan hende ikke nok .... ☐ 3
Ikke som jeg burde ..... ☐ 2 Bryr meg som før ..... ☐ 4

Jeg er rastløs som om jeg stadig må være aktiv 178
Uten tvil svært mye ..... ☐ 1 Ikke så veldig mye ..... ☐ 3
Ganske mye..... ☐ 2 Ikke i det hele tatt ..... ☐ 4

Jeg ser med glede frem til hendelser og ting 179
Like mye som før ..... ☐ 1 Avgjort mindre enn før . ☐ 3
Heller mindre enn før ... ☐ 2 Nesten ikke i det hele tatt ☐ 4

Jeg kan plutselig få en følelse av panikk 180
Uten tvil svært ofte ..... ☐ 1 Ikke så veldig ofte ..... ☐ 3
Ganske ofte ..... ☐ 2 Ikke i det hele tatt ..... ☐ 4

Jeg kan glede meg over gode bøker, radio og TV 181
Ofte ..... ☐ 1 Ikke så ofte ..... ☐ 3
Fra tid til annen ..... ☐ 2 Svært sjelden ..... ☐ 4

UTDANNING

Hvilken utdanning er den høyeste du har fullført?

Grunnskole 7-10 år, framhaldsskole, folkehøgskole ..... 182 ☐ 1
Realskole, middelskole, yrkesskole, 1-2 årig videregående skole..... ☐ 2
Artium, øk.gymnas, allmennfaglig retning i videregående skole ..... ☐ 3
Høgskole/universitet, mindre enn 4 år ..... ☐ 4
Høgskole/universitet, 4 år eller mer ..... ☐ 5

ARBEID

Hva slags arbeidssituasjon har du nå?
Ett eller flere kryss

Lønnet arbeid ..... 183 ☐
Selvstendig næringsdrivende..... ☐
Heltids husarbeid ..... ☐
Utdanning, militærtjeneste ..... ☐
Arbeidsledig, permittert..... ☐
Pensjonist/trygdet..... 188 ☐

Hvor mange timer lønnet arbeid har du i uka? ..... 189 Antall timer

	JA	NEI
Har du skiftarbeid, nattarbeid eller går vakt?		

ALT I ALT

Når du tenker på hvordan du har det for tida, er du stort sett fornøyd med tilværelsen eller er du stort sett misfornøyd?
Bare ett kryss

Svært fornøyd ..... 192 ☐ 1
Meget fornøyd ..... ☐ 2
Ganske fornøyd..... ☐ 3
Både/og..... ☐ 4
Nokså misfornøyd ..... ☐ 5
Meget misfornøyd..... ☐ 6
Svært misfornøyd..... ☐ 7

DIN LEGE

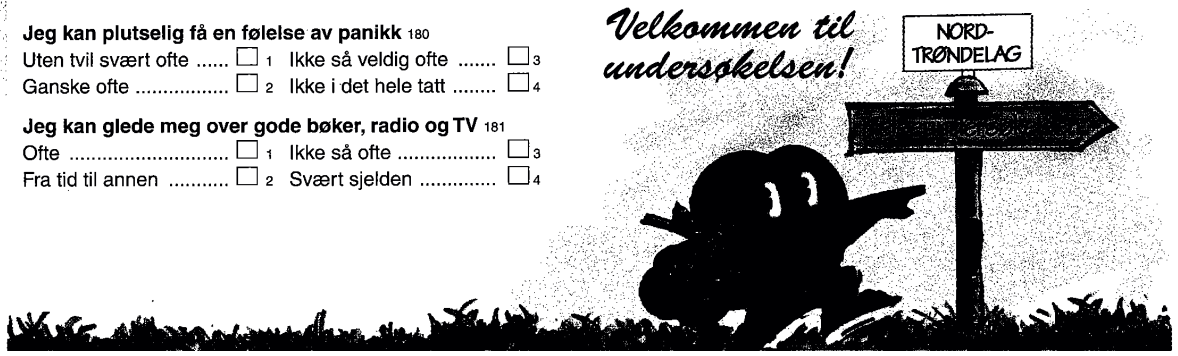
Hvis denne helseundersøkelsen viser at du bør undersøkes nærmere, hvilken allmennpraktiserende lege/kommunelege ønsker du skal foreta undersøkelsen?

Skriv navnet på legen her: 193 *Ikke skriv her*

Takk for utfyllingen!

Nok en gang:

Velkommen til undersøkelsen!



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Appendix
Q2-H2, women

hunt

Helseundersøkelsen i Nord-Trøndelag

SKJEMA FOR KVINNER 20–69 ÅR

Takk for frammøtet til undersøkelsen!
Vi vil også be deg fylle ut dette spørreskjemaet. Opplysningene vil bli brukt i større forskningsarbeider om forebyggende helsearbeid. Noen av spørsmålene likner på spørsmål du har svart på i det skjemaet du fylte ut heime og leverte ved frammøte til helseundersøkelsen. Det er likevel viktig at du svarer på alle spørsmålene også i dette skjemaet. Det utfylte skjemaet returneres i vedlagte svarkonvolutt. Porto er betalt.
Alle opplysningene er underlagt streng taushetsplikt.

Vennlig hilsen
Helsetjenesten i Nord-Trøndelag
Statens Institutt for Folkehelse Statens helseundersøkelser

Hvis du ikke ønsker å besvare spørreskjemaet, sett kryss her og returner skjemaet. Da slipper du purring.
Jeg ønsker ikke å besvare skjemaet ○

UTFYLLING

Dato for utfylling av skjema: / 19 (19)

OPPVEKST

I hvilken kommune bodde du da du fylte 1 år?
*Hvis du ikke bodde i Norge, oppgi **land** i stedet for kommune.*

(24)

ARBEID

Nåværende eller tidligere arbeid:
Hva slags inntektsgivende arbeid har du og event. din ektefelle/samboer? *Hvis du/dere ikke har inntektsgivende arbeid nå: Oppgi det siste yrket.*

	Deg selv	Ektefelle/ samboer
Spesialarbeider eller ufaglært arbeider (25)	☐	☐ (36)
Fagarbeider, handverker, formann	☐	☐
Underordnet funksjonær (f.eks. butikk, kontor, off. tjenester)	☐	☐
Fagfunksjonær (f.eks. sykepleier, tekniker, lærer)	☐	☐
Overordnet stilling i off. eller privat virksomhet	☐	☐
Sjåfør (30)	☐	☐ (41)
Gårdbruker eller skogeier	☐	☐
Fisker	☐	☐
Selvstendig i akademisk erverv (f.eks. tannlege, advokat)	☐	☐
Annen selvstendig næringsvirksomhet	☐	☐
Har ikke vært i inntektsgivende arbeid (35)	☐	☐ (46)

Hvis du NÅ ikke har inntektsgivende arbeid eller du ikke har heltids husarbeid: Gå til BOLIG.

Har du i løpet av de siste 12 månedene hatt sykefravær:	Ja	Nei
med egenmelding (47)	☐	☐
med sykmelding fra lege (48)	☐	☐

Hvis «Ja»: **Hvor lenge *tilsammen*?** *Bare ett kryss*

2 uker eller mindre (49)	☐ 1
2-8 uker	☐ 2
Mer enn 8 uker	☐ 3

Har du i løpet av de siste 12 månedene vurdert å skifte yrke eller arbeidsplass? (50)	Ja	Nei
	☐	☐

Er arbeidet ditt så fysisk anstrengende at du ofte er sliten i kroppen etter en arbeidsdag? *Bare ett kryss* (51)

Ja, nesten alltid	☐ 1	Ganske sjelden	☐ 3
Ganske ofte	☐ 2	Aldri, eller nesten aldri	☐ 4

Krever arbeidet ditt så mye konsentrasjon og oppmerksomhet at du ofte føler deg utslitt etter en arbeidsdag? (52)

Ja, nesten alltid	☐ 1	Ganske sjelden	☐ 3
Ganske ofte	☐ 2	Aldri, eller nesten aldri	☐ 4

Hvordan trives du alt i alt med arbeidet ditt? (53)

Veldig godt	☐ 1	Ikke særlig godt	☐ 3
Godt	☐ 2	Dårlig	☐ 4

BOLIG

Hvem bor du sammen med?
Ett kryss for hver linje og angi antall

	Ja	Nei	Antall
Ektefelle/samboer (54)	☐	☐	
Andre personer over 18 år (55)	☐	☐	
Personer under 18 år (58)	☐	☐	

Hvor mange av barna har plass i barnehage? (61) Antall

Hvilken type bolig bor du i? *Bare ett kryss*

Enebolig/villa (63)	☐	1
Gårdsbruk	☐	2
Blokk/terrasseleilighet	☐	3
Rekkehus/2-4 mannsbolig	☐	4
Annen bolig	☐	5

Hvor stor er din boenhet? (64) kvm

	Ja	Nei
Er det heldekkende tepper i stua? (67)	☐	☐
Er det heldekkende tepper på ditt soverom?	☐	☐
Er det katt i boligen? (69)	☐	☐
Er det hund i boligen?	☐	☐
Er det andre pelskledde dyr eller fugler i boligen?	☐	☐

ØKONOMI

Mottar du noen av følgende *offentlige* ytelser?	Ja	Nei
Sykepenger/sykelønn/rehabiliteringspenger (72)	☐	☐
Ytelser under yrkesrettet attføring	☐	☐
Uførepensjon (74)	☐	☐
Alderspensjon	☐	☐
Sosialstøtte	☐	☐
Arbeidsløshetstrygd	☐	☐
Overgangsstønad	☐	☐
Etterlattepensjon (79)	☐	☐
Andre ytelser	☐	☐

Har det i løpet av det siste året hendt at husholdningen har hatt vansker med å klare de løpende utgifter til mat, transport, bolig og liknende? *Bare ett kryss* (81)

Ja, ofte	☐ 1	Ja, en sjelden gang	☐ 3
Ja, av og til	☐ 2	Nei, aldri	☐ 4

VENNER

Hvor mange gode venner har du? Antall
Regn med de du kan snakke fortrolig med og som kan gi deg god hjelp når du trenger det (82)
Tell ikke med de du bor sammen med, men regn med andre slektninger

	Ja	Nei
Føler du at du har mange nok gode venner? (84)	☐	☐

Hvor ofte tar du vanligvis del i foreningsvirksomhet som f.eks. syklubb, idrettslag, politiske lag, religiøse eller andre foreninger? (85)

Aldri, eller noen få ganger i året	☐ 1	Omtrent en gang i uka	☐ 1
1-2 ganger i måneden	☐ 2	Mer enn en gang i uka	☐ 2

DER DU BOR

Svar ut fra nærmiljøet, dvs. nabolaget/grenda:
Ett kryss for hvert spørsmål

Jeg føler et sterkt fellesskap med de som bor her 86
Helt enig ☐ 1 Delvis enig ☐ 2 Usikker ☐ 3 Delvis uenig ☐ 4 Helt uenig ☐ 5

Selv om noen tar initiativ, er det ingen som blir med på det som settes i gang her 87
Helt enig ☐ Delvis enig ☐ Usikker ☐ Delvis uenig ☐ Helt uenig ☐

Hvis jeg flytter herfra, vil jeg lengte tilbake 88
Helt enig ☐ Delvis enig ☐ Usikker ☐ Delvis uenig ☐ Helt uenig ☐

Man kan ikke stole på hverandre her 89
Helt enig ☐ Delvis enig ☐ Usikker ☐ Delvis uenig ☐ Helt uenig ☐

Når noe skal gjøres her, er det lett å få folk med 90
Helt enig ☐ Delvis enig ☐ Usikker ☐ Delvis uenig ☐ Helt uenig ☐

Det er vanskelig å få kontakt med folk her 91
Helt enig ☐ Delvis enig ☐ Usikker ☐ Delvis uenig ☐ Helt uenig ☐

Det er godt samhold her 92
Helt enig ☐ Delvis enig ☐ Usikker ☐ Delvis uenig ☐ Helt uenig ☐

Ingen orker å ta initiativ til noe lenger her 93
Helt enig ☐ Delvis enig ☐ Usikker ☐ Delvis uenig ☐ Helt uenig ☐

Folk trives godt her 94
Helt enig ☐ Delvis enig ☐ Usikker ☐ Delvis uenig ☐ Helt uenig ☐

Folk her kan ha store problemer uten at naboen vet noe 95
Helt enig ☐ Delvis enig ☐ Usikker ☐ Delvis uenig ☐ Helt uenig ☐

Det er alltid noen som tar initiativ til å løse nødvendige oppgaver her 96
Helt enig ☐ Delvis enig ☐ Usikker ☐ Delvis uenig ☐ Helt uenig ☐

Folk snakker lite med hverandre her 97
Helt enig ☐ 1 Delvis enig ☐ 2 Usikker ☐ 3 Delvis uenig ☐ 4 Helt uenig ☐ 5

SYKDOM I FAMILIEN

Kryss av for de slektningene som har eller har hatt noen av sykdommene. Kryss av for "ingen" hvis ingen av slektningene har hatt denne sykdommen: *Evt. flere kryss på hver linje*

	Mor	*Far*	*Bror*	*Søster*	*Barn*	*Ingen*
Hjerneslag eller hjerneblødning 98	☐	☐	☐	☐	☐	☐
Hjerteinfarkt før 60 års alder..... 104	☐	☐	☐	☐	☐	☐
Astma..... 110	☐	☐	☐	☐	☐	☐
Allergi 116	☐	☐	☐	☐	☐	☐
Kreftsykdom..... 122	☐	☐	☐	☐	☐	☐
Høyt blodtrykk..... 128	☐	☐	☐	☐	☐	☐
Psykiske plager..... 134	☐	☐	☐	☐	☐	☐
Osteoporose (benskjørhet)..... 140	☐	☐	☐	☐	☐	☐
Diabetes (sukkersyke) 146	☐	☐	☐	☐	☐	☐
Alder da de fikk diabetes 152	år	år	år	år	år	

Har du *selv* høysnue eller neseallergi?..... 162 *Ja* ☐ *Nei* ☐

BRUK AV HELSETJENESTER

Har du i løpet av de siste 12 månedene vært hos:
Ett kryss på hver linje — *Ja* *Nei*

- allmennpraktiserende lege (kommunelege, privatpraktiserende lege, turnuskandidat) .....163 ☐ ☐
- bedriftslege..... ☐ ☐
- lege ved sykehus (uten at du var innlagt) ..... ☐ ☐
- annen lege ..... ☐ ☐
- fysioterapeut..... ☐ ☐
- kiropraktor ..... ☐ ☐
- homøopat .....169 ☐ ☐
- annen behandler (naturmedisiner, fotsoneterapeut, håndspålegger, "healer", "synsk", e.l.) ..... ☐ ☐

Ja *Nei*
Har du vært innlagt i sykehus de siste 5 åra?.....171 ☐ ☐

ALKOHOL

Hvis du er totalavholdskvinne: Gå til KOSTHOLD.

Ett kryss for hver spørsmål

Har du noen gang følt at du burde redusere alkoholforbruket ditt?.....172 *Ja* ☐ *Nei* ☐

Har andre noen gang kritisert alkoholbruken din?.....173 *Ja* ☐ *Nei* ☐

Har du noen gang følt ubehag eller skyldfølelse pga. alkoholbruken din?.....174 *Ja* ☐ *Nei* ☐

Har det å ta en drink noen gang vært det første du har gjort om morgenen for å roe nervene, kurere bakrus eller som en oppkvikker? .....175 *Ja* ☐ *Nei* ☐

KOSTHOLD

Hvor mange måltider spiser du vanligvis daglig (middag og brødmåltid)?.....176 Antall

Hvor mange dager i uka spiser du varm middag?

Hva slags type brød (kjøpt eller hjemmebakt) spiser du vanligvis? *Inntil to kryss*

Brødtypen ligner mest på 178	*Loff*	*Fint brød*	*Kneipp-brød*	*Grov-brød*	*Knekke-brød*
	☐	☐	☐	☐	☐

Hva slags *fett* blir vanligvis brukt i din husholdning?
Ett kryss for matlaging og ett kryss for brød

	Til matlaging	*På brød*
Bruker *ikke* smør eller margarin	183 ☐ 1	184 ☐ 1
Meierismør.....	☐ 2	☐ 2
Hard margarin.....	☐ 3	☐ 3
Bløt (soft) margarin	☐ 4	☐ 4
Smør/margarin blanding	☐ 5	☐ 5
Lettmargarin	☐ 6	☐ 6
Oljer	☐ 7	

MEDISINBRUK

Har du i deler av de siste 12 måneder brukt noen medisiner daglig eller nesten daglig? .....185 *Ja* ☐ *Nei* ☐

Hvis «Ja»:
Angi hvor mange måneder du brukte følgende medisiner: *Sett 0 hvis du ikke har brukt medisinene*

	Antall mndr.		Antall mndr.
smertestillende 186		hjertemedisin (ikke blodtrykksmedisin)	
sovemedisin..... 188		annen medisin	
beroligende medisin		*Kosttilskudd:*	
medisin mot depresjon		jerntabletter..... 202	
allergimedisin..... 194		vitamintilskudd	
astmamedisin 196		tran/fiskeoljer 206	

Hvor ofte har du brukt avslappende/beroligende medisin eller sovemedisin den siste måneden? 208
Daglig..... ☐ 1 Sjeldnere enn hver uke ☐ 3
Hver uke, men ikke hver dag . ☐ 2 Aldri..... ☐ 4

HODEPINE

Har du vært plaget av hodepine i løpet av de siste 12 måneder? 209
Ja, anfallsvis (migrene)..... ☐ 1
Ja, annen slags hodepine.... ☐ 2
Nei ..... ☐ 3

Antall anfall siste 12 mndr. 210 ☐☐☐

Hvis «Nei»: Gå til MUSKEL-/SKJELETTPLAGER

Omtrent hvor mange dager i pr. måned har du hodepine?
Mindre enn 7 dager ☐ 1 7 til 14 dager ☐ 2 Mer enn 14 d. ☐ 3

Hvor lenge varer hodepinen vanligvis hver gang? 213
Mindre enn 4 timer ☐ 1 4 timer–3 døgn ☐ 2 Mer enn 3 døgn ☐ 3

Hvor ofte er hodepinen preget av eller ledsaget av:

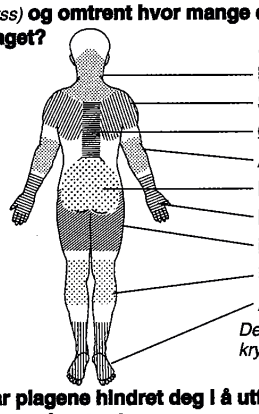
Ett kryss på hver linje	*Sjelden eller aldri*	*Av og til*	*Ofte*
bankende/dunkende smerte 214	☐	☐	☐
pressende smerte	☐	☐	☐
halvsidighet, alltid samme side	☐	☐	☐
halvsidighet, vekselvis h. og v. side	☐	☐	☐
smerter i «hele hodet»	☐	☐	☐
kvalme 219	☐	☐	☐
lys- og/eller lydskyhet	☐	☐	☐
forverring ved fysisk aktivitet.....	☐	☐	☐
synsforstyrrelser *før* hodepine 222	☐	☐	☐

Hvor mange tabletter/stikkpiller har du eventuelt brukt av disse medisinene *alt i alt i løpet av den siste måneden*?
Skriv 0 hvis du ikke har brukt medisinen.
Cafergot 223 ☐☐ Anervan 225 ☐☐ Imigran 227 ☐☐

MUSKEL-/SKJELETTPLAGER

Har du hatt plager (smerter, verk, ubehag) i muskler og/eller ledd i *den siste måneden*? 229 *Ja* ☐ *Nei* ☐

Hvis «Ja»: **Hvor har du hatt disse plagene** *(ett eller flere kryss)* **og omtrent hvor mange dager tilsammen var du plaget?**



Plager (Sett kryss)	Antall dager
Nakke 230	☐☐
Skuldre/aksler..... 233	☐☐
Øvre del av ryggen	☐☐
Albuer 239	☐☐
Korsryggen 242	☐☐
Handledd/hender 245	☐☐
Hofter..... 248	☐☐
Knær..... 251	☐☐
Ankler/føtter 254	☐☐

*Dersom flere kryss: Sett **ring** rundt krysset **der plagen var verst***

Har plagene hindret deg i å utføre daglige aktiviteter den siste måneden?

	Ja	*Nei*
I arbeidet..... 257	☐	☐
I fritida 258	☐	☐

SMERTER I BEINA

	Ja	*Nei*
Har du sår på tå, fot eller ankel som ikke vil gro? 259	☐	☐
Har du smerter i det ene eller i begge beina når du går? 260	☐	☐
Har du oppsøkt lege p.g.a. smerter i beina? 261	☐	☐

Hvis «NEI» på disse spørsmålene: Gå til MENSTRUASJON

	Ja	*Nei*
Kan du gå lenger enn 50 meter? 262	☐	☐
Forsvinner smerten når du står stille en stund? 263	☐	☐
Må du sette deg for at smerten skal gå over? 264	☐	☐

Hvor gjør det mest vondt? *Ett kryss* 265
Fot ☐ Legg ☐ Lår ☐ Hofte ☐

	Ja	*Nei*
Har du smerter i beina når du er i ro? 266	☐	☐
Er smertene verst når du ligger i senga? 267	☐	☐
Blir søvnen forstyrret av smertene? 268	☐	☐
Får du mindre vondt når beinet ligger høyt? 269	☐	☐
Får du mindre vondt når beinet ligger lavt, f.eks. om beinet henger utfor sengekanten? 270	☐	☐
Bedres smertene når du står opp og går litt? 271	☐	☐

MENSTRUASJON

Har du menstruasjon fremdeles? ..... 272 *Ja* ☐ *Nei* ☐

Hvis «Nei»: **Hvor gammel var du da den sluttet?** 273 ☐☐ år

Er du gravid nå? ..... 275 *Ja* ☐ *Nei* ☐ *Vet ikke* ☐

Har du innsatt spiral nå? ..... 276 *Ja* ☐ *Nei* ☐

Når hadde du siste menstruasjon? ..... 277 *Dag Måned År* ☐☐ ☐☐ ☐☐☐☐
Husker du ikke dag, bare angi måned og år, husker du bare år, angi år.

Menstruasjonen din de siste 12 måneder:

Har du det siste året hatt regelmessige menstruasjoner?
At menstruasjonen har vart omtrent like lenge hver gang med omtrent like lange mellomrom ..... 283 *Ja* ☐ *Nei* ☐ *Usikker* ☐

Hvor mange dager hadde du blødning siste gang du hadde menstruasjon? ..... 284 *Antall dager* ☐☐

Hvor mange dager var du uten blødning mellom nest siste og siste menstruasjon? ... 286 *Antall dager* ☐☐

Har menstruasjonen din det siste året uteblitt i mer enn 3 måneder uten at du var gravid? 289 *Ja* ☐ *Nei* ☐

Hvis «Ja»: **Hvor mange måneder i trekk har du vært uten menstruasjonsblødninger?** ..... 290 *Antall mndr.* ☐☐

Hvis «Ja»: **Oppsøkte du lege?** ..... 292 *Ja* ☐ *Nei* ☐

Menstruasjonen tidligere (dvs. før de siste 12 månedene):

Har menstruasjonen din tidligere uteblitt uten at du var gravid? ..... 293 *Ja* ☐ *Nei* ☐

Hvis «Ja»: **Hvor lenge og hvor ofte var den borte sammenhengende?** *Sett kryss eventuelt flere steder*

	1 gang	*2 ganger*	*Oftere*
3–6 måneder..... 294	☐	☐	☐
6–12 måneder.....	☐	☐	☐
Over ett år..... 296	☐	☐	☐

OPERASJONER I UNDERLIVET

	Ja	Nei	Vet ikke
Har du noen gang blitt operert i underlivet? 297	☐	☐	☐
Hvis «Ja»: **Kryss av for hver operasjon:**	Ja	Nei	Vet ikke
Fjernet deler av eller bare én eggstokk 298	☐	☐	☐
Fjernet begge eggstokkene (totalt) 299	☐	☐	☐

Hvis du har fjernet begge eggstokkene, hvor gammel var du da? 300 ____ år

	Ja	Nei	Vet ikke
Operert for endometriose 302	☐	☐	☐
Sterilisert	☐	☐	☐
Utskraping fra livmor (sykehus)	☐	☐	☐
Fjernet hele livmoren 305	☐	☐	☐

Hvis du har fjernet hele livmoren, hvor gammel var du da? 306 ____ år

P-PILLER

	Ja	Nei
Har du noen gang brukt p-piller, minipiller inkludert? 308	☐	☐

Hvis «Ja»: **Hvor gammel var du første gang du brukte p-piller?** 309 ____ år

Hvor lenge har du brukt p-piller i alt? 311 ____ år

Hvis under ett år, antall måneder 313 ____ mndr.

	Ja	Nei
Bruker du p-piller nå?	☐	☐

Hvilket merke bruker du? 316 ____

HORMONBEHANDLING

Utenom p-piller

Har du noen gang brukt medisiner som inneholder østrogen? Vanlige navn på slike medisiner er: Cyclabil, Estraderm, Kilogest, Ovesterin, Progynova, Trisekvens.

	Nå	Før	Aldri
Tabletter eller plaster 318	☐	☐	☐
Krem eller stikkpiller 319	☐	☐	☐

Hvis «Ja»: **Hvor gammel var du første gang du fikk østrogenmedisin, og omtrent hvor mange år brukte du slik medisin?**

	Din alder	Antall år
Tabletter eller plaster 320		
Krem eller stikkpiller 324		

Hvis du bruker østrogenmedisin nå, hvilket merke bruker du? 328 ____

PROBLEMER MED Å BLI GRAVID

	Ja	Nei
Har du noen gang prøvd i mer enn ett år å bli gravid? 329	☐	☐

Hvis «Ja»: **Hvor gammel var du første gang du hadde problemer med å bli gravid?** 330 ____ år

	Ja	Nei
Har du noen gang oppsøkt lege fordi du hadde problemer med å bli gravid? 332	☐	☐

GRAVIDITETER, FØDSLER OG AMMING

Hvor mange ganger har du vært gravid totalt?
Regn med ***alle*** *svangerskap, spontane eller selvbestemte aborter, så vel som fødsler (også dødfødsler)* 333 ____ ganger

Hvor mange barn har du født? 335 ____ barn

Fyll ut for hvert barn (de første 7) opplysninger om fødselsår og omtrent antall måneder du ammet hvert barn og antall måneder menstruasjonen din var borte etter fødselen (fylles ut også for dødfødte eller for barn som er døde senere i livet).

Barn	Fødselsår	Antall måneder med amming	Antall blødningsfrie måneder
1	336 19		
2	342 19		
3	348 19		
4	354 19		
5	360 19		
6	366 19		
7	372 19		

URINLEKKASJE

	Ja	Nei
Har du ufrivillig urinlekkasje? 378	☐	☐

Hvis «Nei»: Gå til KALK I KOSTEN ...

Hvor ofte har du urinlekkasje? 379
- sjeldnere enn en gang pr. måned ☐
- en eller flere ganger pr. måned ☐
- en eller flere ganger pr. uke ☐
- hver dag og/eller natt ☐

Hvor mye urin lekker du vanligvis hver gang? 380
dråper eller lite ☐ små skvetter ☐ større mengder ☐

	Ja	Nei
Har du lekkasje av urin i forbindelse med hosting, nysing, latter, tunge løft 381	☐	☐
Har du lekkasje av urin i forbindelse med plutselig og sterk vannlatingstrang? 382	☐	☐

Hvor lenge har du hatt urinlekkasje? 383
0-5 år ☐ 5-10 år ☐ Over 10 år ☐

	Ja	Nei
Har du søkt lege på grunn av urinlekkasje? 384	☐	☐

Hvordan opplever du lekkasjeplagene dine? 385 *Ett kryss*
- ikke noe problem ☐
- en liten plage ☐
- en del plaget ☐
- mye plaget ☐
- svært stort problem ☐

KALK I KOSTEN OG KOSTTILSKUDD

Hvor mange glass melk (alle sorter, også drikkeyoghurt) drikker du vanligvis daglig? *Bare ett kryss* 386
- Ingen ☐ 1
- Mindre enn ett ☐ 2
- 1–2 glass ☐ 3
- 3 eller mer ☐ 4

Hvor mange brødskiver med kvitost spiser du vanligvis daglig? *Bare ett kryss*
- Ingen ☐ 1
- Mindre enn en ☐ 2
- 1–2 skiver ☐ 3
- 3 eller mer ☐ 4

Bruker du vanligvis noen av disse kosttilskuddene?

	Ja	Nei
vitamin D-tilskudd 388	☐	☐
kalktabletter eller benmel	☐	☐

HUMØR OG TRIVSEL

Ett kryss på hver linje

Angi hvordan du har følt deg den siste måneden:	*Aldri*	*Noen ganger*	*Ganske ofte*	*For det meste*
i godt humør 390	☐	☐	☐	☐
i dårlig humør 391	☐	☐	☐	☐

Er du rask til å oppfatte et humoristisk poeng? 392	*Svært treg*	*Ganske treg*	*Ganske rask*	*Svært rask*
	☐	☐	☐	☐

Er du enig i at det er noe ansvarsløst over folk som stadig prøver å være morsomme? 393

Nei, slett ikke ..... ☐1 Ganske enig ..... ☐3
I noen grad ..... ☐2 Ja, absolutt ..... ☐4

Er du en munter person? 394

Nei, slett ikke ..... ☐1 Ganske munter ..... ☐3
I noen grad ..... ☐2 Ja, absolutt ..... ☐4

SINNE

Sett kryss på det svaret som best beskriver deg i forhold til de to påstandene nedenfor:

Jeg gir uttrykk for mitt sinne, og andre mennesker vet at jeg er sint 395

Nesten aldri ..... ☐1 Ganske ofte ..... ☐3
Noen ganger ..... ☐2 Nesten alltid ..... ☐4

Jeg koker av sinne, men jeg viser det ikke til andre 396

Nesten aldri ..... ☐1 Ganske ofte ..... ☐3
Noen ganger ..... ☐2 Nesten alltid ..... ☐4

HVILE OG AVSLAPPING

Hvor mange timer tilbringer du *vanligvis* i *liggende* stilling i løpet av et døgn?
(nattesøvn, middagshvil) .....397 Antall timer ☐☐

Hvor mange timer tilbringer du *vanligvis* i *sittende* stilling i løpet av et døgn?
(arbeid, måltider, TV, bil etc.) .....399 Antall timer ☐☐

Hvor ofte er du plaget av søvnløshet? 401

Aldri, eller noen få ganger i året ..... ☐1
1–2 ganger i måneden ..... ☐2
Omtrent 1 gang i uka ..... ☐3
Mer enn en gang i uka ..... ☐4

Har du siste år vært plaget av søvnløshet slik at det har gått ut over arbeidsevnen? 402	*Ja*	*Nei*
	☐	☐

Har du i løpet av siste måned hatt innsovningsproblemer? *Bare ett kryss* 403

Nesten hver natt ..... ☐1 Av og til ..... ☐3
Ofte ..... ☐2 Aldri ..... ☐4

Har du i løpet av siste måned våknet for tidlig og ikke fått sove igjen? *Bare ett kryss* 404

Nesten hver natt ..... ☐1 Av og til ..... ☐3
Ofte ..... ☐2 Aldri ..... ☐4

Har du i løpet av siste måned vært plaget av nervøsitet (irritabel, urolig, anspent eller rastløs)? 405

Nesten hele tida ..... ☐1
Ofte ..... ☐2
Av og til ..... ☐3
Aldri ..... ☐4

HVORDAN DU HAR HATT DET

Har det noen gang i løpet av ditt liv vært *sammenhengende perioder på 2 uker eller mer* da du:	*Ja*	*Nei*
følte deg deprimert, trist og nedfor 406	☐	☐
hadde problemer med matlysten eller spiste alt for lite	☐	☐
var plaget av kraftløshet eller mangel på overskudd	☐	☐
virkelig bebreidet deg selv og følte deg verdiløs ...	☐	☐
hadde problemer med å konsentrere deg eller vanskelig for å ta beslutninger	☐	☐
hadde minst tre av de problemene som er nevnt ovenfor samtidig 411	☐	☐

HVORDAN DU SER PÅ DEG SELV

Folk ser på seg selv på ulike måter. Kryss av for hvert utsagn hvor enig eller uenig du er. *Ett kryss på hver linje*

	Svært enig	*Enig*	*Uenig*	*Svært uenig*
Jeg har en positiv holdning til meg selv 412	☐	☐	☐	☐
Jeg føler meg virkelig ubrukelig til tider 413	☐	☐	☐	☐
Jeg føler at jeg ikke har mye å være stolt av 414	☐	☐	☐	☐
Jeg føler at jeg er en verdifull person, i allefall på lik linje med andre 415	☐	☐	☐	☐

	Ja	*Nei*
Synes du at du har funnet et virkelig betydningsfullt innhold i livet ditt? 416	☐	☐
Føler du at du lever fullt ut? 417	☐	☐

HVORDAN DU FØLER DEG NÅ

*Sett kryss i den ruta utenfor det svaret som best beskriver dine følelser **den siste uka.** Bare ett kryss*

Er du vanligvis glad eller nedstemt? 418

Svært nedstemt ..... ☐1
Nedstemt ..... ☐2
Nokså nedstemt ..... ☐3
Både – og ..... ☐4
Nokså glad ..... ☐5
Glad ..... ☐6
Svært glad ..... ☐7

Har du i det store og hele en rolig og god følelse inne i deg? 419

Nesten hele tida ..... ☐1
Ofte ..... ☐2
Av og til ..... ☐3
Aldri ..... ☐4

Føler du deg stort sett sterk og opplagt, eller trøtt og sliten? 420

Meget sterk og opplagt ..... ☐1
Sterk og opplagt ..... ☐2
Ganske sterk og opplagt ..... ☐3
Både – og ..... ☐4
Ganske trøtt og sliten ..... ☐5
Trøtt og sliten ..... ☐6
Svært trøtt og sliten ..... ☐7

Legg det utfylte spørreskjemaet i den vedlagte svarkonvolutten og postlegg den så snart som mulig!
Porto er betalt.

Hjertelig takk for hjelpa!

Steinkjer Trykkeri AS – 74 16 30 00

Appendix
Q2-H2, men

hunt

Helseundersøkelsen i Nord-Trøndelag

SKJEMA FOR MENN 20–69 ÅR

Takk for frammøtet til undersøkelsen!
Vi vil også be deg fylle ut dette spørreskjemaet. Opplysningene vil bli brukt i større forskningsarbeider om forebyggende helsearbeid. Noen av spørsmålene likner på spørsmål du har svart på i det skjemaet du fylte ut heime og leverte ved frammøte til helseundersøkelsen. Det er likevel viktig at du svarer på alle spørsmålene også i dette skjemaet. Det utfylte skjemaet returneres i vedlagte svarkonvolutt. Porto er betalt.
Alle opplysningene er underlagt streng taushetsplikt.

Vennlig hilsen
Helsetjenesten i Nord-Trøndelag
Statens Institutt for Folkehelse Statens helseundersøkelser

Hvis du ikke ønsker å besvare spørreskjemaet, sett kryss her og returner skjemaet. Da slipper du purring.
Jeg ønsker ikke å besvare skjemaet ○

UTFYLLING

Dato for utfylling av skjema: / 19 [19]

OPPVEKST

I hvilken kommune bodde du da du fylte 1 år?
*Hvis du ikke bodde i Norge, oppgi **land** i stedet for kommune.*

[24]

ARBEID

Nåværende eller tidligere arbeid:
Hva slags inntektsgivende arbeid har du og event. din ektefelle/samboer? *Hvis du/dere ikke har inntektsgivende arbeid nå: Oppgi det siste yrket.*

		Deg selv		Ektefelle/ samboer
Spesialarbeider eller ufaglært arbeider	25	☐	☐	36
Fagarbeider, handverker, formann		☐	☐	
Underordnet funksjonær (f.eks. butikk, kontor, off. tjenester)		☐	☐	
Fagfunksjonær (f.eks. sykepleier, tekniker, lærer)		☐	☐	
Overordnet stilling i off. eller privat virksomhet		☐	☐	
Sjåfør	30	☐	☐	41
Gårdbruker eller skogeier		☐	☐	
Fisker		☐	☐	
Selvstendig i akademisk erverv (f.eks. tannlege, advokat)		☐	☐	
Annen selvstendig næringsvirksomhet		☐	☐	
Har ikke vært i inntektsgivende arbeid	35	☐	☐	46

Hvis du NÅ ikke har inntektsgivende arbeid eller du ikke har heltids husarbeid: Gå til BOLIG.

Har du i løpet av de siste 12 månedene hatt sykefravær:		Ja	Nei
med egenmelding	47	☐	☐
med sykmelding fra lege	48	☐	☐

Hvis «Ja»: **Hvor lenge *tilsammen*?** *Bare ett kryss*
2 uker eller mindre [49] ☐1
2-8 uker ☐2
Mer enn 8 uker ☐3

Har du i løpet av de siste 12 månedene vurdert å skifte yrke eller arbeidsplass?	50	Ja ☐	Nei ☐

Er arbeidet ditt så fysisk anstrengende at du ofte er sliten i kroppen etter en arbeidsdag? *Bare ett kryss* 51
Ja, nesten alltid ☐1 Ganske sjelden ☐3
Ganske ofte ☐2 Aldri, eller nesten aldri ☐4

Krever arbeidet ditt så mye konsentrasjon og oppmerksomhet at du ofte føler deg utslitt etter en arbeidsdag? 52
Ja, nesten alltid ☐1 Ganske sjelden ☐3
Ganske ofte ☐2 Aldri, eller nesten aldri ☐4

Hvordan trives du alt i alt med arbeidet ditt? 53
Veldig godt ☐1 Ikke særlig godt ☐3
Godt ☐2 Dårlig ☐4

BOLIG

Hvem bor du sammen med?
Ett kryss for hver linje og angi antall

		Ja	Nei	Antall
Ektefelle/samboer	54	☐	☐	
Andre personer over 18 år	55	☐	☐	
Personer under 18 år	58	☐	☐	

Hvor mange av barna har plass i barnehage? [61] Antall

Hvilken type bolig bor du i? *Bare ett kryss*
Enebolig/villa [63] ☐1
Gårdsbruk ☐2
Blokk/terrasseleilighet ☐3
Rekkehus/2-4 mannsbolig ☐4
Annen bolig ☐5

Hvor stor er din boenhet? [64] kvm

	Ja	Nei
Er det heldekkende tepper i stua? [67]	☐	☐
Er det heldekkende tepper på ditt soverom?	☐	☐
Er det katt i boligen? [69]	☐	☐
Er det hund i boligen?	☐	☐
Er det andre pelskledde dyr eller fugler i boligen?	☐	☐

ØKONOMI

Mottar du noen av følgende *offentlige* ytelser?		Ja	Nei
Sykepenger/sykelønn/rehabiliteringspenger	72	☐	☐
Ytelser under yrkesrettet attføring		☐	☐
Uførepensjon	74	☐	☐
Alderspensjon		☐	☐
Sosialstøtte		☐	☐
Arbeidsløshetstrygd		☐	☐
Overgangsstønad		☐	☐
Etterlattepensjon	79	☐	☐
Andre ytelser		☐	☐

Har det i løpet av det siste året hendt at husholdningen har hatt vansker med å klare de løpende utgifter til mat, transport, bolig og liknende? *Bare ett kryss* 81
Ja, ofte ☐1 Ja, en sjelden gang ☐3
Ja, av og til ☐2 Nei, aldri ☐4

VENNER

Hvor mange gode venner har du?
Regn med de du kan snakke fortrolig med og som kan gi deg god hjelp når du trenger det [82] Antall
Tell ikke med de du bor sammen med, men regn med andre slektninger

		Ja	Nei
Føler du at du har mange nok gode venner?	84	☐	☐

Hvor ofte tar du vanligvis del i foreningsvirksomhet som f.eks. syklubb, idrettslag, politiske lag, religiøse eller andre foreninger? 85
Aldri, eller noen få ganger i året ☐1 Omtrent en gang i uka ☐1
1-2 ganger i måneden ☐2 Mer enn en gang i uka ☐2

DER DU BOR

Svar ut fra nærmiljøet, dvs. nabolaget/grenda.
Ett kryss for hvert spørsmål

Jeg føler et sterkt fellesskap med de som bor her 86
Helt enig ☐ 1 Delvis enig ☐ 2 Usikker ☐ 3 Delvis uenig ☐ 4 Helt uenig ☐ 5

Selv om noen tar initiativ, er det ingen som blir med på det som settes i gang her 87
Helt enig ☐ Delvis enig ☐ Usikker ☐ Delvis uenig ☐ Helt uenig ☐

Hvis jeg flytter herfra, vil jeg lengte tilbake 88
Helt enig ☐ Delvis enig ☐ Usikker ☐ Delvis uenig ☐ Helt uenig ☐

Man kan ikke stole på hverandre her 89
Helt enig ☐ Delvis enig ☐ Usikker ☐ Delvis uenig ☐ Helt uenig ☐

Når noe skal gjøres her, er det lett å få folk med 90
Helt enig ☐ Delvis enig ☐ Usikker ☐ Delvis uenig ☐ Helt uenig ☐

Det er vanskelig å få kontakt med folk her 91
Helt enig ☐ Delvis enig ☐ Usikker ☐ Delvis uenig ☐ Helt uenig ☐

Det er godt samhold her 92
Helt enig ☐ Delvis enig ☐ Usikker ☐ Delvis uenig ☐ Helt uenig ☐

Ingen orker å ta initiativ til noe lenger her 93
Helt enig ☐ Delvis enig ☐ Usikker ☐ Delvis uenig ☐ Helt uenig ☐

Folk trives godt her 94
Helt enig ☐ Delvis enig ☐ Usikker ☐ Delvis uenig ☐ Helt uenig ☐

Folk her kan ha store problemer uten at naboen vet noe 95
Helt enig ☐ Delvis enig ☐ Usikker ☐ Delvis uenig ☐ Helt uenig ☐

Det er alltid noen som tar initiativ til å løse nødvendige oppgaver her 96
Helt enig ☐ Delvis enig ☐ Usikker ☐ Delvis uenig ☐ Helt uenig ☐

Folk snakker lite med hverandre her 97
Helt enig ☐ 1 Delvis enig ☐ 2 Usikker ☐ 3 Delvis uenig ☐ 4 Helt uenig ☐ 5

SYKDOM I FAMILIEN

Kryss av for de slektningene som har eller har hatt noen av sykdommene. Kryss av for «ingen» hvis ingen av slektningene har hatt denne sykdommen. *Evt. flere kryss på hver linje*

	Mor	Far	Bror	Søster	Barn	Ingen
Hjerneslag eller hjerneblødning 98	☐	☐	☐	☐	☐	☐
Hjerteinfarkt før 60 års alder 104	☐	☐	☐	☐	☐	☐
Astma 110	☐	☐	☐	☐	☐	☐
Allergi 116	☐	☐	☐	☐	☐	☐
Kreftsykdom 122	☐	☐	☐	☐	☐	☐
Høyt blodtrykk 128	☐	☐	☐	☐	☐	☐
Psykiske plager 134	☐	☐	☐	☐	☐	☐
Osteoporose (benskjørhet) 140	☐	☐	☐	☐	☐	☐
Diabetes (sukkersyke) 146	☐	☐	☐	☐	☐	☐
Alder da de fikk diabetes 152	år	år	år	år	år	

Har du *selv* høysnue eller neseallergi? 162 Ja ☐ Nei ☐

BRUK AV HELSETJENESTER

Har du i løpet av de siste 12 månedene vært hos :
Ett kryss på hver linje

	Ja	Nei
allmennpraktiserende lege (kommunelege, privatpraktiserende lege, turnuskandidat) 163	☐	☐
bedriftslege	☐	☐
lege ved sykehus (uten at du var innlagt)	☐	☐
annen lege	☐	☐
fysioterapeut	☐	☐
kiropraktor	☐	☐
homøopat 169	☐	☐
annen behandler (naturmedisiner, fotsoneterapeut, håndspålegger, "healer", "synsk", e.l.)	☐	☐

Har du vært innlagt i sykehus de siste 5 åra? 171 Ja ☐ Nei ☐

ALKOHOL

Hvis du er totalavholdsmann: Gå til KOSTHOLD.

Ett kryss for hver spørsmål

Har du noen gang følt at du burde redusere alkoholforbruket ditt? 172 Ja ☐ Nei ☐

Har andre noen gang kritisert alkoholbruken din? 173 Ja ☐ Nei ☐

Har du noen gang følt ubehag eller skyldfølelse pga. alkoholbruken din? 174 Ja ☐ Nei ☐

Har det å ta en drink noen gang vært det første du har gjort om morgenen for å roe nervene, kurere bakrus eller som en oppkvikker? 175 Ja ☐ Nei ☐

KOSTHOLD

	Antall
Hvor mange måltider spiser du vanligvis daglig (middag og brødmåltid)? 176	
Hvor mange dager i uka spiser du varm middag?	

Hva slags type brød (kjøpt eller hjemmebakt) spiser du vanligvis? *Inntil to kryss.*

Brødtypen ligner mest på 178	Loff	Fint brød	Kneipp-brød	Grov-brød	Knekke-brød
	☐	☐	☐	☐	☐

Hva slags *fett* blir vanligvis brukt i din husholdning?

Ett kryss for matlaging og ett kryss for brød	*Til matlaging*	*På brød*
Bruker *ikke* smør eller margarin	183 ☐ 1	184 ☐ 1
Meierismør	☐ 2	☐ 2
Hard margarin	☐ 3	☐ 3
Bløt (soft) margarin	☐ 4	☐ 4
Smør/margarin blanding	☐ 5	☐ 5
Lettmargarin	☐ 6	☐ 6
Oljer	☐ 7	

MEDISINBRUK

Har du i deler av de siste 12 måneder brukt noen medisiner daglig eller nesten daglig? 185 Ja ☐ Nei ☐

Hvis «Ja»:
Angi hvor mange måneder du brukte følgende medisiner: *Sett 0 hvis du ikke har brukt medisinene*

	Antall mndr.		Antall mndr.
smertestillende 186		hjertemedisin (ikke blodtrykksmedisin)	
sovemedisin 188		annen medisin	
beroligende medisin		*Kosttilskudd:*	
medisin mot depresjon		jerntabletter 202	
allergimedisin 194		vitamintilskudd	
astmamedisin 196		tran/fiskeoljer 206	

Hvor ofte har du brukt avslappende/beroligende medisin eller sovemedisin den siste måneden? 208
Daglig ☐ 1 Sjeldnere enn hver uke ☐ 3
Hver uke, men ikke hver dag ☐ 2 Aldri ☐ 4

HODEPINE

Har du vært plaget av hodepine i løpet av de siste 12 måneder? 209
Ja, anfallsvis (migrene)..... ☐ 1
Ja, annen slags hodepine.... ☐ 2
Nei ..... ☐ 3

Antall anfall siste 12 mndr. 210 ☐☐☐

Hvis «Nei»: Gå til MUSKEL-/SKJELETTPLAGER

Omtrent hvor mange dager i pr. måned har du hodepine?
Mindre enn 7 dager ☐ 1 7 til 14 dager ☐ 2 Mer enn 14 d. ☐ 3

Hvor lenge varer hodepinen vanligvis hver gang? 213
Mindre enn 4 timer ☐ 1 4 timer–3 døgn ☐ 2 Mer enn 3 døgn ☐ 3

Hvor ofte er hodepinen preget av eller ledsaget av:

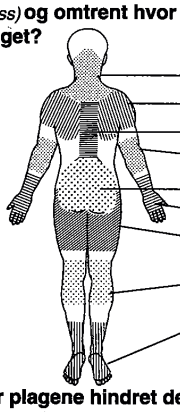
Ett kryss på hver linje	*Sjelden eller aldri*	*Av og til*	*Ofte*
bankende/dunkende smerte 214	☐	☐	☐
pressende smerte	☐	☐	☐
halvsidighet, alltid samme side	☐	☐	☐
halvsidighet, vekselvis h. og v. side	☐	☐	☐
smerter i «hele hodet»	☐	☐	☐
kvalme 219	☐	☐	☐
lys- og/eller lydskyhet	☐	☐	☐
forverring ved fysisk aktivitet.....	☐	☐	☐
synsforstyrrelser *før* hodepine 222	☐	☐	☐

Hvor mange tabletter/stikkpiller har du eventuelt brukt av disse medisinene *alt i alt i løpet av den siste måneden*?
Skriv 0 hvis du ikke har brukt medisinen.
Cafergot 223 ☐☐ Anervan 225 ☐☐ Imigran 227 ☐☐

MUSKEL-/SKJELETTPLAGER

Har du hatt plager (smerter, verk, ubehag) i muskler og/eller ledd i *den siste måneden*? 229 *Ja* ☐ *Nei* ☐

Hvis «Ja»: **Hvor har du hatt disse plagene** *(ett eller flere kryss)* **og omtrent hvor mange dager tilsammen var du plaget?**



Plager (Sett kryss)	Antall dager
Nakke 230	☐☐
Skuldre/aksler..... 233	☐☐
Øvre del av ryggen	☐☐
Albuer 239	☐☐
Korsryggen 242	☐☐
Handledd/hender 245	☐☐
Hofter..... 248	☐☐
Knær..... 251	☐☐
Ankler/føtter 254	☐☐

*Dersom flere kryss: Sett **ring** rundt krysset **der plagen var verst***

Har plagene hindret deg i å utføre daglige aktiviteter den siste måneden?

	Ja	*Nei*
I arbeidet 257	☐	☐
I fritida 258	☐	☐

SMERTER I BEINA

	Ja	*Nei*
Har du sår på tå, fot eller ankel som ikke vil gro? 259	☐	☐
Har du smerter i det ene eller i begge beina når du går? 260	☐	☐
Har du oppsøkt lege p.g.a. smerter i beina? 261	☐	☐

Hvis «NEI» på disse spørsmålene: Gå til URINVEGS...

	Ja	*Nei*
Kan du gå lenger enn 50 meter? 262	☐	☐
Forsvinner smerten når du står stille en stund? 263	☐	☐
Må du sette deg for at smerten skal gå over? 264	☐	☐

Hvor gjør det mest vondt? *Ett kryss* 265
Fot ☐ Legg ☐ Lår ☐ Hofte ☐

	Ja	*Nei*
Har du smerter i beina når du er i ro? 266	☐	☐
Er smertene verst når du ligger i senga? 267	☐	☐
Blir søvnen forstyrret av smertene? 268	☐	☐
Får du mindre vondt når beinet ligger høyt? 269	☐	☐
Får du mindre vondt når beinet ligger lavt, f.eks. om beinet henger utfor sengekanten? 270	☐	☐
Bedres smertene når du står opp og går litt? 271	☐	☐

URINVEGS- OG PROSTATAPLAGER

Ett kryss på hver linje

Har du noen gang blitt fortalt av lege at du har:	*Ja*	*Nei*
forstørret prostata 272	☐	☐
prostatakreft 273	☐	☐

Har du gjennomgått noe av følgende:	*Ja*	*Nei*
sterilisering 274	☐	☐
tatt vevsprøve (biopsi) av prostata 275	☐	☐
kirurgisk fjerning av prostata (helt eller delvis) 276	☐	☐

De neste spørsmålene gjelder siste måned
Bare ett kryss for hvert hver spørsmål

Hvor ofte har du hatt følelsen av at blæren ikke er blitt fullstendig tømt etter avsluttet vannlating? 277
Aldri ..... ☐ 1 Omtrent annenhver gang ... ☐ 4
Omtrent 1 av 5 ganger .... ☐ 2 Omtrent 2 av 3 ganger ..... ☐ 5
Omtrent 1 av 3 ganger .... ☐ 3 Nesten alltid ..... ☐ 6

Hvor ofte har du måttet late vannet på nytt mindre enn 2 timer etter forrige vannlating? 278
Aldri ..... ☐ 1 Omtrent annenhver gang ... ☐ 4
Omtrent 1 av 5 ganger .... ☐ 2 Omtrent 2 av 3 ganger ..... ☐ 5
Omtrent 1 av 3 ganger .... ☐ 3 Nesten alltid ..... ☐ 6

Hvor ofte har du måttet stoppe og starte flere ganger under vannlatingingen? 279
Aldri ..... ☐ 1 Omtrent annenhver gang ... ☐ 4
Omtrent 1 av 5 ganger .... ☐ 2 Omtrent 2 av 3 ganger ..... ☐ 5
Omtrent 1 av 3 ganger .... ☐ 3 Nesten alltid ..... ☐ 6

Hvor ofte syns du det har vært vanskelig å holde igjen når du har følt trang til å late vannet? 280
Aldri ..... ☐ 1 Omtrent annenhver gang ... ☐ 4
Omtrent 1 av 5 ganger .... ☐ 2 Omtrent 2 av 3 ganger ..... ☐ 5
Omtrent 1 av 3 ganger .... ☐ 3 Nesten alltid ..... ☐ 6

Hvor ofte har du hatt svak urinstråle? 281
Aldri ..... ☐ 1 Omtrent annenhver gang ... ☐ 4
Omtrent 1 av 5 ganger .... ☐ 2 Omtrent 2 av 3 ganger ..... ☐ 5
Omtrent 1 av 3 ganger .... ☐ 3 Nesten alltid ..... ☐ 6

Hvor ofte har du måttet trykke eller presse for å begynne vannlatingen? 282
Aldri ..... ☐ 1 Omtrent annenhver gang ... ☐ 4
Omtrent 1 av 5 ganger .... ☐ 2 Omtrent 2 av 3 ganger ..... ☐ 5
Omtrent 1 av 3 ganger .... ☐ 3 Nesten alltid ..... ☐ 6

Hvor mange ganger har du vanligvis måttet stå opp i løpet av natta for å late vannet? 283
Ingen ..... ☐ 1 2 ganger..... ☐ 3 4 ganger ..... ☐ 5
1 gang ..... ☐ 2 3 ganger..... ☐ 4 5 ganger eller mer ☐ 6

Hvis du resten av livet måtte leve med de vannlatings-problemene du har nå, hvordan ville du føle det? 284
Være meget godt fornøyd .. ☐ 1 Være for det meste utilfreds ☐ 5
Være fornøyd ..... ☐ 2 Være misfornøyd ..... ☐ 6
Være for det meste tilfreds . ☐ 3 Ha det forferdelig ..... ☐ 7
Ha blandete følelser ..... ☐ 4

HUMØR OG TRIVSEL

Ett kryss på hver linje

Angi hvordan du har følt deg den siste måneden:	*Aldri*	*Noen ganger*	*Ganske ofte*	*For det meste*
i godt humør 285	☐	☐	☐	☐
i dårlig humør 286	☐	☐	☐	☐

Er du rask til å oppfatte et humoristisk poeng? 287	*Svært treg*	*Ganske treg*	*Ganske rask*	*Svært rask*
	☐	☐	☐	☐

Er du enig i at det er noe ansvarsløst over folk som stadig prøver å være morsomme? 286

Nei, slett ikke ☐1 Ganske enig ☐3
I noen grad ☐2 Ja, absolutt ☐4

Er du en munter person? 289

Nei, slett ikke ☐1 Ganske munter ☐3
I noen grad ☐2 Ja, absolutt ☐4

SINNE

Sett kryss på det svaret som best beskriver deg i forhold til de to påstandene nedenfor:

Jeg gir uttrykk for mitt sinne, og andre mennesker vet at jeg er sint. 290

Nesten aldri ☐1 Ganske ofte ☐3
Noen ganger ☐2 Nesten alltid ☐4

Jeg koker av sinne, men jeg viser det ikke til andre. 291

Nesten aldri ☐1 Ganske ofte ☐3
Noen ganger ☐2 Nesten alltid ☐4

HVILE OG AVSLAPPING

Hvor mange timer tilbringer du *vanligvis* i *liggende* stilling i løpet av et døgn?
(nattesøvn, middagshvil) ....292 Antall timer ☐☐

Hvor mange timer tilbringer du *vanligvis* i *sittende* stilling i løpet av et døgn?
(arbeid, måltider, TV, bil etc.) ....294 Antall timer ☐☐

Hvor ofte er du plaget av søvnløshet? 296

Aldri, eller noen få ganger i året ☐1
1–2 ganger i måneden ☐2
Omtrent 1 gang i uka ☐3
Mer enn en gang i uka ☐4

Har du siste år vært plaget av søvnløshet slik at det har gått ut over arbeidsevnen? 297	*Ja*	*Nei*
	☐	☐

Har du i løpet av siste måned hatt innsovningsproblemer? *Bare ett kryss* 298

Nesten hver natt ☐1 Av og til ☐3
Ofte ☐2 Aldri ☐4

Har du i løpet av siste måned våknet for tidlig og ikke fått sove igjen? *Bare ett kryss* 299

Nesten hver natt ☐1 Av og til ☐3
Ofte ☐2 Aldri ☐4

Har du i løpet av siste måned vært plaget av nervøsitet (irritabel, urolig, anspent eller rastløs)? 300

Nesten hele tida ☐1
Ofte ☐2
Av og til ☐3
Aldri ☐4

HVORDAN DU HAR HATT DET

Har det noen gang i løpet av ditt liv vært *sammenhengende perioder på 2 uker eller mer* da du:	*Ja*	*Nei*
følte deg deprimert, trist og nedfor 301	☐	☐
hadde problemer med matlysten eller spiste alt for lite	☐	☐
var plaget av kraftløshet eller mangel på overskudd	☐	☐
virkelig bebreidet deg selv og følte deg verdiløs ...	☐	☐
hadde problemer med å konsentrere deg eller vanskelig for å ta beslutninger	☐	☐
hadde minst tre av de problemene som er nevnt ovenfor samtidig 306	☐	☐

HVORDAN DU SER PÅ DEG SELV

Folk ser på seg selv på ulike måter. Kryss av for hvert utsagn hvor enig eller uenig du er. *Ett kryss på hver linje*

	Svært enig	*Enig*	*Uenig*	*Svært uenig*
Jeg har en positiv holdning til meg selv 307	☐	☐	☐	☐
Jeg føler meg virkelig ubrukelig til tider 308	☐	☐	☐	☐
Jeg føler at jeg ikke har mye å være stolt av 309	☐	☐	☐	☐
Jeg føler at jeg er en verdifull person, i allefall på lik linje med andre 310	☐	☐	☐	☐

	Ja	*Nei*
Synes du at du har funnet et virkelig betydningsfullt innhold i livet ditt? 311	☐	☐
Føler du at du lever fullt ut? 312	☐	☐

HVORDAN DU FØLER DEG NÅ

*Sett kryss i den ruta utenfor det svaret som best beskriver dine følelser **den siste uka.** Bare ett kryss*

Er du vanligvis glad eller nedstemt? 313

Svært nedstemt ☐1
Nedstemt ☐2
Nokså nedstemt ☐3
Både – og ☐4
Nokså glad ☐5
Glad ☐6
Svært glad ☐7

Har du i det store og hele en rolig og god følelse inne i deg? 314

Nesten hele tida ☐1
Ofte ☐2
Av og til ☐3
Aldri ☐4

Føler du deg stort sett sterk og opplagt, eller trøtt og sliten? 315

Meget sterk og opplagt ☐1
Sterk og opplagt ☐2
Ganske sterk og opplagt ☐3
Både – og ☐4
Ganske trøtt og sliten ☐5
Trøtt og sliten ☐6
Svært trøtt og sliten ☐7

Legg det utfylte spørreskjemaet i den vedlagte svarkonvolutten og postlegg den så snart som mulig!
Porto er betalt.
Hjertelig takk for hjelpa!

Steinkjer Trykkeri AS – 74 16 30 00

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1991

65. Kåre Bergh: APPLICATIONS OF ANTI-C5a SPECIFIC MONOCLONAL ANTIBODIES FOR THE ASSESSMENT OF COMPLEMENT ACTIVATION.
66. Svein Svenningsen: THE CLINICAL SIGNIFICANCE OF INCREASED FEMORAL ANTEVERSION.
67. Olbjørn Klepp: NONSEMINOMATOUS GERM CELL TESTIS CANCER: THERAPEUTIC OUTCOME AND PROGNOSTIC FACTORS.
68. Trond Sand: THE EFFECTS OF CLICK POLARITY ON BRAINSTEM AUDITORY EVOKED POTENTIALS AMPLITUDE, DISPERSION, AND LATENCY VARIABLES.
69. Kjetil B. Åsbakk: STUDIES OF A PROTEIN FROM PSORIATIC SCALE, PSO P27, WITH RESPECT TO ITS POTENTIAL ROLE IN IMMUNE REACTIONS IN PSORIASIS.
70. Arnulf Hestnes: STUDIES ON DOWN´S SYNDROME.
71. Randi Nygaard: LONG-TERM SURVIVAL IN CHILDHOOD LEUKEMIA.
72. Bjørn Hagen: THIO-TEPA.
73. Svein Anda: EVALUATION OF THE HIP JOINT BY COMPUTED TOMOGRAMPHY AND ULTRASONOGRAPHY.

1992

74. Martin Svartberg: AN INVESTIGATION OF PROCESS AND OUTCOME OF SHORT-TERM PSYCHODYNAMIC PSYCHOTHERAPY.
75. Stig Arild Slørdahl: AORTIC REGURGITATION.
76. Harold C Sexton: STUDIES RELATING TO THE TREATMENT OF SYMPTOMATIC NON-PSYCHOTIC PATIENTS.
77. Maurice B. Vincent: VASOACTIVE PEPTIDES IN THE OCULAR/FOREHEAD AREA.
78. Terje Johannessen: CONTROLLED TRIALS IN SINGLE SUBJECTS.
79. Turid Nilsen: PYROPHOSPHATE IN HEPATOCYTE IRON METABOLISM.
80. Olav Haraldseth: NMR SPECTROSCOPY OF CEREBRAL ISCHEMIA AND REPERFUSION IN RAT.
81. Eiliv Brenna: REGULATION OF FUNCTION AND GROWTH OF THE OXYNTIC MUCOSA.

1993

82. Gunnar Bovim: CERVICOGENIC HEADACHE.
83. Jarl Arne Kahn: ASSISTED PROCREATION.
84. Bjørn Naume: IMMUNOREGULATORY EFFECTS OF CYTOKINES ON NK CELLS.
85. Rune Wiseth: AORTIC VALVE REPLACEMENT.
86. Jie Ming Shen: BLOOD FLOW VELOCITY AND RESPIRATORY STUDIES.
87. Piotr Kruszewski: SUNCT SYNDROME WITH SPECIAL REFERENCE TO THE AUTONOMIC NERVOUS SYSTEM.
88. Mette Haase Moen: ENDOMETRIOSIS.
89. Anne Vik: VASCULAR GAS EMBOLISM DURING AIR INFUSION AND AFTER DECOMPRESSION IN PIGS.
90. Lars Jacob Stovner: THE CHIARI TYPE I MALFORMATION.
91. Kjell Å. Salvesen: ROUTINE ULTRASONOGRAPHY IN UTERO AND DEVELOPMENT IN CHILDHOOD.

1994

92. Nina-Beate Liabakk: DEVELOPMENT OF IMMUNOASSAYS FOR TNF AND ITS SOLUBLE RECEPTORS.
93. Sverre Helge Torp: *erb*B ONCOGENES IN HUMAN GLIOMAS AND MENINGIOMAS.
94. Olav M. Linaker: MENTAL RETARDATION AND PSYCHIATRY. Past and present.
95. Per Oscar Feet: INCREASED ANTIDEPRESSANT AND ANTIPANIC EFFECT IN COMBINED TREATMENT WITH DIXYRAZINE AND TRICYCLIC ANTIDEPRESSANTS.
96. Stein Olav Samstad: CROSS SECTIONAL FLOW VELOCITY PROFILES FROM TWO-DIMENSIONAL DOPPLER ULTRASOUND: Studies on early mitral blood flow.
97. Bjørn Backe: STUDIES IN ANTENATAL CARE.
98. Gerd Inger Ringdal: QUALITY OF LIFE IN CANCER PATIENTS.
99. Torvid Kiserud: THE DUCTUS VENOSUS IN THE HUMAN FETUS.
100. Hans E. Fjøsne: HORMONAL REGULATION OF PROSTATIC METABOLISM.
101. Eylert Brodtkorb: CLINICAL ASPECTS OF EPILEPSY IN THE MENTALLY RETARDED.
102. Roar Juul: PEPTIDERGIC MECHANISMS IN HUMAN SUBARACHNOID HEMORRHAGE.
103. Unni Syversen: CHROMOGRANIN A. Phsysiological and Clinical Role.

1995

104. Odd Gunnar Brakstad: THERMOSTABLE NUCLEASE AND THE *nuc* GENE IN THE DIAGNOSIS OF *Staphylococcus aureus* INFECTIONS.
105. Terje Engan: NUCLEAR MAGNETIC RESONANCE (NMR) SPECTROSCOPY OF PLASMA IN MALIGNANT DISEASE.
106. Kirsten Rasmussen: VIOLENCE IN THE MENTALLY DISORDERED.
107. Finn Egil Skjeldestad: INDUCED ABORTION: Timetrends and Determinants.
108. Roar Stenseth: THORACIC EPIDURAL ANALGESIA IN AORTOCORONARY BYPASS SURGERY.
109. Arild Faxvaag: STUDIES OF IMMUNE CELL FUNCTION *in mice infected with* MURINE RETROVIRUS.

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110. Svend Aakhus: NONINVASIVE COMPUTERIZED ASSESSMENT OF LEFT VENTRICULAR FUNCTION AND SYSTEMIC ARTERIAL PROPERTIES. Methodology and some clinical applications.
111. Klaus-Dieter Bolz: INTRAVASCULAR ULTRASONOGRAPHY.
112. Petter Aadahl: CARDIOVASCULAR EFFECTS OF THORACIC AORTIC CROSS-CLAMPING.
113. Sigurd Steinshamn: CYTOKINE MEDIATORS DURING GRANULOCYTOPENIC INFECTIONS.
114. Hans Stifoss-Hanssen: SEEKING MEANING OR HAPPINESS?
115. Anne Kvikstad: LIFE CHANGE EVENTS AND MARITAL STATUS IN RELATION TO RISK AND PROGNOSIS OF CANCER.
116. Torbjørn Grøntvedt: TREATMENT OF ACUTE AND CHRONIC ANTERIOR CRUCIATE LIGAMENT INJURIES. A clinical and biomechanical study.
117. Sigrid Hørven Wigers: CLINICAL STUDIES OF FIBROMYALGIA WITH FOCUS ON ETIOLOGY, TREATMENT AND OUTCOME.
118. Jan Schjøtt: MYOCARDIAL PROTECTION: Functional and Metabolic Characteristics of Two Endogenous Protective Principles.
119. Marit Martinussen: STUDIES OF INTESTINAL BLOOD FLOW AND ITS RELATION TO TRANSITIONAL CIRCULATORY ADAPATION IN NEWBORN INFANTS.
120. Tomm B. Müller: MAGNETIC RESONANCE IMAGING IN FOCAL CEREBRAL ISCHEMIA.
121. Rune Haaverstad: OEDEMA FORMATION OF THE LOWER EXTREMITIES.
122. Magne Børset: THE ROLE OF CYTOKINES IN MULTIPLE MYELOMA, WITH SPECIAL REFERENCE TO HEPATOCYTE GROWTH FACTOR.
123. Geir Smedslund: A THEORETICAL AND EMPIRICAL INVESTIGATION OF SMOKING, STRESS AND DISEASE: RESULTS FROM A POPULATION SURVEY.

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124. Torstein Vik: GROWTH, MORBIDITY, AND PSYCHOMOTOR DEVELOPMENT IN INFANTS WHO WERE GROWTH RETARDED *IN UTERO*.
125. Siri Forsmo: ASPECTS AND CONSEQUENCES OF OPPORTUNISTIC SCREENING FOR CERVICAL CANCER. Results based on data from three Norwegian counties.
126. Jon S. Skranes: CEREBRAL MRI AND NEURODEVELOPMENTAL OUTCOME IN VERY LOW BIRTH WEIGHT (VLBW) CHILDREN. A follow-up study of a geographically based year cohort of VLBW children at ages one and six years.
127. Knut Bjørnstad: COMPUTERIZED ECHOCARDIOGRAPHY FOR EVALUTION OF CORONARY ARTERY DISEASE.
128. Grethe Elisabeth Borchgrevink: DIAGNOSIS AND TREATMENT OF WHIPLASH/NECK SPRAIN INJURIES CAUSED BY CAR ACCIDENTS.
129. Tor Elsås: NEUROPEPTIDES AND NITRIC OXIDE SYNTHASE IN OCULAR AUTONOMIC AND SENSORY NERVES.
130. Rolf W. Gråwe: EPIDEMIOLOGICAL AND NEUROPSYCHOLOGICAL PERSPECTIVES ON SCHIZOPHRENIA.
131. Tonje Strømholm: CEREBRAL HAEMODYNAMICS DURING THORACIC AORTIC CROSSCLAMPING. An experimental study in pigs

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132. Martinus Bråten: STUDIES ON SOME PROBLEMS REALTED TO INTRAMEDULLARY NAILING OF FEMORAL FRACTURES.

133. Ståle Nordgård: PROLIFERATIVE ACTIVITY AND DNA CONTENT AS PROGNOSTIC INDICATORS IN ADENOID CYSTIC CARCINOMA OF THE HEAD AND NECK.
134. Egil Lien: SOLUBLE RECEPTORS FOR **TNF** AND **LPS**: RELEASE PATTERN AND POSSIBLE SIGNIFICANCE IN DISEASE.
135. Marit Bjørgaas: HYPOGLYCAEMIA IN CHILDREN WITH DIABETES MELLITUS
136. Frank Skorpen: GENETIC AND FUNCTIONAL ANALYSES OF DNA REPAIR IN HUMAN CELLS.
137. Juan A. Pareja: SUNCT SYNDROME. ON THE CLINICAL PICTURE. ITS DISTINCTION FROM OTHER, SIMILAR HEADACHES.
138. Anders Angelsen: NEUROENDOCRINE CELLS IN HUMAN PROSTATIC CARCINOMAS AND THE PROSTATIC COMPLEX OF RAT, GUINEA PIG, CAT AND DOG.
139. Fabio Antonaci: CHRONIC PAROXYSMAL HEMICRANIA AND HEMICRANIA CONTINUA: TWO DIFFERENT ENTITIES?
140. Sven M. Carlsen: ENDOCRINE AND METABOLIC EFFECTS OF METFORMIN WITH SPECIAL EMPHASIS ON CARDIOVASCULAR RISK FACTORES.

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141. Terje A. Murberg: DEPRESSIVE SYMPTOMS AND COPING AMONG PATIENTS WITH CONGESTIVE HEART FAILURE.
142. Harm-Gerd Karl Blaas: THE EMBRYONIC EXAMINATION. Ultrasound studies on the development of the human embryo.
143. Noèmi Becser Andersen:THE CEPHALIC SENSORY NERVES IN UNILATERAL HEADACHES. Anatomical background and neurophysiological evaluation.
144. Eli-Janne Fiskerstrand: LASER TREATMENT OF PORT WINE STAINS. A study of the efficacy and limitations of the pulsed dye laser. Clinical and morfological analyses aimed at improving the therapeutic outcome.
145. Bård Kulseng: A STUDY OF ALGINATE CAPSULE PROPERTIES AND CYTOKINES IN RELATION TO INSULIN DEPENDENT DIABETES MELLITUS.
146. Terje Haug: STRUCTURE AND REGULATION OF THE HUMAN UNG GENE ENCODING URACIL-DNA GLYCOSYLASE.
147. Heidi Brurok: MANGANESE AND THE HEART. A Magic Metal with Diagnostic and Therapeutic Possibilites.
148. Agnes Kathrine Lie: DIAGNOSIS AND PREVALENCE OF HUMAN PAPILLOMAVIRUS INFECTION IN CERVICAL INTRAEPITELIAL NEOPLASIA. Relationship to Cell Cycle Regulatory Proteins and HLA DQBI Genes.
149. Ronald Mårvik: PHARMACOLOGICAL, PHYSIOLOGICAL AND PATHOPHYSIOLOGICAL STUDIES ON ISOLATED STOMACS.
150. Ketil Jarl Holen: THE ROLE OF ULTRASONOGRAPHY IN THE DIAGNOSIS AND TREATMENT OF HIP DYSPLASIA IN NEWBORNS.
151. Irene Hetlevik: THE ROLE OF CLINICAL GUIDELINES IN CARDIOVASCULAR RISK INTERVENTION IN GENERAL PRACTICE.
152. Katarina Tunòn: ULTRASOUND AND PREDICTION OF GESTATIONAL AGE.
153. Johannes Soma: INTERACTION BETWEEN THE LEFT VENTRICLE AND THE SYSTEMIC ARTERIES.
154. Arild Aamodt: DEVELOPMENT AND PRE-CLINICAL EVALUATION OF A CUSTOM-MADE FEMORAL STEM.
155. Agnar Tegnander: DIAGNOSIS AND FOLLOW-UP OF CHILDREN WITH SUSPECTED OR KNOWN HIP DYSPLASIA.
156. Bent Indredavik: STROKE UNIT TREATMENT: SHORT AND LONG-TERM EFFECTS
157. Jolanta Vanagaite Vingen: PHOTOPHOBIA AND PHONOPHOBIA IN PRIMARY HEADACHES

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158. Ola Dalsegg Sæther: PATHOPHYSIOLOGY DURING PROXIMAL AORTIC CROSS-CLAMPING CLINICAL AND EXPERIMENTAL STUDIES
159. xxxxxxxxx (blind number)
160. Christina Vogt Isaksen: PRENATAL ULTRASOUND AND POSTMORTEM FINDINGS – A TEN YEAR CORRELATIVE STUDY OF FETUSES AND INFANTS WITH DEVELOPMENTAL ANOMALIES.
161. Holger Seidel: HIGH-DOSE METHOTREXATE THERAPY IN CHILDREN WITH ACUTE LYMPHOCYTIC LEUKEMIA: DOSE, CONCENTRATION, AND EFFECT CONSIDERATIONS.

162. Stein Hallan: IMPLEMENTATION OF MODERN MEDICAL DECISION ANALYSIS INTO CLINICAL DIAGNOSIS AND TREATMENT.
163. Malcolm Sue-Chu: INVASIVE AND NON-INVASIVE STUDIES IN CROSS-COUNTRY SKIERS WITH ASTHMA-LIKE SYMPTOMS.
164. Ole-Lars Brekke: EFFECTS OF ANTIOXIDANTS AND FATTY ACIDS ON TUMOR NECROSIS FACTOR-INDUCED CYTOTOXICITY.
165. Jan Lundbom: AORTOCORONARY BYPASS SURGERY: CLINICAL ASPECTS, COST CONSIDERATIONS AND WORKING ABILITY.
166. John-Anker Zwart: LUMBAR NERVE ROOT COMPRESSION, BIOCHEMICAL AND NEUROPHYSIOLOGICAL ASPECTS.
167. Geir Falck: HYPEROSMOLALITY AND THE HEART.
168. Eirik Skogvoll: CARDIAC ARREST Incidence, Intervention and Outcome.
169. Dalius Bansevicius: SHOULDER-NECK REGION IN CERTAIN HEADACHES AND CHRONIC PAIN SYNDROMES.
170. Bettina Kinge: REFRACTIVE ERRORS AND BIOMETRIC CHANGES AMONG UNIVERSITY STUDENTS IN NORWAY.
171. Gunnar Qvigstad: CONSEQUENCES OF HYPERGASTRINEMIA IN MAN
172. Hanne Ellekjær: EPIDEMIOLOGICAL STUDIES OF STROKE IN A NORWEGIAN POPULATION. INCIDENCE, RISK FACTORS AND PROGNOSIS
173. Hilde Grimstad: VIOLENCE AGAINST WOMEN AND PREGNANCY OUTCOME.
174. Astrid Hjelde: SURFACE TENSION AND COMPLEMENT ACTIVATION: Factors influencing bubble formation and bubble effects after decompression.
175. Kjell A. Kvistad: MR IN BREAST CANCER – A CLINICAL STUDY.
176. Ivar Rossvoll: ELECTIVE ORTHOPAEDIC SURGERY IN A DEFINED POPULATION. Studies on demand, waiting time for treatment and incapacity for work.
177. Carina Seidel: PROGNOSTIC VALUE AND BIOLOGICAL EFFECTS OF HEPATOCYTE GROWTH FACTOR AND SYNDECAN-1 IN MULTIPLE MYELOMA.

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178. Alexander Wahba: THE INFLUENCE OF CARDIOPULMONARY BYPASS ON PLATELET FUNCTION AND BLOOD COAGULATION – DETERMINANTS AND CLINICAL CONSEQUENSES
179. Marcus Schmitt-Egenolf: THE RELEVANCE OF THE MAJOR hISTOCOMPATIBILITY COMPLEX FOR THE GENETICS OF PSORIASIS
180. Odrun Arna Gederaas: BIOLOGICAL MECHANISMS INVOLVED IN 5-AMINOLEVULINIC ACID BASED PHOTODYNAMIC THERAPY
181. Pål Richard Romundstad: CANCER INCIDENCE AMONG NORWEGIAN ALUMINIUM WORKERS
182. Henrik Hjorth-Hansen: NOVEL CYTOKINES IN GROWTH CONTROL AND BONE DISEASE OF MULTIPLE MYELOMA
183. Gunnar Morken: SEASONAL VARIATION OF HUMAN MOOD AND BEHAVIOUR
184. Bjørn Olav Haugen: MEASUREMENT OF CARDIAC OUTPUT AND STUDIES OF VELOCITY PROFILES IN AORTIC AND MITRAL FLOW USING TWO- AND THREE-DIMENSIONAL COLOUR FLOW IMAGING
185. Geir Bråthen: THE CLASSIFICATION AND CLINICAL DIAGNOSIS OF ALCOHOL-RELATED SEIZURES
186. Knut Ivar Aasarød: RENAL INVOLVEMENT IN INFLAMMATORY RHEUMATIC DISEASE. A Study of Renal Disease in Wegener's Granulomatosis and in Primary Sjögren's Syndrome
187. Trude Helen Flo: RESEPTORS INVOLVED IN CELL ACTIVATION BY DEFINED URONIC ACID POLYMERS AND BACTERIAL COMPONENTS
188. Bodil Kavli: HUMAN URACIL-DNA GLYCOSYLASES FROM THE UNG GENE: STRUCTRUAL BASIS FOR SUBSTRATE SPECIFICITY AND REPAIR
189. Liv Thommesen: MOLECULAR MECHANISMS INVOLVED IN TNF- AND GASTRIN-MEDIATED GENE REGULATION
190. Turid Lingaas Holmen: SMOKING AND HEALTH IN ADOLESCENCE; THE NORD-TRØNDELAG HEALTH STUDY, 1995-97
191. Øyvind Hjertner: MULTIPLE MYELOMA: INTERACTIONS BETWEEN MALIGNANT PLASMA CELLS AND THE BONE MICROENVIRONMENT

192. Asbjørn Støylen: STRAIN RATE IMAGING OF THE LEFT VENTRICLE BY ULTRASOUND. FEASIBILITY, CLINICAL VALIDATION AND PHYSIOLOGICAL ASPECTS
193. Kristian Midthjell: DIABETES IN ADULTS IN NORD-TRØNDELAG. PUBLIC HEALTH ASPECTS OF DIABETES MELLITUS IN A LARGE, NON-SELECTED NORWEGIAN POPULATION.
194. Guanglin Cui: FUNCTIONAL ASPECTS OF THE ECL CELL IN RODENTS
195. Ulrik Wisløff: CARDIAC EFFECTS OF AEROBIC ENDURANCE TRAINING: HYPERTROPHY, CONTRACTILITY AND CALCUIM HANDLING IN NORMAL AND FAILING HEART
196. Øyvind Halaas: MECHANISMS OF IMMUNOMODULATION AND CELL-MEDIATED CYTOTOXICITY INDUCED BY BACTERIAL PRODUCTS
197. Tore Amundsen: PERFUSION MR IMAGING IN THE DIAGNOSIS OF PULMONARY EMBOLISM
198. Nanna Kurtze: THE SIGNIFICANCE OF ANXIETY AND DEPRESSION IN FATIQUE AND PATTERNS OF PAIN AMONG INDIVIDUALS DIAGNOSED WITH FIBROMYALGIA: RELATIONS WITH QUALITY OF LIFE, FUNCTIONAL DISABILITY, LIFESTYLE, EMPLOYMENT STATUS, CO-MORBIDITY AND GENDER
199. Tom Ivar Lund Nilsen: PROSPECTIVE STUDIES OF CANCER RISK IN NORD-TRØNDELAG: THE HUNT STUDY. Associations with anthropometric, socioeconomic, and lifestyle risk factors
200. Asta Kristine Håberg: A NEW APPROACH TO THE STUDY OF MIDDLE CEREBRAL ARTERY OCCLUSION IN THE RAT USING MAGNETIC RESONANCE TECHNIQUES

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201. Knut Jørgen Arntzen: PREGNANCY AND CYTOKINES
202. Henrik Døllner: INFLAMMATORY MEDIATORS IN PERINATAL INFECTIONS
203. Asta Bye: LOW FAT, LOW LACTOSE DIET USED AS PROPHYLACTIC TREATMENT OF ACUTE INTESTINAL REACTIONS DURING PELVIC RADIOTHERAPY. A PROSPECTIVE RANDOMISED STUDY.
204. Sylvester Moyo: STUDIES ON STREPTOCOCCUS AGALACTIAE (GROUP B STREPTOCOCCUS) SURFACE-ANCHORED MARKERS WITH EMPHASIS ON STRAINS AND HUMAN SERA FROM ZIMBABWE.
205. Knut Hagen: HEAD-HUNT: THE EPIDEMIOLOGY OF HEADACHE IN NORD-TRØNDELAG
206. Li Lixin: ON THE REGULATION AND ROLE OF UNCOUPLING PROTEIN-2 IN INSULIN PRODUCING ß-CELLS
207. Anne Hildur Henriksen: SYMPTOMS OF ALLERGY AND ASTHMA VERSUS MARKERS OF LOWER AIRWAY INFLAMMATION AMONG ADOLESCENTS
208. Egil Andreas Fors: NON-MALIGNANT PAIN IN RELATION TO PSYCHOLOGICAL AND ENVIRONTENTAL FACTORS. EXPERIENTAL AND CLINICAL STUDES OF PAIN WITH FOCUS ON FIBROMYALGIA
209. Pål Klepstad: MORPHINE FOR CANCER PAIN
210. Ingunn Bakke: MECHANISMS AND CONSEQUENCES OF PEROXISOME PROLIFERATOR-INDUCED HYPERFUNCTION OF THE RAT GASTRIN PRODUCING CELL
211. Ingrid Susann Gribbestad: MAGNETIC RESONANCE IMAGING AND SPECTROSCOPY OF BREAST CANCER
212. Rønnaug Astri Ødegård: PREECLAMPSIA – MATERNAL RISK FACTORS AND FETAL GROWTH
213. Johan Haux: STUDIES ON CYTOTOXICITY INDUCED BY HUMAN NATURAL KILLER CELLS AND DIGITOXIN
214. Turid Suzanne Berg-Nielsen: PARENTING PRACTICES AND MENTALLY DISORDERED ADOLESCENTS
215. Astrid Rydning: BLOOD FLOW AS A PROTECTIVE FACTOR FOR THE STOMACH MUCOSA. AN EXPERIMENTAL STUDY ON THE ROLE OF MAST CELLS AND SENSORY AFFERENT NEURONS

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216. Jan Pål Loennechen: HEART FAILURE AFTER MYOCARDIAL INFARCTION. Regional Differences, Myocyte Function, Gene Expression, and Response to Cariporide, Losartan, and Exercise Training.

217. Elisabeth Qvigstad: EFFECTS OF FATTY ACIDS AND OVER-STIMULATION ON INSULIN SECRETION IN MAN
218. Arne Åsberg: EPIDEMIOLOGICAL STUDIES IN HEREDITARY HEMOCHROMATOSIS: PREVALENCE, MORBIDITY AND BENEFIT OF SCREENING.
219. Johan Fredrik Skomsvoll: REPRODUCTIVE OUTCOME IN WOMEN WITH RHEUMATIC DISEASE. A population registry based study of the effects of inflammatory rheumatic disease and connective tissue disease on reproductive outcome in Norwegian women in 1967-1995.
220. Siv Mørkved: URINARY INCONTINENCE DURING PREGNANCY AND AFTER DELIVERY: EFFECT OF PELVIC FLOOR MUSCLE TRAINING IN PREVENTION AND TREATMENT
221. Marit S. Jordhøy: THE IMPACT OF COMPREHENSIVE PALLIATIVE CARE
222. Tom Christian Martinsen: HYPERGASTRINEMIA AND HYPOACIDITY IN RODENTS – CAUSES AND CONSEQUENCES
223. Solveig Tingulstad: CENTRALIZATION OF PRIMARY SURGERY FOR OVARAIN CANCER. FEASIBILITY AND IMPACT ON SURVIVAL
224. Haytham Eloqayli: METABOLIC CHANGES IN THE BRAIN CAUSED BY EPILEPTIC SEIZURES
225. Torunn Bruland: STUDIES OF EARLY RETROVIRUS-HOST INTERACTIONS – VIRAL DETERMINANTS FOR PATHOGENESIS AND THE INFLUENCE OF SEX ON THE SUSCEPTIBILITY TO FRIEND MURINE LEUKAEMIA VIRUS INFECTION
226. Torstein Hole: DOPPLER ECHOCARDIOGRAPHIC EVALUATION OF LEFT VENTRICULAR FUNCTION IN PATIENTS WITH ACUTE MYOCARDIAL INFARCTION
227. Vibeke Nossum: THE EFFECT OF VASCULAR BUBBLES ON ENDOTHELIAL FUNCTION
228. Sigurd Fasting: ROUTINE BASED RECORDING OF ADVERSE EVENTS DURING ANAESTHESIA – APPLICATION IN QUALITY IMPROVEMENT AND SAFETY
229. Solfrid Romundstad: EPIDEMIOLOGICAL STUDIES OF MICROALBUMINURIA. THE NORD-TRØNDELAG HEALTH STUDY 1995-97 (HUNT 2)
230. Geir Torheim: PROCESSING OF DYNAMIC DATA SETS IN MAGNETIC RESONANCE IMAGING
231. Catrine Ahlén: SKIN INFECTIONS IN OCCUPATIONAL SATURATION DIVERS IN THE NORTH SEA AND THE IMPACT OF THE ENVIRONMENT
232. Arnulf Langhammer: RESPIRATORY SYMPTOMS, LUNG FUNCTION AND BONE MINERAL DENSITY IN A COMPREHENSIVE POPULATION SURVEY. THE NORD-TRØNDELAG HEALTH STUDY 1995-97. THE BRONCHIAL OBSTRUCTION IN NORD-TRØNDELAG STUDY
233. Einar Kjelsås: EATING DISORDERS AND PHYSICAL ACTIVITY IN NON-CLINICAL SAMPLES
234. Arne Wibe: RECTAL CANCER TREATMENT IN NORWAY – STANDARDISATION OF SURGERY AND QUALITY ASSURANCE

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235. Eivind Witsø: BONE GRAFT AS AN ANTIBIOTIC CARRIER
236. Anne Mari Sund: DEVELOPMENT OF DEPRESSIVE SYMPTOMS IN EARLY ADOLESCENCE
237. Hallvard Lærum: EVALUATION OF ELECTRONIC MEDICAL RECORDS – A CLINICAL TASK PERSPECTIVE
238. Gustav Mikkelsen: ACCESSIBILITY OF INFORMATION IN ELECTRONIC PATIENT RECORDS; AN EVALUATION OF THE ROLE OF DATA QUALITY
239. Steinar Krokstad: SOCIOECONOMIC INEQUALITIES IN HEALTH AND DISABILITY. SOCIAL EPIDEMIOLOGY IN THE NORD-TRØNDELAG HEALTH STUDY (HUNT), NORWAY
240. Arne Kristian Myhre: NORMAL VARIATION IN ANOGENITAL ANATOMY AND MICROBIOLOGY IN NON-ABUSED PRESCHOOL CHILDREN
241. Ingunn Dybedal: NEGATIVE REGULATORS OF HEMATOPOIETEC STEM AND PROGENITOR CELLS
242. Beate Sitter: TISSUE CHARACTERIZATION BY HIGH RESOLUTION MAGIC ANGLE SPINNING MR SPECTROSCOPY
243. Per Arne Aas: MACROMOLECULAR MAINTENANCE IN HUMAN CELLS – REPAIR OF URACIL IN DNA AND METHYLATIONS IN DNA AND RNA

244. Anna Bofin: FINE NEEDLE ASPIRATION CYTOLOGY IN THE PRIMARY INVESTIGATION OF BREAST TUMOURS AND IN THE DETERMINATION OF TREATMENT STRATEGIES
245. Jim Aage Nøttestad: DEINSTITUTIONALIZATION AND MENTAL HEALTH CHANGES AMONG PEOPLE WITH MENTAL RETARDATION
246. Reidar Fossmark: GASTRIC CANCER IN JAPANESE COTTON RATS
247. Wibeke Nordhøy: MANGANESE AND THE HEART, INTRACELLULAR MR RELAXATION AND WATER EXCHANGE ACROSS THE CARDIAC CELL MEMBRANE

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248. Sturla Molden: QUANTITATIVE ANALYSES OF SINGLE UNITS RECORDED FROM THE HIPPOCAMPUS AND ENTORHINAL CORTEX OF BEHAVING RATS
249. Wenche Brenne Drøyvold: EPIDEMIOLOGICAL STUDIES ON WEIGHT CHANGE AND HEALTH IN A LARGE POPULATION. THE NORD-TRØNDELAG HEALTH STUDY (HUNT)
250. Ragnhild Støen: ENDOTHELIUM-DEPENDENT VASODILATION IN THE FEMORAL ARTERY OF DEVELOPING PIGLETS
251. Aslak Steinsbekk: HOMEOPATHY IN THE PREVENTION OF UPPER RESPIRATORY TRACT INFECTIONS IN CHILDREN
252. Hill-Aina Steffenach: MEMORY IN HIPPOCAMPAL AND CORTICO-HIPPOCAMPAL CIRCUITS
253. Eystein Stordal: ASPECTS OF THE EPIDEMIOLOGY OF DEPRESSIONS BASED ON SELF-RATING IN A LARGE GENERAL HEALTH STUDY (THE HUNT-2 STUDY)
254. Viggo Pettersen: FROM MUSCLES TO SINGING: THE ACTIVITY OF ACCESSORY BREATHING MUSCLES AND THORAX MOVEMENT IN CLASSICAL SINGING
255. Marianne Fyhn: SPATIAL MAPS IN THE HIPPOCAMPUS AND ENTORHINAL CORTEX
256. Robert Valderhaug: OBSESSIVE-COMPULSIVE DISORDER AMONG CHILDREN AND ADOLESCENTS: CHARACTERISTICS AND PSYCHOLOGICAL MANAGEMENT OF PATIENTS IN OUTPATIENT PSYCHIATRIC CLINICS
257. Erik Skaaheim Haug: INFRARENAL ABDOMINAL AORTIC ANEURYSMS – COMORBIDITY AND RESULTS FOLLOWING OPEN SURGERY
258. Daniel Kondziella: GLIAL-NEURONAL INTERACTIONS IN EXPERIMENTAL BRAIN DISORDERS
259. Vegard Heimly Brun: ROUTES TO SPATIAL MEMORY IN HIPPOCAMPAL PLACE CELLS
260. Kenneth McMillan: PHYSIOLOGICAL ASSESSMENT AND TRAINING OF ENDURANCE AND STRENGTH IN PROFESSIONAL YOUTH SOCCER PLAYERS
261. Marit Sæbø Indredavik: MENTAL HEALTH AND CEREBRAL MAGNETIC RESONANCE IMAGING IN ADOLESCENTS WITH LOW BIRTH WEIGHT
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