Sleep Duration, Cardiometabolic disease and Mortality: Proteomic links with incident diabetes

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Sleep Duration, Cardiometabolic Disease and Mortality

Proteomic links with incident diabetes

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This thesis identifies an association between sleep duration and incident diabetes and incident coronary heart disease (CHD), respectively, and confirms that incident diabetes is a primary risk factor that explains the association between sleep duration and incident CHD. Moreover, this thesis identifies proteomic markers that are associated with sleep duration, and further suggests a biological pathway that links short sleep duration (defined as sleep durations shorter than a referent category) with incident diabetes. One paper in this thesis quantified, in a large-scale analysis on East Asian populations, the sex-specific associations between sleep duration and cardiovascular disease mortality, and the age-specific associations between sleep duration and all-cause mortality thereby contributing to the understanding of how to consider effect modification in population-based studies on sleep duration and mortality.

THOMAS SVENSSON is a medical doctor working as a researcher at the Department of Clinical Sciences Malmö, Lund University. His main research interest is sleep and its association with incident diabetes and coronary heart disease in primarily healthy individuals. Through his research, he aims to identify the biological mechanisms linking aberrant sleep with adverse health outcomes. This knowledge would enable the identification of individuals who would benefit most from personalized lifestyle modifications.

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Thomas Svensson

DOCTORAL DISSERTATION

Doctoral dissertation for the degree of Doctor of Philosophy (PhD) at the Faculty of Medicine at Lund University to be publicly defended on 3 of September at 13.00 in Jubileumsaulan, SUS, Malmö

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Abstract:

Background

Noncommunicable disease, in particular cardiovascular disease (CVD) and diabetes are a major cause of morbidity and mortality in the world. Short and long sleep durations are associated with adverse health outcomes, however, the biological mechanisms for these associations are not fully elucidated. The aim of this thesis is to investigate the association between sleep duration and proteomic markers, respectively, and their associations with incident diabetes, incident coronary heart disease (CHD) and mortality outcomes.

Method

Participants were from the Malmö Diet and Cancer (MDC) study, the MDC cardiovascular cohort (MDC-CC) from the Asia Cohort Consortium (ACC). Paper I investigated the sex-specific associations between sleep duration and incident diabetes and incident CHD in the MDC. Paper II investigated the association between sleep duration and Caspase-8, a proteomic marker of apoptosis, and their respective associations with incident diabetes using the MDC-CC. Paper III investigated the association between sleep duration and mortality outcomes using ACC data. Paper IV investigated the association between sleep duration, proteomic markers, and their respective associations with incident diabetes and incident CHD.

Results

Paper I: sleep durations were associated with incident CHD preceded by incident diabetes in both men and women. Paper II: sleep duration <6 hours was associated with Caspase-8; Caspase-8 was positively associated with incident diabetes and Caspase-8 modified the association between sleep duration and incident diabetes. Paper III: sleep duration was associated with mortality outcomes in both men and women. These associations were modified by age (all-cause mortality) and sex (CVD mortality). Paper IV identified 16 proteomic markers associated with sleep duration quintiles 1, 2, 4, 5 when compared to quintile 3. When combining these proteomic markers to proteomic scores, the score for sleep duration quintile 1 was associated with incident diabetes but not with incident CHD.

Discussion

This thesis suggests a pathway in which short sleep duration through inflammation and apoptotic activity is associated with incident diabetes which in turn increases the risk of future CHD.

Key words: Sleep duration, incident diabetes, incident coronary heart disease, mortality, cardiovascular disease, cardiometabolic disease, prospective cohort study, proteomic markers

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For Aco, Leo, Luca, and Mamusia.
Sleep well and dream big.
Then, in every waking moment,
chase those dreams with all your heart.

Table of Contents

Abbreviations

ADM	Adrenomedullin
AIC	Akaike information criterion
ANS	Autonomic nervous system
BMI	Body mass index
CABG	Coronary artery bypass graft
CAD	Coronary artery disease
CHD	Coronary heart disease
CRP	C-reactive protein
CVD	Cardiovascular disease
CXCL1	C-X-C motif chemokine 1
Caspase-8	Cysteinyl aspartic acid-protease-8
DNA	Deoxyribonucleic acid
Fas	Tumour necrosis factor receptor superfamily member 6
HDL	High-density lipoprotein
HSP27	Heat shock 27 kDa protein
IHD	Ischemic heart disease
JPHC	Japan Public Health Center-based
KIM	Kidney injury molecule
KMCC	Korean Multi-Center Cancer Cohort Study
ICD	International classification of diseases
IL-6	Interleukin-6
LDL	Low-density lipoprotein
MMP-7	Matrix metalloproteinase-7
MMP-9	Matrix metalloproteinase-9

MMP-10	Matrix metalloproteinase-10
MDC	Malmö Diet and Cancer
MDC-CC	Malmö Diet and Cancer Cardiovascular Cohort
MI	Myocardial infarction
NREM	Non-rapid eye movement
OECD	Organisation for Economic Co-operation and Development
OSA	Obstructive sleep apnoea
PCI	Percutaneous coronary intervention
PCR	Polymerase chain reaction
PEA	Proximity extension assay
PSG	Polysomnography
RANKL	Receptor activator of nuclear factor-kb ligand
REM	Rapid eye movement
SCHS	Singapore Chinese Health Study
SD	Standard deviation
SMHS	Shanghai Men's Health Study
SWHS	Shanghai Women's Health Study
TG	Triglycerides
TNF-α	Tumour necrosis factor-α
TNFR1	Tumour necrosis factor receptor 1
t-PA	Tissue-type plasminogen activator
TRAIL	Tumour necrosis factor-related apoptosis-inducing ligand
TRAIL-R2	Tumour necrosis factor-related apoptosis-inducing ligand receptor 2
TRANCE	Tumour necrosis factor-related activation-induced cytokine
TST	Total sleep time
UPAR	Urokinase receptor

List of papers

Paper I

Svensson AK, Svensson T, Kitlinski M, Almgren P, Engström G, Nilsson P, Melander O. Incident diabetes mellitus may explain the association between sleep duration and incident coronary heart disease. Diabetologia. 2018;61(2):331-41.

Paper II

Svensson T, Svensson AK, Kitlinski M, Almgren P, Engström G, Nilsson J, Orho-Melander M, Nilsson P, Melander O. Plasma Concentration of Caspase-8 Is Associated With Short Sleep Duration and the Risk of Incident Diabetes Mellitus. J Clin Endocrinol Metab. 2018;103(4):1592-600.

Paper III

Svensson T, Saito E, Svensson AK, Melander O, Orho-Melander M, Mimura M, Rahman S, Sawada N, Koh WP, Shu XO, Tsuji I, Kanemura S, Park SK, Nagata C, Tsugane S, Cai H, Yuan JM, Matsuyama S, Sugawara Y, Wada K, Yoo KY, Chia KS, Boffetta P, Ahsan H, Zheng W, Kang D, Potter JD, Inoue M. Association of Sleep Duration With All- and Major-Cause Mortality Among Adults in Japan, China, Singapore, and Korea. JAMA Netw Open. 2021;4(9):e2122837.

Paper IV

Svensson T, Svensson AK, Kitlinski M, Engstrom G, Nilsson J, Orho-Melander M, Nilsson PM, Melander O. Very short sleep duration reveals a proteomic fingerprint that is selectively associated with incident diabetes mellitus but not with incident coronary heart disease: a cohort study. BMC Med. 2024;22(1):173.

Papers not included in the thesis

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2. Svensson T, Inoue M, Saito E, Sawada N, Hiroyasu I, Mizoue T, Goto A, Yamaji T, Shimazu T, Iwasaki M, and Tsugane S. The Association Between Habitual Sleep Duration and Mortality According to Sex and Age: The Japan Public Health Center-based Prospective Study. J Epidemiol. 2021;31(2):109-18.
3. Meguro K*, Svensson T*, Chung U-i, Svensson AK. *Both authors contributed equally to the study. Associations of Work-related Stress and Total Sleep Time with Cholesterol Levels in an Occupational Cohort of Japanese Office Workers. J Occup Health. 2021;63(1):e12275.
4. Wilunda C, Abe SK, Svensson T, Sawada N, Tsugane S, Wada K, Nagata C, Kimura T, Tamakoshi A, Sugawara Y, Tsuji I, Ito H, Kitamura T, Sakata R, Mizoue T, Matsuro K, Tanaka K, Lin Y, Inoue M. Sleep duration and risk of cancer incidence and mortality: A pooled analysis of six population-based cohorts in Japan. Int J Cancer. 2022;151(7):1068-80.
5. Svensson T, Madhawa K, Hoang NT, Chung U-i, Svensson AK. Validity and reliability of the Oura Ring Generation 3 (Gen3) with Oura sleep staging algorithm 2.0 (OSSA 2.0) when compared to multi-night ambulatory polysomnography: A validation study of 96 participants and 421,045 epochs. Sleep Medicine. 2024;115:251-63.

Introduction

Burden of major noncommunicable diseases

Noncommunicable diseases constitute a major cause of morbidity and mortality in the world. In 2019, ischemic heart disease (IHD), stroke, and diabetes ranked second, third, and eight among the leading global causes of disability-adjusted life years for all ages(1). Notably, in the age group 50-74, these three causes ranked first, second, and third, respectively(1). In 2021, the number of global deaths attributed to IHD, stroke and diabetes was 8.99 million(2), 7.25 million(3), and 1.66 million(4), respectively. The corresponding numbers for global incident cases of IHD, stroke, and diabetes was 31.9 million(2), 11.9 million(3), and 24.4 million(4), respectively.

IHD and stroke are the two leading causes of the total global burden of cardiovascular disease (CVD)(5). Since the 1960s, the improvement of life expectancy in western Europe, North America, and Japan has predominantly been attributed to reductions in CVD mortality(6). Recent findings, however, show that the declining trends in CVD mortality in high-income countries have slowed down with a possibility of CVD-related mortality rates increasing in the years to come(7). One possible explanation for this reversal are the rising levels of disease-related risk factors. Indeed, this would very well match the disproportionately large increase in the global burden of diabetes between 1990 to 2019(1).

Cardiometabolic disease

A major reason for CVD and diabetes to be grouped together in rankings as well as co-occurring in patients is their sharing of many underlying risk factors. The term “cardiometabolic disease” can therefore be used as a comprehensive term to include coronary artery disease (CAD) (one of the underlying causes of IHD(8)), stroke, and diabetes.

CAD refers to the process of atherosclerotic formation in the coronary arteries involving lesions to the inner lining of the arterial wall, inflammation, accumulation of lipoproteins, oxidation, haemodynamic alterations, and subsequent formation of plaques(9, 10). Atherosclerotic plaques may undergo erosion or subsequent rupture which in turn may lead to fatal or non-fatal myocardial infarction (MI) or stroke.

Major risk factors for CAD include obesity(11), hypertension(12), dyslipidaemia(13), and insulin resistance(14).

Type 2 diabetes mellitus constitutes approximately 96% of prevalent diabetes cases in the world(15). It is a progressive disease characterised by hyperglycaemia in which the early stages are marked by insulin resistance during which insulin-producing beta cells maintain glucose homeostasis by compensating for the increased insulin demand through increased insulin secretion(16). Later stages of the process involve reduced insulin secretion from the beta cells which results in impaired glucose tolerance and subsequently leads to diabetes(16). Major risk factors for diabetes include obesity(11) and physical inactivity(17).

Prevention of cardiometabolic disease

Lifestyle-related factors, including limited physical activity, and poor dietary habits account for a large proportion of the underlying risk factors of cardiometabolic disease. A suboptimal diet may be attributed to 70.3% of new cases of type 2 diabetes(18), whereas the population attributable risks of regular physical activity and daily consumption of fruits and vegetables on acute MI may be in the region of 12.2% and 13,7%, respectively(19). Moreover, physical activity has a positive effect on reducing blood pressure(20), is associated with favourable changes in triglycerides (TG) and high-density lipoprotein (HDL) cholesterol(21), and exercise interventions reduce weight, body mass index (BMI) and visceral fat(22), all of which are risk-factors in the context of cardiometabolic disease.

As mentioned in the previous section, the outlook for the next couple of years with regards to CVD mortality in high-income countries is one of increasing trends. Many countries that have decelerated the reduction of CVD-related mortality have done so without reaching the low levels observed in Japan(7) thereby indicating that additional reduction may be possible.

The last couple of decades have seen a global increase in mean BMI and prevalent obesity(23), and diabetes(15). It is projected that by 2035 the number of adults with a BMI$\geq$30 kg/m^2 in the world will reach 1.53 billion(24), and that by 2050 the number of diabetes cases in the world will reach 1.31 billion(15).

Considering the detrimental implications of a continuation of the trends of the trajectories of cardiometabolic disease and its related risk factors over the next years, it is imperative to identify additional lifestyle-related factors that may be associated with cardiometabolic disease, and to attempt to identify and propose biological mechanisms for such associations.

Sleep

Sleep is a fundamental physiological requirement in all mammals. The amount of required sleep during a 24-hour period differs between mammalian species, but lies in the range between 2 – 20 hours and depends on specific characteristics, for example body weight(25). In humans, total sleep time (TST), i.e., the time spent asleep during a sleep episode can be considered in the context of age where children generally require more sleep than adults, who in turn sleep longer than the elderly (Figure 1)(26). Other age-related differences are also noted for specific sleep parameters as detailed below. The adult human sleep requirement is between 6-9 hours per 24-hour period and over the course of a lifetime the time spent asleep amounts to approximately one third of a human's life. Although the exact function of sleep remains to be fully elucidated, the biological purpose of sleep may be related to neocortical maintenance, neurogenesis, energy conservation, and thermoregulation(25, 27).

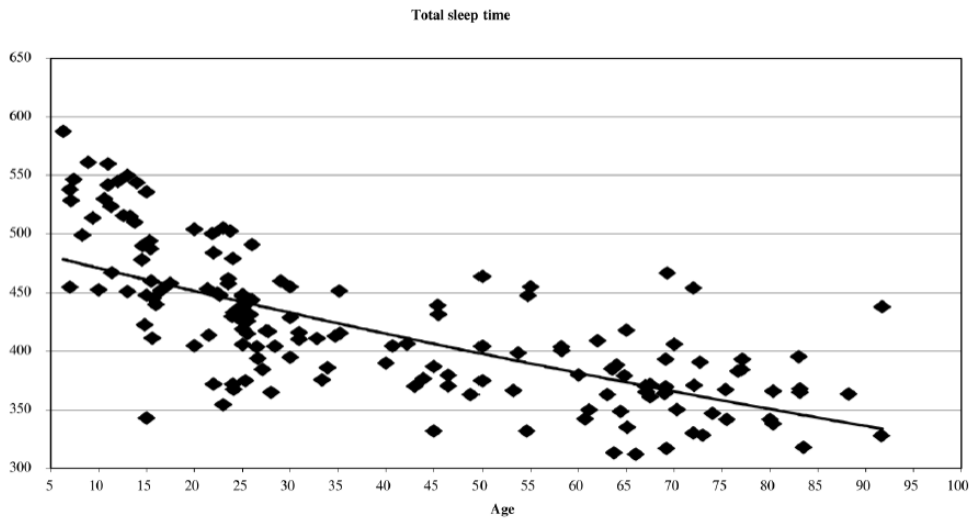

Figure 1. Age-related trends for total sleep time (minutes). Reproduced from Maurice M. Ohayon et al. Meta-Analysis of Quantitative Sleep Parameters From Childhood to Old Age in Healthy Individuals: Developing Normative Sleep Values Across the Human Lifespan. Sleep. 2004;27(7):1255-73 by permission of Oxford University Press on behalf of the Sleep Research Society.

Human sleep is characterised by two distinct phases, non-rapid eye movement (NREM) comprised of sleep stages N1, N2, and N3, and rapid eye movement (REM) sleep comprised of Stage R. The characteristics of the different sleep stages are determined by electroencephalographic, electrooculographic and submental electromyographic activity obtained during polysomnography (PSG). PSG is the collective term for the study and assessment of multiple physiological signals related to sleep, additionally including electrocardiogram (ECG), as well as the

monitoring of airflow, respiratory effort, and oxygen saturation(28). In summary, NREM consists of stages N1, N2, and N3 which can be equated to the depth of sleep (where N1 represents the lightest and N3 the deepest sleep). REM (or R) sleep is characterised by muscle atonia, increased parasympathetic activity and increased temperature compared to NREM sleep. During the course of the typical night, healthy sleepers cycle through the four sleep stages (N1-N3 and R) with each cycle lasting approximately 90 minutes. Although NREM sleep constitutes the largest phase of the night-time sleep episode, the time spent in each of the sleep stages differs between different cycles over the course of the night. Sleep cycles at the start of a sleep episode consist of more sleep in Stage N3 than sleep cycles closer to wake-up, whereas the opposite is true for the time spent in Stage R(29). The proportion of time spent in Stage N3 and Stage R decreases with age, whereas he proportion of time spent in Stage N1 and N2 increases with age (Figure 2)(26). Age-related differences in sleep patterns may be explained by moderating variables such as study methodology (data collection method), physical or mental illness, or external factors such as timing of data collection (e.g., school day vs non-school day in adolescents)(26).

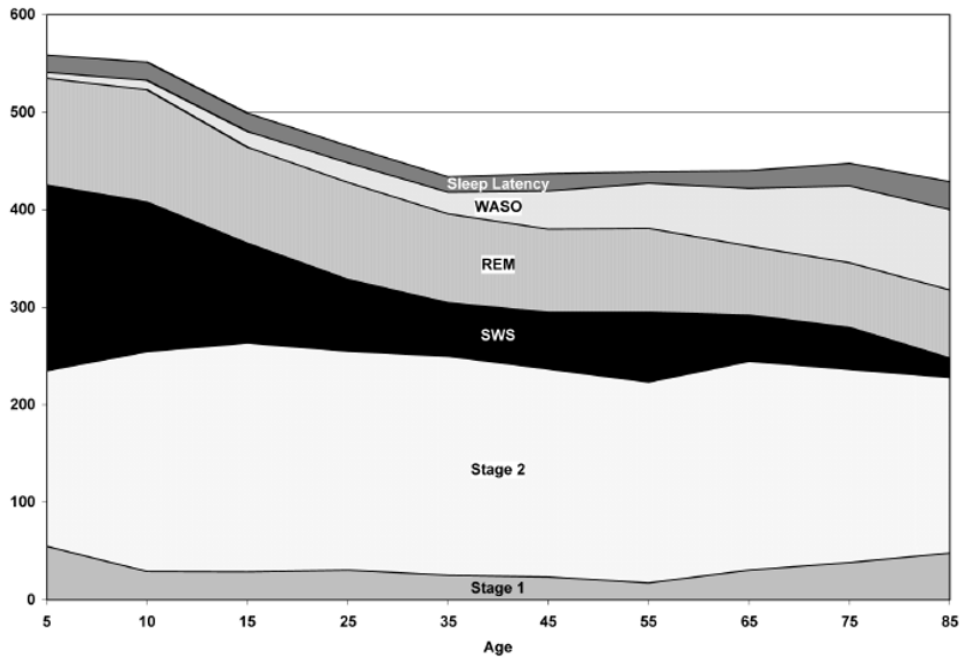

Figure 2. Age-related trends for stage 1 sleep, stage 2 sleep, slow wave sleep (SWS) equivalent to stage 3 sleep, rapid eye movement (REM) sleep, wake after sleep onset (WASO), and sleep latency in minutes. Reproduced from Maurice M. Ohayon et al. Meta-Analysis of Quantitative Sleep Parameters From Childhood to Old Age in Healthy Individuals: Developing Normative Sleep Values Across the Human Lifespan. Sleep. 2004;27(7):1255-73 by permission of Oxford University Press on behalf of the Sleep Research Society.

Measuring sleep time in epidemiological studies

Polysomnography

Based on PSG studies, the indicator for time spent asleep is TST which is the quantification of time spent in any of the sleep stages (N1-N3 and R) during the course of the sleep period. TST differs from sleep period time (SPT) which is the time counted from falling asleep to waking up, i.e., TST plus any periods of wake that occurred during the sleep episode. Although the value of PSG studies to diagnose sleep-related conditions such as obstructive sleep apnoea (OSA) in the clinical setting cannot be overstated, the use of PSG in population-based epidemiological research is limited for several reasons; PSG requires the involvement of specialists to set up equipment and interpret the results, is performed in settings that may not represent the participant's usual sleep environment, is often performed for a single night, and may even impact sleep and thus not be representative of a participant's habitual sleep.

Actigraphy

In settings where objectively measured sleep parameters over several days are of interest, the method of choice would be actigraphy(30). Actigraphs are wrist-born devices that rely on accelerometers to register movement during sleep which can be used to quantify sleep and wake time. Validation studies comparing actigraphy with PSG have found that actigraphy has a high sensitivity but lower specificity, i.e., they perform better at detecting sleep than they do at detecting periods of wake(31). Despite the benefits of actigraphy, for example their cost-effectiveness when compared to PSG, their ability to register sleep over several days, and the possibility for study participants to measure sleep in a natural environment, the use of actigraphs in large-scale population-based epidemiological studies that include tens-of thousands of participants is limited. Such large-scale acquisition, technical setting, participant equipment, and data processing would be too resource demanding.

Self-report questionnaires and interviews

Contrary to the detailed sleep/wake parameters that can be measured using PSG or actigraphy, self-reported sleep time is often denoted as sleep duration. The majority of large population-based cohorts have acquired information about participants' sleep duration using self-report questionnaires or through interviews that often ask for habitual sleep time at night or over a 24-hour period. This question prompts a response that considers the duration between a participant's falling asleep-time and waking up-time, and therefore, when compared to the parameters that can be obtained using objective measures, most closely equates to SPT. Self-reported sleep duration may result in an overestimation, in particular among those who sleep very little(32). Nevertheless, the exposure is suitable for epidemiological research, in particular in very large longitudinal population-based studies that aim to explore the

associations between sleep duration and health outcomes over time. Indeed, sleep duration is emerging as a novel risk factor for a number of health outcomes, including mortality and incident cardiometabolic disease.

Sleep and physiology

Sleep has a profound impact on several systems in the body, including the autonomic nervous system (ANS) and the endocrine system. The resulting physiological changes of sleep vary during different sleep states, i.e., during NREM sleep and REM sleep, respectively. For example, during NREM sleep there is an increase in parasympathetic tone and a decrease in sympathetic nervous system activity resulting in reduced heart rate, blood pressure, and respiratory rate(33). Conversely, during REM sleep, the physiological responses to changes in ANS activity owing to periodic activation of the sympathetic nervous system may be noted as fluctuating heart rate, blood pressure, and respiratory rate(33).

The function of the endocrine system is more complicated as neuroendocrine secretion is not only dependent on sleep but also involves the circadian clock(33). The secretion of certain hormones, e.g., growth hormone and prolactin peaks during sleep whereas the secretion of other hormones, e.g., thyroid-stimulating hormone is inhibited during sleep(33). An example of a hormone with circadian rhythmicity includes cortisol with peak secretion around 7am - 8am(34). Disruption of circadian rhythms, for example through circadian misalignment under experimental conditions, is associated with a reduction in leptin, a hormone secreted during nutritional sufficiency(35), as well as increased levels of plasma glucose and insulin suggesting decreased insulin sensitivity(36). In addition to effects on insulin sensitivity, sleep restriction and circadian misalignment results in increased levels of C-reactive protein (CRP), a marker of inflammation and cardiovascular risk(37).

It is important to note that circadian misalignment is not equivalent to aberrant sleep durations; circadian misalignment can occur with no changes in TST, whereas short and long sleep durations can occur in individuals/groups that are not under environmental influences (e.g., shift work or jet lag) leading to circadian misalignment.

Global sleep duration surveys and sleep duration trends over time

The Organisation for Economic Co-operation and Development (OECD)'s time-use survey of participants aged 15-64 years indicates marked differences in reported sleep durations between nationals of different countries(38); respondents from Japan reported the shortest sleep duration with 7.4 hours whereas South Africa respondents reported the longest sleep duration at 9.2 hours. Indeed, sleep practices can be said to occur in a cultural context(39) within specific norms and expectations.

One example of such a culture-specific sleep practice is the afternoon "siesta" which is part of the sleep culture in the Mediterranean and in Central and South America, or the "inemuri" which is the unintentional daytime nap in Japan that often occurs in public settings(40). Another example of culture-specific sleep practices highlights the opinions among many Japanese families that high-school students have to give up sleep in order to study(40), which is not the prevailing opinion in many other industrialised societies. Indeed, in a study comparing Japanese and Canadian university students, Japanese students recorded shorter sleep durations, idealised a shorter personal sleep duration, while recognising a shorter ideal sleep duration from a cultural perspective(41).

Human night-time sleep patterns may have changed over time. Although one study on pre-industrialized equatorial societies showed that nocturnal sleep occurred unsegmented(42), there are other indications suggesting that pre-industrialised societies, including pre-industrial Europe, relied on bimodal sleep(43). Moreover, it has been argued that the aspirations towards one consolidated nightly sleep episode are the consequences of technological advancements, including artificial lights(43, 44) and may even be "unnatural"(44). Although such discussions are beyond the scope of the present thesis, it would be of interest to review if habitual sleep durations have changed over recent years or decades. Indeed, a recent systematic review(45), based predominantly on time-use surveys, found that trends in sleep duration differ by country; over a period of several decades some countries (e.g., Austria, Belgium, Finland, Germany, Japan and Russia) have decreased sleep durations, whereas other countries (e.g., Britain, Bulgaria, Canada, France, Korea, Netherlands, and Poland) have increased sleep durations. The largest decrease was noted in Japan with approximately 24 minutes per day whereas the largest increase was noted in Bulgaria with approximately 7.8 hours per week. If sleep is instead categorised into short (≤6 hours) and long (>9 hours) sleep durations, one study focusing on secular changes of time-use surveys in 10 countries indicated that the prevalence of short sleep had increased in two countries (Italy and Norway), and decreased in three countries (Sweden, the United Kingdom and the United States)(46). Conversely, long sleep durations had increased in 5 countries (Australia, Finland, Sweden, the United Kingdom, and the United States) and decreased in two countries (Canada and Italy)(46).

Two recent longitudinal studies indicate trends of decreasing sleep duration over time in China, where sleep duration decreased from 8.2 hours to 7.8 hours over a period of 11 years(47), and in Finland, where only slight decreases in sleep duration among men (from 7.57 hours to 7.39 hours) and women (from 7.69 hours to 7.37 hours) were noted between the years 1975 and 2011(48). A large study of US adults found decreasing mean sleep durations between 1985 (7.4 hours) and 2012 (7.2 hours) with no significant change between 2004 and 2012(49). However, the questions asking about habitual sleep duration in these studies may be more relevant than time-use surveys given that they are more similar to the exposures used in the

studies investigating associations between sleep duration and health outcomes(50), as further detailed below. The underlying causes for any trends indicating shorter habitual sleep durations over time could be due to changes in employment, including longer working hours, caffeine intake, or the use of technology (e.g., television or artificial lighting) before bedtime(50). It could also be due to ageing populations (where older individuals report shorter sleep durations), or even cohort effects.

Sleep duration and health outcomes

Sleep duration and mortality

In 1964, E Cuyler Hammond published the pioneer article showing that there is an association between sleep duration and all-cause mortality(51). The analyses were based on a prospective study that included over 1,000,000 participants who had answered questionnaires about lifestyle factors. The study was thereby not only the first to investigate sleep duration and its association with mortality, but it remains to date one of the largest studies to investigate this association. Considering the large study sample, Hammond was able to categorise sleep into eight hourly sleep duration categories ranging from less than 4 hours of sleep to more than 10 hours of sleep per night, and to calculate the mortality rates for each of the categories. The overall conclusion was that individuals with approximately 7 hours of sleep per night presented with the lowest mortality rates. Moreover, those with 10 or more hours of sleep per night had above-average mortality rates and those with less than 5 hours of sleep had the highest mortality rates.

The study by Hammond, despite its many strengths, was limited by its univariable analyses. Although the study considered stratification by age, it did not consider other important factors as confounders for the association, for example sex, past medical history, physical activity, or other lifestyle factors. A number of studies have been published since Hammond's pioneer study that do take into account many of the factors that are relevant for the association between sleep duration and mortality outcomes.

Sleep duration and all-cause mortality

Subsequent prospective studies indicate that both short and long sleep durations are overall significantly and positively associated with all-cause mortality. In particular, short sleep duration, i.e., sleep duration shorter than a referent value, shows a wide range of effect sizes depending on the study with hazard ratios ranging between 1.11–2.62(52-70) when compared to the referent sleep duration category.

Similar significant and positive associations exist also for long sleep duration when compared to a referent sleep duration, with hazard ratios ranging between 1.13–2.88(52-55, 58-65, 67-84).

Notably, although there are studies that report no significant associations between short(71-76, 78-87) or long(57, 66, 85-87) sleep durations with all-cause mortality, the results of several meta-analyses indicate that both short(88-93) and long(88, 90-94) sleep durations should be considered as lifestyle risk factors with regards to all-cause mortality outcomes.

Sleep duration and mortality from cardiovascular disease

Research on associations between sleep duration and mortality has included also cause-specific mortality outcomes, including CVD. Short sleep duration is associated with increased risk of mortality from IHD(75, 85), CHD(68, 95, 96), heart disease(54), stroke (64, 75, 97) and CVD (53, 58, 60, 64, 70). Long sleep duration is also associated with increased risk of mortality from CHD(95, 96), heart disease (54), stroke(68, 70, 97), cerebrovascular disease(59), and CVD (58, 60, 68, 69, 76, 80, 81, 83).

There are also prospective studies that find no associations between short sleep duration and mortality from stroke (68), cerebrovascular disease(59), or CVD (57, 69, 76, 80, 81, 83, 84). Similarly, there are studies that report no association between long sleep duration and mortality from IHD (85), CHD (68) or CVD (53, 57, 64, 84).

Irrespectively, meta-analyses show that both short and long sleep durations(91) or that long but not short sleep duration(88, 93) are associated with CVD mortality. For CVD-specific causes, both short and long sleep durations are associated with mortality from stroke and CHD (91).

Limitations of existing studies investigating sleep duration and mortality outcomes

Prospective studies investigating the associations between sleep duration and all-cause, or cause-specific mortality outcomes have considered similar referent sleep duration categories. Most studies consider 7 hours(51-53, 55, 56, 58, 59, 61, 63-69, 72, 73, 75, 79, 81, 83, 86, 87, 95, 97), 7-8 hours(54, 57, 60, 70, 71, 76, 82, 84), or 7-9 hours(74) as the referent sleep duration to which other sleep duration categories are compared. However, alternative referent categories have also been used, e.g., 6-7 hours(85) or <10 hours(77). Despite the overall consistency between studies, several limitations remain to be considered.

Varying definitions of short and long sleep durations

One limitation are the inter-study differences in what constitutes short and long sleep durations, respectively. The definitions of short sleep duration vary from <4 hours(96), ≤4 hours(84), ≤5 hours(52, 62, 67, 69, 81, 95, 97), <6 hours(53, 55, 78, 80, 83, 85, 86),≤6 hours(60, 75, 87), <7 hours(56, 57, 61, 65, 68, 71, 74), and ≤7

hours(70) whereas the definitions of long sleep duration vary from >7 hours(56, 65, 68), ≥8 hours(69, 78), >8 hours(53, 57, 80, 86, 96), ≥9 hours(52, 55, 64, 67, 70, 71, 83, 84, 87, 95, 97), >9 hours(74, 85), and ≥10 hours(62, 81). Some studies use the terms short sleep duration(63, 79) or long sleep duration(63, 75, 79) without prior definition whereas other studies consider separate definitions for men and women(76) and yet other studies use different definitions of long sleep duration even within the same study(61). Consequently, the term short sleep duration in one study is equivalent to a referent sleep duration in another study with the same limitation being true for definitions of long sleep duration. This heterogeneity between studies constitutes a problem when aiming to consolidate findings with the purpose of defining sleep duration recommendation guidelines.

Variations in follow-up time and sample size

Another limitation is the large variations in follow-up time. Of the prospective studies mentioned above, follow-up ranges from 3 years(62) to 35 years(60). A very short follow-up time does not allow for a sufficient number of outcome events unless the sample size is very large. For example, of the prospective studies above, sample sizes range from 724 participants(56) to over 1,000,000 participants(51, 59) which is the third limitation of consideration. Indeed, approximately 50% of the above studies have sample sizes smaller than 15,000 participants(54-57, 61, 62, 64, 66, 67, 71-74, 76-78, 80-83, 85, 86) and only 20% of the above studies analysed samples that were larger than 100,000 participants(51, 52, 58, 59, 65, 70, 87, 96).

Reverse causation bias

A fourth limitation is the high probability of reverse causation bias; whereas certain studies have adjusted for prevalent disease at baseline or consider analyses stratified by prevalent disease(55, 56, 59, 61, 64, 65, 67, 69, 71-78, 80, 81, 86, 87, 96, 98), this approach may question certain statistical assumptions including the one of proportional hazards given the high risk of mortality with past history of CVD, cancer or diabetes. It would therefore be more prudent to exclude participants with prevalent disease at baseline either in the main analysis or in sensitivity analyses(52-54, 58, 60, 63, 66, 68, 79, 83-85, 95).

Moreover, many studies do not consider the exclusion of participants who died within a certain pre-determined follow-up period(56, 57, 59, 60, 63, 64, 67, 70, 71, 74, 75, 78, 80, 83-87) which increases the risk of reverse causation bias.

Effect modification

A fifth limitation is that the association between sleep duration and mortality outcomes seems to differ between men and women(52) and between younger and older age groups(69). However, of the studies that performed analyses stratified by sex(52, 54, 56, 57, 59-61, 64-68, 76, 77, 81, 83, 84) or age(60, 61, 64, 67, 69, 84, 98), only four studies(61, 67, 69, 84) considered all the necessary statistical tests to

confirm if indeed such stratified analyses were justified. Some results suggest that the association between sleep duration and all-cause mortality does not differ by sex(58, 61, 63, 71, 72, 84, 86) or age(61, 71, 84). However, the results of meta-analytic studies suggests a possibly stronger effect of long sleep duration on all-cause mortality among older participants(92). Finally, there appear to be differences in results from East Asian populations when compared to results obtained from European or North American populations(92).

Taken together, the combination of limitations of the population-based studies investigating the association between sleep duration and mortality outcomes preclude the investigation of effect modification by important characteristics without the risk of reverse causation bias.

Sleep duration and cardiometabolic risk factors

Sleep duration is associated with several cardiometabolic risk factors, including BMI and obesity(99), hypertension(100), and impaired glucose tolerance(101). Indeed, results from one meta-analysis, albeit on cross-sectional studies, indicate that sleep durations <5 hours are positively associated with obesity and where incremental hourly increases of sleep duration are inversely associated with BMI(99). Similarly, a meta-analysis on the association between sleep duration and hypertension in prospective studies found that sleep durations <5 hours and <6 hours, respectively, (when compared to 7 hours) are associated with hypertension(100). The same study also reported that incremental hourly increases of sleep duration were inversely associated with hypertension(100). Although the association between sleep duration and impaired glucose tolerance is yet to be quantified in a meta-analysis, independent studies show that sleep duration <6 hours (when compared to 6-7 hours)(101), sleep durations of 6 hours and ≥9 hours (when compared to 7-8 hours)(102) are associated with impaired glucose tolerance. Sleep duration <6 hours (when compared to 6-8 hours) is also associated with impaired fasting glucose(103). The combination of associations between sleep duration and cardiometabolic risk factors prompts the further question if indeed sleep duration is associated with incident diabetes and incident CHD.

Sleep duration and incident diabetes and coronary heart disease

Sleep duration and incident diabetes

The number of prospective studies that have investigated the association between sleep duration and incident diabetes using time-to-event analyses are few, but those results indicate a significant and positive association between short (<6 hours when compared to 7 hours)(104) and long (≥8 hours when compared to 7-7.9 hours)(105) sleep durations with incident diabetes albeit in different populations. Remaining

prospective studies have investigated the association between sleep duration and incident diabetes without using time-to-event analyses(106-114). Despite methodological differences, the results of these studies confirm positive associations between short sleep durations of <5 hours (when compared to 7 hours)(106), ≤5 hours (when compared to 7 hours)(114), ≤5 hours (referent sleep duration not specified)(108), ≤5 hours (when compared to 7 hours)(112), ≤5 hours (when compared to >7 hours)(110), <6 hours (when compared to 7 hours)(111), ≤6 hours (when compared to 7-8 hours)(113), ≤7 hours (when compared to 8 hours)(109) as well as long sleep durations of ≥7.5 hours (when compared to 7 hours)(106), >8 hours (when compared to 7 hours)(111), ≥9 hours (when compared to 8 hours)(107), ≥9 hours (when compared to 7 hours)(112), ≥9 hours (when compared to 7-8 hours)(113) with incident DM. One prospective study on Swedish women with 32 years of follow-up time found no association between sleep duration and incident diabetes(115). Overall, meta-analyses support a J-shaped or U-shaped association between sleep duration and incident type 2 diabetes(116-118).

Sleep duration and incident coronary heart disease and myocardial infarction

Similarly to the studies on sleep duration and incident diabetes mellitus, the number of prospective studies that have investigated the association between sleep duration and incident CHD using time-to-event analyses are few. The results from these studies indicate that sleep durations of <6 hours (when compared with 7-8 hours)(119), ≤6 hours (when compared with 7 hours)(120) and ≥8 hours (when compared to 7 hours) are associated with incident MI(121).

Sex-stratified analyses found associations between sleep duration ≤5 hours (when compared to 8 hours) and incident MI in women but not in men(122). The same study reported no associations with incident MI for sleep durations ≥9 hours(122).

Two of these studies did not find an association between sleep durations of ≥9 hours (when compared to 7-8 hours)(119) or >8 hours (when compared to 7 hours) and incident CHD(120). One study did not find any association between sleep duration and incident MI(63), albeit with a relatively short follow-up period of approximately 4 years.

One study that did not use time-to-event analyses reported that sleep durations ≤5 hours and ≥9 hours (when compared to 8 hours) were associated with total CHD (including fatal MI and non-fatal CHD) in women(123).

Limitations of existing studies

A major strength of the above studies is that they have all adjusted for prevalent diabetes at baseline. However, given the importance of diabetes as a major risk factor for CHD(124), it is inevitable that those who are diagnosed with diabetes during follow-up most certainly have a change in their baseline hazard of future CHD events. For this reason, it is necessary to consider incident cases of diabetes to

better understand if indeed incident diabetes is on the pathway between sleep duration and incident CHD.

Putative mechanisms for the association between sleep duration and cardiometabolic disease

The exact mechanisms and biological pathways through which sleep duration is associated with cardiometabolic disease is not fully elucidated. Studies have identified associations between sleep duration and leptin and ghrelin (a hormone related to energy insufficiency(35)) in which sleep duration is positively associated with serum concentrations of leptin and inversely associated with serum concentrations of ghrelin(125). Similarly, studies implementing experimental conditions found that sleep restriction resulted in reduced levels of leptin(126, 127) and increased levels of ghrelin(127). This would suggest a pathway whereby short sleep duration leads to increased hunger and appetite(127), resulting in increased energy intake and subsequent weight gain. Indeed, this would be consistent with the observed increased risks of weight gain and obesity in short sleepers (≤6 hours) as compared to average sleepers (7-8 hours)(128). However, weight gain is also seen over time in long sleepers (≥9 hours) when compared to average sleepers(128), thereby raising valid questions about possible differential mechanisms, including reverse causation, between short and long sleep durations and metabolic and cardiometabolic outcomes.

The suggestion of systemic inflammatory markers (e.g., CRP, interleukin-6 (IL-6), and tumour necrosis factor-α (TNF-α)) as possible mechanisms of the association between sleep duration and cardiometabolic outcomes are not unfounded considering the known associations between CRP(129), IL-6(130), respectively, and CHD. However, additional research is warranted as the results of individual studies on the association between sleep duration and markers of inflammation seem inconclusive; one study reported on a positive association between habitual sleep duration and levels of CRP and IL-6, and an inverse association between PSG sleep duration and TNF-α (131), whereas another study found that sleep duration was inversely associated with CRP and IL-6(132). The results of a meta-analysis do not support such findings of linear associations(133). Instead, the meta-analysis recognised, when comparing long sleep duration (>8 hours) with referent 7-8 hours, a positive association between long sleep duration and CRP and IL-6, respectively and no association between sleep duration and TNF-α. It should be noted that any interpretation of the results of the meta-analysis must be considered in the context of time of blood sampling; although the time of blood collection does not appear to influence variability of CRP and IL-6(134), TNF-α, on the other hand exhibits a diurnal rhythm(135).

Overall, markers of systemic inflammation are nonspecific to sleep duration or to the underlying cause of inflammation and may therefore not provide a comprehensive picture of the pathways that possibly link sleep duration with cardiometabolic outcomes. Moreover, there is a high probability of reverse causation when, for example, considering the somnogenic actions of TNF-α and its implication in the regulation of time spent in NREM sleep(135).

The next step in the investigation of an association between sleep duration and markers of inflammation would be to consider proteomic markers that may serve to elucidate possible biological pathways for the association between sleep duration and cardiometabolic outcomes. Sleep duration is independently associated with five proteomic markers of cardiovascular risk, follistatin, matrix metalloproteinase 9 (MMP-9), kidney injury molecule (KIM), adrenomedullin (ADM), and urokinase receptor (UPAR)(136). One further step will be to investigate the association between sleep duration-specific markers and cardiometabolic outcomes. Moreover, the suggestion that sleep deprivation may play a regulatory role in TNF-α production(135) and function(137) combined with the implication of activated enzymes specific to the tumour necrosis factor receptor 1 (TNFR1) pathway, e.g., Cysteinyl aspartic acid-protease-8 (Caspase-8)(138, 139) in beta cell apoptosis(140) warrants further targeted investigation of this enzyme in the context of sleep duration and incident diabetes.

Aims

The overall aim of this thesis is to investigate the associations between sleep duration and incident cardiometabolic disease; to investigate incident diabetes as a risk factor on the pathway between sleep duration and incident CHD; and to identify proteomic markers that may help elucidate the biological pathways responsible for the found associations. The aim is also to quantify the specific associations of sleep duration categories with mortality outcomes in a very large cohort of East Asians with the consideration of possible effect modification through important characteristics.

Specific aims

Paper I: To investigate, in sex-specific analyses, the role of incident diabetes as the possible biological mechanism for the reported association between short/long sleep duration and incident CHD.

Paper II: To elucidate if sleep duration is associated with plasma concentrations of Caspase-8 and whether measured plasma concentrations of Caspase-8 are associated with incident diabetes. The secondary aim of paper II was to investigate if plasma concentrations of Caspase-8 modify the known association between sleep duration and incident diabetes.

Paper III: To conduct a large individual-level analysis on the sex-specific association between sleep duration and all-cause- and major-cause mortality in a pooled longitudinal cohort. A secondary objective of paper III was to investigate sex-stratified effect modification of age and BMI on mortality outcomes.

Paper IV: To identify proteomic markers associated with specific sleep duration quintiles. Secondary aims of paper IV were to create weighted proteomic sleep scores based on any proteomic markers predictive of each sleep duration category and to further investigate associations between proteomic sleep scores and incident diabetes and incident CHD, respectively.

Methods

Study populations

Malmö Diet and Cancer Study

The Malmö Diet and Cancer (MDC) Study is a population-based, prospective study in the city of Malmö, Sweden. Between the years 1991-1996, men and women between the ages 45-73 years were randomly selected and recruited for examination. The baseline examination collected anthropometric data (including weight, height, and blood pressure), blood samples, and a detailed questionnaire with items related to heredity, socioeconomic variables, social network, occupation, physical activity, smoking, alcohol consumption, past and current diseases, and medication. Details of the study have been described elsewhere(141).

The baseline study population consisted of 30,447 participants. For the purpose of analyses in paper I, participants were excluded if they, at baseline, had prevalent occurrence of the outcomes of interest (diabetes and/or CHD) (Figure 3). Participants were also excluded if they had not provided information about the main exposure of interest (sleep duration), or if their reported information represented outlier values. A total of 16,344 participants (6966 men and 9378 women) were included in the analysis of paper I. Participants were followed from starting point until December 31, 2010, with person-years calculated from starting point to the date of incident event, loss to follow-up, or end of follow-up period, whichever came first. Data from MDC was used in paper I.

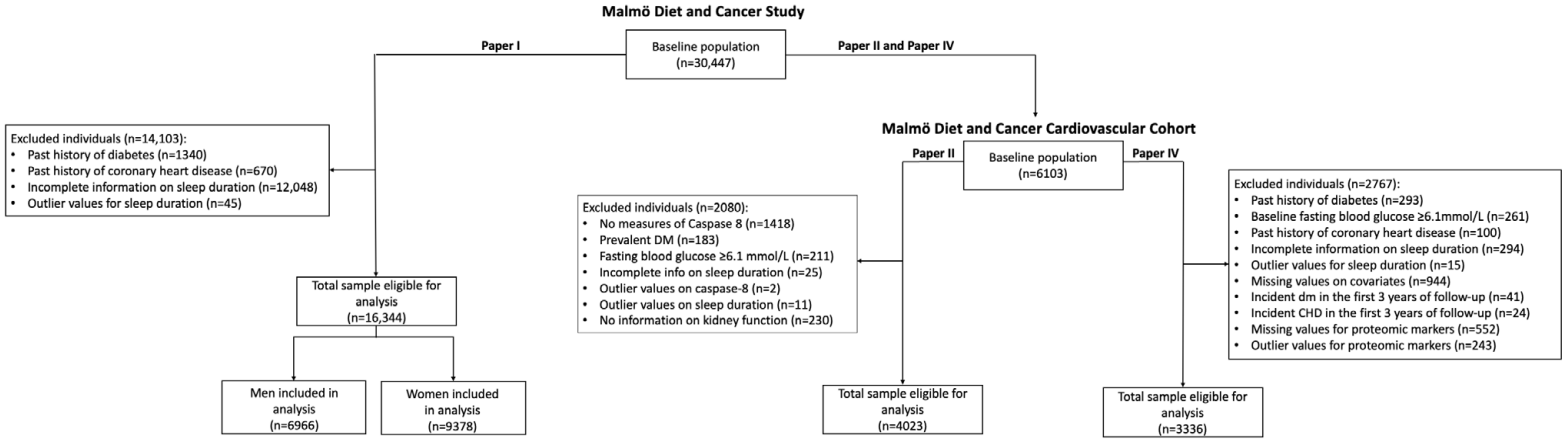

Figure 3. Flowchart of participant inclusion and exclusion for paper I, paper II, and paper IV using the Malmö Diet and Cancer Study and the Malmö Diet and Cancer Cardiovascular Cohort.

Malmö Diet and Cancer Study – Cardiovascular Cohort

The Malmö Diet and Cancer Study – Cardiovascular Cohort (MDC-CC) is a sub-cohort of the MDC Study with the purpose to investigate the epidemiology of carotid artery disease(142). The MDC-CC is comprised of 6103 participants that were randomly selected from the MDC Study between 1991-1994. MDC-CC participants underwent detailed examinations, including ultrasonography of the carotid artery, and provided plasma for the measure of novel proteomic markers. Fasting plasma specimens obtained from MDC-CC participants were frozen to -80°C immediately following blood sampling. Data from MDC-CC was used in paper II and paper IV.

For the purpose of analyses in paper II, baseline MDC-CC participants were included if they had measured plasma levels of the first main exposure (Caspase-8), had complete information on the second main exposure (sleep duration) as well as data on kidney function. From these, participants were excluded if they had prevalent occurrence of the outcome of interest (diabetes) at baseline or if their data on the two main exposures represented outlier values. A total of 4023 MDC-CC participants were included in the analysis of paper II.

For the purpose of analyses in paper IV, baseline MDC-CC participants were excluded they if they prevalent diabetes or CHD at baseline, incomplete information on the first main exposure (sleep duration) or where the main exposure constituted an outlier value, had incomplete information on covariates used in analyses, had missing data on the second main exposure (proteomic markers) or where the plasma concentrations of proteomic markers constituted outlier values. Additionally, participants with an outcome event of interest (incident diabetes or incident CHD) occurring in the first three years of follow-up were also excluded. A total of 3336 MDC-CC participants were included in the analysis of paper IV.

Asia Cohort Consortium

The Asia Cohort Consortium (ACC)(143) is a consortium of more than 30 cohorts from Asia, including Bangladesh, China, India, Iran, Japan, Korea, Malaysia, Mongolia, Singapore, and Taiwan. The purpose of the ACC is to investigate associations between environmental exposures, genetics, including their interactions, and the aetiology/mortality of diseases. The establishment of the ACC was done to allow for sufficient sample size and power for these investigations.

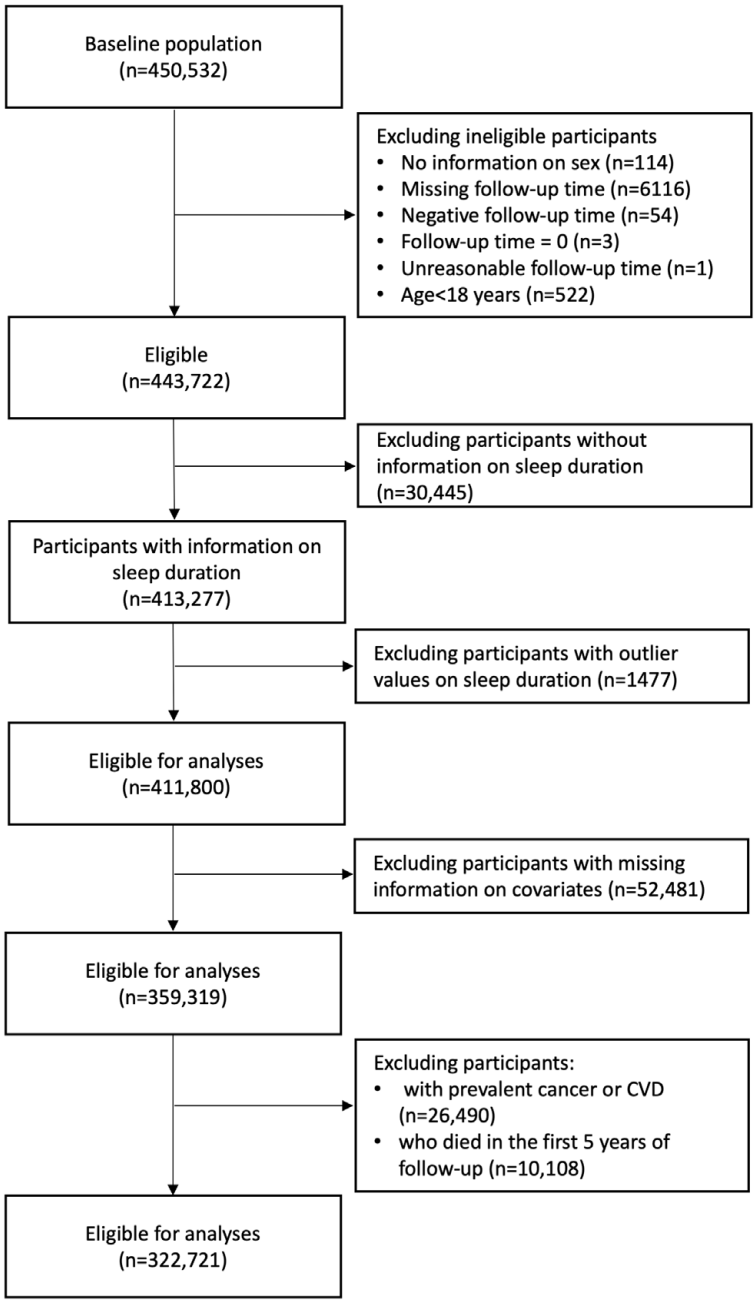

Figure 4. Flowchart of participant inclusion and exclusion for paper III using data from the Asia Cohort Consortium. From Thomas Svensson et al. Association of Sleep Duration With All- and Major-Cause Mortality Among Adults in Japan, China, Singapore, and Korea, JAMA Network Open, 4(9):e2122837 (2021). Reproduced under the Creative Commons Attribution License (CC-BY).

Paper III included data from nine ACC cohorts with a total of 450,532 eligible participants from four countries: five cohorts from Japan (Japan Public Health Center-based prospective (JPHC) Study 1 and JPHC Study 2, Miyagi Cohort Study, Ohsaki National Health Insurance Cohort Study, and the Takayama Study), two cohorts from China (Shanghai Men's Health Study (SMHS), and Shanghai Women's Health Study (SWHS)), one cohort from Singapore (the Singapore Chinese Health Study (SCHS)), and one cohort from Korea (the Korean Multi-Center Cancer Cohort Study (KMCC)). Cohorts were included based on the availability of information about the main exposure (sleep duration). For the purpose of paper III, participants were excluded if they had prevalent CVD or cancer at baseline, had missing information on the main exposure (sleep duration) or where the main exposure constituted an outlier value in cohorts with continuous measurements, had missing or unreasonable information on covariates or follow-up time, or if participants were younger than 18 years of age (Figure 4). Participants were also excluded if they died within five years of follow-up. A total of 322,721 ACC participants (144,179 men and 178,542 women) were included in the analysis of paper III.

Sleep duration definitions

The main exposure in papers I to IV was self-reported habitual sleep duration. Information on sleep duration was obtained through self-report questionnaires.

Malmö Diet and Cancer Study

For papers I, II, and IV, habitual sleep duration was assessed through two open questions: "How many hours do you usually sleep per night during a typical week (Monday-Friday)?" and "How many hours do you usually sleep per night during a typical weekend (Saturday-Sunday)?" The questions thus asked participants how long they slept on weekdays and weekends. The information on weekday and weekend sleep, respectively, was used to calculate a weighted average sleep duration:

$$\frac{weekday \times 5 \; + \; weekend \times 2}{7}$$

The weighted average sleep duration was a continuous variable. Any self-reported sleep duration that represented an outlier value of more than 3 interquartile ranges below or above the first and fourth quartiles respectively, was excluded from analyses.

In paper I and paper II, continuous weighted average sleep duration was categorised into five sleep duration groups (<6 hours, 6 – 7 hours, 7 – 8 hours, 8 – 9 hours, and

≥9 hours). The referent sleep duration was set at 7 – 8 hours, and short and long sleep durations were defined as <6 hours and ≥9 hours, respectively.

In paper IV, continuous weighted average sleep duration was categorised into quintiles: Quintile 1: 4.00 – 6.57 hours; Quintile 2: 6.64 – 7.14 hours; Quintile 3: 7.21 – 7.57 hours; Quintile 4; 7.64 – 8.00 hours; Quintile 5: 8.14 – 11.00 hours. The reason for this categorization was based on limited sample size; the categorisation of sleep duration into discrete hourly categories (as in paper I and paper II) would in paper IV result in groups that were unbalanced with regards to number of participants and number of outcome measures. This, in turn would preclude the relevant statistical analyses. Quintile 3 was chosen as the reference category to allow comparisons with other studies that often use 7 – 8 hours as the referent group, and to allow for the investigation of J-shaped or U-shaped associations that are often reported between sleep duration and the respective outcomes e.g.,(117, 144). Short and long sleep durations were defined as quintile 1 and quintile 5, respectively.

Asia Cohort Consortium

For paper III, habitual sleep duration was assessed on a cohort-by-cohort basis where three cohorts (KMCC, SCHS, and the Takayama Study) asked about sleep duration as a categorical variable, and six cohorts (JPHC Study 1 and JPHC Study 2, Miyagi Cohort Study, Ohsaki National Health Insurance Cohort Study, SMHS, and SWHS) asked about sleep duration as a discrete value (in hours). The questions for each cohort were as follows: JPHC Study 1 and JPHC Study 2: "How many hours do you usually sleep?"; Miyagi Cohort Study: "How many hours on average do you sleep per day?"; Ohsaki National Health Insurance Cohort Study: "How many hours on average do you sleep per day?"; Takayama Study: "How long did you sleep on average during the past year? (including naptime)?"; SMHS: "In the past year, how many hours did you sleep each day (including sleeping at day and night, but not including the time you woke up between two periods of sleep)?" SWHS: "In the past 2 years, how many hours did you sleep each day (including sleeping during the day and night, but not including time if you woke up between two periods of sleep)?; SCHS: "On the average, during the last year, how many hours did you sleep in a day, including naps?"; KMCC: "During the past year, on average per day, how many hours of sleep have you had (including nap time)?"

For sleep duration as a discrete value, outlier values of more than 3 interquartile ranges below or above the first and fourth quartiles respectively, were excluded from analyses. The information obtained from the cohort-specific questionnaires was subsequently harmonized into one categorical sleep duration variable that was used in analyses. The harmonized variable consisted of six groups: ≤5 hours, 6 hours, 7 hours, 8 hours, 9 hours, and ≥10 hours. The referent category was set at 7 hours.

Sleep duration was assessed at the time of the baseline survey for all cohorts with the exception of SWHS, where sleep duration was assessed at the third follow-up survey.

Outcome definitions

Incident diabetes

Incident diabetes was an outcome measure in paper I, paper II, and paper IV. New onset diabetes in participants without prevalent diabetes at baseline was identified either through linkage of the Swedish 10-digit personal identification number with local and national registers(145-149) or if participants had fasting plasma glucose concentration ≥7 mmol/l or a 120-min plasma glucose value of >11.0 mmol/l during study follow-up(150, 151).

Incident coronary heart disease

Incident CHD was an outcome measure in paper I, and paper IV. Incident events were defined as a first fatal or non-fatal MI, coronary artery bypass graft (CABG) or percutaneous coronary intervention (PCI) and identified through linkage of the Swedish personal identification number with three registries(152, 153). Fatal or non-fatal MI or death due to CHD was defined according to the International Classification of Diseases, ninth (ICD-9) and tenth (ICD-10) revisions corresponding to codes 410, 412, and 414 (ICD-9), and I21-I23 and, I25 (ICD-10). CABG was classified using the national classification of surgical procedures operation codes (KKÅ or Op6): 3065, 3066, 3068, 3080, 3092, 3105, 3127, 3158, and PCI was classified using codes FNG02 and FNG05.

Additional outcomes of interest

The investigation of incident diabetes as an event occurring on the temporal pathway between sleep duration and incident CHD required the consideration of additional endpoints.

Incident coronary heart disease preceded by incident diabetes

The first related outcome was any incident CHD that was preceded by incident diabetes (Figure 5a). 'non-diabetes CHD' defined as an incident CHD event occurring in participants without incident diabetes or in those where incident diabetes occurred after incident CHD, and 2) 'diabetes-CHD' defined as incident CHD diagnosed on the same day, or following a diagnosis of incident diabetes.

Incident coronary heart disease without preceding incident diabetes

The second related outcome was incident CHD that occurred in individuals without any incident diabetes diagnosed during the follow-up period leading to incident CHD (Figure 5b)

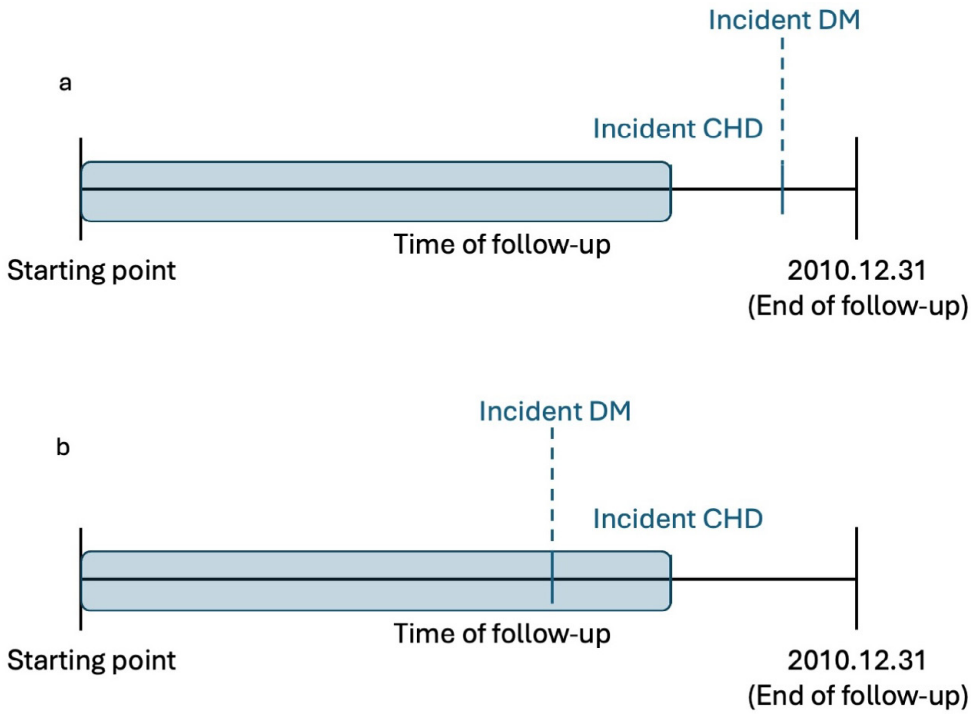

Figure 5. The temporal consideration of incident diabetes (DM) in relation to incident coronary heart disease (CHD) for the definition of additional outcome variables of interest in paper I.

Mortality

Mortality was an outcome measure in paper III and was determined using death certificates and categorised according to ICD-9 and ICD-10 classifications. Causes of death were attributed to all-cause mortality (ICD-9: 001-999, ICD-10: A00-G99, I00-N99, Q00-T98, V00-Y99) or to cause-specific mortality from CVD (ICD-9: 410-414 and 430-438, ICD-10: I20-I25 and I60-I69), cancer (ICD-9: 140-208, ICD-10: C00-C96), other causes (mortality other than CVD or cancer). For the purpose of this thesis, only results related to mortality from all-causes and CVD are presented in the result section.

Proteomic markers

Paper IV used 92 proteomic markers from the Olink Proseek Multiplex CVD 1 panel. Paper II focused on one marker (Caspase-8) from the same CVD 1 panel. Plasma concentrations of the proteomic markers were quantified using a validated high-specificity immunoassay, the Proximity Extension Assay (PEA). PEA is a method that simultaneously binds matched pairs of deoxyribonucleic acid (DNA)-labelled antibodies to a target protein(154). The simultaneous binding to the target protein allows for proximal positioning of the matched antibodies with resulting hybridization and polymerase-dependent extension of their DNA labels(155). The resulting DNA ligation is amplified through polymerase chain reaction (PCR) with the amount of ligated DNA proportional to the target protein's concentration(155).

All proteomic markers were measured in stored fasting plasma specimens from the MDC-CC baseline examination frozen to -80°C following collection.

Concentrations of proteomic markers were provided on a logarithmic (log2) scale. Any markers that were below the limit of detection were provided a missing value.

Study-specific considerations

In paper IV, 14 proteomic markers that were below the limit of detection for 413 study participants (≥10% of the study population) were excluded from analyses, leaving 78 markers for analysis. In both paper II and paper IV, markers were standardized, where the standard score represents the number of standard deviations (SD) above or below the proteomic marker's mean concentration.

Candidate proteomic markers for the construction of a proteomic score were selected based on four separate cross-fit partialing out lasso logistic regressions (described under "Statistical analysis" below, and in detail in paper IV). One cross-fit partialing-out lasso logistic regression was performed for each sleep duration quintile as it was compared with the referent quintile 3. The resulting p-values and ß coefficients of each analysis provided information on which markers to retain ($p<0.05$) and their corresponding weight (ß coefficients) in the proteomic score. The ß coefficient was multiplied with the standardized concentration (denoted as 'standconc' in the equation below) of the significant markers e.g.:

$$\beta_{marker1} \times standconc_{marker1} + \beta_{marker2} \times \text{standconc}_{marker2} + \beta_{marker3} \times \text{standconc}_{marker3} \ldots$$

The proteomic scores can be considered as potential predictors of habitual sleep duration. This was tested by comparing 15 independent linear regression models with continuous sleep duration as the dependent variable and with all possible combinations of the four proteomic scores as the independent variables (four models including one score each; six models combining variations of two scores; four models combining variations of three scores; and one model including all scores).

The Akaike Information Criterion (AIC) was used to identify the best predictors of habitual sleep duration by taking into consideration the goodness of fit and model complexity. The proteomic scores from the model with the lowest AIC were retained for the semi-parametric survival analyses.

In Paper II, the distribution of log2 Caspase-8 was positively skewed and transformed with the square root throughout. Square root-transformed standardised Caspase-8 was used both as a continuous variable (incremental increases per 1 SD) and as a binary variable in analyses. The binary variable was constructed in several steps: step 1 was to divide the transformed Caspase-8 into quartiles. Step 2 was to calculate incidence rates of diabetes mellitus for each quartile. Step 3 was to select a suitable cut-off based on diabetes mellitus incidence rates of each quartile. The incidence rates of diabetes were similar in quartiles 1, 2, and 3, thus allowing for a binary variable with the cut-off determined at quartile 4.

Statistical analysis

Cross-sectional analyses

Paper II and paper IV considered cross-sectional analyses to investigate possible predictors involving habitual sleep duration and proteomic markers. Multilinear regression with backward elimination (retention: $p<0.05$) was used in paper II to identify predictors of Caspase-8. The variables age, sex, cystatin C, education, physical activity, smoking, alcohol intake, shift work, waist circumference, low-density lipoprotein (LDL) cholesterol, HDL cholesterol, TG, fasting blood glucose, hypertension, sleep quality and sleep duration were entered into the initial model. The variable with the largest p-value was removed first with the new model tested against the preceding model.

In paper IV, least absolute shrinkage and selection operator (lasso) was used to account for the multicollinearity of the 78 available proteomic markers when selecting suitable markers predictive of habitual sleep duration quintiles. Specifically, ten-fold cross-fit partialing-out lasso logistic regression(156) controlling for age and sex was used to identify proteomic markers for the association between the respective sleep duration quintiles Q1, Q2, Q4, and Q5 with Q3 (retention: $p<0.05$). This lasso method shrinks the coefficients of certain proteomic markers to '0' thereby producing sparse models that are more easily interpretable(157, 158) while also producing ß coefficients and standard errors that allow testing of significance(159).

Survival analysis

The primary analyses of the papers included in this thesis are time-to-event analyses allowing for the estimation of hazard ratios (HR) and their 95% confidence intervals for the association between the main exposure(s) and the main outcome(s). Paper I, paper II, and paper IV used Cox proportional hazards regression; Paper III used Cox proportional hazards regression with shared frailty models to account for any unobserved between-study heterogeneity(160) at the cohort level.

Paper IV also determined mediating effects of the proteomic score by fitting a flexible parametric survival model that included only the main exposure (the relevant sleep duration quintile) and the probable mediator (proteomic score). Stata's "standsurv" post-estimation command was used on the fitted model. The fitted models used three degrees of freedom for the baseline hazard and considered the sleep duration quintile as a time varying effect with three degrees of freedom.

In papers I-IV, follow-up time was defined as the number of person-years from the start of follow-up until the end of the follow-up period. Statistical models were performed in multivariable models that considered adjustment for important covariates of the associations between the main exposure(s) and main outcome(s).

Table 1. Details of studies included in the thesis

Characteristic	Paper I	Paper II	Paper III	Paper IV
Study cohort	Malmö Diet and Cancer Study	Malmö Diet and Cancer Study – Cardiovascular Cohort	Asia Cohort Consortium	Malmö Diet and Cancer Study – Cardiovascular Cohort
Study design	Prospective cohort	Prospective cohort	Prospective cohort	Prospective cohort
Sample size for analysis	16,344 (6966 men; 9378 women)	4023	322,721 (144,179 men: 178,542 women)	3336
Main exposure(s)	Sleep duration	Sleep duration, plasma concentrations of Caspase-8	Sleep duration	Sleep duration, proteomic sleep scores
Main outcome(s)	Incident diabetes, Incident coronary heart disease	Incident diabetes	Mortality	Incident diabetes, Incident coronary heart disease
Primary statistical method	Cox proportional hazards regression	Cox proportional hazards regression	Cox proportional hazards regression with shared frailty models	Cox proportional hazards regression
Secondary statistical method	-	Multilinear regression with backward elimination	-	10-fold cross-fit partialing out lasso logistic regression

Results

Paper I

The main aim of this study was to investigate the sex-specific associations between habitual sleep duration with incident diabetes and incident CHD, respectively. The secondary aim of the study was to investigate if incident diabetes could be considered an explanatory variable (i.e., that incident diabetes occurs on the pathway) for the association between sleep duration and incident CHD.

Incident diabetes

The results of the multivariable adjusted models for men show that, when excluding the first three years of follow-up, sleep durations <6 hours (HR=1.35, 95% CI: 1.01, 1.80) and sleep durations ≥9 hours (HR=1.37, 95% CI: 1.03, 1.83) were significantly and positively associated with incident diabetes when compared to the referent sleep duration (7 – 8 hours) (Figure 6). For women, the corresponding models showed that only sleep duration <6 hours (HR=1.53, 95% CI: 1.16, 2.01) was significantly associated with incident diabetes (Figure 7).

Incident coronary heart disease

The results of the multivariable adjusted models for men show that, when excluding the first three years of follow-up, sleep durations <6 hours (HR=1.41, 95% CI: 1.06, 1.89) and sleep durations ≥9 hours (HR=1.33, 95% CI: 1.01, 1.75) were significantly and positively associated with incident CHD when compared to the referent sleep duration (7 – 8 hours) (Figure 6). For women, the corresponding models showed that only sleep duration <6 hours (HR=1.46, 95% CI: 1.03, 2.07) was significantly associated with incident CHD (Figure 7).

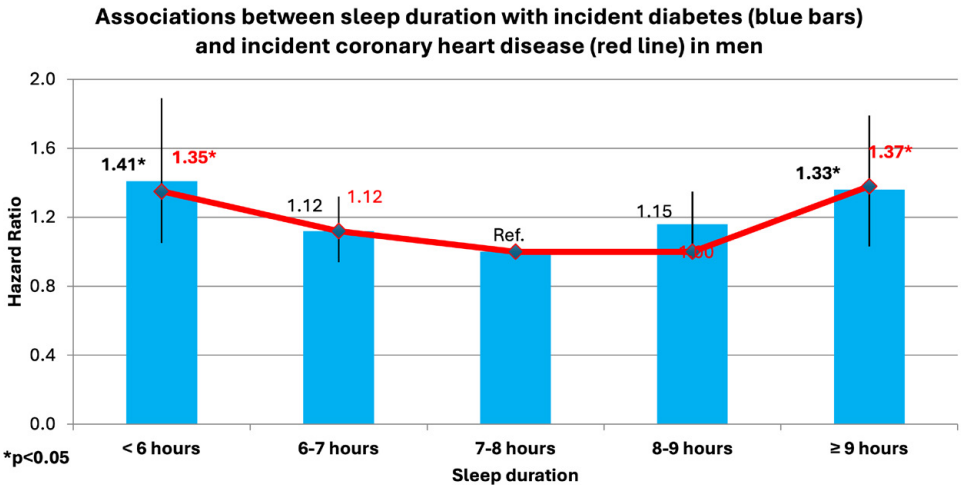

Figure 6. The association between sleep duration and incident diabetes and incident coronary heart disease in men.

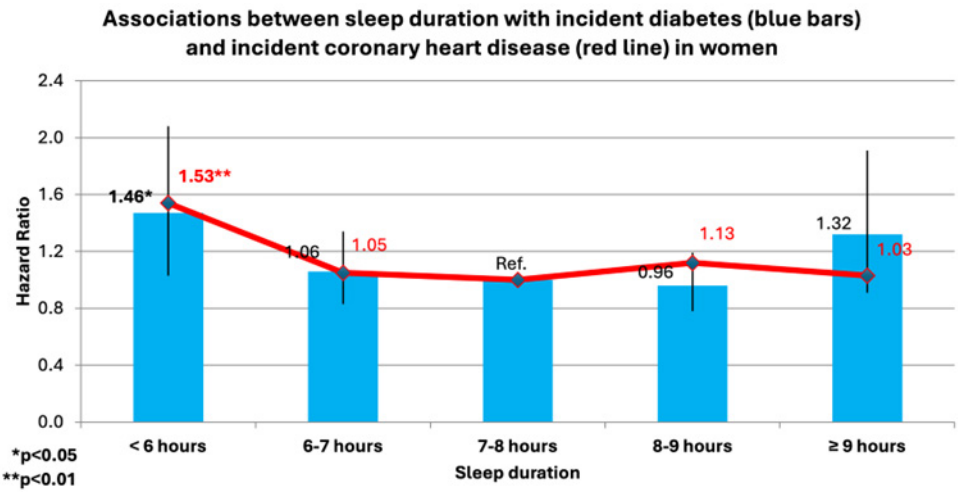

Figure 7. The association between sleep duration and incident diabetes and incident coronary heart disease in men.

Incident coronary heart disease with preceding incident diabetes

In the multivariable adjusted model for men excluding the first three years of follow-up, sleep durations <6 hours (HR=2.34, 95% CI: 1.20, 4.55) and sleep durations ≥9 hours (HR=2.10, 95% CI: 1.11, 4.00) were significantly and positively associated with incident CHD that was preceded by incident diabetes. For women, the corresponding models showed that only sleep duration <6 hours (HR=2.88, 95% CI:1.37, 6.08) was significantly associated with incident CHD preceded by incident diabetes.

Incident coronary heart disease without preceding incident diabetes

Sleep duration was not associated with incident coronary heart disease without preceding diabetes mellitus.

Paper II

The main aim of this study was to investigate the associations and interactions between sleep duration and Caspase-8 with incident diabetes. The results of this study can be broken down into three steps: the first step was to investigate if sleep duration was associated with plasma concentrations of Caspase-8. The second step was to investigate if plasma concentrations of Caspase-8 were associated with incident diabetes. The third step was to investigate if Caspase-8 modifies the association between sleep duration and incident DM.

First step: the stepwise backward elimination showed that sleep duration <6 hours was significantly and positively associated with Caspase-8 in a multilinear logistic regression model that also included age, male sex, Cystatin C, waist circumference, LDL cholesterol, and fasting blood glucose.

Second step: Plasma concentrations of Caspase-8 were significantly and positively associated with incident diabetes in two separate models; one model investigated per 1 SD incremental increases of concentrations of Caspase-8 (HR per 1 SD incremental increase=1.24, 95% CI: 1.13–1.36, whereas the other model investigated a binary variable comparing high plasma concentrations (quartile 4; HR=1.44, 95% CI: 1.19–1.74) compared to low plasma concentrations (quartiles 1-3; reference).

Third step: The interaction between dichotomised plasma concentrations of Caspase-8 and sleep duration was significant for the association with incident diabetes (P for interaction=0.007) thus indicating effect modification by Caspase-8 on the association between sleep duration and incident diabetes.

Paper III

The main aim of this study was to investigate, in a pooled analysis of individual-level data, the association between self-reported sleep duration with all-cause mortality and cause-specific mortality outcomes in a sufficiently large sample of East Asian participants to allow for further investigation of possible effect modifiers of these associations.

Men

The results of the multivariable models in men show that, when compared with the referent 7 hours, sleep durations were associated with all-cause mortality (≤5 hours (HR=1.15, 95% CI: 1.07, 1.23), 8 hours (HR=1.06, 95% CI: 1.03, 1.10), 9 hours (HR=1.13, 95% CI: 1.07, 1.20), and ≥10 hours (HR=1.34, 95% CI: 1.26, 1.44)), and CVD mortality (≤5 hours (HR=1.32, 95% CI: 1.14, 1.52) and ≥10 hours (HR=1.48, 95% CI: 1.27, 1.71).

Women

The results of the multivariable models in women show that, when compared with the referent 7 hours, sleep durations were associated with all-cause mortality (≤5 hours (HR=1.07, 95% CI: 1.00, 1.15), 6 hours (HR=1.06, 95% CI: 1.01, 1.11), 8 hours (HR=1.07, 95% CI: 1.02, 1.12), 9 hours (HR=1.17, 95% CI: 1.09, 1.25), and ≥10 hours (HR=1.48, 95% CI: 1.36, 1.61)), and CVD mortality (≤5 hours (HR=1.21, 95% CI: 1.05, 1.40), 8 hours (HR=1.18, 95% CI: 1.07, 1.30), 9 hours (HR=1.37, 95% CI: 1.18, 1.60),and ≥10 hours (HR=1.41, 95% CI: 1.16, 1.71)).

Effect modification

All-cause mortality

In sex-stratified analyses, age was a significant effect modifier among men only ($p<0.001$). Compared to the referent category of 7 hours, all sleep duration categories were significantly and positively associated with all-cause mortality in men younger than 65 years, whereas among men older than 65 years only sleep durations of 9 hours and ≥10 hours were positively associated with the outcome. BMI was not an effect modifier of all-cause mortality in men or women.

Cardiovascular disease mortality

Sex was a statistically significant effect modifier of the association between sleep duration and CVD mortality ($p=0.02$). In sex-stratified analyses, neither age nor BMI were effect modifiers for the association between sleep duration and CVD mortality.

Paper IV

The main aim of this study was to investigate if proteomic scores based on sleep duration quintiles were associated with incident diabetes and incident CHD, respectively, independently of sleep duration. The results of the study are therefore broken down into four main steps: the first step was to identify specific proteomic

markers that are associated with specific sleep durations quintiles. The second step was to construct sleep quintile-specific proteomic scores and investigate if a combination of these scores could be predictive of habitual sleep duration. The third step was to investigate the association between sleep duration quintiles and incident diabetes and incident CHD, respectively. The fourth step was to investigate if proteomic scores were associated with incident diabetes and incident CHD, respectively, independently of the original sleep duration quintiles.

First step: the study identified 16 unique proteomic markers that were significantly associated with sleep duration quintile 1, quintile 2, quintile 4, and quintile 5 when compared to the referent sleep quintile 3 (Table 2). Of these proteomic markers, tumour necrosis factor-related apoptosis-inducing ligand receptor 2 (TRAIL-R2) was associated with sleep duration quintiles 1 and 2, matrix metalloproteinase-7 (MMP-7) was associated with sleep duration quintiles 1, 4, and 5, and tumour necrosis factor receptor superfamily member 6 (Fas) was associated with sleep duration quintiles 2, 4, and 5. The remaining 13 proteomic markers were each associated with only one sleep duration quintile.

Second step: the combination of proteomic scores for sleep duration quintile 1 and quintile 5 resulted in the model with the lowest AIC for the prediction of habitual sleep duration using linear regression models.

Third step: For diabetes, the final multivariable model showed that, when compared to sleep duration quintile 3, sleep duration quintile 1 (HR= 1.32, 95% CI: 1.00–1.74), Q2 (HR=1.33, 95% CI: 1.00–1.76), and quintile 5 (HR=1.48, 95% CI: 1.09–2.00) were significantly and positively associated with incident diabetes. For incident CHD, the final multivariable model showed that only sleep duration quintile 1 (HR= 1.37, 95% CI: 1.01–1.86) was significantly and positively associated with the outcome.

Fourth step: Inclusion of the proteomic scores for sleep duration quintile 1 and quintile 5 for mutual adjustment with sleep duration for incident diabetes as the outcome, resulted in a final multivariable model in which all sleep duration quintiles but one (quintile 5) were attenuated, and in which the proteomic score for sleep duration quintile 1 (HR=1.27, 95% CI: 1.06–1.53) was significantly and positively associated with incident diabetes.

For incident CHD as the outcome, the inclusion of proteomic scores for sleep duration quintile 1 and quintile 5 resulted in a final multivariable model in which sleep duration quintile 1 remained significantly and positively associated with the outcome and where neither proteomic score for sleep duration quintile 1 nor quintile 5 were associated with incident CHD.

Table 2. Proteomic markers significantly associated with specific sleep duration quintiles when compared to referent sleep duration (quintile 3) using 10-fold cross-fit partialing out lasso logistic regression analyses

Sleep duration quintile	Proteomic marker	Beta coefficient	SE	z	P-value	95% confidence interval
Quintile 1 vs Quintile 3	TRANCE	-0.199	0.084	-2.37	0.018	-0.363 – -0.035
	MMP-7	0.193	0.082	2.37	0.018	0.033 – 0.353
	MMP-10	-0.160	0.069	-2.32	0.020	-0.295 – -0.025
	Follistatin	0.206	0.091	2.25	0.024	0.027 – 0.385
	E-selectin	0.201	0.091	2.22	0.027	0.023 – 0.378
	TRAIL-R2	0.266	0.133	2.00	0.046	0.005 – 0.528
Quintile 2 vs Quintile 3	TRAIL-R2	0.431	0.143	3.02	0.0026	0.151 – 0.711
	Fas	-0.457	0.156	-2.92	0.0035	-0.763 – -0.150
	Kallikrein-6	-0.284	0.111	-2.57	0.010	-0.500 – -0.067
	U-PAR	-0.302	0.140	-2.16	0.031	-0.576 – -0.0280
Quintile 4 vs Quintile 3	Fas	-0.452	0.135	-3.34	0.00084	-0.717 – -0.187
	Renin	0.186	0.070	2.64	0.0082	0.048 – 0.324
	HB-EGF	-0.329	0.135	-2.44	0.015	-0.594 – -0.064
	MMP-7	0.189	0.081	2.34	0.019	0.031 – 0.348
	CXCL6	0.210	0.097	2.16	0.031	0.019 – 0.400
Quintile 5 vs Quintile 3	Prolactin	0.261	0.081	3.22	0.0013	0.102 – 0.420
	MMP-7	0.296	0.097	3.05	0.0023	0.106 – 0.486
	CXCL1	0.245	0.100	2.44	0.015	0.0483 – 0.441
	Fas	-0.402	0.170	-2.37	0.018	-0.735 – -0.070
	t-PA	0.256	0.110	2.32	0.020	0.0395 – 0.472
	HSP27	-0.284	0.130	-2.19	0.029	-0.538 – -0.030

Abbreviations: TRANCE: tumour necrosis factor-related activation-induced cytokine, MMP-7: Matrix metalloproteinase-7, MMP-10: Matrix metalloproteinase-10, TRAIL-R2: tumour necrosis factor-related apoptosis-inducing ligand receptor 2, Fas: tumour necrosis factor receptor superfamily member 6, U-PAR: urokinase plasminogen activator surface receptor, HB-EGF: proheparin-binding epidermal growth factor-like growth facto, CXCL6: C-X-C motif chemokine 6, CXCL1: C-X-C motif chemokine 1, t-PA: tissue-type plasminogen activator, HSP27: heat shock 27 kDa protein.

From Thomas Svensson et al. Very short sleep duration reveals a proteomic fingerprint that is selectively associated with incident diabetes mellitus but not with incident coronary heart disease: a cohort study, BMC Med 22, 173 (2024). Reproduced under the Creative Commons Attribution License (CC-BY).

Discussion

Summary

This thesis identifies an association between sleep duration and incident diabetes and incident CHD, respectively, and confirms that incident diabetes is a primary risk factor that explains the association between sleep duration and incident CHD. Moreover, this thesis identifies proteomic markers that are associated with sleep duration and further suggests a biological pathway that links short sleep duration (defined as sleep durations shorter than a referent category) with incident diabetes. One paper in this thesis quantified, in a large-scale analysis on East Asian populations, the sex-specific associations between sleep duration and CVD mortality, and the age-specific associations between sleep duration and all-cause mortality thereby contributing to the understanding of how to consider effect modification in population-based studies on sleep duration and mortality.

Sleep duration and mortality

The findings from paper III show that, in a very large population of East Asian participants, there is a clear association between sleep duration and mortality from all-causes, CVD, cancer, and other causes. The study findings further show that these associations exist in both men and women for all outcomes of interest. For the purpose of this thesis, only the results related to all-cause mortality and CVD mortality will be discussed in detail.

Sleep duration and all-cause mortality

The association between sleep duration and all-cause mortality was not modified by sex in a large cohort of East Asian participants. This finding is in accord with two previous studies(61, 84) and one study published after paper III(161). We have similarly confirmed that sex is not an effect modifier in a Japanese cohort(162). Contrary to these findings, two recently published studies did confirm a modifying effect of sex on the association between sleep duration and all-cause mortality(163, 164); the results of one study predominantly identified that sleep durations ≤5 hours and 6 hours (when compared to 7 hours) are positively associated with all-cause

mortality among men but not women(163) whereas the other identified a higher risk with sleep duration ≤5 hours among women but not among men(164).

The results of our study must be put into a wider perspective. First, the results signify that stratification by sex alone may be insufficient for all-cause mortality. Indeed, the association between sleep duration and all-cause mortality may be so robust that it does not show any sex-specific associations unless considered in the context of other characteristics. In paper III, age was an effect modifier of the sex-specific associations between sleep duration and all-cause mortality in men only. Men younger than 65 years maintained a J-shaped association between sleep duration and all-cause mortality whereas older men were at increased risk only with sleep durations of 9 hours or ≥10 hours (when compared to 7 hours). Our findings confirm those reported in one previous study(69) which found increased risks of all-cause mortality among those younger than 65 years with sleep durations ≤5 hours and ≥8 hours (when compared to 7 hours). The results also differ from those reported in one previous study(67) in which a U-shaped association was maintained only among the older participants. However, those analyses were not concomitantly stratified by sex, they considered different age strata, and with markedly smaller samples. The second point that is important to consider when aiming to explain the found associations between sleep duration and all-cause mortality is that the term all-cause mortality includes not only deaths from lifestyle-related noncommunicable diseases, but also includes self-harm, accidents, and causes attributed to communicable diseases. This heterogeneity of causes of death further necessitates a focus on more homogeneous groupings to allow for more in-depth interpretations. The increase in mortality risk with "short" sleep duration may be explained by an increased CVD risk(137). For this reason, and considering the overall purpose of the thesis, attention will therefore centre on CVD mortality before continuing with cardiometabolic disease.

Sex-specific associations between sleep duration and cardiovascular disease mortality

Taken together, the findings of paper III indicate that sex is a significant effect modifier of the associations between sleep duration and mortality from CVD. Indeed, our findings related to CVD mortality indicate slightly higher risks for men compared to women at both extremes of sleep duration (i.e., ≤5 hours and ≥10 hours, respectively), whereas women, on the other hand, seem to have a dose-dependent association with steadily increasing, and statistically significant, risks with sleep durations of 8 hours and 9 hours, respectively, when compared to the referent 7 hours. These dose-dependent associations for sleep durations exceeding the referent 7 hours do not appear for men.

The approach of stratifying CVD-related outcomes according to sex is a well-accepted practice in cardiovascular research. Of the studies published prior to paper III that included also CVD-related mortality outcomes in sex-stratified analyses, our results confirm the findings of a large Japanese cohort that reported a significantly increased risk of CVD mortality for men who slept ≥10 hours and a dose-response for women with sleep durations of 8 hours, 9 hours, and ≥10 hours (when compared to 7 hours)(68). It is noteworthy that this cohort is not part of the ACC which further strengthens the validity of our findings. Another publication using data from the large Shanghai Women's and Men's Health Studies(52), both of which are included in the pooled analyses of paper III, found that, among women, only sleep duration ≥10 hours (when compared to 7 hours) was significantly associated with CVD mortality. However, the same study also confirmed a linear trend for the association between sleep duration and CVD mortality among women, but not among men, thus indicating the possibility of a dose-dependent association. It is possible that even larger samples are required to find associations between specific sleep duration categories and CVD mortality outcomes among women. Indeed, our own study using the JPHC cohorts, which are also part of the ACC, found no association between any sleep duration categories and CVD mortality outcomes among women, but a significant and positive association with 9 hours and ≥10 hours of sleep (when compared to 7 hours) among men in the fully adjusted model(162). However, when excluding deaths occurring within the first 5 years of follow-up, those associations were abrogated thereby indicating either reverse causation or a loss of statistical power as the reason for the loss of significance. Another study(54) found, albeit with very wide confidence intervals, an increased risk of mortality from heart diseases for men with sleep durations <6 hours and for women with sleep durations of ≥9 hours when compared to 7.0-7.9 hours. That study also reported a significantly decreased risk of stroke mortality for men with sleep durations of 8.0-8.9 hours and a significantly increased risk of stroke mortality for women with sleep durations of 6.0-6.9 hours. Although the results of that study confirm the positive associations with the most extreme sleep durations, the study did not test if sex was an effect modifier, which may have been important given their very wide confidence intervals. Moreover, this study as well as other studies(60, 65) have further considered CAD and stroke-related outcomes separately. This approach was not considered in paper III as it would necessitate further classification of stroke into haemorrhagic and non-haemorrhagic causes. Despite the very large sample size in paper III, the consideration of CVD mortality from three separate outcome perspectives, i.e., CAD, haemorrhagic and ischemic strokes, respectively, would not allow for sufficient statistical power to conduct analyses stratified on two characteristics. Other studies with sex-stratified analyses found that sleep durations ≥9 hours when compared to 7 hours were positively associated with CVD mortality only in men(83) or only women with sleep durations ≤5 hours or ≥9 hours (when compared to 7-8 hours)(60). Yet additional studies have reported no associations

between sleep duration and CVD mortality in men or women(57, 64, 84), or reported ambiguous results(59).

Four recent studies (i.e., those published after paper III) on US and UK populations have confirmed the overall non-linear association between sleep durations ≤5 hours(161, 163, 165), 6 hours(163), 8 hours(161, 165) and ≥9 hours(161, 163, 165) when compared to 7 hours and CVD mortality. Two additional recent studies further confirmed that extremes of sleep duration, 5.5 hours and ≥8.5 hours, respectively, when compared to 6.5-7.5 hours(166) and <5 hours and ≥9 hours, respectively, when compared to 6 hours(164) are associated with CVD mortality. It should be noted, however, that three of these studies found no effect modification by sex for the association between sleep duration and CVD mortality(161, 163, 164) which is contrary to our own results. This may be due to differences in population characteristics, ethnicity, or study methodology.

Age and sex-specific associations between sleep duration and cardiovascular disease mortality

The sex- and age-stratified associations (i.e., above 65 years of age) found in paper III are similar to those reported in one study of elderly Taiwanese community residents(76): sleep durations of 8 hours, 9 hours, and ≥10 hours among older women were positively associated with CVD mortality whereas only the extreme sleep duration of ≥10 hours was associated with CVD mortality among older men. Our study further reported significant and positive associations for older women with sleep durations of ≤5 hours and 6 hours, respectively, and for older men with sleep durations ≤5 hours. One reason for the discrepant results related to short sleep duration between paper III and the study by Lan et al(76), could be their small sample size and consolidation of the shortest sleep duration category to include all sleep durations <7 hours. Our results also partially confirm the findings from a study on elderly (aged 65-85 years) Japanese where a sleep duration ≥10 hours (compared to 7 hours) among men was positively associated with CVD mortality(81). Our publication using data only from the JPHC cohorts found no effect modification of age in men or women, albeit with an age cut-off at the median age of 50 years to allow for a sufficient number of cases in the respective age strata(162). Consequently, this cut-off may not be aligned with corresponding changes related to age that may impact sleep duration, whether they be physiological or environmental (e.g., retirement).

One study published after paper III did not find any effect modification by age of the association between sleep duration and CVD mortality(163). However, this study, despite its very large study sample, did not additionally test for effect modification by age in sex-stratified analyses. One argument speaking in favour of conducting multi-layered stratification are the results in paper III that relate to sleep

duration and cancer mortality. Granted, this outcome is not the main focus of this thesis, however, the results highlight the importance of stratification according to both sex and age when considering mortality as the primary outcome. Although, at first glance, the sex-specific analyses on sleep duration and cancer mortality do not reveal any significant associations among men, further stratification by age-group (i.e., younger and older than 65 years of age) reveals not only a significant effect modification by age, but also that the risks of cancer mortality among men younger than 65 years are higher for all but one (6 hours) of the sleep duration categories when compared to the referent 7 hours of sleep. This particularly emphasizes the need to conduct analyses stratified by specific characteristics of interest and strengthens the overall purpose of the paper.

Overall, the impact of both short and long sleep durations on CVD-related events cannot be understated; population attributable fractions estimate that in the USA alone, the number of CVD events related to sleep durations shorter or longer than 7 hours are expected to exceed 1,000,000 cases over a 10-year period(161). However, these analyses do not consider participants free from diabetes or heart disease at baseline which may explain why the majority of these events would be due to sleep durations exceeding 7 hours.

Cardiovascular disease mortality driving all-cause mortality?

CVD mortality may in fact be the driver for the association between sleep duration and all-cause mortality. Although the number of CVD-related deaths in paper III are notably smaller than the corresponding numbers for cancer mortality and other-cause mortality, the obtained hazard ratios for the association between sleep duration and CVD mortality closely reflect those for the association between sleep duration and all-cause mortality. Indeed, the results of recent publications show comparable similarities(161, 163, 165, 166) even when analyses are stratified by sex(163).

A link between sleep duration and increased risk of cardiovascular disease and all-cause mortality

Despite the very large dataset available in paper III, the study itself did not allow for further conclusions about the mechanisms or pathways that may be responsible for the observed associations between sleep duration and CVD mortality on the one hand, and for the sex- and age-specific differences on the other hand. Excess risk of both all-cause mortality and CVD mortality is significantly increased in men with new onset diabetes and/or incident non-fatal CVD(167) which implicates incident disease as intermediate steps in a suggested pathway between sleep duration and mortality outcomes. Paper I and paper II aimed, using the MDC and MDC-CC studies, to further investigate if indeed a pathway including incident diabetes can be considered for the association between short sleep duration and incident CHD.

Sleep duration and incident diabetes and incident coronary heart disease

Incident diabetes emerges as the link between sleep duration and incident coronary heart disease

The reasons for the findings between sleep duration and risk of mortality, in particular from CVD, and any suggestion of underlying pathways that may help understand such associations are further investigated in paper I, paper II, and paper IV. Paper I was the first study of its kind to investigate if incident diabetes is on the path between sleep duration and incident CHD. The study findings must be taken into consideration in the context of its three main findings: first, that sleep duration is independently associated with incident diabetes; second, that sleep duration is independently associated with incident CHD; and third, that sleep duration is associated specifically with incident CHD preceded by incident diabetes (diabetes-CHD) but not with incident CHD occurring before incident diabetes. Moreover, these patterns were consistent in both men and women. Indeed, among men, sleep durations <6 hours and ≥9 hours were positively associated with diabetes-CHD whereas among women a sleep duration <6 hours was positively associated with the outcome. The hazard ratios for sleep durations <6 hours were comparable for men and women with more than a twofold increase in risk of diabetes-CHD for men and a nearly threefold increase in risk for women. Although no formal statistical test was conducted to confirm the sex-specific differences of our results, the decision to conduct sex-stratified analyses was done not only because it is an established approach in cardiovascular research, but also due reported sex-specific differences involving diabetes outcomes(124, 168) and related risk factors(169, 170), including sleep duration(171). Notwithstanding the above effect sizes, the main difference between men and women in paper I was that sleep duration ≥9 hours was associated with incident diabetes-CHD in men, but not women. Although the study itself does not allow to further detail the mechanisms behind this difference, the context of diabetes risk must be considered both in terms of biological (e.g., BMI and metabolic syndrome) and psychosocial factors (e.g., socioeconomic status, psychological stress and health behaviours)(172). Paper I was well-adjusted for many of these important confounders, yet sleep duration emerged as an independent risk factor of incident diabetes and incident diabetes-CHD.

The finding of incident diabetes as part of the pathway between sleep duration and incident CHD was further confirmed in paper IV despite its methodological differences when compared to paper I. For example, due to a primary focus on the exploration of biomarkers associated with sleep duration, paper IV utilised a subset of the study population in paper I. Consequently, the sample size was smaller which necessitated combined analyses for men and women as well as consideration of statistical cut-offs for the definition of sleep duration categories. Notwithstanding

these differences, when compared to the referent quintile 3, one extreme of sleep duration (quintile 1) was positively associated with both incident diabetes and incident CHD. However, when considering incident diabetes as a time-varying covariate in the same models, the association between sleep duration quintile 1 and incident CHD was entirely abrogated. The combined results of paper I and paper IV thus point toward an overall picture in which incident diabetes is an important intermediate step that may explain the association between short sleep duration (whether defined as <6 hours or as 4.00 – 6.57 hours) when compared to a referent category. Paper II further confirms an increased risk of incident diabetes with sleep durations of 6-7 hours and 8-9 hours (when compared to 7-8 hours).

Paper I and paper IV, however, have certain differences with respect to the found associations between their respective definitions of the extremes of long sleep duration with both incident diabetes and incident CHD. Whereas paper I identified a positive association between ≥9 hours of sleep with both incident diabetes and incident CHD, paper IV substantiated only the association between sleep duration quintile 5 (8.14 hours – 11.00 hours) and incident diabetes. These discrepant findings could be due to the methodological differences discussed in the previous paragraph.

Short sleep duration and proteomic markers

Paper II focused on a targeted investigation of the association between sleep duration and Caspase-8. Paper IV, on the other hand, focused on exploratory analyses using a double machine learning technique to identify markers that were associated with specific sleep duration quintiles when compared to a referent sleep duration quintile. The analyses in paper IV identified six proteomic markers that were significantly associated with sleep duration quintile 1 when compared to sleep duration quintile 3.

Caspase-8

Caspase-8 is an initiator of apoptosis activated through the extrinsic (or receptor-mediated apoptosis) with upstream binding of TNF to TNFR1, Fas ligand to Fas, or tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) to the receptors TRAIL-R1 and TRAIL-R2(173) with further recruitment of proteins which leads to activation of Caspase-8(174, 175). To our knowledge, the finding in paper II of a direct association between sleep duration and plasma concentrations of Caspase-8 has not been previously identified. Indeed, the association was confirmed in two models: 1) when using a categorical definition of sleep duration where specifically <6 hours (when compared to 7-8 hours) was significantly and positively associated with plasma concentrations of Caspase-8, and 2) when considering sleep duration as a continuous variable where there was a significant inverse association with plasma concentrations of Caspase-8. These findings indicate that plasma

concentrations of Caspase-8 are upregulated with shorter sleep durations. This requires further interpretation in the context of the pathway in which Caspase-8 is involved. Consequently, Caspase-8 may serve as a proxy marker for the extrinsic apoptotic pathway that is activated by TNF-α; TNF-α in turn is implicated in bidirectional associations with sleep(135).

Follistatin

Follistatin is a modulator of the transforming growth factor-beta (TGF-ß) signalling pathway(176). Paper IV found that plasma concentrations of follistatin were upregulated with sleep duration quintile 1 when compared to sleep duration quintile 3. This finding partially supports one previous study that found an L-shaped association between sleep duration and follistatin levels(136), indicating up-regulation predominantly with short sleep duration. Few other studies have linked follistatin to sleep-related outcomes or exposures, but the findings of one study suggests that concentrations of circulating follistatin follow a circadian rhythm (177). Although follistatin's overall function is largely unknown, it is suggested to be involved in the regulation of energy metabolism(178) with plasma concentrations of follistatin increased during exercise(179). Follistatin has also been implicated in the role of a stress-response protein(180) which, although without any specific studies on sleep deprivation, may allow for speculation as to the reason for its up-regulation with sleep duration quintile 1.

E-selectin

E-selectin is a cellular adhesion molecule(181) mediating cell-to-cell adhesion with particular involvement in rolling and adhesion of leucocytes to endothelial cells(182). Paper IV found that plasma concentrations of E-selectin were upregulated with sleep duration quintile 1 when compared to sleep duration quintile 3. This finding supports one previous study where sleep deprivation was associated with increased concentrations of soluble E-selectin(183). Other studies with sleep-related outcomes or exposures have reported increased concentrations in OSA(184). There are, however, also studies that have found no significant associations between sleep duration and E-selectin (185, 186). Concentrations of E-selectin may adjust rapidly to alterations in sleep dynamics given that treatment with positive airway pressure results in lower concentrations of E-selectin(184, 187).

Tumour necrosis factor-related activation-induced cytokine

Tumour necrosis factor-related activation-induced cytokine (TRANCE) is also known as receptor activator of nuclear factor-kb ligand (RANKL). RANKL has been implicated in vascular inflammation, vascular calcification, and angiogenesis(188). Paper IV found that plasma concentrations of TRANCE were downregulated with sleep quintile 1 when compared to sleep quintile 3. No previous studies have linked TRANCE with sleep duration or other sleep-related outcomes or exposures.

Matrix metalloproteinase-10

Matrix metalloproteinase (MMP)-10 is involved in the resolution of acute inflammation(189). Paper IV found that plasma concentrations of MMP-10 were downregulated in sleep duration quintile 1 when compared to sleep duration quintile 3. To our knowledge no other studies have linked MMP-10 with sleep duration or other sleep-related outcomes or exposures.

Matrix metalloproteinase 7

MMP-7 has been linked with both proapoptotic and cell proliferative pathways through cleavage of the ligand FasL and its involvement in the Fas/FasL pathway(190). Moreover, it is possibly implicated in TNF-α release through the pre-processing of the TNF-α precursor(191). Paper IV found that plasma concentrations of MMP-7 were upregulated in sleep duration quintile 1 when compared to sleep duration quintile 3. Although no other studies have reported links between sleep duration and MMP-7, a recent study found a U-shaped association between sleep duration and MMP-9(136), a matrix metalloproteinase that has also been mentioned in the context of TNF-α precursor pre-processing and TNF-α release(191).

Tumour necrosis factor-related apoptosis-inducing ligand receptor 2

As mentioned under Caspase-8, above, TRAIL-R2 triggers caspase-dependent apoptosis through the binding of TRAIL(173), and soluble TRAIL-R2 is released with apoptosis activated through the Fas/FasL pathway(192). Paper IV found that plasma concentrations of TRAIL-R2 were upregulated in sleep duration quintile 1 when compared to sleep duration quintile 3. This result partially supports the findings from paper II and further links short sleep duration with Caspase-dependent apoptosis.

Taken together, the findings from paper II and paper IV implicate an up-regulation of proteomic markers involved in the extrinsic apoptotic pathway, including Caspase-8, TRAIL-R2, and MMP-7 given its role as a pre-processor of the TNF-α precursor.

Long sleep duration and proteomic markers

The analyses in paper IV identified six proteomic markers that were significantly associated with sleep duration quintile 5 when compared to sleep duration quintile 3.

Prolactin

Prolactin is involved in lactation, reproduction, immune response and angiogenesis(193). Paper IV found that plasma concentrations of Prolactin were upregulated in sleep duration quintile 5 when compared to sleep duration quintile 3. These results are in line with previous studies that have shown an increased release

of Prolactin during sleep(194-197) and that partial sleep deprivation is inversely associated with prolactin levels(198). Moreover, increased serum Prolactin levels have been mentioned in the context of excessive daytime sleepiness(199) which is also in line with the directions of our results.

Matrix metalloproteinase-7

Paper IV found that plasma concentrations of MMP-7 were upregulated in sleep duration quintile 5 when compared to sleep duration quintile 3. It is therefore the only one of the proteomic markers discussed in detail in this thesis that was upregulated with both quintile 1 and quintile 5. No previous studies have linked sleep duration outcomes or exposures with MMP-7 for which reason it is not possible to discuss this seemingly contradictory finding in relation to sleep duration. One possibility, however, is that MMP-7 fulfils dual roles or functions which cannot be understood through the measurement of only plasma concentrations. Future studies with additional knowledge of the specific pathways involved are required to elucidate the roles of MMP-7 with relation to short and long sleep durations.

C-X-C motif chemokine 1

C-X-C motif chemokine 1 (CXCL1) is a chemoattractant cytokine involved in neutrophile recruitment in inflammation and the promotion of angiogenesis(200). Paper IV found that plasma concentrations of CXCL1 were upregulated in sleep duration quintile 5 when compared to sleep duration quintile 3. No previous studies have linked CXCL1 to sleep duration or sleep-related exposures or outcomes.

Tissue-type plasminogen activator

Tissue-type plasminogen activator (t-PA) plays a role in endogenous fibrinolysis(201). Paper IV found that plasma concentrations of t-PA were upregulated in sleep duration quintile 5 when compared to sleep duration quintile 3. Overall, our findings are in line with those of previous studies reporting reduced acute release of t-PA in men who habitually sleep shorter than 7 hours (when compared to men who sleep between 7.0 – 8.1 hours)(202). Although our results may seem contrary to studies reporting that t-PA is positively associated with desaturation events during sleep(203) as well as with moderate to severe OSA(184, 203), our finding of an upregulation of t-PA with the longest sleep quintile may suggest a link between OSA and long self-reported sleep duration.

Tumour necrosis factor receptor superfamily member 6

Tumour necrosis factor receptor superfamily member 6 (Fas) is a cell-surface receptor involved in caspase-dependent apoptosis(138). Paper IV found that plasma concentrations of Fas were downregulated in sleep duration quintile 5 when compared to sleep duration quintile 3. No previous studies have directly linked Fas with sleep exposures or outcomes, however, this result lends additional credence to

the findings of paper II; the inverse associations between sleep duration and plasma concentrations of Caspase-8 may, by extension be a consequence of downregulation of Fas with long sleep duration.

Heat shock 27 kDa protein

Heat shock 27 kDa protein (HSP27) functions as an antioxidant and an anti-apoptotic agent during oxidative- and chemical stress, respectively(204). Paper IV found that plasma concentrations of HSP27 were downregulated in sleep duration quintile 5 when compared to sleep duration quintile 3. Although no other studies have linked HSP27 with sleep duration or sleep-related exposures or outcomes, the findings that long sleep duration is associated with an antiapoptotic process would be in line with the findings in paper II.

Proteomic markers and cardiometabolic disease

Caspase-8

Paper II found that plasma concentrations of Caspase-8 were positively associated with incident diabetes. This association held both when Caspase-8 was considered as a continuous variable and when dichotomised into high vs low concentrations. It is noteworthy that these associations were independent of conventional risk factors of diabetes, including but not limited to age, physical activity, waist circumference, and fasting blood glucose levels. These results indicate a possibility of the involvement of Caspase-8 or the extrinsic apoptotic pathway in the pathogenesis of diabetes. Caspase-8 is associated with coronary events(205), with the combined findings of paper II and paper IV further implicating incident diabetes as the explanatory pathway for downstream associations with incident coronary outcomes. The exact mechanisms through which Caspase-8 and/or the extrinsic apoptotic pathway are linked with diabetes remain to be elucidated. However, Caspase-8 has been discussed in beta cell apoptosis, in the maintenance of beta cell mass, and in the regulation of insulin secretion, albeit in experimental models only(206). Moreover, a more recent study has reported that Caspase-8 protein is increased in cultured human adipocytes from type 2 diabetics as compared to non-diabetics and suggested a role of Caspase-8 in adipose tissue inflammation and glucose intolerance(207). It is, however, difficult to make direct comparisons between experimental studies and the results obtained in paper II and paper IV given that such comparisons would be between tissue-specific expressions and non-specific plasma concentrations of Caspase-8.

The other main finding of paper II was that plasma concentrations of Caspase-8 were an effect modifier of the association between sleep duration and incident diabetes. Indeed, it was only among participants with a high (quartile 4) plasma concentration of Caspase-8 where sleep duration was associated with incident diabetes. It would be of interest for future studies to not only replicate these findings,

but to further investigate if sleep-specific interventions impact and thus are causally linked with plasma concentrations of Caspase-8.

Proteomic scores and cardiometabolic disease

Paper IV further combined the respective markers of quintiles 1 and 5 into quintile-specific proteomic scores and tested their associations with incident diabetes and incident CHD, respectively. These models were mutually adjusted for the original phenotype (sleep duration quintiles) and their respective proteomic scores to investigate any possibility of a mediating effect of the proteomic scores on the respective outcomes of interest.

Proteomic score for sleep quintile 1

The proteomic score for sleep duration quintile 1 remained significantly associated with incident diabetes in the final multivariable model. Paper IV did not investigate the association between the individual markers comprising the proteomic score for sleep duration quintile 1 and incident diabetes. However, previous research has found that follistatin(208), E-selectin(209), MMP-7(210), and TRAIL-R2(192) are associated with the risk of diabetes whereas RANKL is inversely associated with prevalent diabetes(211). Our findings thus support known associations while taking one additional step by combining proteomic markers to a composite score specific for the phenotype of interest. Indeed, the inclusion of proteomic scores abrogated the association between the sleep duration quintiles 1 and 2 and incident diabetes. Moreover, when further investigating the possibilities of this proteomic score mediating the association between the original phenotype (sleep duration quintile 1) and incident diabetes, the phenotype-specific score was found to mediate approximately 40% of the association. Given the observational nature of the study, it is not possible to make any inferences of causality between the proteomic score and incident diabetes. However, the overall results speak in favour of the phenotype-specific score providing information about a probable explanatory mechanism linking short sleep duration with incident diabetes.

The proteomic score for sleep duration quintile 1 was not associated with incident CHD in paper IV. Previous studies focusing on individual markers have reported that follistatin and TRAIL-R2, but not MMP-7, MMP-10 or TRANCE, are associated with incident CHD in those without prevalent diabetes(212), and that there is no association between E-selectin and incident CHD(213, 214) or incident MI(215). Moreover, the proteomic score for sleep duration quintile 1 did not attenuate the association between its phenotype and incident CHD which indicates the that such associations may need to be considered through proteomic markers other than those available in the assay used in paper IV. Indeed, the proteomic assays available today allow for the quantification of thousands of proteomic markers,

some of which may provide suggestions of specific pathways that are relevant for this association.

Proteomic score for sleep quintile 5

The proteomic score for sleep duration quintile 5 was not associated with incident diabetes or incident CHD. Moreover, this proteomic score did not markedly attenuate the association between its phenotype (sleep duration quintile 5) and incident diabetes. This may be explained through residual confounding whereby long sleep duration is a proxy for other unadjusted comorbidities or lifestyle-related variables. Alternatively, and similar to the discussion in the previous paragraph, there may be proteomic markers available in large-scale proteomic datasets that could provide suggestions of relevant proteomic pathways.

Overall summary

The results from papers I-IV provide, in the context of existing research, a suggested pathway between short sleep duration (defined as sleeping less than an appropriate referent category) and increased risk of mortality. Although far from being complete, one suggested pathway for the association between short sleep duration and incident CHD can thus be described as increased hunger and appetite(127) through decreased leptin and increased ghrelin(125), with subsequent weight gain(128) or obesity(216), metabolic syndrome(167), upregulation of proteomic markers related to predominantly apoptosis and inflammation (paper IV), incident diabetes (papers I, II, and IV) increasing the risk of incident CHD (paper I and IV), with further downstream implications of significantly increased sex- and age-specific risks of CVD mortality (paper III) with all-cause mortality (paper III). This pathway is far from complete and requires additional mechanistic studies to elucidate the missing components that may help connect the dots.

Major limitations

The papers included in the thesis share many limitations that deserve to be discussed in detail. Some of these limitations can be considered in the context of future research.

A single measure of sleep duration

As mentioned in the introduction to this thesis, there are several methodological approaches that allow researchers to obtain information about an individual's sleep duration, including PSG, accelerometers, and through self-report questionnaires. The subjective nature of the definition of sleep duration that has been used

throughout papers I-IV is, although limited, not the primary concern of this exposure. The main consideration that should be discussed is instead the single measure of sleep duration upon which the study results rely. Sleep duration, or total sleep time, represents a dynamic measure that changes not only with age(26) but also with external influence, including occupational expectations or life circumstance such as child rearing. Consequently, papers I-IV work under the assumption that habitual sleep duration remains consistent throughout the defined follow-up times of each study. Indeed, one may question if this is indeed possible given that follow-up times of the individual papers vary from an average of 13.4 – 14.0 years (paper III) to 21.8 – 22.4 years (paper IV).

Sex-specific expressions of proteomic markers

The results of paper III identify both age and sex as important modifiers of the associations between sleep duration and CVD mortality. Indeed, although paper IV investigated and found the interaction between sleep duration and sex non-significant with regards to the outcomes, it is possible that this is a result of the limited sample size. There may be differences between men and women with regards to diabetes risk factors(217) with diabetes, in turn, appearing as a stronger risk factor for incident CHD in women compared to men(124). Moreover, there is a possibility of sex-specific associations between proteomic markers and beta-cell function and insulin sensitivity, respectively(218). Based on proteomic profiles, one study has suggested that men and women may respond differently to CVD risk factors(219). It is therefore not inconceivable that sleep duration may result in sex-specific expressions of proteomic markers which could not be investigated in paper II or paper IV.

Additional sleep parameters of interest

This thesis has focused on one sleep-related parameter: sleep duration. This is not a limitation in itself given that it is a valid measure that has shown robust associations with health-related outcomes across many different study populations. However, it may be of interest to consider additional sleep parameters that may add additional dimensions to the understanding of sleep in the context of health. Such measures would include sleep quality which takes into account the subjective experience of sleep. A recent consensus statement of the National Sleep Foundation(220) discussed the importance of two sleep-related parameters: catch-up sleep to compensate for nights with insufficient sleep, and sleep regularity which focuses on the variability of sleep duration and sleep timing. Variability in sleep duration and sleep timing is significantly and positively associated with metabolic syndrome(221) and CVD (222) independently of sleep duration. Sleep regularity is also associated with risk of all-cause mortality and cardiometabolic mortality

independently of sleep duration(223). In order to accurately assess sleep regularity, objective measures of sleep duration are required, which is the focal point of future perspectives.

Future perspectives

Future research on the association between sleep and outcomes related to cardiometabolic disease and mortality, respectively, is suggested to focus on aspects that have not been considered in this thesis.

It is recommended that future research focuses on objectively measured sleep parameters, including but not limited to TST. Indeed, several studies have been published using accelerometer data to define sleep duration, sleep timing, or sleep regularity in the context of cardiometabolic disease and mortality. The number of prospective studies with large sample sizes are, however, limited. Given the advent of consumer wearable technologies and the emergence of wearable devices, it is recommended that sleep research aims to incorporate these new technologies under the condition that the devices meet the required standards set out by sleep researchers. Wearable technologies have been discussed for the development of sleep-related biomarkers(224). One very important standard that must be considered, and which was discussed at the Sleep Research Society's workshop(224), is the validation of wearable technologies. Indeed, part of our work has been to validate two different consumer wearables that have been used in our related research, one wrist-worn device(225) and one ring-type device(226), the latter of which was compared to PSG. Overall, validation studies point towards an improvement in the sensitivity, and specificity in wearable devices' detection of sleep. Although several question marks remain to be answered with regards to data privacy, the proprietary algorithms of said devices, the possibility to conduct studies in naturalistic settings with validated repeat measurements over weeks, or even months of sleep parameters, including TST, time in bed, sleep onset latency, sleep efficiency, and minutes spent in different sleep stages has never been more readily available.

Another consideration for future studies is to not only utilise objectively derived repeated measures of sleep parameters, but to also consider repeated measures of proteomic markers. Such data would allow for a better understanding of any changes that occur with regards to the expression of proteomic markers based on concomitant changes in sleep parameters.

Taken together, the focus of future studies should be on repeated measures of both digital markers and biomarkers over extended periods of time. This approach will guide our understanding of individual-level differences in both phenotype and proteomic expression.

Conclusion

The work in this thesis has identified noteworthy associations between short sleep duration and proteomic markers, which in turn are significantly associated with incident diabetes. The work thus suggests a pathway in which short sleep duration through inflammation and apoptotic activity is associated with incident diabetes which in turn increases the risk of future CHD. The work has also quantified the risk of mortality in a population of East Asians and through this highlighted sex and age as effect modifiers of such associations. The work thus adds to the understanding of how future risks of mortality may differ according to sex and age, and that sleep duration recommendations ought to consider these characteristics when formulating sleep duration recommendation guidelines.

Populärvetenskaplig sammanfattning

Sömn är en fysiologisk nödvändighet för människan. Sömnbehovet är individuellt, men studier har visat att en vuxen människa i genomsnitt bör sova mellan 6 och 9 timmar under en 24-timmarsperiod. Trots detta sover många av oss kortare eller längre än vad som är rekommenderat. Sådana avvikelser kan bero på ålder, där yngre sover längre än äldre, eller på yttre faktorer som till exempel livsstilsval och arbetstid. Kortare perioder med sömnbrist kan leda till märkbara, om än tillfälliga, konsekvenser som exempelvis uttalad trötthet eller försämrad prestationsnivå vilka snabbt kan återställas efter återställd sömnängd. Konsekvenserna av uttalade perioder med avvikande sömnmängd är betydligt allvarligare. Forskning har visat att för kort eller för lång sömn kan kopplas till inte bara diabetes och hjärtkärlsjukdom, men också till förtidig död.

År 2021 uppgick antalet nya fall av diabetes i världen till närmare 24 miljoner. Trender visar att antalet diabetesfall ökar och det uppskattas att antalet individer med diabetes kommer att uppgå till närmare 1.3 miljarder år 2050. Diabetes är en sjukdom i sig, men konsekvenserna sträcker sig längre än så. Diabetes är också en mycket viktig riskfaktor för framtida hjärtkärlsjukdom och förtidig död. Sjukdomsförebyggande åtgärder är således det viktigaste steget för att förbättra folkhälsan och på sikt minska antalet nya sjukdomsfall.

Diabetes- och hjärtkärlsjukdomsförebyggande åtgärder har fokuserat mycket på livsstilsförändringar med huvudsaklig inriktning mot kost och motion. Viktnedgång, förbättrade kolesterol- och blodsockernivåer har i sin tur varit mätbara resultat på framgångsrika livsstilsförändringar. Under senare år har forskning emellertid visat att avvikande sömnmängd, i synnerhet för kort sömn, också kan räknas till gruppen modifierbara livsstilsfaktorer för framtida sjukdomsrisk. Till exempel är för kort sömn kopplad till två hormoner som är kan öka hunger och aptit vilket i sin tur kan innebära att för kort sömn potentiellt kan leda till ökat energiintag med viktökning och efterföljande sjukdom som följd.

De exakta mekanismerna genom vilka avvikande sömnnivåer är kopplade till framtida diabetes- och hjärtkärlsjukdom är emellertid inte helt klarlagda. En tänkbar mekanism är ökad inflammation; studier har visat att kort sömn är kopplad till ett flertal markörer som talar för systemisk inflammation. Dessa inflammationsparametrar har även visat sig vara riskfaktorer för hjärtkärlsjukdom. Problemet med dessa parametrar är de är förhöjda också vid inflammatoriska tillstånd samt vid infektioner; de är med andra ord inte specifika för sömn.

Under senare år har ny teknologi gett forskare möjligheten att mäta proteinmarkörer i blodprover. Dessa proteinmarkörer erbjuder nya infallsvinklar för studier som bättre vill förstå sambandet mellan livsstilsfaktorer och sjukdom då de kan ge en inblick i möjliga biologiska signalvägar. Få forskare har fokuserat på sömnmängd och proteinmarkörer, och ännu färre har vidare kunnat undersöka hur dessa sömnspecifika proteinmarkörer är kopplade till nyinsjuknande i diabetes- och kranskärlssjukdom. Nyinsjuknande kan mätas i studier som följer deltagare under flera år och där insamlade data om livsstilsfaktorer (t.ex. sömn) och blodmarkörer (t.ex. proteinmarkörer) kopplas till information om sjukdom som hämtas från nationella registerdatabaser. Sådan forskning är unik för Sverige och de nordiska länderna.

Denna avhandling baseras på fyra studier som har två huvudsakliga mål. Det första målet är att öka förståelsen för vilken roll kön och ålder spelar i sambandet mellan sedvanlig sömnmängd och risken att dö. Det andra målet är att öka förståelsen för sambandet mellan sömn och nyinsjuknande i diabetes respektive kranskärlsjukdom. Ett viktigt delmål i det sistnämnda målet är att undersöka om antalet timmar sömn kan kopplas till specifika proteinmarkörer samt om det är möjligt, baserat på uppmätta proteinmarkörer, att föreslå signalvägar för sambandet mellan sömnmängd och nyinsjuknande i diabetes respektive kranskärlssjukdom.

Resultaten av en i avhandlingen ingående studie visar att kön inte spelar roll för sambandet mellan sömnmängd och död i de fall där dödsorsaken inte utgör huvudfokus. Däremot spelar ålder en viktig roll för detta samband: män yngre än 65 år har ökad risk för död när antalet timmar sömn är färre eller fler än 7 timmar. Hos män som är äldre än 65 år ökar risken för död endast om sömnmängden är lika med eller större än 9 timmar. Vidare visar samma studie att kön spelar en viktig roll för sambandet mellan antalet timmar sömn och död från kardiovaskulära sjukdomar.

Resultatet i avhandlingen visar också att sambandet mellan sömnmängd och nyinsjuknande i kranskärlssjukdom föregås av insjuknande i diabetes. Antalet timmar sömn är också kopplat till flera proteinmarkörer vilka tillsammans talar för att de tänkbara och föreslagna signalvägarna som kan koppla för lite sömn till nyinsjuknade i diabetes i huvudsak representerar signalvägar relaterade till inflammation och programmerad celldöd. Samma proteinmarkörer är i våra studier inte direkt kopplade till kranskärlssjukdom. Detta styrker ytterligare hypotesen att för lite sömn kan kopplas till diabetesinsjuknande vilket i sin tur ökar risken för insjuknande i kranskärlssjukdom.

Fynden i denna avhandling talar för att sömnmängd är en viktig livsstilsfaktor som direkt kan kopplas till diabetes och indirekt till kranskärlssjukdom. Upptäckten av föreslagna signalvägar möjliggör för fortsatta studier som förhoppningsvis kommer att kunna besvara frågan om orsakssamband. Ur ett folkhälsoperspektiv talar resultaten i denna avhandling för vikten att bibehålla en rekommenderad sömnmängd runt ca 7 timmar.

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