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Insomnia and risk for cardiovascular disease

Thesis for the degree of Philosophiae Doctor

Trondheim, April 2012

Norwegian University of Science and Technology

Faculty of Medicine

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Norwegian University of
Science and Technology

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ISBN 978-82-471-3477-1 (printed ver.)

ISBN 978-82-471-3478-8 (electronic ver.)

ISSN 1503-8181

Doctoral theses at NTNU, 2012:100

Printed by Skipnes kommunikasjon

Insomni og risiko for hjerte- og karsykdom

Forskning som har blitt utført det siste tiåret har gitt resultater som taler for at dårlig søvn og søvnlidelser bidrar til utvikling av hjerte- og karsykdom. Insomni er definert som en subjektiv opplevelse av å ha problemer med innsoving, for tidlig oppvåkning om morgenen og/eller dårlig søvnkvalitet, og insomni ansees for å være den vanligste søvnforstyrrelsen. Forekomsten av minst ett av symptomene kan være så høy som 33% i den generelle befolkningen. Foreløpig er det få studier på sammenhengen mellom insomni og risiko for framtidig hjerte- og karsykdom. Insomni henger sammen med en ugunstig livsstil og utvikling av metabolsk syndrom, som betyr at personer med insomni ofte har høyere blodtrykk, kolesterol og høyere kroppsmasseindeks enn personer uten slike søvnplager. En annen mulighet for sammenhengen er at søvnproblemene bidrar til økt utskillelse av inflammatoriske stoffer i blodet som kan øke risikoen for hjerte- og karsykdom. Om det er en klar sammenheng mellom metabolsk syndrom og inflammasjon, er heller ikke kjent, og spesielt er dette uavklart i yngre aldersgrupper.

Studiene i avhandlingen tok utgangspunkt i den andre Helseundersøkelsen i Nord-Trøndelag (HUNT-2) som foregikk i perioden 1995-97. Alle personer over 20 år i fylket ble invitert, og deltakerne fylte ut spørreskjema, gjennomgikk en klinisk undersøkelse, og det ble tatt blodprøver. I spørreskjemaet ble det blant annet spurt om søvnplager. Fra undersøkelsen startet til og med 2008 ble det fortløpende registrert førstegangs hjerteinfarkt eller hjertesvikt, hvor opplysningene enten ble hentet fra sykehusjournaler i Helse-Nord Trøndelag eller fra Dødsårsaksregisteret. I en undergruppe bestående av 10 000 deltakere ble det målt høy-sensitivt C-reaktivt protein (hsCRP) i blod, som er et mål på graden av inflammasjon i kroppen.

Resultatene viste at personer med symptomer på insomni hadde en moderat økt risiko for førstegangs hjerteinfarkt og økt risiko for hjertesvikt sammenliknet med personer uten søvnproblemer. Vi fant også at sjansen for å få hjerteinfarkt eller hjertesvikt var høyere jo flere symptomer på insomni som var tilstede samtidig. Vi tok høyde for betydningen av andre faktorer som kunne påvirke resultatene, og i de statistiske analysene justerte vi for forskjeller i alder, kjønn, ekteskapsstatus, utdanningsnivå, skiftarbeid, blodtrykk, kolesterol, diabetes, vekt, fysisk aktivitet, alkohol og røyking. I tillegg justerte vi for symptomer på depresjon og angst, som begge kan medføre søvnplager. De justerte analysene viste at disse mulig konfunderende faktorene ikke påvirket resultatene i nevneverdig grad, noe som styrker sannsynligheten for at våre funn har en underliggende biologisk årsak. I en annen studie fant vi at den positive sammenhengen mellom metabolsk syndrom og hsCRP var like tydelig i alle aldersgrupper. Til tross for at personer med insomni oftere har metabolsk syndrom, fant vi ingen holdepunkter for at personer med insomni har økt utskillelse av hsCRP. Disse funnene taler imot at inflammasjon, indikert av høy hsCRP, kan forklare sammenhengen mellom insomni og framtidig hjerte- og karsykdom.

Til tross for at insomni er forbundet med en moderat risikoøkning for hjerte- og karsykdom, er insomni så vanlig i befolkningen at søvnplager kan spille en viktig rolle for hjertehelse. I tillegg er insomni en lett gjenkjennbar og potensielt håndterbar

tilstand. I forebygging av hjerte- og karsykdom bør derfor søvnplager tas med i vurderingen. Det kreves mer forskning på området, slik at man får en bedre forståelse av de underliggende mekanismene.

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Biveiledere: Lars Vatten og Rune Wiseth

Finansieringskilde: Samarbeidsorganet mellom Helse Midt-Norge og NTNU

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Acknowledgement

This thesis is based on the work carried out during the years 2009-2012 at the Department of Public Health and General Practice, Faculty of Medicine, Norwegian University of Science and Technology (NTNU). This work was financed through a research fellowship granted by the Liaison Committee between the Central Norway Regional Health Authority and NTNU.

This work is based on data from the second Nord-Trøndelag Health Study (HUNT 2), and I want to thank the HUNT research Centre for providing the data. The Nord-Trøndelag Health Study is the result of important contribution from many individuals, including the participants. I also want to thank the Department for Research and Development and clinicians at the Medical Department, Nord-Trøndelag Health Trust, for extracting data from the patient records.

Many individuals contributed to this thesis, and I wish to express my deepest gratitude to all those who made this work possible, in particular:

Researcher Imre Janszky, my supervisor, for your excellent supervision, encouragement and enthusiasm. I really admire your deep scientific knowledge, your careful approach and high scientific discipline. I have really enjoyed working with you these years!

Without your knowledge, encouragement and critical advice this work would not have been possible.

Professor Lars Vatten, my co-supervisor, for introducing me to the field of epidemiology, for believing in me and guiding me, for your always careful and excellent comments in my manuscripts, and for good ideas and constructive criticisms to my work.

Professor Rune Wiseth, my co-supervisor, thank you for the excellent introduction to cardiology and medical research, for always being helpful since I stepped into your office with the claim to start scientific work as a student in the fifth year of my medical studies.

Professor Pål Richard Romundstad for your invaluable help with paper IV and answering all my questions in methodology, software and statistical analyses.

Professor Kristian Hveem for your excellent help with paper III and IV.

Carl Platou, PhD student and MD, for organizing the data collection on myocardial infarction and heart failure cases from medical records from the hospitals in Nord-Trøndelag County.

Bjørn Olav Åsvold, postdoctor and MD, for your important contribution to paper IV, for all the interesting discussions and for your great help and good advices.

Johan Håkon Bjørngaard, postdoctor, for your invaluable help and great ideas on paper III, for teaching me the multiple imputation method, and for always having time to answer my questions.

Linn Beate Strand, PhD student, for being a good friend and college, for your contribution to paper II, and for the trips we did together to different courses in epidemiology and to the congress in Dubai.

Bjørn Mørkedal, PhD, former Phd student, for scientific cooperation and for teaching me STATA software and providing wonderful do-files.

I want to thank my parents for encouraging education and curiosity, and my wife Tonje for her endless love and support and great patience during this work.

Trondheim, April 2012

Lars Erik Sande Laugsand

List of Papers

This thesis is based on the following original papers, which will be referred to in the text by their Roman numerals.

I. Laugsand LE, Vatten LJ, Platou C, Janszky I. **Insomnia and risk of acute myocardial infarction: A population study.** Circulation. 2011; 124: 2073-2081.

II. Laugsand LE, Strand LB, Vatten LJ, Platou C, Janszky I. **Insomnia and the risk of incident heart failure: A population study.** Submitted; not published.

III. Laugsand LE, Vatten LJ, Bjørngaard JH, Hveem K, Janszky I. **Insomnia and high-sensitivity C-reactive protein: The HUNT study, Norway.** Psychosomatic Medicine. 2012; 74: 543-553.

IV. Laugsand LE, Åsvold BO, Vatten LJ, Romundstad PR, Wiseth R, Hveem K, Janszky I. **Metabolic factors and high-sensitivity C-reactive protein: the HUNT study.** European Journal of Cardiovascular Prevention and Rehabilitation. 2011 July 20; in press.

List of abbreviations

AMI	Acute myocardial infarction
BMI	Body mass index
CHD	Coronary heart disease
CI	Confidence interval
CRP	C-reactive protein
CVD	Cardiovascular disease
DSM	Diagnostic and Statistical Manual of Mental Disorders
HDL	High-density lipoprotein
HF	Heart failure
HPA-axis	Hypothalamic-pituitary-adrenal axis
HR	Hazard ratio
HsCRP	High-sensitivity C-reactive protein
HUNT	Nord-Trøndelag Health Study
ICD	International Classification of Disease
IL-6	Interleukin-6
LDL	Low density lipoprotein
NREM	Non-rapid eye movement
REM	Rapid eye movement
SCN	Suprachiasmaticus nucleus
TNF-α	Tumor-necrosis factor- α

1 Introduction

Cardiovascular disease (CVD) is the leading cause of death worldwide. According to the estimations of the World Health Organization, more than 17 million people died of CVDs in 2008, which accounts for 31% of all deaths.[1] Among the most common forms of CVD are coronary heart disease (CHD) and heart failure (HF). CHD occurs when the supply of blood to the heart muscle cells is hampered due to the narrowing of the coronary vessels, while HF is usually defined as the inability of the heart to supply sufficient blood flow to meet the needs of the body.

A large proportion of CVDs is preventable through the reduction of potentially modifiable risk factors, including tobacco smoking, overweight, dyslipidemia, hypertension, physical inactivity, poor diet, and psychosocial factors.[2] Over the last two decades, cardiovascular mortality rates have declined substantially in high-income countries.[3] There is clear evidence that both population-wide primary prevention and individual health-care intervention strategies have contributed to the decline in CVD mortality.[4] However, a large proportion of CHD patients do not have any of the established conventional risk factors,[5] and because CHD is still by far the leading cause of death in the industrialized countries, it is important to learn more about the underlying causes and to reveal other potentially preventable risk factors.

Research over the last decades has provided increasing evidence that poor sleep and sleep disorders may contribute to the development of CVD. First, obstructive sleep apnea was recognized as a strong risk factor for both acute myocardial infarction (AMI) and for cardiovascular disease in general.[6,7] Obstructive sleep apnea is a primary sleep

disorder that is common in middle age. It is caused by repetitive episodes of airway obstruction during sleep that leads to frequent awakenings during the night, loud snoring and increased sleepiness during the day. In relation to other sleep problems, there is considerable less knowledge about their associations with CVD.

Among the different sleep disorders, we have concentrated on insomnia in this thesis. Insomnia is a subjective feeling of having difficulty initiating or maintaining sleep or having a feeling of non-restorative sleep.[8] It is a highly prevalent condition in the industrialized world and is the most common primary sleep disorder. It has been estimated that the prevalence of at least one insomnia symptom could be as high as 33% in the general population.[9] Little is known about the cardiovascular effects of insomnia. Therefore, our main aim was to prospectively investigate the association of insomnia with risk for acute myocardial infarction and heart failure in a large population-based study. Another aim was to explore possible mechanisms related to insomnia and cardiovascular disease, with emphasis on inflammatory and metabolic factors.

Below, we first outline the atherosclerotic process and its clinical manifestations. Then we briefly describe the physiology and pathophysiology of sleep. Finally, we summarize the current knowledge related to the association of insomnia and AMI and HF, and we attempt to review the possible underlying mechanisms for such an association.

1.1 Atherosclerosis and cardiovascular disease

The underlying disease process in the blood vessels that results in cardiovascular disease is known as atherosclerosis, which refers to hardening of blood vessels. Atherosclerosis typically develops in the arterial wall over a period of decades with distinct chronological phases.[10,11]

1.1.1 The development of atherosclerosis

Initial phase. The endothelium, the inner lining of blood vessels, is exposed to several harmful factors in blood. Such factors include elevated and modified levels of LDL (Low Density Lipoprotein), free radicals, as well as microbial pathogens. During the initial phase of atherosclerosis the endothelium attempts to repair itself by attracting immune cells to the injured site. When the reparative process fails, the endothelium becomes permeable and lymphocytes and monocytes migrate into the deep layer of the intima where a series of reactions occur attracting LDL particles to the site. These fatty LDL particles are engulfed by monocytes, before being transformed into macrophages or foam cells. The accumulation of lipid-laden foam cells constitutes the initial pathological manifestation of the atherosclerotic process, the so-called fatty streak formation. The fatty streaks appear commonly as early as in the first decade of life, but at the early stage, the process is still reversible.

Progressive phase. If the elimination of harmful factors is not complete, the inflammatory process continues, and the atherosclerotic lesion develops further. As the attempt at endothelial repair progresses, a fibrous cap consisting of smooth muscle and

collagen is formed. At the same time, the macrophages and monocytes involved in the original reaction are destroyed, which results in the formation of a lipid pool covered by the fibrous cap. At this stage the initial fatty streaks transform, with the contribution of the immune cells, to an advanced atheromatous lesion or plaque. The progressive phase is generally considered to occur during young adulthood through middle age.

Late phase. While the plaque increases in size, the arterial wall compensates by expanding. If the intrinsic compensating capacity of the arteries is exhausted, the atheromatous lesion can intrude into the lumen and alter blood flow, and thereby cause clinical symptoms. In addition, the atherosclerotic plaque can cause clinical symptoms by facilitating thrombosis. The surface of the plaque is in itself thrombogenic and the rupture of a plaque may also facilitate thrombosis. Immune activity may facilitate both the rupture of the plaque and thrombosis by digestion of the fibrous cap or by changing the hemostatic properties of the blood. This latter phase is typically known as atherothrombosis, and occurs in middle-aged or elderly individuals.

It is important to note that the immune system is believed to play a central role at all stages of atherosclerosis.[12,13] Recently, it has become apparent that atherosclerosis is a chronic low-grade inflammatory process affecting large- and medium-sized arteries throughout the cardiovascular system.[14] A large number of studies have determined that several pro-inflammatory biomarkers can give prognostic information about risk of CVD beyond that available from traditional risk factors (see 1.1.2).[10] Much research has focused on high-sensitivity C-reactive protein (hsCRP), which is a biomarker of innate immunity. HsCRP is a reliable and relatively cheap biomarker of the underlying systemic inflammation, and appears to reflect the activity of atherosclerosis from the

early stages that are free of symptoms to the late clinical stages. Increased levels of hsCRP have been associated with increased cardiovascular morbidity and mortality in population-based cohort studies[15-17], and with poor prognosis among survivors of acute coronary events.[18,19] Therefore, the American Heart Association has suggested that hsCRP should have a place in individual risk evaluation related to CHD.[20] It is debated, however, whether increased levels of C-reactive protein (CRP) should be regarded a cause or a consequence of CVD. Some have suggested that CRP is causally linked to CVD through pro-inflammatory effects that increase cardiovascular risk by initiating the atherosclerotic process.[21-23] Another explanation could be reverse causation, since cytokines may leak from atherosclerotic plaques into the circulation, with subsequent hepatic synthesis of CRP.[24] It is also possible that metabolic factors (for example obesity) in themselves may cause higher CRP. Interleukin-6 (IL-6) is the primary cytokine that is involved in hepatic CRP synthesis, and 30% of the total IL-6 production takes place in visceral and subcutaneous fat (Figure 1).[25] Thus, CRP may be a bystander in the association of various risk factors with CVD. It is also not known whether CRP is related to obesity and other cardiovascular risk factors at a young age, when the burden of atherosclerotic plaques is low. As we discuss below (see 1.3.5), both metabolic factors and inflammation are biologically plausible pathways for adverse effects of insomnia. Therefore, one aim of the thesis was to clarify how hsCRP is associated with metabolic factors at different ages (Aim 4, see 1.4).

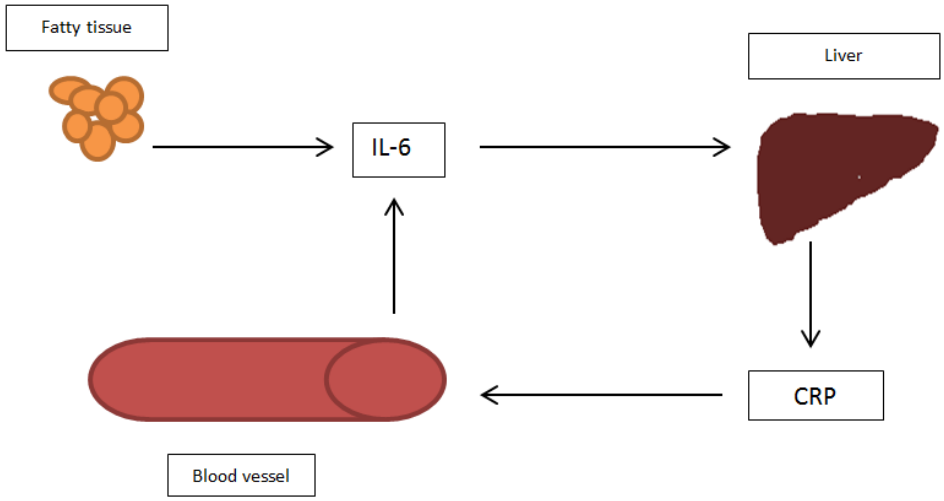


Figure1. The secretion of CRP from the liver is induced by the messenger cytokine interleukin-6 (IL-6) produced in fatty tissue and in atherosclerotic plaques.

1.1.2 Acute myocardial infarction

Myocardial ischemia, the main manifestation of CHD, is caused by an imbalance between the supply of oxygen and other essential myocardial nutrients and myocardial demand for these substances. Myocardial ischemia occurs most commonly as a result of obstructive atherosclerosis. AMI is the most important manifestation of atherosclerosis and CHD, and it occurs when cardiac myocytes die due to myocardial ischemia. AMI can be diagnosed on the basis of an appropriate clinical history of chest pain, electrocardiography and elevated biochemical markers.[26]

It has been estimated that in Norway, 12,000 to 15,000 myocardial infarctions occurs annually, and a total of 5000 deaths (12.5% of all deaths) are due to CHD each year.[27] There has been a consistent reduction in the number of deaths from CHD in

middle age in Norway. The prognosis after an AMI has also improved during the last decades, and 90% of hospitalized cases of AMI survive the acute phase.[28] However, among those who survive there is an increased subsequent risk for heart failure, stroke or sudden death.[29-31]

Atherosclerosis and AMI have a multifactorial but common origin. Certain lifestyle habits promote atherogenic traits in genetically susceptible persons. A number of risk factors are known to predispose to the condition (Table 1). Some of these, including age, sex, race and family history, cannot be changed, whereas other major risk factors, such as serum cholesterol, smoking habits, diabetes and hypertension, can be modified.[32]

Table 1. Causes and risk factors for coronary heart disease

Non-modifiable	**Modifiable**
Age	Hyperlipidemia
Male sex	Cigarette smoking
Positive family history	Hypertension
Genetic factors	Diabetes mellitus
	Physical inactivity
	Obesity
	Poor diet

Age. CHD rates increase with age. Atherosclerosis is rare in childhood, except in familiar hyperlipidemia, but is often detectable in young men between 20 and 30 years of age.[33-35] The condition is almost universal among the elderly in western societies.[36]

Sex. Men have considerably higher incidence of CHD than women, especially before women's menopause.[37] The reason for this sex difference is not clearly understood, but it is probably related to hormonal factors and to lifestyle differences.[38]

Family history and genetic factors. CHD is often found in several members of the same family, and family history is important for CHD risk.[39] Most common forms of CHD are believed to be a result of several genes acting in combination with environmental factors.[40]

Cigarette smoking. There is a strong association of cigarette smoking with the risk of developing CHD, both related to the number of cigarettes per day, and the duration of smoking during the lifetime.[41] There is a large body of evidence showing the beneficial effect of smoking cessation on CHD mortality.[4] It has been shown that those who quit smoking between 35 and 44 years of age have the same life expectancy as those who have never smoked.[42]

Hyperlipidemia. High serum cholesterol, especially when associated with a low high-density lipoprotein (HDL), is strongly associated with CHD risk.[1,43,44] HDL is the fraction of cholesterol that removes cholesterol from the blood vessels. High serum triglyceride is also linked with CHD.[45] Serum cholesterol levels can be reduced by drugs, physical activity and by dietary changes, in particular by a reduction in the consumption of saturated fat.[2]

Hypertension. Both systolic and diastolic hypertension are associated with an increased risk of CHD.[4,46]

Diabetes mellitus is strongly associated with AMI risk. Early detection and treatment with strict control of blood glucose is vital for prevention of CHD.[4,47]

Poor diet. A diet that is high in saturated fat is associated with increased risk of CHD, and similar associations have been shown for low intake of fruit, vegetables and fish.[48-51] There is evidence from clinical trials that modification of the diet may have a significant beneficial impact on the risk of CVD in both primary and secondary prevention settings.[52]

Obesity. People who are overweight or obese are at increased risk of AMI.[53] The adverse effect is more pronounced when the fat is concentrated in the abdomen. This is known as central obesity and can be identified by a high waist to hip ratio.

Physical inactivity. A sedentary lifestyle with lack of exercise is an important and modifiable risk factor for CHD. It is recommended that adults participate in a minimum of 150 minutes of moderate intensity activity every week and in muscle-strengthening activities on at least two days a week.[54]

1.1.3 Heart failure

Heart failure is a complex syndrome that can result from any structural or functional cardiac disorder that impairs the ability of the heart to function as a pump to support the circulation. The clinical syndrome manifests itself when cellular respiration is impaired due to the failure of the heart to pump enough blood to support the metabolic demands

of the body, or when normal cellular respiration can only be maintained with an elevated left ventricular filling pressure.[55]

The prevalence of heart failure is approximately 2% in Norway (i.e. 80,000 to 100,000 persons) and the incidence increases with age.[56] About 75% of heart failure cases occur after 75 years of age.[57] The clinical syndrome is one of the most frequent causes of hospitalizations in the elderly, and it is the main problem among 20% of patients admitted to departments of general internal medicine.[58] The prognosis of heart failure has improved over the past 10 years, but the mortality rate is still high, and only about 50% of the patients are expected to be alive after 5 years.[59]

Heart failure is diagnosed on the basis of its symptoms and signs as well as objective evidence of cardiac dysfunction at rest.[55] Typical symptoms of heart failure include difficulties breathing, coughing, frequent need to urinate at night and generalized fatigue. Common signs are increased rate of breathing, crackles in the lung bases and a galloping heart-rhythm. A physical examination can also reveal peripheral and central edema. The most frequently used diagnostic tests of heart failure include echocardiography, chest radiography and electrocardiography.

Coronary artery disease is the most frequent cause of heart failure in western countries.[57] Following an initial myocardial infarction; there is a three- to six fold increase in the risk of heart failure.[29,60] However, more than 60% of heart failure cases are due to other causes than CHD (Table 2).[61]

Table 2. Causes and risk factors of heart failure

Ischaemic (35-40 %)	**Non-Ischaemic (60-65 %)**
The same causes as in table 1.	Hypertension
	Valvular heart disease
	Cardiomyopathies
	-Familial
	-Toxic (alcohol, chemotherapy, cocaine)
	-Infectious (viral, bacterial, fungus, Chagas disease)
	-Autoimmune (hypersensitivity, connective tissue disorders)
	-Metabolic (diabetes, overweight, hypo -and hyperthyroidism)
	-Nutrition (thiamin-, carnitine-, selen deficiency)
	-Hematologic (haemochromatosis, thalassemia)
	Atrial fibrillation and other untreated arrhythmias
	Congenital heart disease
	Pulmonary causes
	Myocarditis (viral, bacterial, autoimmune)
	Pericardial diseases
	Endocarditis
	Anaemia
	Increased heart rate and decreased heart rate variability
	Increased activity in the sympathetic nervous system
	Unknown

1.2 Normal sleep, circadian rhythm and cardiovascular physiology

Sleep is a natural process that is crucial for the maintenance and restoration of homeostasis. Sleep, like many other aspects of mammalian behavior and physiology, including physical activity, alertness, hormone levels, body temperature, immune function, and digestive activity, shows circadian rhythmicity. The circadian rhythms are controlled by a single tiny brain area, the suprachiasmaticus nucleus (SCN).[62]

Physiological sleep can be divided into non-rapid eye movement (NREM) and rapid eye movement (REM) stages. These stages appear in a cyclical manner during sleep, with 4 to 5 NREM/REM cycles occurring every night. As sleep progresses, each recurring REM episode gets gradually longer. REM sleep is characterized by vivid dreams, loss of muscle tone, and rapid eye movement.[63] NREM is a deeper stage of sleep with the body being least metabolically active.[64]

Sleep is an important modulator of cardiovascular function, as sleep may exert effects on the autonomic nervous system, cardiac function, systemic hemodynamics, endothelial function, coagulation, metabolism and the immune system.

1.2.1 Effects on the autonomic nervous system and hemodynamics

Cardiac contractility, cardiac output and peripheral vascular resistance are all under autonomic nervous control. The autonomic nervous system is affected by the rhythmic patterns in homeostatic regulatory mechanisms initiated by SCN. Sympathetic activity is higher during the day, while parasympathetic activity is more pronounced at night.[65] As a result, there is also cyclical variation in cardiovascular hemodynamics over a 24 hours cycle, with the lowest heart rate and blood pressure observed during the night, followed by an early morning increase in heart rate and blood pressure. In association with specific sleep phases, NREM is characterized by a progressive decrease in sympathetic activity with an accompanying parasympathetic predominance, which leads to decreases in heart rate, cardiac output, peripheral vascular resistance, and blood

pressure. Contrary to this, REM sleep is accompanied by sympathetic activity leading to elevated blood pressure and heart rate, similar to levels during wakefulness.[66]

1.2.2 Effects on endothelial function and coagulation

Brachial artery flow-mediated endothelium-dependent vasodilatation is decreased and coronary artery vascular tone is markedly increased in the early morning after awakening compared with that during sleep, and subsequently recovers by late morning.[67,68] Similarly, increased platelet aggregation, blood viscosity and coagulability, increased secretion of tissue plasminogen activator, and fibrinolytic activity have been measured in early morning.[69,70] These changes are mainly caused by increased activity in the sympathetic nervous system.[71,72]

1.2.3 Effects on metabolism

It is believed that during normal sleep the metabolic rate is reduced by around 15% and reaches a minimum in the morning in a standard circadian pattern.[73] When we are awake our brain cells use a considerable amount of glucose and the intracellular glycogen stores become depleted during the day. During sleep this process is reversed so that glucose is available during the next period of wakefulness. It has been shown that glucose utilization in normal subjects is at its highest during the wakeful state and lowest in NREM sleep and intermediate in REM sleep.[74] During the NREM sleep,

energy is conserved and body temperature drops. These metabolic changes seem to be controlled by reproducible changes in the release of growth hormone and cortisol.[75]

Sleep-wake behavior has also been linked with neurohormonal regulation of appetite. Ghrelin and leptin are two hormones that regulate energy balance in a reciprocal fashion.[76] Leptin is an adipocyte hormone that signals satiety to the brain and increases energy expenditure. Ghrelin is produced mainly in the stomach and it signals hunger to the brain. The interplay of these two hormones is important for regulation of sleep. In essence, leptin down-regulates NREM sleep and total sleep time, while ghrelin promotes NREM sleep.[77]

1.2.4 Effects on the immune system

Sleep is also a restorative process that is important for immune system functioning. Evidence from research in animals and humans suggests a bidirectional communication between the immune and the neuroendocrine system.[78] Cytokines may represent the link between these systems, and these immunologically active peptides influence the sleep-waking cycle of the brain and different stages of sleep, either by promoting or inhibiting sleep. However, the exact mechanisms of cytokine-induced signaling of the brain are not well understood.

1.3 Disturbed sleep and its cardiovascular effects

During the 20th century many changes in lifestyle have taken place, which influence both the length of sleep and sleep quality. Today we have longer work days and an increasing number of people are working in shifts. Use of television, personal computers, internet and artificial lights curtail our sleep and extend our active phase. Sleep deprivation is becoming increasingly common. It has been suggested that the average sleep duration has decreased from about 9 hours per night to about 7.5 hours during the 20th century.[79]

Self-reported short sleep duration and sleep complaints have been associated with increased cardiovascular morbidity and mortality in epidemiological studies.[80] Short sleep duration has also been associated with hypertension,[81] obesity,[82] and increased risk of developing diabetes mellitus.[83,84] However, the exact mechanisms underlying the link between short sleep duration and increased cardiovascular risk are largely unknown. Short-term experimental studies on sleep deprivation have tried to reveal possible pathophysiological mechanisms behind these associations.

1.3.1 Short-term, experimental sleep deprivation

Several studies have found that experimental sleep deprivation leads to increased blood pressure, which has been related to increased activity in the sympathetic nervous system and decreased parasympathetic activation.[85-87] Similar physiological changes in the autonomic nervous system and hemodynamics are seen during stress and increased work expenditure. It has also been proposed that the increased activity in the

sympathetic nervous system leads to decreased endothelial function and increased coagulability.[88,89]

Sleep restriction also induces metabolic dysregulation, as release of anabolic hormones involved in the metabolism is altered by sleep restriction.[64,77,90] During sleep deprivation, the sleep-associated growth hormone pulse at the onset of sleep is substantially decreased,[91] while cortisol levels are elevated during the evening.[79] The combined effect of these hormonal changes is slowed glucose metabolism. Sleep deprivation also down-regulates the satiety hormone, leptin, and up-regulates the appetite-stimulating hormone, ghrelin, which leads to increased hunger and appetite.[92] The combination of slowed glucose metabolism and increased appetite may lead to insulin resistance and weight gain.[93]

Less is known about the effects of sleep loss on immune function, but acute sleep deprivation seems to facilitate the diurnal production of pro-inflammatory cytokines IL-6, tumor necrosis factor-α (TNF-α) and CRP.[94-99] Studies suggest that these cytokines may be important in sleep regulation and for the sleep architecture, and play an important role in causing daytime sleepiness and fatigue of sleep-deprived individuals.[78,100] As described in 1.1.1, the sustained elevation of these pro-inflammatory cytokines is also a good predictor of cardiovascular morbidity and mortality.

The available experimental data suggest that with short-term sleep deprivation, blood pressure, autonomic tone, hormones, metabolism, inflammation and coagulability are all altered in a direction that potentially facilitates atherosclerosis. However, the

changes seen in experimental settings of acute sleep deprivation are mild and resolve quickly with recovery of sleep. There is much less knowledge about whether chronic sleep problems and habitual insomnia symptoms lead to the same pathophysiological changes.

1.3.2 Insomnia

1.3.2.1 Definition and Prevalence

Insomnia is defined as a subjective feeling of having difficulty initiating or maintaining sleep or having a feeling of non-restorative sleep.[8] Insomnia is not synonymous with short sleep or sleep restriction, as it also covers the quality of sleep and not only its duration. People with insomnia symptoms could have normal or long sleep duration.[101]

Most adults have experienced insomnia or sleeplessness at one time or another in their lives and it has been estimated that the prevalence of at least one insomnia symptom could be as high as 33% in the general population.[9] Insomnia is considered to be the most common sleep disorder. It is especially a highly prevalent condition in the industrialized world, and about 10% of the general population present chronic insomnia complaints and seek medical help for insomnia.[102] However, insomnia is an under-recognized and therefore under-treated problem, since about 60% of people suffering from insomnia never talk to their physicians about their difficulties related to sleep.[103]

1.3.2.2 Sociodemographic correlates of insomnia

Insomnia has several sociodemographic correlates.

Age. Insomnia affects all age groups. Between 15 and 44 years of age the prevalence is stable around 20 %, but it increases from 45 years of age. In elderly individuals above 65 years the prevalence is close to 50 %.[104] It has been suggested that chronic age-related disorders may explain some of the association of age with insomnia.[105]

Sex. Insomnia affects women more often than men, and the difference increases with age.[106]

Socio-economic status. Insomnia is more frequent among persons who are separated, divorced or widowed, and among people with low educational level and/or low economic status, and among people who are unemployed.[9]

1.3.2.3 Comorbid conditions

Numerous chronic medical conditions can initiate or maintain insomnia symptoms. Nearly 50% of people suffering from insomnia have recurrent or persistent health problems.[107]

Medical disorders with chronic pain, like arthritis or back pain; cardiovascular diseases, especially heart failure; obstructive respiratory diseases; gastrointestinal disorders; chronic renal insufficiency; endocrine conditions, especially thyroid

dysfunction, and certain neurological disorders are known to be associated with chronic insomnia.[108]

Psychological distress. There is also considerable overlap between insomnia and psychological distress, especially depressive and anxiety symptoms.[109] In persons with a current major depressive episode, the presence of insomnia symptoms was found in nearly 80% of the patients, and the prevalence was nearly 90% when an anxiety disorder was concomitantly present.[110]

Substance use. Use, abuse or withdrawal of psycho-active substances is also an important cause of insomnia symptoms. Use of drugs, such as beta-blockers, some serotonin re-uptake inhibitors, some neuroleptics and amphetamines may result in insomnia.[9] Withdrawal of hypnotics and anxiolytics may cause rebound insomnia.[111]

Alcohol is a central nervous system depressant known for its important effects on sleep and wakefulness. Chronic alcoholism is strongly associated with insomnia.[112] Alcohol is also often used as a sleeping aid in the general population, although alcohol in the long-term is associated with sleep disruption and creates a sense of non-refreshed sleep in the morning.[113] Smoking and caffeine has also been found to be positively associated with insomnia symptoms.[114,115]

1.3.2.4 Treatment of insomnia

It is important to find any comorbid causes of insomnia, as treatment of the underlying cause can in itself treat insomnia.[116] If no specific cause is identified or elimination of

the cause is not possible, there are several treatment options available that may result in reliable and durable changes among persons who suffer from chronic insomnia (see Table 3).[116-118] The combination of pharmacological and non-pharmacological interventions provides the optimal treatment.[119]

Table 3. Treatment options for insomnia

Non-pharmacological therapies
Adherence to sleep hygiene rules
Curtail time in bed Avoid the use of clock in the bedroom Exercise in the late afternoon or early evening Avoid coffee, alcohol, and nicotine Regularize the bedtime Eat a light bedtime snack Avoid daytime napping Monitor use of hypnotics
Progressive muscle relaxation
Stimulus control
Helps to re-associate the bed and bedroom with the rapid onset of sleep.
Sleep restriction
Its objective is to reduce time in bed to lower the chance of fragmented and poor-quality sleep.
Pharmacological therapies
Benzodiazepines
Antidepressants
Melatonine

1.3.3 Insomnia and coronary heart disease

Only a few prospective studies have investigated insomnia in relation to risk for CHD, and the results have been inconsistent.[120-131] Previous studies were small with numbers ranging from 416 to 10,308 participants. Only one of these previous studies assessed the

three aspects of insomnia simultaneously.[124] Most studies concentrated on difficulty in initiating and/or maintaining sleep, and generally found a moderately increased risk for CHD associated with these symptoms.

In many studies the outcomes were poorly defined and often based on self-report. Only a few studies included AMIs that were verified by modern standards, including diagnostic information based on electrocardiography and cardiac enzymes.[122,128,129] The authors combined verified AMI with other less well-defined cardiovascular outcomes, and no separate estimates for the verified AMIs were reported. In other studies, outcomes were cardiovascular death and/or self-reported cardiovascular disease.[120,124,125,127]

There is considerable overlap between insomnia and psychological distress, especially depressive symptoms.[109] There is also some evidence that depression and anxiety are associated with increased risk for AMI.[132] However, in prospective studies of insomnia and AMI, only a few have evaluated and adjusted for depressive symptoms in the analyses.[121,122,124]

Many common chronic somatic disorders cause sleep problems and are also related to AMI. Only a relatively small study attempted to address the possibility that chronic disorders could explain the insomnia-AMI association.[121]

One of the aims of this thesis (Aim 1, see 1.4) was to investigate prospectively the association of insomnia symptoms with the risk of acute myocardial infarction in a large population-based study, taking into the account the effects of established cardiovascular risk factors, psychological distress, and chronic somatic disorders.

1.3.4 Insomnia and heart failure

Insomnia symptoms are highly prevalent among HF patients. Recent studies indicate that the prevalence of these symptoms among HF patients ranges from 23% to 73%.[133-135] However, it is largely unknown whether insomnia is associated with later risk of HF among individuals who were free from heart failure at baseline. There is a lack of large prospective studies of insomnia and risk of HF, and therefore, another aim of this thesis was to investigate the association of insomnia symptoms with the risk of subsequent HF in a large population based study (Aim 2, see 1.4).

1.3.5 Possible pathophysiological mechanisms linking insomnia to CVD

The pathophysiology of insomnia and its link to CVD are not fully understood. Insomnia is considered to be a disorder of hyperarousal experienced throughout the entire day.[136] This hyperarousal may exhibit itself as a state of hypervigilance during the day and difficulty initiating and maintaining sleep at night. The hyperarousal in persons suffering from insomnia is accompanied by chronic activation of stress responses with increased activity in the hypothalamic-pituitary-adrenal axis (HPA axis) and sympathetic nervous system.[136,137] Cortisol-releasing hormone and cortisol, products of the hypothalamus and the adrenals, respectively, are known to cause arousal and sleeplessness to humans and animals.[102] This stress response is also accompanied by increased metabolic rate, increased heart rate, decreased heart rate variability and increased blood pressure, secretion of catecholamines and pro-inflammatory

cytokines.[102,138,139] Thus, abnormalities in the autonomic nervous system and neuroendocrine system may represent a biologically plausible causal link between insomnia and CVD.

Insomnia has also been associated with an unhealthy lifestyle, and insomnia is associated with an increased prevalence of obesity, physical inactivity and cigarette smoking.[140] Consequently, insomniacs are more likely to have hypertension, unfavorable lipid levels and impaired fasting glucose compared to persons without insomnia symptoms.[141,142]

Insomnia symptoms also seem to promote unfavorable metabolic changes. The increased sympathetic activation and hypercortisolemia in persons with insomnia have been implicated in the pathophysiology of insulin resistance and the metabolic syndrome. The metabolic syndrome is closely linked to insulin resistance and comprises a clustering of various metabolic abnormalities in a given individual that increase the risk of diabetes mellitus, cardiovascular disease and premature death.[143-146] These include the presence of central obesity, an adverse lipid profile (high triglycerides and low HDL cholesterol), raised blood pressure and glucose intolerance.[147] In a recent study, difficulties initiating sleep and non-restorative sleep were associated with increased risk of developing metabolic syndrome over a 3-year period.[148] Several prospective population-based studies have reported that both difficulties initiating sleep and maintaining sleep are associated with increased risk of developing type 2 diabetes and hypertension.[149-151]

1.3.5.1 Insomnia in relation to inflammation

Only a few studies have examined chronic sleep problems or habitual insomnia symptoms in relation to inflammation, and the results have been inconsistent.[152-155] Most studies were small and few had information on established cardiovascular risk factors and other important covariates, including psychological distress or common chronic disorders that are known to be associated with both inflammation and insomnia.

One of the aims of this thesis was to evaluate the associations of insomnia symptoms and inflammation measured as hsCRP in a large population **(see Aim 3, 1.4)**.

1.4 Objective

The aim of this thesis is to contribute to a better understanding of the complex web of causes that links insomnia to cardiovascular disease (see figure 2). Therefore, our main objective was to investigate prospectively the association of insomnia and risk of acute myocardial infarction and heart failure in a large population-based study (Aims 1 and 2). We also wanted to explore possible mechanisms for such an association, mainly the role of inflammation (Aim 3). As a consequence, we investigated the association of insomnia with hsCRP in a subset of the population. It is largely established that insomnia is associated with an unhealthy lifestyle and with the metabolic syndrome. However, as mentioned earlier (section 1.1.1), the association of hsCRP and metabolic factors is not clear from the literature, and to shed light on the complex relations between insomnia, metabolic factors and inflammation, we also investigated this question (Aim 4).

In summary, our aims were to examine

Aim 1 - the association of insomnia with risk of acute myocardial infarction

Aim 2 - the association of insomnia with risk of heart failure

Aim 3 - the association of insomnia with hsCRP

Aim 4 - the association of metabolic factors with hsCRP

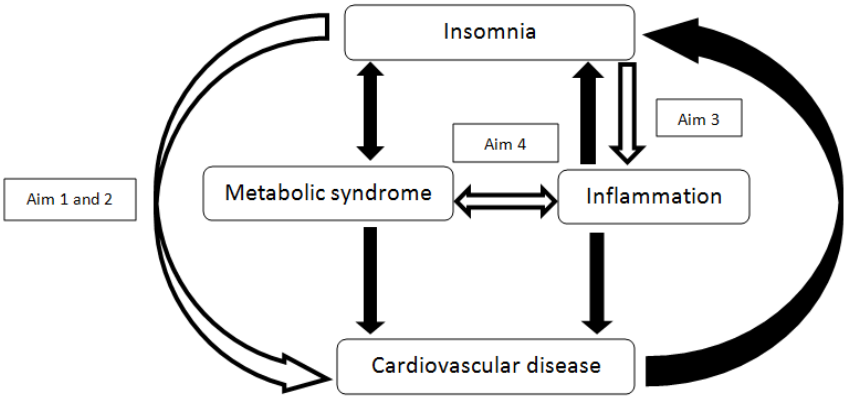


Figure 2. Possible relations between insomnia, metabolic syndrome, inflammation and cardiovascular disease. White arrows refer to associations proposed and investigated in this thesis. Black arrows indicate established associations or associations not examined in this thesis.

2 Methods

2.1 The Nord-Trøndelag Health Study (HUNT)

Nord-Trøndelag is one of 19 Norwegian counties and is located in the central part of Norway. The county is divided into 24 administrative areas, i.e. municipalities (Figure 3). Nord-Trøndelag County is considered to be fairly representative of Norway regarding geography, economy, and industry, sources of income, age distribution, morbidity and mortality.[156] The population is stable at about 131,000 inhabitants (2010), with a net migration out of the county of 0.3% per year (1996-2000). It is also a homogenous population, as less than 3% of the population is non-Caucasian. The population is served by two local hospitals, in Levanger and in Namsos.

The adult population of the county was the study base for the three phases of the Nord-Trøndelag Health Study (HUNT). This thesis analyzed data from the second phase (HUNT 2).

All inhabitants in Nord-Trøndelag aged 20 years or older were invited to participate in HUNT 2. In total, 94,187 individuals were eligible to participate, and 65,215 (69%) attended.

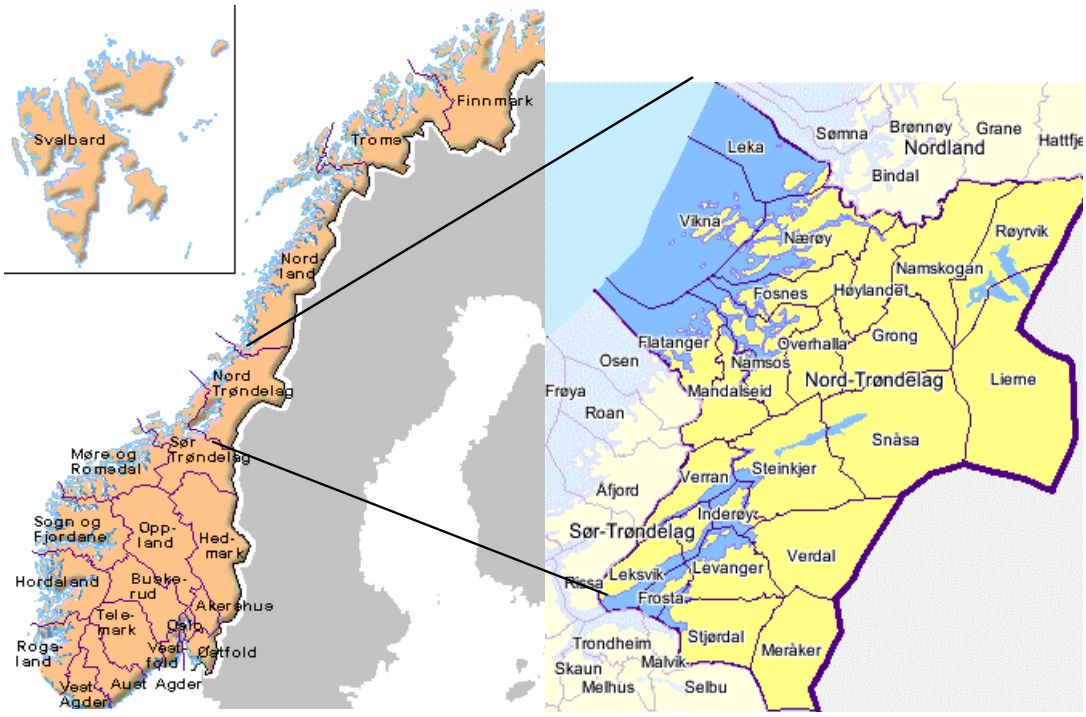


Figure 3. Norway and Nord-Trøndelag County. Adapted from Holmen et al, Norwegian Journal of Epidemiology 2003.[156]

A questionnaire (questionnaire 1) was attached to the invitation letter. This questionnaire was to be completed prior to the clinical examination. A second questionnaire (questionnaire 2) was handled out at the clinical examination (see below) and was to be completed and returned by mail in a pre-stamped envelope.

The HUNT study is a collaboration between the HUNT Research Centre, The Faculty of Medicine, the Norwegian University of Science and Technology, the Norwegian Institute of Public Health, Nord-Trøndelag County Council, and the Central Norwegian Regional Health Authority. The Norwegian Board of Health, the Regional Committee for Ethics in Medical Research, and the Norwegian Data Inspectorate

approved this study. Participation in HUNT was voluntary, and each participant signed a written consent regarding the survey and subsequent follow-up, and to the use of data for research purposes.

A more comprehensive description of the second HUNT study is published elsewhere.[156]

2.2 Study variables

Information on cardiovascular diseases as endpoints came from the medical records from the two hospitals in the county and from the National Cause of Death Registry. All other information came from HUNT 2.

2.2.1 Definition and ascertainment of CVD (papers I and II)

After participating at the baseline examination in HUNT, the participants were followed up for a first AMI and incident HF failure from baseline examination until December 31th, 2008, either identified at hospitals or by the National Cause of Death Registry.

Hospitalizations for AMI and HF were identified through linkage with medical records from the two hospitals of Nord-Trøndelag. AMI and HF were defined and diagnosed according to the European Society of Cardiology/American College of Cardiology consensus guideline. Criteria for AMI included (1) certain symptoms according to case history information, (2) specified changes in the blood levels of cardiac enzymes, and (3) specified ECG changes.[26] Criteria for HF included symptoms

and signs of HF. On the majority of patients echocardiography, radiological examinations and biochemical measurements were done.[157] The overall quality of the hospital diagnoses of AMI and HF in Nordic countries is high.[158,159] As there is no unequivocal definition of HF, the HF diagnosis in Nordic registers appears slightly less precise than for acute myocardial infarction. Accordingly, for the purpose of population-based research, only those with a primary diagnosis of HF in the hospital discharge register should be regarded as definite cases. Consequently, we included only those with a primary diagnosis of HF in the hospital discharge register as definite cases.

Deaths due to AMI or HF were identified by the National Cause of Death registry. Reporting of deaths by physicians and public health officers to the national Cause of Death Registry in Norway is mandatory. The data are collected and organized by Statistics Norway. The classification is based on International Classification of Diseases (ICD) codes. Both the primary and secondary causes of death are reported. The unique 11-digit identification number of every Norwegian citizen enables linkage of data from the Cause of Death Registry with other data, such as data from the HUNT study and the local hospitals in the county. For the analyses, the information on causes of death was complete through December 31th, 2008. Deaths from CVD were defined as AMI (ICD 9th Revision code 410; ICD 10th Revision codes I21 and I22) and HF (ICD-9: 428; ICD-10: I50.0, I50.1 and I50.9) as one of the causes of death, respectively.

2.2.2 Questionnaires

Insomnia (papers I-III). The second HUNT questionnaire included 3 items related to insomnia. One question was related to difficulty in initiating sleep ("Have you had difficulties falling asleep in the last month?" with the following response options: never/occasionally/often/almost every night). The second question was related to difficulty in maintaining sleep ("During the last month, have you woken up too early and not been able to get back to sleep?" with the following response options: never/occasionally/often/almost every night). The third question was related to having a feeling of non-restorative sleep ("How often do you suffer from poor sleep?" with the following response options: never or a few times a year/1-2 times per month/about once a week/more than once a week). The last question was restricted to individuals 20 to 69 years of age. Apart from insomnia symptoms, participants 20 to 69 years of age were also asked whether symptoms related to sleep influenced their work situation ("During the last year, have you been troubled by insomnia to such a degree that it influenced your work performance?" with the response options "yes" or "no").

Alcohol intake. The participants were asked about their usual intake of wine, beer, and spirits. In papers I-III, we categorized participants according to their alcohol consumption indicated by their usual number of drinks over a 2-week period as abstainers, light drinkers (0-1 drinks per day), moderate drinkers (>1 but ≤ 2 drinks per day), or heavy drinkers (≥ 2 drinks per day). In paper IV, the alcohol intake was categorized as less than one drink a month versus one or more drinks per month.

Level of physical activity. The participants were also asked about their level of physical activity. Light physical activity was defined as activity that does not involve

sweating or a feeling of breathlessness. In paper I-III, the participants were classified as (1) inactive if they reported less than 1 hour of hard and less than 3 hours of light activity per week, (2) moderately active if they reported 1 to 3 hours of hard or > 3 hours of light activity per week, and (3) physically active if they reported ≥ 3 hours of hard activity per week.

S*moking.* Responses to questions related to smoking were categorized as current, previous or never smoking in papers I-III. In paper IV, smoking was categorized as current vs. no smoking.

Education. In papers I-II, education was categorized as low (≤ 9 years), medium (between 10 and 12 years), or high (≥ 12 years). In paper III, education was categorized as (1) primary school, 7-10 years (2) high school or intermediate school (3) university qualifying examination or junior college (4) university or other post-secondary education, less than 4 years (5) university/college, more than 4 years.

Marital status was dichotomized as living alone or not (papers I-III).

Shift work. Participants between 20-69 years of age were asked whether they worked in shifts (papers I-III).

Sleep medication/sedatives (papers I-III). In questionnaire 2, participants were asked about their use of sleep medication/sedatives ("How often have you taken tranquilizers/sedatives or sleep medication in the last month?" with the following response options: daily/every week but not every day/less than once a week/never).

Common chronic disorders (papers I-III). Participants extensively assessed and reported their medical history regarding common chronic disorders, such as previous AMI, stroke, asthma, angina pectoris, diabetes mellitus, goiter, hypothyroidism, hyperthyroidism, fibromyalgia, arthritis, rheumatism, ankylosing spondylitis, cancer, epilepsy, or osteoporosis.

Hormonal status among women was assessed in questionnaire 2. Female participants were asked whether they used hormone replacement therapy or oral contraception. They were also asked about menopausal status and ongoing pregnancy. In paper III, hormonal status among women was categorized into 4 categories: premenopausal not using oral contraception; premenopausal using oral contraception; menopausal not using hormone therapy; or menopausal using hormone therapy.

Depression and anxiety (paper I-III). The Hospital Anxiety and Depression Scale was used to assess symptoms of anxiety and depression. A 14 four-point Likert-scaled item was included in questionnaire 1, 7 for anxiety and 7 for depression. Scores on both the anxiety and depression subscales ranged from 0 to 21, and increasing score indicated increased symptom load. No somatic items or items regarding sleeping difficulties were included. The Hospital Anxiety and Depression Scale has been found to have good testing properties in the assessment of symptom severity of anxiety and depression both in primary health care and in hospital settings.[160] The psychometric properties have been validated previously in HUNT 2.[161]

2.2.3 Clinical examination

The clinical examination was conducted by trained nurses and included standardized assessment of blood pressure, weight, height, and waist and hip circumference. *Systolic* and *diastolic blood pressures* were measured with a Dinamap 845XT (Criticon/GE Healthcare) sphygmomanometer based on oscillometry, and the average of the second and third measurements was used in the analysis. *Height* and *weight* were recorded with participants wearing light clothes without shoes; height was measured to the nearest centimeter and weight to the nearest 0.5 kilogram. *Waist circumference* was measured to the nearest centimeter at the level of umbilicus. *Body mass index* (BMI) was computed as weight (in kilograms) divided by the squared value of height (in meters).

2.2.4 Laboratory measurements

A non-fasting serum sample was drawn from each participant at the clinical examination, and analyzed at the Central Laboratory, Levanger Hospital, Nord-Trøndelag, with a Hitachi 911 Autoanalyzer (Mito, Japan). Time between the last meal and the venipuncture was recorded, and the samples were sent to the laboratory on the same day (some samples drawn on Friday were sent the following Monday).

Serum concentrations of *total serum cholesterol*, *HDL cholesterol* and *triglycerides* (papers I-IV) were analyzed in all participants, applying reagents from Boehringer Mannheim. The day-to-day coefficients of variation were 1.3% to 1.9%, 2.4%, and 0.7% to 1.3%, respectively. Total and HDL cholesterol were measured by an

enzymatic colorimetric cholesterol esterase method, and HDL was measured after precipitation with phosphotungsten and magnesium ions. Triglycerides were measured with an enzymatic colorimetric method.

In paper IV, we also calculated *non-HDL cholesterol* as the difference between total serum cholesterol and HDL cholesterol and used it as a measure of the lipid content of atherogenic lipoproteins.

All participants from four of 24 municipalities in Nord-Trøndelag County were selected for *high-sensitivity C-reactive protein measurement* (papers III and IV), including 9,995 persons. HsCRP was analyzed using an ultrasensitive assay with particle-enhanced immunological agglutination. The degree of particle agglutination was measured turbidimetrically and the quantification range was 0.1- 20 mg/l. The detection limit was 0.03 mg/l, and samples without detectable hsCRP were assigned this value. Serum was stored at -80°C and hsCRP levels were measured approximately 2 years after the serum was collected. The analysis was performed at an accredited, biomedical laboratory using their standard assay for CRP analysis, the CRP (Latex) US (Hoffman- La Roche, Switzerland). The assay provider (Hitachi/Roche) has tested reproducibility both within run (% coefficient of variation, CV, 0.43-1.34) and between days (% CV 2.51-5.70) as well as method comparison (r =0.996). The tested sample concentrations were between 0.11 and 18.63mg/l. The age and sex distribution of hsCRP values in our studies were largely comparable to those of other large population-based studies from Europe.[162]

2.3 Statistical analyses

All statistical analyses were conducted with STATA 10.1 for windows (Stata Corp.).

2.3.1 Prospective analyses (papers I and II)

In papers I and II, we used Cox proportional hazard models to examine the association of insomnia symptoms with subsequent risk of AMI and heart failure, respectively. We calculated hazard ratios (HRs) with 95% confidence intervals (CIs). We assessed the influence of each insomnia symptom using the original 4 response categories. Each category of reported insomnia symptoms was compared with reporting no insomnia complaints. For tests of trend, we assigned a numeric value of 0 to 3 to the insomnia categories, with 0 having no insomnia complaints, treating the categories as a continuous variable.

Insomnia symptoms were also dichotomized, and the highest categories, i.e., difficulty initiating sleep almost every night, difficulty maintaining sleep almost every night, and non-restorative sleep more than once a week, were compared with the rest of the categories. In a separate analysis, we calculated the risk associating with the increasing number of dichotomized insomnia symptoms when those without any symptom constituted the reference category.

The associations of insomnia symptoms and risk for AMI were assessed in different multivariable models. We included age, sex, education, shift work, and marital status as potentially confounding factors in our models. Established cardiovascular risk

factors such as high blood pressure, low physical activity, high BMI, smoking, abstinence from alcohol and heavy drinking, dyslipidemia, diabetes mellitus and history of previous AMI (in paper II) may act as both confounding and mediating factors for the association of sleep disorders with cardiovascular risk. We therefore analyzed the data both with and without the factors included in the analyses. It is also not clear whether psychological distress is a cause or a consequence of sleep disorders. Thus, in separate analyses, we additionally adjusted for depression and anxiety.

We also conducted several stratified analyses to examine whether the association of insomnia symptoms and cardiovascular diseases could be modified by other factors. We formally tested the homogeneity of stratum-specific relative risks. For these tests of interaction, we used the insomnia trend variables as defined above.

In sensitivity analyses, we dealt with other possible confounders such as chronic somatic disorders and sleep medication/sedatives, either by adjusting for them in the analyses, or by restricting the study population. To address the possibility of reverse causation as an explanation for the observed associations, we excluded the first 5 years of follow-up and repeated the analyses. We also restricted the analyses to CVD cases that were confirmed at the hospital; thus, we excluded cases whose diagnosis was based on death certificates alone. In paper II, we also tested whether AMI during follow- up influenced our estimates of the association of insomnia and HF, and included AMI during follow-up as a time-dependent variable.

We tested the proportionality of hazards using log-log curves and formal tests of interaction with time or log-time. There was no evidence against the proportionality assumption (P>0.10).

2.3.2 Cross- sectional analyses (paper III and IV)

The HsCRP levels were log-transformed due to skewed distribution. The association of hsCRP levels with insomnia symptoms (paper III) and metabolic factors (paper IV) were assessed in different multivariable models using linear regression with hsCRP as the dependent variable. The multivariable models included potential confounders. The inclusion of these potentially confounding covariates was based on prior knowledge. To address the robustness of our findings, hsCRP was also analyzed after categorization. Analyses were conducted separately for men and women, based on previous studies suggesting sex differences.

2.3.2.1 Insomnia and hsCRP (paper III)

The association of insomnia symptoms with hsCRP levels was assessed in four different models. In model 1, we adjusted for age only. In model 2, we adjusted for age, education, marital status, shift-work, smoking, alcohol, physical activity, blood pressure, BMI, diabetes mellitus, serum lipids, and hormonal status for women. Model 3 was identical to model 2, with additional adjustment for depression and anxiety

symptoms and chronic pain. Model 4 was the same as model 3, but the analyses were restricted to participants without a history of chronic disease.

We calculated standardized and unstandardized regression coefficients with 95% confidence intervals for each category of insomnia symptoms, using people with no insomnia complaint as the reference. For tests of linear trend, we assigned a numeric value of 0 to 3 to the insomnia categories, with 0 having no insomnia complaints, treating the categories as a continuous variable. In separate analyses, a quadratic term was included to assess non-linear trends. We also calculated the regression coefficients associated with the cumulative number of dichotomized insomnia symptoms using those without any symptoms as the reference category.

Multiple imputations were performed on participants selected for hsCRP measurement but who did not respond to questions concerning insomnia or who had missing covariate information. Imputation was performed using the ICE-solution in Stata and 20 imputed datasets were created to achieve maximum accuracy. All the variables in the analyses, including the dependent variable, were used to impute missing values, as well as other HUNT variables not included in the present analysis. In addition, we also performed complete case analyses.

2.3.2.2 Metabolic factors and hsCRP (paper IV)

The associations of each metabolic factor (BMI, mean systolic and diastolic blood pressure, HDL cholesterol, non-HDL cholesterol, and triglycerides) with mean hsCRP were assessed in three different models: (1) adjusted for age; (2) adjusted for non-

metabolic confounders (age, smoking, alcohol, and, in females; estrogen hormone replacement therapy, oral contraception, or ongoing pregnancy); and (3) with additional adjustment for the other metabolic factors. We estimated the adjusted and unadjusted percentage difference in hsCRP (with 95% confidence interval) per one standard deviation (SD) difference in each metabolic factor. In stratified analyses, we analyzed younger (20-34 years), middle-aged (35-59 years), and older (60 years and above) participants separately.

In a separate analysis, the participants were placed in three categories of metabolic risk factors. We defined the following cut-off points to indicate increased metabolic risk: waist circumference ≥ 94 cm for men and ≥ 80cm for women, serum triglycerides ≥ 1.7 mmol/l, HDL < 1.0 mmol/l for men and < 1.3 mmol/l for women, systolic blood pressure ≥ 130 mmHg or diastolic blood pressure ≥ 85 mmHg, and prevalent diabetes mellitus. Participants without any unfavorable level of metabolic factors were classified as low risk, those with unfavorable levels of one or two metabolic factors were classified as intermediate risk, and those with three of more metabolic factors with unfavorable values were classified as high risk individuals. We estimated geometric mean hsCRP within each 10-years age group for each of these metabolic groups and assessed whether hsCRP differed between the groups. Linear regression modeling was also applied to test differences across metabolic risk categories for hsCRP in different age groups.

3 Results

3.1 Paper I: Insomnia and the risk of acute myocardial infarction: A population study

We assessed insomnia symptoms and risk of acute myocardial infarction in 52,610 participants with no history of previous AMI and answering one or more of the questions related to insomnia. Prevalences of having difficulties maintaining sleep almost every night, having difficulties maintain sleep almost every night and having non-restorative sleep more than once a week were 3.3 %, 2.5 %, and 8.0 %, respectively.

Table 4 displays characteristics of the study population according to difficulties initiating sleep. High frequency of insomnia symptoms was most prevalent among older participants and was more frequent among women than men. In general, insomnia symptoms were associated with cardiovascular risk factor in a dose-dependent manner. There was also a strong association between insomnia symptoms and depression, anxiety, and the use of sleep medication/sedatives. The other two insomnia symptoms, difficulty maintaining sleep and feeling of non-restorative sleep, showed largely similar associations to these characteristics.

Table 4. Baseline characteristics of the HUNT-2 participants, free of history of AMI at baseline, according to difficulties initiating sleep (Paper I)

		Never	Sometimes	Often	Almost every night
Variable	**N**	**% (n)**	**% (n)**	**% (n)**	**% (n)**
Total	51,982	55.1 (28,630)	36.2 (18,818)	5.5 (2,846)	3.3 (1,691)
Sex (male)	23,226	48.3 (14,008)	41.2 (7,754)	33.0 (939)	31.0 (525)
Diabetes mellitus	1,411	2.4 (690)	2.8 (522)	3.4 (95)	6.2 (104)
Current smoking	14,611	25.1 (7,145)	30.5 (5,718)	39.9 (1,130)	36.7 (618)
Physical inactive	18,109	36.0 (9,439)	36.0 (6,738)	46.4 (1,167)	55.8 (765)
Shift work*	7,673	21.2 (4,249)	23.8 (2,847)	25.5 (433)	22.4 (144)
Living alone	20,154	37.8 (10,806)	39.6 (7,421)	40.9 (1,162)	45.3 (765)
Education <=9 years	17,546	31.6 (8,707)	37.5 (6,740)	44.7 (1,205)	58.5 (894)
Use of sleep medicine/sedatives daily	2,021	1.4 (348)	4.2 (711)	11.5 (299)	42.9 (663)
	N	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**
Age (years)	51,982	47.7 (16.6)	50.8 (16.8)	52.1 (16.4)	59.4 (18.1)
BMI (kg/m^2)	51,682	26.3 (4.0)	26.4 (4.1)	26.5 (4.4)	26.6 (4.3)
Systolic BP (mmHg)	51,822	136.8 (21.2)	138.1 (21.9)	138.0 (22.1)	142.6 (24.6)
Diastolic BP (mmHg)	51,822	79.9 (12.0)	80.5 (12.2)	80.8 (12.0)	81.5 (12.7)
Total cholesterol (mmol/L)	51,884	5.8 (1.2)	6.0 (1.3)	6.1 (1.3)	6.3 (1.3)
HDL cholesterol (mmol/L)	51,871	1.4 (0.4)	1.4 (0.4)	1.4 (0.4)	1.4 (0.4)
Triglycerides (mmol/L)	51,884	1.7 (1.1)	1.8 (1.1)	1.8 (1.2)	2.0 (1.2)
Depressive symptoms	50,554	2.8 (2.7)	3.9 (3.1)	5.3 (3.7)	6.0 (4.0)
Anxiety symptoms	49,827	3.4 (2.8)	4.9 (3.3)	6.9 (4.1)	7.3 (4.5)

* Those who answered yes to the question: "Do you have shift work, night work or standing by duties?"

During 11.4 years of follow-up a total of 2,368 participants had a first AMI. A total of 1,813 were diagnosed at hospital admission and 555 cases were identified based on information from the National Cause of Death Registry.

Table 5 presents the multivariable adjusted hazard ratios for AMI in relation to insomnia symptoms. Having difficulty initiating sleep, difficulty maintaining sleep, and having the feeling of non-restorative sleep were associated with a moderately increased

risk of AMI compared with those who reported never or almost never to have these symptoms. After adjustment for established cardiovascular risk factors, the effect sizes of the associations were attenuated slightly, however, the estimates of effect were not further attenuated after additional adjustment for depression or anxiety, respectively. Among the insomnia symptoms, difficulties initiating sleep appeared to have the strongest and most robust association with AMI.

As shown in Table 6, when we combined the insomnia symptoms, a dose-dependent association was seen between the number of insomnia symptoms and AMI risk.

Furthermore, we performed stratified analyses in subgroups to ensure that our results were consistent. When compared with men, women appeared to have somewhat higher relative risks of AMI associated with difficulties initiating sleep almost every night and cumulative insomnia symptoms (P for homogeneity 0.009 and 0.015, respectively). We found no statistical evidence for any effect modification by other stratifying factors, i.e., age, BMI, cholesterol, education, shift work, blood pressure, smoking status, and the association of insomnia with AMI risk was largely consistent in these subgroup analyses.

Table 5. Hazard ratios for AMI according to insomnia symptoms (paper I)

Variable	Events/ Person time	Model 1 95 % CI	Model 2 95 % CI	Model 3 95 % CI	Model 4 95 % CI	Model 5 95 % CI
Difficulty initiating sleep						
Never	1166/ 324352	Ref.	Ref.	Ref.	Ref.	Ref.
Occasionally	840/ 210079	1.01 (0.92-1.10)	1.04 (0.94-1.14)	1.02 (0.91-1.13)	1.02 (0.92-1.14)	1.04 (0.93-1.16)
Often	140/ 31300	1.19 (1.00-1.42)	1.18 (0.97-1.43)	0.98 (0.78-1.22)	0.98 (0.78-1.24)	1.06 (0.84-1.34)
Almost every night	162/ 17059	1.58 (1.34-1.87)	1.53 (1.27-1.84)	1.45 (1.18-1.80)	1.48 (1.18-1.84)	1.53 (1.21-1.92)
P for trend		<0.0001	<0.0001	0.019	0.018	0.005
Difficulty maintaining sleep						
Never	718/ 283601	Ref.	Ref.	Ref.	Ref.	Ref.
Occasionally	1265/ 247383	1.06 (0.96- 1.16)	1.02 (0.92- 1.13)	1.06 (0.95-1.18)	1.06 (0.95-1.19)	1.10 (0.98-1.23)
Often	228/ 39960	1.11 (0.96- 1.30)	1.08 (0.92- 1.26)	1.10 (0.91-1.32)	1.15 (0.95-1.39)	1.22 (1.01-1.48)
Almost every night	116/ 13110	1.39 (1.14- 1.70)	1.33 (1.07- 1.65)	1.30 (1.01-1.68)	1.32 (1.01-1.72)	1.46 (1.11-1.90)
P for trend		0.003	0.033	0.049	0.025	0.002
Feeling of non-restorative sleep						
Never, few times a year	742/ 350581	Ref.	Ref.	Ref.	Ref.	Ref.
1-2 times per month	177/ 83777	0.94 (0.80- 1.10)	0.92 (0.77- 1.09)	0.91 (0.76-1.09)	0.91 (0.76-1.09)	0.95 (0.79-1.15)
Once a week	97/ 35435	1.18 (0.96- 1.45)	1.18 (0.95- 1.46)	1.04 (0.82-1.33)	1.05 (0.82-1.34)	1.15 (0.90-1.47)
More than once a week	133/ 39801	1.30 (1.08- 1.57)	1.32 (1.09-1.60)	1.27 (1.03-1.57)	1.25 (1.00-1.55)	1.41 (1.13-1.76)
P for trend		0.007	0.006	0.074	0.115	0.006

For models, see Table 6.

Table 6. Hazard ratios for AMI according to the number of insomnia symptoms (paper I)

		Model 1		Model 2		Model 3		Model 4		Model 5	
Number of symptoms	**Events / person time**	**HR**	**95 % CI**	**HR**	**95 % CI**	**HR**	**95 % CI**	**HR**	**95 % CI**	**HR**	**95 % CI**
0	997/ 463131	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.
1	212/ 37710	1.24	1.00- 1.54	1.28	1.03-1.59	1.19	0.94-1.50	1.19	0.94-1.51	1.30	1.02-1.65
2	81/ 12727	1.47	1.07-2.02	1.44	1.04-2.00	1.39	0.98-1.97	1.34	0.94-1.93	1.47	1.02-2.13
3	13/ 2417	1.92	1.11-3.33	1.73	0.98-3.06	1.89	1.04-3.44	1.73	0.92-3.25	2.12	1.13-4.00
HR for each symptom increase		1.24	1.11-1.38	1.22	1.10-1.37	1.20	1.06-1.35	1.18	1.04-1.34	1.25	1.08-1.45
P for trend			<0.0001		<0.001		0.003		0.011		0.001

Model 1: adjusted for age and sex.
Model 2: model 1 + marital status, education, and shift-work.
Model 3: model 2 + systolic blood pressure, total cholesterol, diabetes mellitus, BMI, physical activity and smoking.
Model 4: model 3 + depression.
Model 5: model 3 + anxiety.

Several sensitivity analyses were conducted to assess the robustness of our findings. The results did not materially change by excluding the first 5 years of follow-up, by restricting outcomes to hospital-verified AMI, or excluding users of sleep medication/sedatives. In another sensitivity analyses, we excluded participants with known chronic disorders. The association of insomnia with AMI risk among these individuals was similar to that observed in the entire cohort.

3.2 Paper II: Insomnia and risk of incident heart failure: A population study

We assessed the prospective association of insomnia symptoms with the risk of incident heart failure in 54,279 men and women who were free of known heart failure at baseline and who answered one or more of the questions related to insomnia.

Table 7 displays characteristics of the study population according to the cumulative number of insomnia symptoms. Older participants were more likely to have insomnia symptoms and symptoms were more frequent in women than men. In general, insomnia symptoms were associated with cardiovascular risk factors in a dose-dependent manner. There was also a strong association between insomnia symptoms and depression, anxiety, and the use of sleep medication/sedatives.

A total of 1,489 cases of heart failure occurred during 11.3 years of follow-up, 1,004 cases were diagnosed at hospital admission, and 408 cases were identified based on information from the National Cause of Death Registry.

Individual insomnia symptoms were associated with increased risk of incident HF in our models adjusted for age, sex, education, shift-work, and marital status. After further adjustment for established cardiovascular risk factors, and previous AMI, the strength of the associations was attenuated. The multiadjusted hazard ratios for HF in Model 3 were 1.32 (95% CI 1.01-1.72) for people with difficulties initiating sleep almost every night, 1.26 (CI 0.93-1.72) for those with difficulties maintaining sleep almost every night, and 1.08 (CI 0.72-1.61) for those with a feeling of non-restorative sleep more than once a week compared with people who never experienced these sleep problems. The estimates of effect were further attenuated after adjustment for depression and anxiety.

As shown in Table 8, the cumulative number of insomnia symptoms was associated with increased risk of HF in a dose-dependent manner in all models.

We conducted several stratified analyses to assess whether the associations of insomnia symptoms and HF could be modified by other factors. Compared to men, women appeared to have a higher relative risk of HF associated with non-restorative sleep and with the cumulative symptoms of insomnia (P for homogeneity of HR 0.004 and 0.016, respectively). In other stratified analyses we found no statistical evidence for effect modification.

Table 7. Baseline characteristics of the HUNT-2 participants free of HF at baseline according to cumulative number of insomnia symptoms (paper II)

Number of insomnia symptoms		**0**	**1**	**2**	**3**
Variable	**N**	**% (n)**	**% (n)**	**% (n)**	**% (n)**
Total	44,047	91.1 (40,134)	2.7 (2,711)	2.2 (978)	0.5 (224)
Sex (male)	20,205	47.0 (18,870)	34.2 (926)	34.8 (340)	29.9 (67)
Diabetes mellitus	770	1.7 (666)	2.4 (66)	3.1 (30)	3.6 (8)
Current smoking	13,423	29.6 (11,858)	38.4 (1,037)	45.2 (440)	39.6 (88)
Physical inactive	15,140	35.2 (13,456)	46.6 (1,145)	49.3 (436)	52.6 (103)
Shift work*	7,685	22.3 (7,108)	22.9 (420)	20.9 (129)	21.7 (28)
Living alone	16,496	37.7 (15,108)	34.8 (941)	36.8 (359)	39.3 (88)
Education <=9 years	12,703	27.7 (10,905)	47.1 (1,230)	48.2 (455)	52,8 (113)
Use of sleep medicine/sedatives daily	1,235	1.8 (663)	10.8 (269)	24.4 (221)	39.0 (80)
Previous myocardial infarction	709	1.5 (609)	2.2 (60)	3.3 (32)	3.6 (8)
	N	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**
Age (years)	44,047	44.1 (13.2)	49.5 (12.5)	50.1 (13.2)	53.0 (12.0)
BMI (kg/m^2)	43,916	26.1 (4.0)	26.7 (4.6)	26.8 (4.6)	26.5 (4.6)
Systolic BP (mmHg)	43,922	133.5 (18.7)	135.9 (19.7)	134.2 (19.7)	136.9 (20.7)
Diastolic BP (mmHg)	43,922	79.1 (11.6)	80.9 (11.6)	79.9 (11.7)	81.9 (11.3)
Total cholesterol (mmol/L)	43,966	5.7 (1.2)	6.1 (1.3)	6.1 (1.3)	6.3 (1.4)
HDL cholesterol (mmol/L)	43,954	1.4 (0.4)	1.4 (0.4)	1.4 (0.4)	1.4 (0.4)
Triglycerides (mmol/L)	43,966	1.7 (1.1)	1.8 (1.2)	1.9 (1.3)	2.0 (1.2)
Depressive symptom score	43,513	3.1 (2.8)	5.1 (3.7)	6.1 (3.9)	7.0 (4.3)
Anxiety symptom score	43,286	4.1 (3.1)	6.6 (4.0)	7.7 (4.3)	9.0 (4.8)

* Those who answered yes to the question: “Do you have shift work, night work or standing by duties?”

Table 8. HRs and 95% CIs for heart failure according to cumulative number of insomnia symptoms (paper II)

		Model 1		Model 2		Model 3		Model 4		Model 5	
Number of symptoms	**Events / person time**	**HR**	**95 % CI**	**HR**	**95 % CI**	**HR**	**95 % CI**	**HR**	**95 % CI**	**HR**	**95 % CI**
0	270/471292	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.
1	28/31515	1.17	0.79-1.73	1.20	0.80-1.80	0.96	0.57-1.61	0.91	0.59-1.52	0.95	0.55-1.62
2	18/11037	1.92	1.19-3.11	1.88	1.13-3.13	1.35	0.72-2.50	1.20	0.64-2.26	1.43	0.76-2.69
3	7/2490	2.95	1.39-6.27	2.69	1.19-6.08	4.53	1.99-10.31	3.83	1.66-8.85	5.25	2.25-12.22
HR for each symptom increase		1.37	1.15-1.62	1.35	1.12-1.62	1.29	1.04-1.61	1.23	0.98-1.54	1.34	1.06-1.68
P for trend			<0.001		0.001		0.021		0.077		0.013

Model 1, adjusted for age and sex
Model 2, Model 1 + marital status, education, and shift-work
Model 3, Model 2 + systolic blood pressure, total cholesterol, diabetes mellitus, BMI, physical activity, smoking, alcohol and previous AMI.
Model 4, Model 3 + depression
Model 5, Model 3 + anxiety

Several sensitivity analyses were performed, and the results did not materially change by excluding the first five years of follow-up, restricting follow-up to HF cases that were confirmed at the hospital, or adjusting for use of sleep medication/sedative. Adjustment for chronic diseases only slightly attenuated the association of insomnia with HF risk compared to the results of the main analyses. No appreciable change in our estimates occurred after adjustment for incident AMI as a time-dependent covariate during follow-up.

3.3 Paper III: Insomnia and high-sensitivity C-reactive protein: The HUNT study, Norway

A total of 8,547 men and non-pregnant women answered one or more of questions related to insomnia out of 9,995 participants with available hsCRP measurement in the HUNT study.

Among men, using multivariable linear regression analyses of the logarithm of hsCRP, difficulties initiating sleep and non-restorative sleep were associated with increasing hsCRP levels after adjustment for age. However, after additional adjustment for cardiovascular risk factors the associations were attenuated. In men, HsCRP was not associated with having difficulties maintaining sleep, insomnia with influence on work or with the cumulative number of insomnia symptoms (Table 9).

Table 9. Regression coefficients with 95% confidence interval for the association of hsCRP and cumulative number of insomnia symptoms (multiple imputation) (paper III)

	Model 1			Model 2			Model 3			Model 4		
Variable	**B***	**95% CI**	**β****	**B***	**95% CI**	**β****	**B***	**95% CI**	**β****	**B***	**95% CI**	**β****
For women												
0	Ref		Ref	Ref		Ref	Ref		Ref	Ref		Ref
1	0.02	-0.14 to 0.18	0.01	0.01	-0.14 to 0.15	0.00	0.01	-0.14 to 0.16	0.01	0.00	-0.19 to 0.20	0.00
2	-0.05	-0.23 to 0.13	-0.03	-0.09	-0.25 to 0.08	-0.06	-0.08	-0.25 to 0.09	-0.05	-0.04	-0.28 to 0.20	-0.02
3	0.07	-0.23 to 0.13	0.04	-0.03	-0.25 to 0.19	-0.02	-0.01	-0.24 to 0.22	-0.01	-0.25	-0.59 to 0.08	-0.16
Linear trend	0.00	-0.06 to 0.06		-0.02	-0.08 to 0.03		-0.02	-0.08 to 0.04		-0.05	-0.13 to 0.04	
P for trend	*0.92*			*0.46*			*0.60*			*0.27*		
P for quadratic trend	*0.72*			*0.93*			*0.90*			*0.33*		
n	*4,378*			*4,378*			*4,378*			*2,977*		
For men												
0	Ref		Ref	Ref		Ref	Ref		Ref	Ref		Ref
1	0.20	0.02 to 0.36	0.13	0.13	-0.03 to 0.30	0.09	0.15	-0.02 to 0.32	0.10	0.14	-0.06 to 0.34	0.09
2	0.09	-0.13 to 0.32	0.06	0.03	-0.19 to 0.25	0.02	0.06	-0.17 to 0.29	0.04	0.03	-0.26 to 0.32	0.02
3	0.10	-0.28 to 0.32	0.06	0.00	-0.37 to 0.37	0.00	0.04	-0.33 to 0.42	0.03	0.14	-0.37 to 0.66	0.09
Linear trend	0.07	-0.01 to 0.15		0.03	-0.05 to 0.11		0.04	-0.04 to 0.13		0.05	-0.05 to 0.16	
P for trend	*0.10*			*0.46*			*0.30*			*0.33*		
P for quadratic trend	*0.17*			*0.24*			*0.23*			*0.55*		
n	*3,901*			*3,901*			*3,901*			*2,969*		

*Unstandardized beta coefficient
** Standardized beta coefficient
Model 1 age adjusted; Model 2 adjusted for age, marital status, education, shift-work, systolic– and diastolic blood pressure, triglycerides, HDL- and total cholesterol, diabetes mellitus, BMI, physical activity, smoking and alcohol intake, and hormonal status in women; Model 3 adjusted for the same variables as in model 2 and depression score, anxiety score and chronic pain; Model 4 adjusted for the same variables as in model 3, but restricted to those without a chronic somatic disorder.

In women, there was no evidence for an association of individual insomnia symptoms with hsCRP levels.

The associations of insomnia and hsCRP were largely the same in the complete case analyses as those obtained performing multiple imputations.

3.4 Paper IV: Metabolic factors and high-sensitivity C-reactive protein: the HUNT study

A total of 4,503 men and 4,411 women without missing data on hsCRP and metabolic factors were included in our analyses.

In the multivariable analyses, all the metabolic factors were significantly associated with hsCRP, with the exception of systolic blood pressure in women and non-HDL cholesterol in men (Table 10A and B). Among the metabolic risk factors, BMI appeared to be the most strongly associated factor, and showed a strong positive association with hsCRP also after adjustment for the other metabolic factors, with similar associations in women and men. HsCRP was 37.6% higher per standard deviation increase in BMI for men and 48% higher for women after multivariable analyses. Stratifying the analysis according to age groups showed that the associations between metabolic factors and hsCRP were fairly robust. The associations were generally slightly stronger in younger than in older age groups. In the youngest age group, hsCRP was 54.3% higher per standard deviation increase in BMI for men and 62% higher for women after multivariable adjustment, and the corresponding

differences were 39.3% and 57.8% in the middle aged group, and 28.9% and 28.0% in the oldest age group.

In a secondary analysis, using hsCRP in categories the results were essentially similar to those of the primary analysis.

Table 10A. Percentage difference in mean hsCRP per standard deviation difference with a 95% confidence interval in each metabolic risk factor among females (n=4381) estimated in linear regression (paper IV)

Metabolic risk factor	Model 1	Model 2	Model 3
Body mass index[a]			
per S.D. (=4.1)	57.0 (50.8 to 63.4)	59.0 (53.0 to 65.4)	48.0 (41.9 to 54.5)
Mean systolic BP[n]			
per S.D. (=21.9)	23.4 (17.0 to 30.2)	22.3 (15.4 to 28.8)	-0.1 (-6.9 to 7.1)
Mean diastolic BP[n]			
per S.D. (=12.2)	22.1 (16.4 to 28.2)	22.0 (16.4 to 28.0)	10.0 (3.0 to 17.2)
Triglycerides			
per S.D. (=1.12)	47.7 (39.8 to 56.0)	42.5 (34.9 to 50.4)	10.7 (3.9 to 18.2)
HDL cholesterol			
per S.D. (=0.39)	-24.2 (-27.5 to -20.7)	-24.8 (-28.0 to -21.5)	-13.6 (-17.6 to -9.4)
Non HDL cholesterol			
per S.D. (=1.26)	16.2 (10.5 to 22.1)	14.0 (8.5 to 19.8)	-6.5 (-11.2 to -1.5)

Values are percentage in mean hsCRP per standard deviation difference (95% confidence interval). The percentage change is on the linear scale. a: n =4353. n: n=4379. In addition to previous exclusions, 28 and 2 women with missing information on BMI and blood pressure measurements respectively were excluded from this part of the analysis.
Model 1, adjusted for age; Model 2, adjusted for age, smoking, alcohol intake, estrogen hormone replacement therapy, oral contraception or ongoing pregnancy; Model 3, model 2 + the other metabolic risk factors.

Table 10B. Percentage difference in mean hsCRP per standard deviation difference with a 95% confidence interval in each metabolic risk factor among males (n=4491) estimated in linear regression (paper IV)

Metabolic risk factor	Model 1	Model 2	Model 3
Body mass index[a]			
per S.D. (=4.1)	41.1 (34.2 to 48.1)	45.5 (38.5 to 52.8)	37.6 (30.5 to 45.1)
Mean systolic BP[n]			
per S.D. (=21.9)	5.2 (1.00 to 11.2)	6.1 (0.5 to 12.0)	-9.7 (-16.1 to -2.7)
Mean diastolic BP[n]			
per S.D. (=12.2)	12.3 (6.9 to 17.8)	13.2 (7.9 to 18.8)	13.5 (6.2 to 21.3)
Triglycerides			
per S.D. (=1.12)	10.0 (6.0 to 14.2)	9.5 (5.5 to 13.7)	-5.2 (-9.1 to -1.0)
HDL cholesterol			
per S.D. (=0.39)	-23.0 (-26.7 to -19.3)	-23.2 (-26.8 to -19.3)	-17.2 (-21.4 to -12.7)
Non HDL cholesterol			
per S.D. (=1.26)	13.0 (7.6 to 18.6)	12.7 (7.4 to 18.3)	2.3 (-2.9 to 7.9)

Values are percentage difference in mean hsCRP per standard deviation difference (95% confidence interval). The percentage change is on the linear scale. a: n=4482. n: n=4488. In addition to previous exclusions 9 and 3 men with missing information on BMI and blood pressure measurements respectively were excluded from this part of the analysis. Model 1, adjusted for age; Model 2, adjusted for age, smoking, and alcohol intake; Model 3, model 2 + the other metabolic risk factors

In a separate analysis, we placed the participants in three categories of metabolic risk factors within each 10-years age group (Figure 4A and B). We found statistical evidence for an association between hsCRP and metabolic risk across all age groups in both genders. Participants with high metabolic risk had consistently higher hsCRP values compared to the rest of the population and hsCRP appeared to be higher in the intermediate metabolic group compared to the low-risk group.

Figure 4A. Distribution of hsCRP among women according to metabolic risk (paper IV)

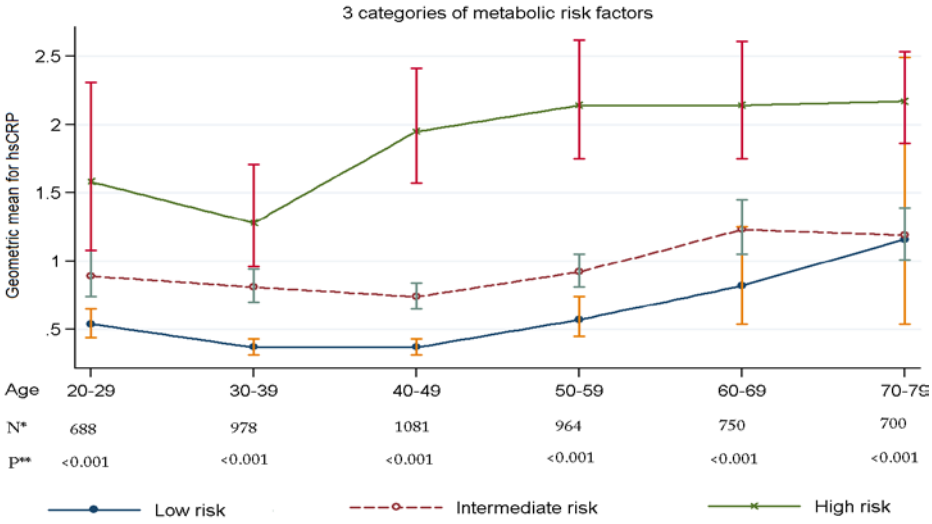
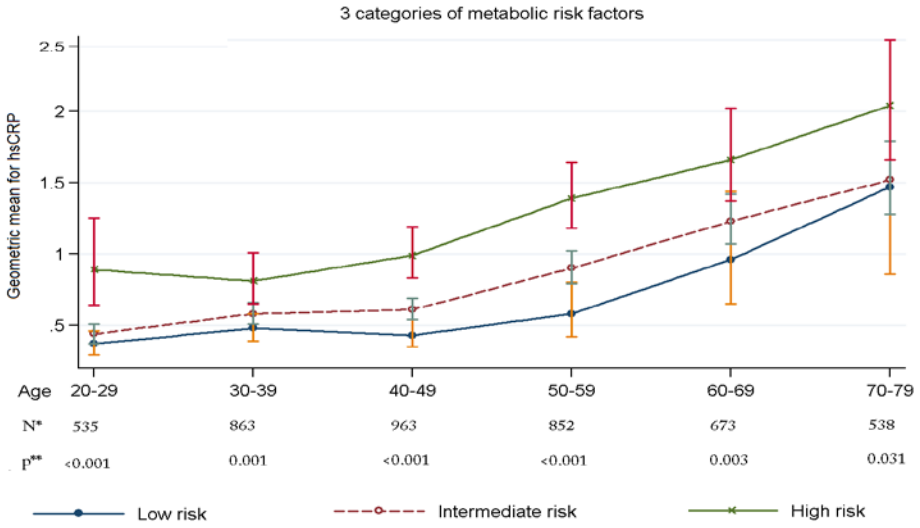


Figure 4B. Distribution of hsCRP among men according to metabolic risk (paper IV)



*Total number of participants in each age group; ** p value for test of homogeneity of the hsCRP values across three categories of metabolic risk (linear regression).

4 Discussion

Our overall aim was to investigate the prospective associations of insomnia with risk for AMI and HF, and to explore possible mechanisms behind these associations. Briefly, our principal findings can be summarized as follows:

- Insomnia was associated with a moderately increased risk for AMI.
- Insomnia was associated with an increased risk of incident heart failure.
- There were no consistent associations between insomnia symptoms and hsCRP levels.
- Metabolic factors, especially body mass index, had a relatively strong association with hsCRP at all ages both in men and women.

4.1. Strengths and Limitations

Below we discuss the methodological strengths and limitations of this thesis. We systematically review the typical sources of error in epidemiology, i.e. the difference between the observed and the causal effect, and discuss the possibilities of these errors in our studies.

4.1.1 Random error

The observed associations in epidemiological studies may always be influenced by chance or unexplained variability in the data. Random error can be defined as fluctuation in the data caused by any factors that randomly affect the result of the measurement.[163] Analogously, the opposite of random error is precision, and an estimate with little random error is described as precise. In our study, we indicated the precision of the estimates by 95% confidence intervals.

We had an ample sample size compared to most previous studies and therefore, the estimates generally showed high precision, with relatively narrow confidence intervals. The large sample size also made these studies relatively well powered to detect subgroup differences.

4.1.2 Systematic error (lack of internal validity)

Systematic error, often referred to as bias, is the other main type of error in epidemiology. The opposite of bias is validity, and an estimate with little systematic error is considered to be valid. Systematic error can be defined as any process or effect that leads to nonrandom deviation of results from the true, i.e. causal values. In contrast to random error that is decreasing with increasing size of the study, systematic error does not depend on sample size.[163] Most violations of the internal validity of a study can be classified in three categories; confounding, selection bias, and information bias.

4.1.2.1 Confounding

Confounding can be thought of as a mixture of the effect of the exposure with effects of other factors. Confounding can lead to overestimation, underestimation, and even to a change in the direction of the estimated effect of interest.[164] The clearest form of confounding is seen when the factor is a common cause for both the exposure and the outcome. A factor must fulfill at least two criteria to be regarded as a confounding factor. Firstly, a confounder must be related to the exposure under study, but it must not be affected by the exposure. Secondly, a confounder must be related to the outcome, but not be affected by it. Although both these criteria are necessary, they are not sufficient.[163]

It is important to note that a factor that is associated with both the exposure and the outcome may be mediating the effect of the exposure, and therefore, it is on the causal pathway between the exposure and the outcome, and such mediating factors should not be regarded as confounders. In the assessment of which factors should be regarded as potential confounders, the basis should be prior knowledge about their relations with the exposure and the outcome; in other words, the inclusion of confounders in the analysis should not be assessed on statistical grounds only.[164]

The HUNT2 study had comprehensive clinical, demographic and psychosocial data on each participant, and therefore, we could control for many confounding factors in the statistical analyses. However, we cannot exclude the possibility of uncontrolled confounding, i.e. that factors for which we had no information could have a confounding effect. Nevertheless, any remaining confounder potentially able to

influence our results considerably would need to be strongly associated with both the exposure and the outcome, and be unrelated to the other factors that were included in the analysis.

For example, we had no information on the prevalence of sleep apnea syndrome, which is a possible confounder for the association of insomnia with CVD, since sleep apnea syndrome is a well-established risk factor for cardiovascular disorders.[6] Also, apnea patients often complain about difficulties in initiating or maintaining sleep, and they often suffer from early awakenings.[165] However, the strength of the association between sleep apnea and insomnia is not clear, and it has been suggested that the association could be explained, at least in part, by confounding by age and depression.[165,166] In the analyses, we adjusted for age and depression, as well as for blood pressure and BMI, i.e., for strong correlates of sleep apnea syndrome and CVD. Therefore, it appears unlikely that sleep apnea alone could explain the higher risk for AMI and HF among people with insomnia symptoms.

4.1.2.2 Selection bias

Selection bias is a distortion that results from erroneous selection of the participants in the study.[163,167] The common element of such biases is that the relation of the exposure to the outcome is different for those who participate and those who theoretically would be eligible for the study.

The participation rate in the HUNT study was generally higher than in most other large population studies. However, 31 % of those who were invited did not

participate in HUNT 2. The participation rate among participants 20 to 25 years of age was considerably lower than among older age groups. Therefore, caution is needed, for example, when interpreting our finding concerning the association of metabolic factors and hsCRP among the youngest age group in paper IV. However, a comprehensive non-participation study was conducted after HUNT 2, and the major reason for not participating among younger people was the lack of time.[156] Therefore, there is no reason to believe that a health-related selection mechanism is the reason for the lower participation among the youngest age groups.

Biased follow-up could potentially distort the results of prospective studies, and potentially lead to selection bias (paper I and II). Data on emigration provided by Statistics Norway enabled us to censor participants who moved away from Nord-Trøndelag, however, the population is stable with a low net migration out of the county.[156] Loss to follow-up, which is often a major problem in prospective cohort studies of the general population,[163] is therefore unlikely to play an important role in our studies.

4.1.2.3 Information bias

Information bias occurs whenever the collected information on the participants is erroneous. Such misclassification, either related to exposure or to outcome, can be differential or non-differential. Misclassification of exposure is non-differential if it is unrelated to the outcome; and differential if the misclassification of exposure is dependent on the outcome. Similarly, misclassification of outcomes is non-differential

if unrelated to exposure; and otherwise, it is differential. Differential misclassification can either exaggerate or underestimate an effect. In contrast, non-differential misclassification typically tends to produce estimates of the effect that are closer to the null.[163]

Insomnia symptoms were self-reported and therefore subject to misclassification. Similar to other relevant studies, we did not assess sleep objectively, for example, by performing a polysomnography. However, polysomnography is not routinely used for evaluation of insomnia,[168] because difficulty initiating sleep or maintaining sleep, or non-restorative sleep, cannot be objectively measured. In fact, insomnia may be present even in the absence of any sign of an objective sleep disturbance from a polysomnographic evaluation.[168] Furthermore, we did not rely on a formal diagnosis of insomnia, and in the analyses, we assessed the severity of symptoms both separately, and in combination, in relation to the outcome of interest. However, our evaluation of insomnia symptoms largely reflected the current diagnostic criteria used in the 4th edition of the Diagnostic and Statistical Manual of Mental Disorders.[8]

Prospective cohort studies are generally less prone to biased exposure ascertainment than other study designs. However, during long follow-up periods, measured values at baseline may change. Insomnia was evaluated only once at the beginning of the follow-up; thus, we could not examine the possible effects of time-dependent changes in the severity of insomnia. However, lack of repeated measurements of insomnia cannot possibly explain the observed association of insomnia with the risk of later CVD.

The identification and ascertainment of AMI and HF as outcomes could also be prone to misclassification. However, the overall quality and reliability of the hospital discharge diagnosis of AMI and HF is high in Nordic countries.[158,159] As there is no unequivocal definition of HF, the HF diagnosis in Nordic registers appears slightly less precise than for acute myocardial infarction. To further increase the specificity of the diagnosis we considered only primary diagnoses of HF, as recommended.[158] The reliability of the diagnosis due to deaths of CVD from the Cause of Death Registry is somewhat lower than the reliability of hospital discharge diagnosis, largely because of the low autopsy rates in Norway.[169] However, we obtained similar results by restricting follow-up to hospital confirmed cases of CVD; thus, it seems unlikely that low reliability of CVD deaths could explain the higher risk for CVD among people with insomnia symptoms.

The non-fasting nature of the blood samples could also introduce misclassification. Triglyceride concentrations are especially sensitive to eating whereas total serum cholesterol and HDL cholesterol levels may be less affected.[170] In study IV, we hypothesized that applying stricter criteria, using fasting blood samples, would most likely result in even stronger associations between the respective metabolic factors and hsCRP.

4.2 Generalizability (external validity)

Generalizability refers to whether, and to which degree, our results apply to people outside of the population we studied. We see no relevant biological differences between

the adult population of Nord-Trøndelag and other adult populations that would make the effects of insomnia on CVD risk markedly different in other populations. However, the estimated effects of insomnia may vary between countries because of different latitudes, different underlying rates of AMI and HF and different sleeping/circadian habits in the populations. Moreover, the question related to non-restorative sleep in our studies was restricted to people younger than 70 years of age, and therefore, our results concerning this particular variable, and the cumulative number of insomnia symptoms, cannot readily and directly be generalized to elderly populations.

4.3 Comparison with previous studies

4.3.1 Insomnia and the risk of AMI

In our study, insomnia symptoms were associated with a moderate increase in the risk for AMI. The results were fairly robust in different multivariable models and several sensitivity analyses. Although the observed relative risk of insomnia with AMI was only moderate, insomnia is a frequent, easily recognizable, and potentially manageable condition for most patients. The association of insomnia and AMI risk is in line with a few smaller studies which concentrated on difficulty in initiating and/or maintaining sleep.[120-130]

Several studies have suggested that women are more prone to insomnia than men,[9] and that there is a sex difference related to cardiovascular risk and mortality. In the MONICA study,[122] the association of insomnia with CHD risk was stronger in

women, whereas the opposite was found by Mallon et al.[121] Many previous studies were restricted to either men[120,125,127,128] or women.[126] In the present study, there was no compelling evidence for a sex difference. The relative risk of AMI associated with difficulties initiating sleep and for the cumulative insomnia symptoms was slightly higher in women than in men. However, the difference should be interpreted with caution; it does not necessarily suggest that insomnia is more dangerous for women. Instead, the sex difference might be explained by the lower baseline AMI risk among women.

Prior reports of insomnia and CVD have been criticized for lack of adequate control for depression and anxiety, as well as for failing to include important confounders such as co-morbid chronic somatic disorders.

In the present study, adjustment for depression did not substantially change the estimated associations. This corresponds to the findings of the few studies that could evaluate depressive symptoms in their analyses.[121,122,124] Inclusion of anxiety in our models somewhat strengthened the association of insomnia complaints with AMI risk. This was an unexpected and unexplained result. To the best of our knowledge, no previous study included adjustment for anxiety, and future studies are warranted to confirm or refute our finding.

Previously, one small study attempted to address the possibility that chronic disorders could explain the association between insomnia complaints and risk for AMI.[121] Similar to our findings, there was no evidence in that study suggesting that chronic disorders could explain the observed association. We also addressed the

possibility of reverse causation as an explanation for the observed associations by excluding the first five years of follow-up, but this did not substantially change the results. Therefore, our study, together with most previous studies, seems to support a possible causal role for insomnia in the pathogenesis of CHD.

4.3.2 Insomnia and the risk of HF

We found that insomnia is associated with an increased risk of HF. To our knowledge, this is the largest study to date that has assessed insomnia in relation to the risk of HF.

Previously, the association has been investigated in a few smaller studies.[171,172] In a recent prospective study by Ingelsson et al., 282 out of 2,314 middle-aged men were hospitalized with HF over 30 years of follow-up.[171] Similar to our findings, they reported that difficulties initiating sleep were associated with a moderately increased risk of HF (HR 1.22, 95% CI 1.03-1.43), and difficulties maintaining sleep with a slight increase in risk of HF (HR 1.14, 95% CI 0.96-1.36). The investigators adjusted for several established risk factors for HF, including myocardial infarction during follow-up, but they did not adjust for other somatic disorders or psychological distress. Newman et al. found that daytime sleepiness at baseline predicted incident HF and cardiovascular mortality in 5,888 participants above 65 years of age over a five year follow-up.[172] However, the investigators did not adjust for several important established risk factors for HF.

In contrast to previous studies, which assessed some selected insomnia symptoms, we included all aspects of sleep problems that are typically used to identify

insomnia.[9] Therefore, we could also investigate the joint effect of insomnia symptoms. Similar to previous studies, we found a moderately increased risk related to individual symptoms, but among people with all three insomnia symptoms simultaneously; the risk was particularly high even after adjustment for established cardiovascular risk factors and psychological distress. Our finding may suggest that malfunctioning of some aspects of sleep may be somehow compensated, and the net effect on cardiovascular disease may be limited. For example, having difficulties falling asleep might be compensated by a satisfactory depth and a good continuity of sleep. However, if the initiation of sleep is poor and combined with repeated awakenings and superficial sleep, there may not be any compensatory mechanisms.

CHD is an important risk factor for HF. However, most HF cases are not preceded by a coronary event.[173] In the analyses, we adjusted for a history of AMI at baseline and also for incident AMI during follow-up. These adjustments did not change the association of insomnia with incident HF. Moreover; when we analysed the joint effects of insomnia symptoms, the association of insomnia with incident HF was much stronger than for incident AMI. For example, having all three insomnia symptoms compared with none, was associated with more than three-fold higher risk (317% increased risk) of HF, as compared to less than two-fold higher risk (85% increase) of AMI after adjustment for cardiovascular risk factors. Thus, it seems unlikely that the association between insomnia and HF observed in this study can be due to the association between insomnia and AMI.

Although we found that insomnia is associated with increased risk of heart failure in our study, more population-based studies are needed to better establish the HF

risk associated with insomnia and to reveal the possible underlying pathophysiological mechanisms.

4.3.3 Insomnia and hsCRP

In this large population based study, we found no consistent associations between insomnia symptoms and hsCRP levels in either women or men after controlling for established cardiovascular risk factors, psychosocial distress, chronic pain, and after taking into consideration the role of chronic somatic disorders.

Women are more prone to insomnia,[9] and in general, women have higher hsCRP levels than men.[162,174] Moreover, both experimental studies on the effect of sleep deprivation on inflammatory markers,[94] and studies examining the association of habitual insomnia and inflammation have suggested differences by sex.[152,153] Therefore, it seems plausible that the association between insomnia and inflammation could also differ by sex, and we therefore conducted separate analyses for men and women. Among men, we found that difficulty initiating sleep and having non-restorative sleep were associated with higher hsCRP levels, but only in the age-adjusted models. Among women, we found no associations between insomnia symptoms and hsCRP levels.

Several short-term experimental studies have demonstrated that acute sleep deprivation leads to increased levels of inflammatory markers, including hsCRP.[94-99] However, only a few studies have examined whether chronic sleep problems or habitual insomnia symptoms are associated with inflammation.[152-155]

In a young Finnish birth cohort including 2,104 men and 1,907 women, there was a positive association between an aggregated sleep disturbance variable and hsCRP levels, but the association was restricted to men.[153] The authors excluded participants with depression, but adjusted for several cardiovascular risk factors. In another study, difficulty initiating sleep and having non-restorative sleep were associated with a higher level of hsCRP and IL-6 among 95 healthy non-smoking women without any known chronic disorders.[152] The analyses were adjusted for several cardiovascular risk factors and measures of psychological distress. Among the 115 men in the study, there was no association of sleep disturbances with hsCRP. In two other small studies, including 188 and 43 individuals, difficulty initiating and maintaining sleep and non-restorative sleep were associated with higher hsCRP levels.[154,155] However, in these studies there was no information on important cardiovascular risk factors.

As we found no strong evidence for any association between insomnia and hsCRP, further studies are needed to explore the possible pathophysiological mechanisms linking insomnia to the risk of CVD.

4.3.4 Metabolic factors and hsCRP

Insomnia appears to be associated with an unhealthy lifestyle and with the metabolic syndrome. However, the association of hsCRP and metabolic factors, especially in younger age groups, is not clear from the literature. In our study, we found that all the measured metabolic factors were relatively strongly associated with hsCRP at all ages

both in men and women. Among the metabolic factors, BMI displayed the strongest association.

To our knowledge, we have conducted the largest population-based study on this topic. Only a small number of previous studies have examined metabolic factors and hsCRP, and included both sexes in all age groups.[175-177] Those studies were mainly conducted in middle-aged or older populations, and generally, it was reported relatively higher levels of hsCRP in individuals with metabolic syndrome in those studies. In the NHANES III, 8570 men and women older than 20 years of age were included, but specific findings on the association of metabolic syndrome with hsCRP in young participants were not reported.[175] Another study assessed the association in young adults (24 to 43 years) and found that participants with metabolic syndrome had higher hsCRP levels.[176] Similar to our study, the authors reported that BMI was the component of the metabolic syndrome that was most strongly associated with hsCRP. Similar findings among adolescents have also been reported.[177]

The associations between metabolic factors and hsCRP were slightly stronger in young people (20-34 years) than among older individuals. Other studies have shown strong correlations between the extent of atherosclerosis in adolescence and early adulthood and cardiovascular risk factors, including an adverse lipid profile, high blood pressure, and cigarette smoking, that are strongly predictive of the severity of atherosclerosis later in life.[33,178,179] As atherosclerotic plaques at a young age are substantially less active than later in life, our findings may be regarded as indirect evidence against the hypothesis of reverse causality, i.e. the hypothesis that cytokines may leak from atherosclerotic plaques into the circulation with subsequent hepatic

synthesis of CRP (see Figure 1 on page 18). In contrast, it supports the hypothesis that metabolic factors may in themselves cause higher hsCRP. Thus, CRP may not only be a bystander in the relationship between the established risk factors and CVD.

However, as our study is cross-sectional, the temporal relation between the development of the metabolic syndrome and hsCRP cannot be inferred. Therefore, the clinical usefulness of our findings in relation to coronary risk stratification of young adults remains to be addressed in prospective studies.

5 Conclusion

In this thesis we investigated the interrelations between insomnia, metabolic syndrome and inflammation, and the relation of these factors to the risk of CVD. Our findings are summarized in Figure 5.

Our data support the potential importance of insomnia in the development of AMI and HF, two of the most common forms of CVD. Insomnia is a frequent, easily recognizable, and potentially manageable condition for most patients. However, especially the association of insomnia with increased risk of HF needs to be confirmed in other prospective population-based studies. Clinical trials are also needed to determine whether improving sleep may improve cardiovascular risk factor levels and outcomes. If our results are supported in other studies and causation is proved, evaluation of insomnia symptoms should be included in the clinical risk assessment and could be useful in cardiovascular prevention.

We tried to explore possible pathophysiological mechanisms behind the association of insomnia with CVD risk. We found no association of insomnia symptoms with hsCRP levels after controlling for established cardiovascular risk factors, psychosocial distress, chronic pain, and after taking into consideration the role of chronic somatic disorders. Therefore, our results do not support that inflammation, as reflected by elevated levels of hsCRP, is an important factor linking insomnia to CVD. It is largely established that insomnia is associated with an unhealthy lifestyle and with the metabolic syndrome. However, the association of hsCRP and metabolic factors is not clear from the literature. We found a relatively strong association of metabolic factors with hsCRP levels at all ages, and both in men and women. As atherosclerotic plaques at a young age are substantially less active than later in life, our findings may be regarded as indirect evidence against the reverse causation hypothesis, i.e. the increased hsCRP levels are only caused by active atherosclerotic plaques. Our results support the hypothesis that the metabolic factors in themselves may cause increased levels of hsCRP. Thus, CRP may not only be a bystander in the relationship between the established risk factors and CVD. Although our findings shed further light on the complex interrelation between insomnia and CVD, further studies are needed to disentangle the complex web of surrogates, correlates and causative factors.

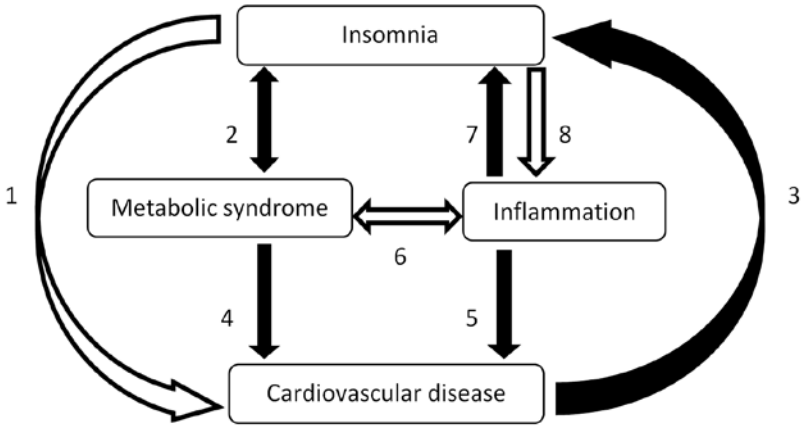


Figure 5. Suggested interrelationships between insomnia, metabolic syndrome, inflammation, and cardiovascular disease. White arrows refer to associations proposed and investigated in this thesis. Black arrows indicate established relationships, which are not examined here.

1. Insomnia might increase CVD risk: supported by our results.
2. Insomnia and the metabolic syndrome are related: relatively well-established in previous studies, not investigated in this thesis.
3. CVD causes insomnia: established in previous studies, not investigated in this thesis.
4. Metabolic syndrome increases CVD risk: well-established, not investigated in this thesis.
5. Inflammation increases CVD risk: well-established, not investigated here.
6. The metabolic syndrome and inflammation are related: supported by our findings.
7. and 8. Inflammation and insomnia are related: not confirmed by our results.

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