PSYCHOSOCIAL EXPOSURES AT WORK, PHYSIOLOGICAL STRESS RESPONSE AND DEVELOPMENT OF ATHEROSCLEROSIS

– RESULTS FROM TWO COHORT STUDIES.

NANNA HURWITZ ELLER, 2012

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Denne afhandling er af Det Sundhedsvidenskabelige Fakultet ved Københavns Universitet antaget til offentligt at forsvares for den medicinske doktorgrad.

København, den 4/12 2012.

Ulla Wewer,
dekan.

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PREFACE

First of all, I wish to thank all co-authors, collaborators and the participants in the Atherosclerosis and Work-project and the Organization, Stress and Health-project studies. However, a few individuals have to be mentioned for being especially helpful: DMSc Bo Netterstrøm for planning the Atherosclerosis and Work-project, secretary Ina Sørensen for all contact to the participants of the project, chief physician Carsten Hædersdal for instruction and rendering technical assistance in connection with the ultrasound scans, senior researcher Åse Marie Hansen and senior researcher Jesper Kristiansen at The Danish National Research Centre for the Working Environment for analysis of hormones and HRV, respectively, MD Birgitta Malmberg, Lund University Hospital, Sweden for introducing me to the HRV-method, and professor Søren Feodor Nielsen for statistical assistance.

The Atherosclerosis and Work-project received financial support from "Master builder Lauritz Peter Christensen and wife Kirsten Sigrid Christensen's Fund", the Region 3-fund, and the Danish Heart Foundation. The National Research Centre for the Working Environment financed the analysis of hormones in 1998. The Danish Working Environment Research Fund funded the Organization, Stress and Health-project.

FORORD

I slutningen af mit medicinske studium fik jeg midt i 1980'erne mulighed for at opholde mig en måned på Arbejdsmedicinsk Klinik på Rigshospitalet. Jeg blev modtaget med åbne arme af specialets første professor, overlæge dr.med. Finn Gyntelberg og følte mig straks godt tilpas i arbejdsmedicinen. Finn satte mig i gang med mine første videnskabelige opgaver og har i det afsluttende arbejde før indlevering af disputatsen opmuntret og bidraget med konstruktiv kritik. En arbejdsmedicinsk ansættelse blev det dog først til, da jeg i 1996 blev reservelæge ved Arbejdsmedicinsk Klinik på Hillerød Hospital hos overlæge dr.med. Bo Netterstrøm. Han havde nogle år forinden forsvaret sin disputats "Psykosocial arbejdsbelastning og iskæmisk hjertesygdom", og det var mig en gåde, hvordan en psykisk belastning skulle kunne medføre en fysisk sygdom som akut myokardieinfarkt. Nu er jeg ikke i tvivl om, at psykisk (arbejds)belastning er af stor betydning for vores helbred, fysisk såvel som psykisk. Det daglige arbejde på klinikken lærte mig meget om psykisk arbejdsmiljø og stress. Tak til alle de kolleger jeg mødte der for en dejlig arbejdsplads gennem mange år.

Da ARA-projektet påbegyndtes i 1998, planlagde Bo studiet og indgød mig troen på, at jeg ville kunne bidrage med noget nyt. Sekretær Ina Sørensen stod for kontakten til projektets deltagere og det daglige overblik. Projektet var lokalt forankret, og jeg fik opbakning fra flere afdelinger på Hillerød Sygehus. Jeg kunne låne en ultralydsscanner kvit og frit på Klinisk Fysiologisk Afdeling, hvor ledende overlæge Carsten Hædersdal vejledte mig og ydede teknisk assistance, en bogstavelig talt ubetalelig hjælp. I 1998 fik jeg lov at bruge et lokale i det nye kardiologiske ambulatorium på Medicinsk Afdeling B i 3 måneder, i 2002 kunne jeg have ultralydsscanneren på mit kontor, og i 2008 kunne jeg være på Klinisk Fysiologisk Afdeling efter dagarbejdstid. Fra Neurologisk Afdeling stillede neuropsykolog Peter Bruhn sin Interferenstest og bånd med PASAT-testen til min rådighed. På Det Nationale Forskningscenter for Arbejdsmiljø, NFA, forestod seniorforsker Åse Marie Hansen alle hormonanalyser, og seniorforsker Jesper Kristiansen oparbejdede HRV-data fra ARA2008 og fra OSH. Læge Birgitta Malmberg, Lunds Universitetshospital introducerede mig til HRV-metoden i 2002 og hjalp med oparbejdning af data fra ARA2002. Professor i statistik, Peter Allerup, Danmarks Pædagogiske Universitet, har bidraget til artikel III, mens professor i statistik Søren Feodor Nielsen, Copenhagen Business School har analyseret og fortolket data og bistået med manuskriptet til artikel V. I forbindelse med OSH-projektet har overlæge Morten Blønd, Arbejdsmedicinsk klinik, Nykøbing Falster Hospital, forestået indsamling af data og kliniske undersøgelser sammen med henholdsvis arbejdsmediciner Anette Rosenthal og afdelingslæge ph.d. Martin Lindhardt Nielsen. Endelig har deltagerne i de to studier stillet sig selv og deres tid til rådighed; tusind tak til alle!

ARA-projektet gennemførtes i 1998 med støtte fra Murermester Lauritz Peter Christensen og hustru Kirsten Sigrid Christensens Fond, mens Arbejdsmiljøinstituttet bekostede hormonanalyserne. I 2002 støttedes projektet af Region 3-Fonden, og frikøb til sammenskrivning i 2006 blev finansieret af Hjerteforeningen. OSH-projektet støttedes af Arbejdsmiljøforskningsfonden.

Til sidst tak til Stig for din daglige opbakning og til vores sønner Mathias, Povl og Lauritz, fordi I lever jeres liv med så stort engagement.

Nanna Eller
Januar 2013
Arbejds- og Miljømedicinsk Afdeling, Bispebjerg Hospital

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LIST OF PAPERS:

The thesis is based on the following 7 papers, referred to in the text by their Roman numerals.

I Eller, N. H., and Netterstrøm, B. Psychosocial factors at home and at work and four year progression in intima media thickness. International Journal of Behavioural Medicine 2007; 14 (1):21-29. #

II Eller, N.H., Netterstrøm, B., and Hansen, Å.M. Psychosocial factors at home and at work and levels of salivary cortisol. Biological Psychology 2006; 73:280-287.

III Eller, N.H., Kristiansen, J. and Hansen, Å.M. Long-term effects of psychosocial factors of home and work on biomarkers of stress. International Journal of Psychophysiology 2011; 79:195-202.

IV Eller, N.H., Nielsen, S.F., Blønd, M., Nielsen, M., Hansen, Å.M. and Netterstrøm, B. Effort reward imbalance and salivary cortisol in the morning. Biological Psychology 2012; 89:342-348.

V Eller, N.H. Kristiansen, J., Blønd, M., Nielsen, M., and Netterstrøm, B. Effort reward imbalance is associated with vagal withdrawal in Danish public sector employees. International Journal of Psychophysiology 2011; 81:218-224.

VI Eller, N. H., Netterstrom, B., and Allerup, P. Progression in intima media thickness - the significance of hormonal biomarkers of chronic stress. Psychoneuroendocrinology 2005; 30:715-723.

VII Eller, N. H., Malmberg, B., and Bruhn, P. Heart rate variability and intima media thickness. International Journal of Behavioural Medicine 2006; 13(3):201-213.

 # Erratum
Paper I: In the section "Strategy for the analysis" a paragraph is missing: The text should read:
Baseline levels of all other variables (psychosocial factors at home, physiological measures and behavioural measures) were used as independent variables in a series of simple linear regression analyses using IMTprogression as the dependent variable. Also, IMTmean (1998) was included as a covariate.

ABBREVIATIONS USED:

ABU . . . . Anstrengelse-Belønnings-Ubalance, den danske betegnelse for ERI.
ACR . . . . awakening cortisol response, increase in salivary cortisol during the first 30 minutes after waking
AMI . . . . acute myocardial infarction
AW . . . . Atherosclerosis and Work, name of the primary project
CAD . . . . coronary artery disease
CCA . . . . common carotid artery
CI . . . . confidence interval
DC . . . . demand control
ECG . . . . electrocardiogram
ERI . . . . effort reward imbalance
FDG . . . . fluor-18 labelled 2-deoxy-D-glucose
HbA_{1c} . . . . glycated haemoglobin
HDL . . . . cholesterol, high-density lipoprotein cholesterol
HF . . . . high frequency power, high frequent variance in heart rate variability during a time period, e.g. 5 minutes (0.18-0.40 Hz)
HFnu . . . . HF / (TP-VLF)
HPA-axis . . . . hypothalamic-pituitary-adrenal axis
HRV . . . . heart rate variability
IHD . . . . ischemic heart disease
IHS . . . . iskæmisk hjertesygdom
IMT . . . . intima media thickness
KIDHs . . . . Kupio Ischemic Heart Disease study
KK . . . . krav kontrol
LF . . . . low frequency power, low frequency variance in heart rate variability during a time period, e.g. 5 minutes (0.04-0.18 Hz)
LF / $HF_{difference}$ . . . . . . the difference between LF / HF during test and LF / HF during sleep
LFnu . . . . LF / (TP-VLF)
Ln . . . . natural logarithm
NRCWE . . . . the National Research Centre for the Working Environment
OSH . . . . Organisation, Stress and Health, name of the secondary project
PET . . . . positron emission tomopgraphy
RMSSD . . . . the square root of the mean of the sum of the squares of the differences between adjacent intervals
S1 . . . . salivary cortisol in the first saliva sample collected immediately after waking
SX . . . . salivary cortisol in the saliva sample collected X minutes after waking
SA-node . . . . sino-atrial node
SA-system . . . . sympathico-adrenal system
SD . . . . standard deviation
SDNN . . . . standard deviation of all NN intervals (NN is the duration between two normal heart beats)
TP . . . . total power, the total variance in heart rate variability during a time period, e.g. 5 minutes (<0.40 Hz)
VLF . . . . very low frequency power, low frequent variance in heart rate variability during a time period, e.g. 5 minutes (<0.04 Hz)
WWH . . . . weekly working hours

SUMMARY

Among the many risk factors for ischaemic heart disease (IHD) work-related psychosocial factors increase the risk of IHD independently of the conventional coronary risk factors. The pathophysiological explanation for this association has not been entirely identified, but it has been suggested that long-term or repeated physiological stress may be atherogenic. The possible atherogenic effect of psychosocial factors has been difficult to examine in population studies because a feasible measure of the level of physiological stress has not been available. Additionally, the use of acute myocardial infarction as an endpoint in stress research has been problematic. During recent decades, technical advances have made research in stress physiology and early atherosclerosis in population studies possible. The physiological stress response can be measured non-invasively using salivary cortisol and analysis of heart rate variability (HRV), and the individual's level of atherosclerosis can be directly estimated in the form of carotid intima media thickness (IMT) on the common carotid artery (CCA).

The aim of this thesis was to evaluate the significance of work-related psychosocial factors for the pathophysiology of stress and atherosclerosis, testing the following hypothesis: a) Work-related psychosocial factors influence the physiological stress response, b) which in itself is atherogenic. The hypothesis was studied in three parts, including the link between work-related psychosocial factors and IMT. Furthermore, a) the link between the work-related psychosocial factors and the physiological stress response, and b) the link between the stress response and IMT were studied. The partial hypothesis a) was studied in two cohorts.

The two cohorts were The Atherosclerosis and Work study (the AW study), and The Organisation, Stress, and Health study (the OSH study). The AW study included 130 working people (30-59 years) in 1998, of whom 95 were re-examined in 2002 and 70 in 2008. The OSH study included 502 individuals in 2006, of whom 391 were re-examined in 2008. Early atherosclerosis was examined in the AW study and estimated by means of IMT measures obtained at both CCAs of an individual at the transition to the bulbous. As the participants were ultrasound-scanned in both 1998 and 2002, a study of progression in IMT was possible. Progression in IMT may mirror the effect of a factor better than IMT itself, as IMT is determined by genetics and the effect of all atherogenic factors experienced until the measurement. In both cohorts, the participants' physiological stress states were measured using cortisol in the saliva (at awakening and after 20 or 30 minutes (ACR) and in the AW study, 4 more samples during the day) and analysis of HRV (spectral analysis including the measures of total power (TP), low frequency power (LF), and high frequency power (HF)).

The term "work-related psychosocial factors" includes a large variety of measures. The factors included were the dimensions from the Demand Control model (DC model) and the Effort Reward Imbalance model (ERI model). Furthermore, a number of background variables were included in the AW study, i.e., social status, civil status (single or co-habiting), the number of children in the home, the number of hours used weekly looking after the home in 2002, the experience of time pressure in daily life in 2002, and the number of working hours per week (WWH).

In general, the data were analysed separately for women and men by linear regression, analysis of variance, and repeated measures analysis of covariance. The OSH data were analysed by means of a mixed effects model, including subject as a random effect. The thesis includes a number of supplementary interaction analyses to reveal possible gender differences in connection with the physiological stress response and early atherosclerosis.

WORK-RELATED PSYCHOSOCIAL FACTORS AND IMT

Associations between psychosocial factors and progression in IMT (paper I) could be demonstrated among the women in the form of an association between high effort and large IMT progression, in the same way as co-habiting women had larger IMT progression than single women. Large IMT progression among the men was connected to high age, fewer children and high effort reward imbalance (ERI). Adjustment for baseline levels of conventional coronary risk factors did not change the results significantly. An analysis of the interaction between gender and ERI confirmed the gender difference. Furthermore, effort and overcommitment were associated with IMT progression in both genders. These findings indicate an association between the ERI model and progression in IMT over four years.

WORK-RELATED PSYCHOSOCIAL FACTORS AND THE PHYSIOLOGICAL STRESS RESPONSE

The AW study: The co-habiting women exhibited higher cortisol levels throughout the entire day than the single women. A high level of time pressure was associated with a large ACR, as well as with high cortisol levels during the day among women. Among the men, high levels of effort, ERI and overcommitment were connected with high ACR and high cortisol levels throughout the day (paper II). There was a significant interaction between the ERI model and gender, suggesting that this effect was more pronounced in men. Neither ERI nor other psychosocial factors had a significant association with cortisol in the long-term analysis (paper III). However, a long-term effect of the ERI model on HRV data in men was shown in the form of a significant association between effort and ERI and low TP, whereas reward was significantly associated with high TP. Also in this analysis, a significant interaction between gender and the ERI model was revealed, meaning that the ERI model was associated with TP in men but not in women.

The OSH study: The dimensions of the ERI model were only nearly significant for cortisol. However, cortisol in the form of the awakening-level and ACR increased significantly in the cohort from the baseline in 2006 to the follow-up in 2008 (paper IV). In contrast, the ERI model was significantly associated with HRV, i.e., effort and ERI were associated with decreased levels

of TP and HF, whereas reward was associated with increased TP and HF. ERI was also significantly associated with increased LF/HF (paper V). The year of measurement was not of significance to the HRV data. Therefore, it was suggested that HRV (the autonomic nervous system) responds to stressors perceived at the time of measurement. There were no interactions between gender and the ERI model in these data.

THE PHYSIOLOGICAL STRESS RESPONSE AND IMT

Among the women, a strong association was found between a high rise in cortisol in the first 20 minutes in the morning and a large progression in IMT (paper VI). A similar connection was not found among the men in the sex-stratified analyses. However, sex and salivary cortisol did not interact, and when all participants were included in the analysis, a cortisol increase in the first 20 minutes in the morning was positively and significantly associated with IMT progression. Among the men, a tendency was found towards a low HRV being associated with high IMT. A large difference between LF / HF during test and sleep, i.e., having a sympathetic-dominated autonomous tonus during test and a parasympathetic dominance during sleep, was significantly associated with low IMT (paper VII). Sex and HRV did not interact.

DISCUSSION:

The methodology of the AW study may be criticised in several ways. First, the number of participants in the AW study included to examine the progression in IMT was small. This problem was enlarged since all of the analyses were performed separately for each sex, and hereby further reducing the size of the groups in the analyses in which IMT progression was the dependent variable. This small size will invariably lead to weaker associations. The population was self-selected and experienced little psychosocial strain, especially in relation to the DC model. This selection bias might be expected to minimise the chance of finding any association between psychological strain and atherosclerosis. The significance of psychological workload may thus be larger than the AW study showed, and the results should not be used to dismiss a possible significance of strain in relation to the DC model. Similarly, the spread of the covariate used was small, which may explain why the study found only weak associations with the conventional coronary risk factors. The work-related psychosocial factors were estimated by means of a questionnaire filled in at two time points. The salivary cortisol, HRV, and IMT were measured at the same time. However, IMT develops over months and years. A given measurement of physiological stress may mirror the actual workload but may be of limited relevance with respect to IMT development. A major strength of the study was its prospective design.

The OSH study included data on the ERI model, cortisol and HRV measured simultaneously. The ERI model was significantly associated with HRV while the ERI model did only tend to be associated with ACR. These results imply that the two allostatic systems respond differently to a given stressor.

CONCLUSION:

Although the women and men participating in the AW study did not belong to a risk population, an association between psychological working environments, physiological stress response and early atherosclerosis could be demonstrated. High ERI was associated with high IMT progression, high ACR, and low HRV in men. The effect of the ERI model on the physiological stress response was confirmed in the OSH study with respect to HRV, but the association between the ERI model and cortisol was not significant.

Work-related psychosocial factors, especially those covered by the ERI model, were associated with activity in both the HPA axis and the autonomic nervous system and with increased IMT progression. The psychological quality of the working environment may be associated with the risk of atherosclerosis.

DANSK RESUMÉ

Blandt de mange risikofaktorer for iskæmisk hjertesygdom (IHS) er arbejdsrelaterede psykosociale faktorer, som uafhængigt af konventionelle koronare risikofaktorer øger risikoen for IHS. Den patofysiologiske forklaring på denne sammenhæng er ikke fuldstændigt identificeret, men det er foreslået, at langvarig eller gentagen fysiologisk stress kan være aterogen. En mulig aterogen effekt af psykosociale faktorer har været vanskelig at undersøge i populationsstudier, idet håndterbare metoder til måling af det fysiologiske stressrespons ikke har været tilgængelige. Samtidig har brugen af akut myokardieinfarkt som effektmål i stressforskningen været problematisk. I løbet af de seneste årtier har den tekniske udvikling muliggjort forskning i stressfysiologi og tidlig aterosklerose i populationsstudier. Det fysiologiske stressrespons kan måles non-invasivt ved brug af spytkortisol og hjerterytmevariabilitet (HRV), og det enkelte individs grad af aterosklerose kan måles direkte i form af intima media tykkelsen (IMT) på arteria carotis communis (ACC).

Formålet med afhandlingen var at undersøge betydningen af arbejdsrelaterede psykosociale faktorer for det fysiologiske stressrespons og tidlig aterosklerose. Hypotesen var: a) Arbejdsrelaterede psykosociale faktorer påvirker det fysiologiske stressrespons, b) som i sig selv er aterogent. Hypotesen blev undersøgt i tre trin, hvoraf det første var sammenhængen mellem arbejdsrelaterede psykosociale faktorer og progression i IMT. Desuden undersøgtes a) sammenhængen mellem arbejdsrelaterede psykosociale faktorer og det fysiologiske stressrespons og b) sammenhængen mellem stressresponset og IMT. Delhypotese a) undersøgtes i to kohorter.

De to kohorter var Arbejdsmiljø og aterosklerose (ARA) og Organisation, Stress, og Helbred (OSH). ARA inkluderede 130 personer i arbejde (30- 59 år) i 1998, hvoraf 95 blev genundersøgt i 2002 og 70 i 2008. OSH inkluderede 502 personer i 2006, hvoraf 391 genundersøgtes i 2008. Tidlig aterosklerose blev undersøgt i ARA og estimeret ved IMT-målinger fra begge ACC ved overgangen til bulbus. Idet deltagerne blev scannet både i 1998 og i 2002, var det muligt at studere progression i IMT. Progression i IMT afbilleder muligvis bedre effekten af en given påvirkning end IMT selv, idet IMT er genetisk bestemt og påvirket af alle de aterogene faktorer, individet har været udsat for indtil undersøgelsen. I begge kohorter, måltes individets grad af stress ved brug af spytkortisolmålinger (ved opvågning og efter 20 (30) minutter (ACR), samt i ARA, desuden 4 prøver i løbet af dagen) og analyse af HRV (spectralanalyse med målene total power (TP), low frequency power (LF) og high frequency power (HF)).

Termen "arbejdsrelaterede psykosociale faktorer " inkluderer en række forskellige mål. De faktorer, der inkluderedes i de nævnte studier var dimensionerne fra Krav-Kontrol-modellen (KK-modellen) og Anstrengelse-Belønnings-modellen (AB-modellen). I ARA inkluderedes desuden social status, civil status (enlig eller samboende), antal børn i hjemmet, antal timer brugt på husligt arbejde i 2002, oplevelse af tidspres i hverdagen i 2002 og ugentlige arbejdstimer.

Generelt analyseredes data separat for kvinder og mænd med brug af lineær regression, varians- og covarians-analyse. OSH-data blev analyseret med brug af mixed effects model med individ som random effect. Afhandlingen inkluderer en række supplerende interaktionsanalyser for at afdække mulige kønsforskelle i forbindelse med psykosociale faktorer, stressrespons og IMT.

PSYKOSOCIALE FAKTORER I ARBEJDET OG IMT

Der fandtes association mellem stor anstrengelse og IMTprogression hos kvinder, ligesom samboende kvinder havde større IMTprogression end enlige kvinder (artikel I). Stor IMTprogression var hos mænd associeret til høj alder, færre børn og stor ubalance mellem anstrengelse og belønning (ABU). Kontrol for konventionelle koronare risikofaktorer målt ved baseline ændrede ikke resultaterne signifikant. En interaktionsanalyse mellem køn og ABU bekræftede kønsforskellen. Anstrengelse og overforpligtigelse var associeret med IMTprogression i begge køn. Fundene indikerer, at arbejde med ABU er associeret med progression i IMT gennem 4 år.

PSYKOSOCIALE FAKTORER I ARBEJDET OG DET FYSIOLOGISKE STRESS RESPONS

ARA-studiet: Samlevende kvinder havde højere kortisolværdier gennem dagen end enlige kvinder. Oplevelse af tidspres var associeret med ACR og med høje kortisolværdier dagen igennem. Blandt mænd var stor anstrengelse, ABU og overforpligtigelse associeret med høj ACR og høje kortisolværdier gennem dagen (artikel II). Der var en signifikant vekselvirkning mellem ABU-modellen og køn, som tydede på, at ABU var af større betydning for mænd end for kvinder. Hverken ABU eller andre psykosociale faktorer var associeret med kortisol i form af målinger fra både 2002 og 2008 (artikel III). Der fandtes imidlertid en langvarig virkning af ABU-modellen på HRV-målinger fra både 2002 og 2008 hos mænd i form af en signifikant association mellem anstrengelse og ABU og lav total power (TP), mens belønning var signifikant associeret med høj TP. Her var der også en signifikant vekselvirkning mellem køn og ABU-modellen, således at ABU-modellen var associeret med TP hos mænd, men ikke hos kvinder.

OSH-studiet: Dimensionerne fra ABU-modellen var kun nær-signifikant associeret med kortisol, men kortisolniveauet ved opvågning og ACR steg signifikant for hele kohorten fra 2006 til 2008 (artikel IV). Anstrengelse og ABU var associeret med nedsat TP og HF, mens belønning var associeret med forhøjet TP og HF. ABU var også signifikant associeret med forhøjet LF/HF. Året for måling var ikke af betydning for HRV (artikel V). Dette indikerer, at HRV (det autonome nervesystem) reagerer samtidigt med oplevelse af stressorerne. Der var ikke vekselvirkninger mellem køn og ABU-modellen i disse data.

DET FYSIOLOGISKE STRESS RESPONSE OG IMT

Blandt kvinder fandtes en signifikant sammenhæng mellem stor stigning i kortisol de første 20 minutter om morgenen og en stor progression i IMT (artikel VI). En tilsvarende sammenhæng fandtes ikke blandt mænd i de kønsstratificerede analyser.

Imidlertid var der ingen vekselvirkning mellem køn og kortisol. Når alle deltagere inkluderedes i samme analyse med køn inkluderet som faktor, var kortisolstigning de første 20 minutter om morgenen positivt og signifikant associeret med IMTprogression de følgende fire år.

Blandt mænd var der en tendens til en sammenhæng mellem lav HRV og høj IMT. En stor forskel mellem LF / HF under stresstest og under søvn, dvs. at have sympatisk dominans under stresstest og parasympatisk dominans under søvn var associeret med lav IMT (artikel VII). Køn og HRV vekselvirkede ikke.

DISKUSSION:

Metodemæssigt kan ARA kritiseres på en række områder. For det første var studiet lille, når effektmålet var progression i IMT. Dette problem forværredes ved brug af køns-stratificerede analyser, idet grupperne blev meget små. Dette fører samtidig til svagere associationer. Populationen var selv-selekteret og oplevede kun lille belastning, specielt i relation til KK-modellen. Denne selektionsbias må forventes at mindske chancen for at finde en association mellem psykosocial belastning og aterosklerose. Betydningen af psykosocial belastning kan således være større, end den ARA viste, og resultaterne kan ikke bruges til at afvise KK-modellen. Tilsvarende var spredningen i de covariate variable lille, hvilket muligvis forklarer, at studiet kun fandt begrænset betydning af de konventionelle koronare risikofaktorer. De psykosociale faktorer på arbejdet blev målt ved brug af spørgeskemaer udfyldt på to tidspunkter, samtidig med måling af spytkortisol, HRV og IMT. Imidlertid udvikler IMT sig over måneder og år. Et givent mål for fysiologisk stress kan afspejle den aktuelle belastning uden at være af betydning for IMT. Det er en styrke, at studiet var prospektivt.

OSH inkluderede data vedrørende ERI modellen, kortisol og HRV målt på samme tid. ERI modellen var signifikant associeret med HRV mens ERI modellen kun viste en ikke-signifikant association med ACR. Disse resultater peger på, at de to allostatiske systemer reagerer forskelligt på en given stressor.

KONKLUSION:

På trods af at deltagerne i ARA-studiet ikke tilhørte en risikopopulation, fandtes en sammenhæng mellem psykosocial arbejdsmiljø, fysiologisk stressrespons og tidlig aterosklerose. Stor ABU var associeret med IMTprogression, høj ACR og lav HRV hos mænd. Betydningen af ABU-modellen for det fysiologiske stressrespons blev bekræftet i OSH-studiet i forbindelse med HRV, men sammenhængen med kortisol var ikke signifikant.

Psykosociale faktorer specielt belyst af ABU-modellen var associeret med aktivitet i HPA-aksen og i det autonome nervesystem og med øget IMTprogression hos mænd. Kvaliteten af det psykiske arbejdsmiljø kan være associeret med risiko for aterosklerose.

INTRODUCTION

Ischaemic heart disease (IHD) is one of the Western world's most widespread diseases and causes of death (Braunwald, 1997, Cooper et al. 2000). Among the many risk factors for this condition, work-related psychosocial factors increase the risk of IHD independently of the conventional coronary risk factors (Eller et al. 2009, Kivimaki et al. 2006b). The pathophysiological explanation of an association between psychosocial factors and IHD has not been entirely elucidated. Because psychological stressors activate the physiological stress response (Dickerson et al. 2004, Kovács et al. 2005), a patho-physiological explanation may be that long-term or repeated physiological stress due to the psychological working environment may be atherogenic (Dickerson et al. 2004, Kovács et al. 2005, Rozanski et al. 1999).

A patho-physiological explanation of how work-related psychosocial factors may lead to IHD has been difficult to examine in population studies because feasible physiological measures of the level of physiological stress have not been available. Currently, the physiological stress response can be measured non-invasively through the use of salivary cortisol (Clow et al. 2004, Kirschbaum et al. 1994, Pruessner et al. 1997) and analysis of heart rate variability (HRV) (Kristal-Boneh et al. 1995, Malik, 1996).

The use of acute myocardial infarction (AMI) as an endpoint in stress research has been problematic. AMI occurs predominantly in elderly men and even then rarely; therefore, studies using AMI as an effect measurement must include a large number of subjects who should be followed for several years. However, today, the individual's level of atherosclerosis can be directly estimated by ultrasound examination of the walls of the arteries in the neck, and the intima media thickness of the vessel wall (IMT) may be considered to be a valid alternative for cardiovascular events as outcome (Bots et al. 2003, Mancini et al. 2004, Peters et al. 2011c).

Due to these advances, research in stress physiology and atherosclerosis is proceeding.

OVERVIEW

The purpose and hypotheses of the thesis based on papers I-VII are stated. The methods used are presented, as are the two cohort studies from which results are included, the Atherosclerosis and Work study (AW study) and the Organisation, Stress, and Health study (OSH study). The results are described in light of the hypotheses and the literature. Finally, the methodological challenges, statistical considerations, and biological plausibility of the results are discussed, directions for future research are suggested, and a conclusion is drawn.

THE PURPOSE OF THE STUDY

The aim of the study was to evaluate the significance of work-related psychosocial factors in the pathophysiology of atherosclerosis.

HYPOTHESES

The hypothesis was that a) work-related psychosocial factors influence the physiological stress response, b) which in itself is atherogenic, reformulated as follows:

OVERALL HYPOTHESIS

1 Adverse work-related psychosocial factors are positively associated with progression in IMT (paper I, prospective study).

PARTIAL HYPOTHESES RELATED TO THE MAIN HYPOTHESIS, PART A

2 Adverse work-related psychosocial factors are positively associated with reactivity in the hypothalamic-pituitary-adrenal axis (HPA axis) in the morning (papers II, cross-sectional study; III, prospective study, and IV, mixed effect study).

3 Adverse work-related psychosocial factors are associated with decreased HRV (papers III, prospective study, and V, mixed effect study).

4 Work-related psychosocial factors measured at baseline are associated with biomarkers of stress in follow-up studies (paper III).

PARTIAL HYPOTHESES RELATED TO THE MAIN HYPOTHESIS, PART B

5 High reactivity in the HPA axis in the morning is associated with high IMT progression (paper VI, prospective study).

6 Low HRV is associated with high IMT and IMT progression (paper VII, cross-sectional study).

SUMMARY OF THE METHODS USED

WORK-RELATED PSYCHOSOCIAL FACTORS:

The term "work-related psychosocial factors" includes a large variety of measures originating in various working environments and cultures (Eller et al. 2009). Psychological stressors can be evaluated objectively (e.g., the number of weekly working hours (WWH)) or subjectively in the form of self-reported data. Furthermore, the so-called ecological method uses reports from people with particular types of jobs to describe their exposure to psychosocial factors in those types of jobs (aggregate data). This method prevents individual experiences of strain from influencing the measure of exposure. Theoretical stress models are frequently used as the bases for questionnaires, i.e., the Demand Control Model (DC model), and the Effort Reward Imbalance Model (ERI model), as these models (particularly the DC model) have been frequently examined, and several papers have shown associations between psychosocial load according to the models and different endpoints (Eller et al. 2009, Netterstrom et al. 2008). In the AW study, one objective was to describe the psychosocial working environment in as much detail as possible. At the time of the baseline study in 1998, this goal included the use of an objective measure, WWH, as well as the DC model and the ERI model. All of the measures were self-reported. However, the DC model was found to have no associations with the effects measured (Netterstrom et al. 2010b). Accordingly, in the papers including data from the OSH study, only the ERI model was used as the measure of psychological load at work.

The DC model by Karasek (1979) includes two main dimensions: demands and control (decision latitude and skill discretion) (Karasek R et al. 1981). The model is based on the notion that demands per se are not stressful if they are coupled with adequate control over work and the work environment. By combining the two main dimensions, four working conditions are possible: jobs with high demands and high control are termed "active" (e.g., lawyers and general practitioners), jobs combining high demands and low control are termed "strained" (e.g., assembly line workers and bus drivers), jobs with low demands and high control are termed "relaxed" (e.g., craftsmen), and jobs with both low demands and low control are termed "passive" (e.g., attendants). According to the DC model, individuals working under job conditions characterised as "strained" have a greater risk of developing stress-related diseases (Karasek et al. 1988). Social support has been shown to modify the strain that might lead to stress, and in several studies, social support is used together with the DC model (Johnson et al. 1989). Working conditions that include strain and low social support, iso-strain, are the worst.

The ERI model was proposed by Siegrist in 1996 and can be seen as a model complementary to the DC model. The key concept of this model is social reciprocity. The model refers to the individual's experience of the balance between the effort made and the reward received (Siegrist, 1996, Siegrist et al. 2004). According to the ERI model, the most stressful condition occurs when the effort made is not followed by sufficient reward. Reward is not only a financial matter but also includes the esteem associated with the work, as well as the security of the work and future prospects. An effort-reward imbalance (ERI) according to the model, may lead to stress. People with personalities characterised by overcommitment are more likely to accept such an imbalance and face greater risk of becoming stressed (Siegrist et al. 2004).

The use of theoretically based stress models may be a strength of a design, but at the same time, it is important that the models do not rule out the exploration of other stressors. During the last 20 years or more, the labour market has changed, and fewer people are employed in production (e.g., industry and farming), whereas more people are employed in education, administration, health care, and knowledge production. This shift means that the stress models used in the 1980s might require further development to be used today. In the 1970s, when the DC model was developed, job strain showed stronger association with disease than did demands and control separately (Eller et al. 2009). The increased education level of the working population and the increased demands on the labour market necessitate adjustments in the way exposure is measured. Different occupations have different types of psychological demands, and higher-status occupations tend to be associated with higher psychological demands (Marmot et al. 1997). This methodological challenge is discussed by Kristensen et al., who suggest that researchers in the field should distinguish between intensification (faster work pace, mostly relevant for blue-collar workers) and extensification (longer working hours and deadlines, mostly relevant for white-collar workers) of demands (Kristensen et al. 2004).

IMT:

When an artery is scanned by ultrasound at right angles to the vessel wall, a characteristic double line is observed. Pathological anatomical studies have shown that the width of this double line corresponds to the distance from the endothelium (intima) to the transition between the media and adventitia (Pignoli et al. 1986). The distance is called IMT. The IMT can be measured with great precision (Salonen et al. 1993a), a precision that is increased with the number of measurements (Schmidt C et al. 1999). Since the basic original paper by Pignoli et al., IMT has been widely used as a measure of the degree of coronary atherosclerosis. This usage has been criticised as not necessarily measuring the actual atherosclerosis but possibly only an adaptive response in the vessel wall, due to increased strain. However, these changes are presumably an expression of the earliest stages of atherosclerosis, consistent with the finding that the associations between the risk factors for atherosclerosis and IMT are linear, without threshold value and associated with increased cardiovascular risk (Bots et al. 1997).

A surrogate endpoint can be defined as a laboratory measurement or physical sign used as a substitute for a clinical meaningful outcome that is a direct measure of how a patient functions or survives and that is expected to predict the effect of therapy (Peters et al. 2011c). Peters et al. have discussed the legitimacy of using IMT as a surrogate measure for IHD according to the criteria proposed by Prentice. The criteria includes a comparison with a gold standard (fulfilled by the work of Pignoli et al., 1986); adequate reproducibility; cross-sectional relations with established risk factors and prevalent disease; relations with severity of atherosclerosis elsewhere in the arterial system; relations with future events; ability for a biomarker to change over time; ability to be affected by interventions over time; and relations between change in biomarker level over time and change in risk (Prentice, 1989).

The reproducibility of the IMT is believed to be very high (Kanters et al. 1997, Salonen et al. 1993a). There are significant differences in the methods used for measuring IMT. One can group the methods based on the anatomical location of the IMT measurements, whether the measurements are manual or computer-based, and whether the IMT measurement is based on maximum measurement, random measurement or an average over longer sections of the vessel (Simon et al. 2002). An average of computer-based measurements of the carotid artery has been seen as the most reproducible value (Crouse et al. 1995, Kanters et al. 1997, Schmidt C et al. 1999), as IMT measured manually by ultrasound scanning has a large interobserver variability but small intraobserver variability. However, when the interobserver variability is taken into consideration, perhaps by employing the same observer and using unambiguous instructions for scanning and measuring, the reproducibility is high (Bots et al. 2003, Crouse et al. 1995, Kanters et al. 1997, Salonen et al. 1993a). This view has been confirmed by a recent comparison of manual and semi-automated edge detection of IMT (Peters et al. 2011b).

IMT has been shown to be associated with risk factors for cardiovascular disease (CVD) (Crouse, III et al. 2002, Gnasso et al. 1996, Heiss et al. 1991, Lynch et al. 1995, Prati et al. 1992, Rosvall et al. 2002, Salonen et al. 1989, Salonen et al. 1991), with the levels of atherosclerotic changes in the coronary vessels (Black et al. 2002, Craven et al. 1990, Crouse et al. 1995, Geroulakos et al. 1994, Heiss et al. 1991, Salonen et al. 1993b), and with atherosclerosis elsewhere (Cerne et al. 2002, Howard et al. 1994, Lekakis et al. 2000, Lekakis et al. 2005). Additionally, IMT has been found to predict future CVD in several studies (Engstrom et al. 2009, Lorenz et al. 2006, Lorenz et al. 2007, Rosvall et al. 2005, Salonen et al. 1993b). A meta-analysis by Lorenz et al. reports that the age- and sex-adjusted overall estimates of the relative risk of AMI were 1.15 (95% CI, 1.12 to 1.17) per 0.10-mm IMT difference in the common carotid artery (CCA), whereas the age- and sex-adjusted relative risks of stroke were 1.18 (95% CI, 1.16 to 1.21) per 0.10-mm IMT difference in the CCA (Lorenz et al. 2007).

A given IMT is, however, an expression of the total effects of all of the risk factors to which the individual has been exposed up to the time of the examination, including, not least, genetic disposition. The progression of IMT may be a more precise estimate of the significance of a risk factor (Crouse, III, 2001, Fujii et al. 2003, Mackinnon et al. 2004). An association has been demonstrated between risk factors for IHD and progression in IMT, that is equivalent to the association between manifest IHD and IMT (Agewall et al. 1996, Barnett et al. 1997, Everson et al. 1997, Lynch et al. 1997a, Lynch et al. 1997b), as well as between progression in IMT and incidence of IHD (Crouse, III, 2001, Hodis, 1995). Several studies have attempted to demonstrate the effects of treatment on IMT, and regression of IMT has been shown after antihypertensive treatment (Wang et al. 2006), use of statins (Espeland et al. 2005), and life style change (Markus et al. 1997). The effect of IMT regression on the risk of future IHD is still being discussed. However, recent reviews suggest that this association is also present (Lorenz et al. 2007, Peters et al. 2011a).

SALIVARY CORTISOL:

Cortisol is easily measured in the saliva. Saliva samples can be stored at room temperature for a long time before the cortisol decays, making this method appropriate for use in epidemiological studies (Kirschbaum et al. 1994). Cortisol circulates in the blood, partially bound to cortisol binding protein but also including an unbound fraction. It is this free fraction that is biologically active. The free cortisol in the saliva is linearly associated with the free cortisol in the blood, although the exact value is lower in the saliva than in the blood (Kirschbaum et al. 1994).

However, cortisol production varies significantly during the day, and the hormone is secreted in a pulsatile manner. Accordingly, the use of cortisol as a measurement of activity in the HPA axis has methodological problems (Hjortskov et al. 2004). Furthermore, there is a large inter-individual variation in the level of cortisol (plasma concentration: 40 –180 ng/ml; secretion rates: 8-25 mg/day) (Fulford et al. 2005). Thus, one cannot use the cortisol level in a random saliva test as a measurement of the HPA axis activity. Within the first half-hour after waking in the morning, cortisol secretion increases by 50 – 75 %. This increase has a good intra-individual stability over days (Pruessner et al. 1997). The morning rise in cortisol secretion is distinct from the circadian rise in the HPA-axis activity (Wilhelm et al. 2007) and is suggested to be related to the sleep / wake transition activating the body, to meet demands when awake (Pruessner et al. 1997). Schulz et al. examined 100 people (average age 25.7 years) with questionnaires about work overload in relation to daily tasks on two days, with a week in between. Subsequently, salivary cortisol in the morning was measured on three separate days within a 7-day period. The individuals who reported a high degree of work overload exhibited larger rises in salivary cortisol than those without work overload. Furthermore, a sex difference was found, in that women with a high work overload had a greater rise in salivary cortisol than men (Schulz et al. 1998). The rise in cortisol in the first 30 minutes after waking is called the Awakening Cortisol Response (ACR, used in this thesis), or the Cortisol Awakening Response (CAR) (Clow et al. 2004) and is found to be increased in connection with different types of strain, both stated objectively (e.g., when caring for others (Davis et al. 2004)) and as subjectively experienced stress (Kudielka et al. 2003b, Kunz-Ebrecht et al. 2004b, Pruessner et al. 1999, Steptoe et al. 2000a, Steptoe et al. 2003). Associations have also been found between the ACR and other measurements of cortisol activity in the morning and different measurements of health. Kudielka et al. have found an association between health complaints and ACR, in that individuals who were not healthy exhibited higher cortisol on waking, but lower relative elevation, in contrast to healthy individuals (Kudielka et al. 2003b).

Research using salivary cortisol as either an effect measure or an explanatory variable has recently been reviewed. The conclusion was that study design is of the utmost importance. The deviation measures, i.e., ACR or diurnal slope (decrease in cortisol from morning to evening), appear to be rather consistently associated with psychological factors, whereas a flat diurnal curve (low morning cortisol and high evening cortisol) appears to be associated with ill-health (The Scancort Group, 2011).

HRV:

HRV is defined as the spontaneous fluctuations in the sinus rhythm due to processes in the body (Kristal-Boneh et al. 1995). Beat -to-beat variations in the heart rhythm are mainly caused by changes in the autonomous nervous system. Parasympathetic and sympathetic nerve fibres richly innervate the sinoatrial node (SA node). Parasympathetic stimulation hyperpolarises the SA node and lowers the heart rhythm, whereas the release of noradrenaline from the sympathetic nerve endings and adrenaline from the suprarenal gland stimulate the heart rhythm. Because acetylcholine from the parasympathetic nerve endings is quickly hydrolysed, the SA node reacts more rapidly to parasympathetic than to sympathetic stimulation (Kristal-Boneh et al. 1995, Malik, 1996).

By analysing the variability of heart rhythms observed in an electrocardiogram (ECG), the activity in the autonomous nervous system can be evaluated. HRV analysis covers time-domain and spectrum analysis. Based on physiological and mathematical relationships, these two methods are, to a great extent, correlated. Time-domain analysis yields measurements for the heart rhythm and its variations, as increased heart rhythm leads to reduced time between each heartbeat and thus reduced HRV (Kristal-Boneh et al. 1995, Malik, 1996). The measurement used is standard deviation of all intervals (SDNN), which is affected by both parasympathetic and sympathetic tone and the root mean square of differences between adjacent intervals (RMSSD), which is thought to be more specific for parasympathetic tone.

Spectral analysis is an analysis of the changes in the heart frequency from beat to beat, which can be presented in the form of a tachogram, a diagram in which the number of beats is indicated on the x-axis and the RR interval on the y-axis (Kristal-Boneh et al. 1995). After a Fourier transformation, the frequency area within the given period is reproduced on the x-axis and the ”Power spectrum density” (ms2) is measured on the y-axis. The total power (TP) during the period in question is the area under the curve, the total variance. By considering the power (variance) equivalent to the specific frequency areas, the distribution of frequency changes can be calculated. In this way, spectrum analysis can be used to express the sympathovagal balance as the ratio between low-frequency changes (LF: 0.04-0.18 Hz) and high-frequency changes (HF: 0.18-0.4 Hz) in heart rhythm. The HF changes are caused by changes in vagal tone, whereas LF changes are the results of both sympathetic and parasympathetic tone. The cause of the very-low-frequency changes (VLF: 0-0.04 Hz) is unclear, but these changes are thought to be related to slow thermo-regulatory oscillations and cannot be interpreted in analyses of short duration (Malik, 1996). TP is the sum of VLF, LF and HF, measurements in ms2. LF and HF can be standardised as follows: LFnu = LF / (TP-VLF) and HFnu = HF / (TP-VLF).

HRV, in the form of reduced values for time-domain analysis, is known to be associated with, among other things, heart disease (Kristal-Boneh et al. 1995, Liao et al. 1996) and to predict illness and death (Dekker et al. 2000, Huikuri et al. 1999, Liao et al. 1996, Liao et al. 2002). Psychosocial strain has also been found to be correlated with reduced HRV, and particularly with a reduced extent of high-frequency changes, pointing to a reduced vagal tone (Delaney et al. 2000, Hanson et al. 2001, Vrijkotte et al. 2000). In this way, both epidemiological results and neurophysiology indicate that HRV may be seen not merely as an indicator of an individual’s reaction to a psychosocial strain but also as a possible pathophysiological explanation for the significance of psychosocial strain in the development of IHD.

THE AW STUDY

The AW study was a prospective study on psychosocial factors, stress physiology and IMT, consisting of a baseline examination in 1998 and re-examinations in 2002 and 2008. Table 1 shows the physiological and psychosocial factors for the participants in 1998, 2002 and 2008.

PARTICIPANTS

The study included 130 working subjects (30 - 60 years of age) who were recruited via advertisements at Hilleroed Hospital and articles in newspapers. The population comprised 88 women and 42 men. One man had experienced an AMI several years earlier, and five men and four women were being treated with anti-hypertension medicine. No individuals suffered from diabetes or depression. In 2002, 95 of the original 130 participants participated in the follow-up study. The reasons for dropping out were moving, pressure of work and lack of interest. The absentees were significantly younger and smoked significantly less than the participants. They also tended to have a better coronary risk profile than the participants. There were no significant differences with respect to psychosocial factors. Six women were being treated for hypertension, and 6 men were being treated for hypertension or IHD (of these, two women and three men were also being treated in 1998). Ninety per cent of the women were in the workforce, and 97 % of these were employed in the health service, predominantly working as secretaries, midwives, physiotherapists or nurses. Almost 86 % were co-habiting. The majority, 73 %, had normal menstrual cycles. Of the men, 84 % were working, of which 41 % were employed in the health service as doctors, nurses and technical personnel. Other positions included childcare worker, head teacher, police officer, and development consultant. Approximately 78 % of the men were co-habiting.

In 2008, 58 women and 28 men were re-examined in a third study. Three women and seven men were not working, and eight women and six men were medicated with antihypertensive medicine.

METHODS

In 1998, 2002, and 2008, the participants filled in an identical questionnaire on health and working environment. At all three examinations, measurements of waist and hip width, weight and height, ultrasound scanning of neck arteries, and blood and saliva tests to analyse for cortisol were performed. In 1998, urine samples were taken for analysis for cortisol and catecholamines. In 2002, HRV was measured and an acute stress test was performed, and in 2008 HRV was measured for 15 minutes in the supine position.

IMT:

The same examiner conducted all of the ultrasound examinations. An Acuson ultrasound scanner was used for all of the examinations, specifically, the Acuson Xp 128 in 1998 and the Acuson Aspen in 2002 and 2008. The transducer used was a broadband, linear 7 MHz transducer. The ultrasound scanner was set at the pre-programmed "carotid" programme. The log compression was set to 60-70 dB and was reduced in examinations of overweight participants, with a view to improving the picture quality. The CCA was identified with the examination subject lying on his/her back and with the head turned at approximately 30° towards the opposite side. After the definition of the transition to the bulbous, the vessel was scanned longitudinally and perpendicular to this transition, by which means the far wall of the vessel was visualised in the form of a characteristic double-line echo. The vessel's near wall was frequently pictured in a similar way, but this measurement was not used in the analysis, as the measurement of a vessel's near wall depends on the gain setting of the ultrasound scanner (Bots et al. 2003, Salonen et al. 1993b). The IMT was measured three times on both the right and left CCAs at the transition to the bulbous, perpendicular to a tangent in the artery's lengthwise direction. Thus, for each participant, six values were measured for IMT (Eller et al. 2001a).

SALIVARY CORTISOL:

In 1998, salivary cortisol was measured on waking, after 20 minutes, after 60 minutes and at 18:00 on a working day. In 2002, this variable was measured on waking, after 20, 30 and 60 minutes, after 8 hours and at 18:00 on a working day. In 2008, salivary cortisol was measured on waking, after 30 minutes and at 18:00 on a working day. Saliva testing tubes (Salivette®, polyester swabs) were sent to participants with instructions on how and when the test should be performed. The participants were instructed to fast and to refrain from smoking or brushing their teeth before and between testing in the morning and during the hour before testing in the daytime. The participants were also instructed to take the first test immediately after waking, while they were still in bed. Finally, the participants had to note the identification information on each test.

The participants delivered the saliva samples in connection with the clinical examination. The saliva samples were stored at -20 ° C until analysis. After thawing, the sample tubes were centrifuged at 3000 rpm for five minutes. The cortisol in saliva was analysed in a competitive radioimmunoassay from Orion Diagnostica, Espoo, Finland. The analysis was performed according to the manufacturer's instructions. The cross-reactivity to cortisone was <0.2 %. A 1470 Wizard gamma counter (Wallac, Turku, Finland) was used for measuring the radioactivity. A method evaluation of a certified reference material in water performed by the laboratory at the National Research Centre for the Working Environment (NRCWE) showed no bias in the method, i.e., the re-finding was 97 % [CI: 94%; 100.9%]. The detection limit was 1.59 nmol/l. The variation co-efficient was 19 % with 11.5 nmol/l and 16 % with 49.2 nmol/l (Hansen et al. 2003).

HRV:

In 2002, the participants had their ECG measured from the beginning of the clinical examination (starting between 12:00 and 17:00) until the following morning. The ECG data were gathered digitally and processed by computer with the use of software from Danica Biomedical, Sweden. In 2002, the data extraction was performed at the Department of Occupational Medicine, University Hospital Lund, Sweden, whereas in 2008, the data extraction was performed at NRCWE. All R-R intervals were first processed automatically and then run through manually with inspection of the R-R intervals. After the inspection, the ectopic heartbeats or artefacts could be replaced with normal heartbeats by the software. Only recordings with sinus rhythm throughout the test period and for the first hour after the participants reported switching off the light at night were included (N=78).

Because the ECG recordings were of different lengths, two 5-minute recordings were selected for analysis. These recordings comprised 5 minutes during the stress test and 5 minutes during the first 30 – 60 minutes of the sleep period (VII). During the stress test, the 5 minutes in which the TP was lowest were selected to express the most indicative stress response and the highest heartbeat frequency. Conversely, the 5-minute period with the greatest TP during the first 30 – 60 minutes of the sleep period was selected, based on the assumption that the participant was most relaxed in this period. In these two periods, a number of variables from time-domain analysis (SDNN, RMSSD) and spectrum analysis (TP, HF, LF, HFnu, LFnu or LF/ HF) were selected.

STRESS TEST:

A brief stress test was included in the re-examination in 2002. The purpose of this addition was to stress the participants at a low level to compare the data for HRV. For ethical reasons, actual psychologically stressing tests, e.g., provocation of earlier trauma, were avoided. The test was formulated in two parts, which were conducted immediately after one another. The first test was a memory interference test (VII), and the other was a mathematical test, the Paced Auditory Serial Addition Test (Wiens et al. 1997). In connection with both tests, a correct answer was ignored, while whereas errors received a comment of "No!" Failure to answer was met with encouragement to make an effort. The two tests together lasted approximately 15 minutes.

THE OSH STUDY

The OSH study was a prospective study on possible changes in health in relation to the huge reorganisation of the public administration in the Danish counties and municipalities occurring on January 1st 2007 (Netterstrom et al. 2010a), with a baseline examination in 2006 and re-examination in 2008. Table 2 shows the physiological and psychosocial factors assessed for the participants in 2006.

PARTICIPANTS

In November 2004, Statistics Denmark identified 2030 potential participants through an extraction of data regarding places of employment and salary code. The identified participants were employed in the public administration in one of the five municipalities (Helsinge, Hillerød, Hørsholm, Karlebo, and Skævinge) or the two counties (Frederiksborg and Nordjylland) included in the study. Four municipalities and one of the counties were merging with others, whereas one municipality and one county remained unmerged with only minor changes in task. In spring 2006, the identified employees were invited to participate in the questionnaire study, and 1379 individuals returned the questionnaire (response rate 68%). Of these respondents, 262 had left the work place of interest, i.e., were retired or had taken a job in the private sector, and were therefore excluded from the study. Along with the invitation to participate in the questionnaire study, the individuals had also received an invitation to participate in the sub-study including biological measures. Of the remaining 1117 individuals, 502 individuals volunteered to participate in the sub-study on biomarkers. The 616 sub-study non-participants and the 502 participants did not differ significantly in age, sex, training, perceived stress or sick leave in 2005. In 2008, 391 participants were re-examined.

Approximately 90 % of the OSH participants were white-collar workers, e.g., 40 % of the women were clerks or secretaries, and 15 % were social workers, whereas 34 % of the men were engineers with an academic degree or architects, and 14 % worked as IT consultants. The men were significantly older and more obese than the women, and the men exhibited higher alcohol consumption than the women. The proportion of smokers decreased significantly, from 15 % in 2006 to 3-4 % in 2008. The level of physical activity did not change between the two years but was different between the two sexes, as 53.1 % of the women and 38.1 % of the men reported a level of physical activity of up to 4 hours per week. The mean levels of total cholesterol and glycated hemoglobin (HbA_{1c}) were within the reference values but increased between the two years in both sexes.

METHODS

SALIVARY CORTISOL:

Saliva samples were collected in the same manner in 2006 and 2008, namely at awakening and 30 minutes post-awakening on a working day. The equipment for the saliva samples (two Salivette ®, Saarsted, cotton swabs) was given to the participants at the clinical examination together with oral and written instructions about sampling. The instructions on sampling were the same as in the AW study. The analysis was performed at NRCWE. The staff, and methods were the same as for the AW study.

Thirty-one participants did not deliver saliva samples at all in 2006. In 2008, the participants (N = 391) were examined in the same order and in the same months as in 2006, and the follow-up time was approximately two years for every subject. Three hundred fifty six participants (255 women and 101 men) delivered all four saliva samples and information on sampling time, and in total, 480 participants were represented by at least two saliva samples.

HRV:

A total of 236 individuals had their ECG taken over approximately 18 hours, starting during the clinical examination between 9:00 and 12:00 at the work place and ending the next morning. The following morning, the participant was allowed to remove the logger and return the logger to the work place along with a short diary reporting when they were seated reading or watching television at home after work. In 2008, the procedure was repeated with 195 individuals. In 2006, 205 individuals delivered adequate diary information (seated rest) concomitant with technically satisfactory ECGs during the relevant 15 minute period and were included in the study. In 2008, 170 were included based on the same criteria. Finally, 170 women and 61 men delivered contemporary data on seated-rest HRV and the psychosocial work environment in either 2006 (N = 77) or 2008 (N = 28) or both years (N = 126).

The HRV data in paper V included the averaged values of the spectral analysis during three 5- minute periods (a total of 15 minutes) of sitting while reading or watching television during leisure time. The mean (standard deviation, SD) starting times of measurement were 20:32 (1:48) and 20:40 (1:31) in 2006 and 2008, respectively. A three-lead LifeCard CF Holter monitor recorded the ECG data (Delmar Reynolds Medical Inc., Irvine, CA, USA). Only recordings of the sinus rhythm were included in the study. Artefacts and abnormal beats in the ECG segments were autodetected by commercial software and verified by visual inspection. Before calculating the HRV metrics, the RR-intervals were filtered for possible outliers (ectopic beats, falsely detected beats, and missed beats) using the algorithm published by Moody (Moody, 1993), resampled with a frequency of four Hz and linearly detrended. This refinement of the ECG data was performed at NRCWE.

▌STRATEGY OF ANALYSIS AND STATISTICAL CONSIDERATIONS

GENDER / SEX-SEPARATED ANALYSIS AND ANALYSIS OF INTERACTION:

In these papers, most of the analyses were performed separately for the two genders / sexes. Sex is defined as "the classification of living things generally as either male or female, according to their reproductive organs and functions assigned by the chromosomal complement," whereas gender is "a person's self-representation as male or female, or how that person is responded to by social institutions" (Messing et al. 1998). The separation of the analyses was determined based on the well-known differences with regard to gender roles, aims in life and different perceptions of psychosocial strain (Lundberg, 2005, Sandanger et al. 2004, Vermeulen et al. 2000) and on the fact that there are substantial differences between the sexes in connection with the development of atherosclerosis (Barrett-Connor, 1997, Vaccarino et al. 2011). The participants in both cohort studies had different distributions with respect to social status and jobs between the genders. This factor should be taken into consideration, as conducting the analysis as a modelling with an interaction term may conceal the exposure-effect relationships among women and men (Messing et al. 1998, Messing et al. 2003). The analyses, therefore, are suggested to include both gender-separated analyses and an analysis including an interaction term between sex and the variable in question (i.e., the psychosocial factor) (Messing et al. 2003). Because not all of these analyses had been included in all the papers, further analyses of the data have been conducted, and the results of these further analyses are presented in the appendix.

TRANSFORMATION OF THE VARIABLES:

In the analyses, it was originally assumed that the connection was linear and that the dependent variables were normally distributed. Particularly considering the data in paper II, these variables were re-analysed, as it may be questionable to rely on a visually based decision that the data are normally distributed. All of the HRV data were logarithmically transformed.

"CHANGE" AS A DEPENDENT VARIABLE

The papers refer to "change" with respect to two dependent variables, the ACR and the IMT progression. In both cases, the measures were already established as differences. A change can be calculated as a percentage or as a simple difference between the two measurements. The supplementary analyses did not reveal advantages obtained from the expression of the increase in cortisol as a percentage in connection with the data from paper II (data not shown). The percentage increase in cortisol was used in paper VI. The IMT progression was expressed as a difference.

STATISTICAL METHODS:

Due to the small amount of data, an ongoing strategy has been to perform a number of simple, linear regression analyses with each individual independent variable, followed by multiple or stepwise regression performed with stepwise inclusion of variables, instead of the simultaneous inclusion of all variables and computer-based selection of significant variables (I, II, VI -VII). The physiological stress measurements, cortisol and HRV, were measured several times during the day. Repeated measures analysis of variance was therefore used, in which cortisol, TP or HF was involved as the repeated measure and the different psychosocial factors as independent variables (II and III). However, it was realised that most of the data included in the two studies were of the type "repeated inter-correlated data". This designation indicates that the repeated measures analysis of variance was not appropriate to analyse the data. Subsequently, in the two most recent papers, a random intercept model, i.e., a mixed effects model with subject as the random effect was used (IV and V). This model fully utilises the repeated measurements from the same individuals. The incorporation of the individual specific cortisol (or HRV) levels allows the generalisation of the results to other samples that are comparable to this sample (Zuur et al. 2009).

MULTIPLE COMPARISONS:

The statistical method described, conducting series of simple linear regression analyses, increases the risk of finding significant associations simply due to multiple comparisons. If the level of significance is set to be $p < 0.05$, one out of 20 analyses may be expected to turn out significant by chance. This problem can be solved by means of a Bonferroni correction, which lowers the significance level in each individual test. In general, if n tests are performed, the level of significance after Bonferroni correction is 0.05/n (Peacock et al. 2011). In paper I, this change would indicate that p should be corrected to 0.05/13, as 13 different psychosocial factors were examined. However, this paper is exploratory, i.e., examines the significance of a multitude of psychosocial factors to determine which factors are of interest. Therefore, publication of all results should be seen as a strength and as being of importance to bring science forward (Moran, 2003, Nakagawa, 2004). In paper III, the number of psychosocial factors was diminished, and in papers IV and V, only the ERI model was examined. This reduction in number of factors indicates that the need for Bonferroni correction was managed by selecting the variables of interest. Finally, as significant associations consistently point to the ERI model, the findings regarding this effect are likely to be valid.

The analyses were performed using SPSS, versions 8 –19, and in paper IV, by means of R (Pinheiro et al. 2000, Pinheiro et al. 2010, R Development Core Team, 2009).

WORK-RELATED PSYCHOSOCIAL FACTORS AND IMT

Though the use of IMT as a proxy measure for coronary atherosclerosis is theoretically a great advance, the method has been used in relatively few studies that include work-related psychosocial factors. The Kupio Ischaemic Heart Disease studies (KIDHs) introduced the method in epidemiological cohort studies, and several of the earliest IMT studies that include psychosocial factors were published based on this cohort of Finnish high-risk men. The inspiration to conduct the AW study was the papers by Lynch and Everson from KIDH (Everson et al. 1997, Lynch et al. 1997b). In the first study, which included 940 Finnish men, Lynch et al. found that those with high demands and low income had significantly greater IMT progression in comparison with those that had low demands and high income (Lynch et al. 1997b). To the extent that one can compare the reported conditions of low or high demands in the workplace with effort, this study appears to show an association between lack of social reciprocity and IMT progression. Everson et al. studied the progression in IMT, revealing an interaction between cardiovascular reactivity and workplace demands among 591 men between 42-60 years of age. The men who had an increase in blood pressure of 20 mmHg or more in connection with preparing for a cycle test and who reported large demands at work experienced the greatest progression in IMT over four years (Everson et al. 1997).

In the AW study, IMT progression was associated with the ERI model in both genders (I). An interaction analysis showed that in ERI was significantly and positively associated with IMT progression in men, whereas this factor was of no significance to IMT progression in women (appendix, table I-1). Overcommitment was found to be associated with IMT progression (I). In the gender-separated analysis, the association was most pronounced in the women. The possible association between overcommitment and IMT progression has not been examined in other studies, although, in a cross-sectional study, Xu et al. found that women with overcommitment had greater IMT compared with women without overcommitment (Xu et al. 2010).

More studies have examined the association between IMT and social status. In the AW study, social status was not found to be associated with IMT value or IMT progression. This result is likely because the population examined was small and exhibited little social variation (e.g., no manual labourers among the men). Lynch et al. found that education, occupation and income were associated with IMT, so that a longer education, employment as lower/middle management and high income was associated with lowest IMT, after controlling for conventional coronary risk factors (Lynch et al. 1995). IMT progression is certainly found to be significantly greater in people at lower social levels than in those with higher social status, both in Sweden (Rosvall et al. 2006) and in the USA (Ranjit et al. 2006), just as office clerks are shown to have greater IMT values than other groups of women (Gallo et al. 2003). However, the socioeconomic position in childhood was not found to be associated with IMT at the ages of 24-39 (Kivimaki et al. 2006a) most likely because the individuals examined were so young that the significance of their upbringing had not been able to affect them yet.

With respect to the DC model, the AW study revealed that greater demands were associated with a tendency towards a greater IMT progression among women, but not among men (I). In a cross-sectional study of 2658 Swedish men and women, aged 46-65, Roswall et al. found that women with higher job demands and lower control had greater IMT in CCA and more plaques, but did not show that strain was associated with greater IMT among men (Rosvall et al. 2002). Women with high demands but with feelings of control exhibited IMT values at the same level as women with strain. Hintsanen et al. examined 478 men and 542 women (also a cross-sectional study) and found the opposite, that strain in men and not in women was associated with greater IMT (Hintsanen et al. 2005).

The AW study included a number of private psychosocial factors that have not been examined in other studies in connection with IMT or IMT progression, i.e., children at home, hours of housework and time pressure. An increasing number of children were found to have an association with smaller IMT progression among men, independently of age (significant interaction between gender and children, appendix table I-1). The reason for this preventative effect of children on men might be the positive psychological effects of being with and playing with children, including physical activity. Steptoe et al. found that blood pressure, which is an important risk factor for IMT, decreased more in parents compared with others from work to the evening, and with no sex difference (Steptoe et al. 2000b). The measure 'hours of housework' may have a similar effect. Hours of housework were not associated with IMT progression in women, but in men, hours of housework showed a tendency to an association (beta = – 0.302, p = 0.10; I). This measure and that of time pressure need to be further developed and validated, as the current measures are believed to be too vaguely defined.

The values measured in the AW study for IMT agree with the values that are found internationally (Aminbakhsh et al. 1999, Howard et al. 1993, Kivimaki et al. 2006a, Salonen et al. 1993b). The magnitude of IMT progression is somewhat greater in the AW study than has generally been reported. In the AW study, mean progression rates of 0.030 mm / year and 0.048 mm / year were found for women and men, respectively, whereas in their review from 2003, Bots et al. found a median progression for both men and women from different studies of 0.015 mm / year (Bots et al. 2003). The difference between our findings and those cited may be explained by methodological differences. In the AW study, measurements are obtained in the immediate vicinity of the bulbous, proximally to this anatomical feature. Because atherosclerosis arises in the vessels' places of division, i.e., among others, in the bulbous, and from there progresses towards the heart (Strong JP, 1992), even a little progression is measured by the method used in the AW study, whereas the most frequently used methods of measurement of IMT are based on multiple measurements over 1 cm of CCA or possibly over a larger part of the vessel tree, where progression will be distinguished much later in the process. Such methods were used in the studies in which the referred measurements for IMT progression originate (Bots et al. 2003). Similarly, progression in the IMT in the internal carotid artery is found to be greater than in the common carotid artery (Mackinnon et al. 2004). The progression rate for IMT is presumably also different in different populations. Thus, Paternetti found progression rates in a French population of 0.01 – 0.02 mm / year (Paterniti et al. 2001), whereas studies from KIDHs have shown progression rates of 0.08 – 0.19 mm / year (Krause et al. 2000, Lakka et al. 2001, Lynch et al. 1997b). Recently, results on IMTprogression from a European multicenter study have been reported revealing gender-specific differences in IMT and its progression (Kozakova et al. 2011). The study included 366 men and 422 women, all apparently healthy. IMTprogression was not related to baseline anthropometric parameters, blood pressure, plasma lipids, glucose and insulin or to their 3-year changes, in either men or women. However, the sub-population in which the Framingham score increased during follow-up experienced increased IMTprogression when compared to those with unchanged Framingham score. This study supports the findings in the AW study as IMTprogression was not related to conventional coronary risk factors is this study either.

Table 3 summarises the design, analysis and findings of the few studies that have used progression in IMT as the outcome variable and work-related psychosocial factors as the explanatory variable. Cross-sectional studies are also presented. Three of the prospective studies originate from the KIDHs and one from the Coronary Risk in Young Finns Study (Rosenstrom et al. 2011). The four Finnish studies are all large, high-quality cohort studies. The two early studies have only included men and examine the effect of work stress using new measures of exposure. The study by Krause et al. uses an objective measure of workload in the form of working hours, whereas Rosenström et al. focus on the DC model, including both genders. Paper I includes both genders and examines the effect of several exposures. The paper reports on the association between ERI and IMT progression in men, and the effect of effort and overcommitment in both genders. Although the study includes rather few participants, it is important as it highlights the possibility of private, as well as work-related, stressors being of significance to IMT progression.

WORK-RELATED PSYCHOSOCIAL FACTORS AND THE PHYSIOLOGICAL STRESS RESPONSE

WORK-RELATED PSYCHOSOCIAL FACTORS AND SALIVARY CORTISOL

The measurement of salivary cortisol was developed during the 1980s and was soon viewed as a promising tool for assessing the levels of physiological stress in individuals (Kirschbaum et al. 1994). The first wave of psychological research using salivary cortisol was primarily engaged in detecting possible differences in levels of cortisol in connection with acute stressors, such as computer tests (Schreinicke et al. 1990) or the Triers Social Stress Test (Kirschbaum et al. 1992, Kirschbaum et al. 1994). Along with this research, of course, basic methodological questions were raised and discussed, introducing the use of salivary cortisol and ACR as feasible measures of stress in epidemiological studies (Pruessner et al. 1997, Pruessner et al. 1999, Wüst et al. 2000). Researchers from the Whitehall Studies contributed with several papers with promising results in connection with the notion that work stress might result in changes in basic physiology (Kunz-Ebrecht et al. 2004a, Kunz-Ebrecht et al. 2004b, Steptoe et al. 2003, Steptoe et al. 2004a), and in line with this, several authors have reported a larger cortisol increase in the morning on working days compared to with free days (Kunz-Ebrecht et al. 2004a, Maina et al. 2009, Schlotz et al. 2004).

Objectively assessed job demands have been found to increase cortisol levels measured in different manners (Fischer et al. 2000, Fox et al. 1993, Zeier et al. 1996). Lundberg et al. studied salivary cortisol on a day off in 227 women that worked either full-time, with up to 10 hours weekly overtime or with more than 10 hours weekly overtime (Lundberg et al. 2002). The women with the most overtime work had significantly higher salivary cortisol on a day off than the other women. In the AW study, using a dichotomous analysis, WWH below 37 hours / week was associated with high ACR in women, but in the repeated measures analysis of variance, there was no association. The published data from the AW study do not include measurements on leisure days. However, greater time pressure was found to have a connection with greater ACR in women (II). The concept of perceived time pressure in daily life has only been used in the AW study.

Fox et al. studied subjectively assessed workload and found that low control in combination with high subjective workload was associated with increased averaged cortisol levels (Fox et al. 1993). In the Whitehall study, Steptoe et al. reported an inverse association between job strain and salivary cortisol measured one time on a non-specified time during the day (Steptoe et al. 1998). The great diurnal variation in cortisol explains this finding. Kunz-Ebrecht et al. studied job demands and control and salivary cortisol in a group of 97 men and 84 women from Whitehall (Kunz-Ebrecht et al. 2004b). In this analysis, job control was not found to be associated with ACR in both sexes, but high job demands had an association with a higher ACR in women. An interaction with socio-economic status was observed; women with low social status and high job demands had increased cortisol levels during the day, whereas women with low social status and low job demands had low cortisol levels.

Correspondingly to the lack of an association between job strain and a random assessment of salivary cortisol (Steptoe et al. 1998), Hanson et al. did not find an effect of the ERI model on cortisol levels, which may be due to the first saliva sample being obtained at 8:00 a.m. rather than at awakening (Hanson et al. 2000). The Whitehall study included questions on the DC model, but to estimate ERI, it was necessary to construct a proxy measure (Kuper et al. 2002). In this manner, Steptoe et al. studied the ERI model and salivary cortisol in 86 men and 79 women from the Whitehall II cohort. The authors did not find an association between the ERI proxy measure and cortisol (Steptoe et al. 2004b). This discrepancy from other studies may be due to using the proxy measure as items. Similarly demands-items were used to estimate effort and items describing social support and job satisfaction were used to describe reward. Still Steptoe et al. reported significant associations between overcommitment and high levels of salivary cortisol in men (Steptoe et al. 2004b).

Due to the sparse findings in the above cited studies, we chose to examine cortisol at awakening (S1 and after 30 minutes) and during the day (6 measurements). In addition we chose to record a number of psychosocial factors at home and at work (II). Our hypothesis was that a high psychological load might be associated with higher cortisol levels.

The findings were that the women experiencing a high degree of time pressure had high ACR and a tendency to high levels of cortisol throughout the day ($p = 0.06$), whereas ERI was significantly associated with ACR. Among the men, a high degree of time pressure and a high ERI were associated with high salivary cortisol on waking. In addition, high effort, ERI and overcommitment were associated with high ACR and high cortisol levels throughout the day. However, the DC model was not associated with cortisol levels, although, unexpectedly good support from colleques and superiors was associated with high cortisol throughout the day in men (II).

Paper II was the first paper to examine the associations between the full ERI model and salivary cortisol in a cohort study, although on a small scale. Additionally, paper II suggested that salivary cortisol would likely be influenced by not only work-related factors but also by factors originating in private life circumstances, in particular the concept of time pressure. Because the analyses used in the paper could be criticised, the data have been re-analysed. The first re-analysis was performed like the analysis in the paper where logarithmically transformed cortisol measures as the dependent variable was used (appendix, table II-1). Furthermore, an analysis of the interactions between gender and the psychosocial factors was carried out (appendix, table II-2). It was revealed that the ERI has a different association with cortisol level between the two genders in this cohort. ERI appears to be strongly positively correlated to cortisol levels in men but to a lesser degree in women. Additionally, overcommitment was significantly and positively associated with cortisol levels, especially in men. In Whitehall, Steptoe et al. also found that overcommitment was associated with higher cortisol among men, whereas no connection was found between the model and physiological measurements among women (Steptoe et al. 2004b). The data from the AW study supported the association between overcommitment and cortisol levels in men.

Paper III (AW data), using data from the examinations in 2002 and 2008, reports that the level of load (psychosocial factors) measured at the baseline was significantly associated with the level of salivary cortisol measured at the baseline and after 6 years. In a large part of the scientific litterature reporting on psychosocial factors and the incidence of IHD, the psychosocial factors have only been measured at the baseline. The hypothesis was, therefore, that the baseline measures of psychosocial factors might be of significance to levels of salivary cortisol measured in both 2002 and 2008. The population under examination was small, but a significantly positive association between ERI and salivary cortisol was revealed in men in gender-separated analyses (III). A supplementary analysis of interaction between gender and ERI was, however, negative. The supplementary analysis including both genders did not reveal an association between the baseline measures of the psychosocial variables and the levels of cortisol (appendix, table III-1).

The literature on salivary cortisol and psychosocial factors has increased, and several reviews have concluded that work-related psychosocial factors are associated with salivary cortisol levels (Chandola et al. 2010, Chida et al. 2009, The Scancort Group, 2011). Some researchers consider work stress to be a rather low stressor and so with presumably little physiological effect. Subsequently, the positive association between psychological strain at work and increased levels of cortisol may be interpreted as a normal physiological activation (Karlson et al. 2011). The reason for the in-consistent findings in the litterature may be due to methodological differences in measuring and analysing of the data. The ACR is a single measure, but measuring the salivary cortisol several times a day (and preferably for several days) demands a statistical method sophisticated enough to address inter-correlated data, such as the mixed effects model.

In accordance with these statistical considerations, we conducted the analyses reported in paper IV (OSH data). This paper focuses on the ERI model and the levels of cortisol at awakening and 30 minutes thereafter, and the findings are believed to support the observations in paper II. The ERI model showed a tendency towards an association with the ACR in a healthy subsample with increases in cortisol levels during the first 30 minutes in the morning. However, the cohort's cortisol levels increased significantly from 2006 to 2008, which may have concealed the possible effect of ERI conditions. The results thus confirm that other factors than the measured work-related factors may be of significance for salivary cortisol levels. An obvious flaw would be if the cortisol analyses were defective may be due to the use of different laboratories in the two years, change in the methods used in the laboratory, too long storage of the samples in 2006, or change in the equipment used for sampling. However, the exact same equipment, method of storage and analysis, staff and laboratory were used in both years. To show equivalence between different runs, natural saliva samples (5.9 nmol/l and 24.5 nmol/l) were used as control materials and analyzed together with the samples. Westgard control charts were used to document that the trueness and the precision of the analytical methods remained stable (Westgard, 1981). The performance of the methods has been further validated by participation in interlaboratory comparison schemes (Garde et al., 2003; Hansen et al., 2003). Therefore, the increase in levels of cortisol seen in the OSH study occuring from 2006 until 2008 seem reliable. The data used for the analyses were collected one year before and one year after the major re-organisation of the non-state public offices, the Danish "Kommunal-reform" January 1st, 2007. Because the participants were working in public offices, the reorganisation might explain why all of the participants experienced an increase in salivary cortisol levels. Health effects of changes in organisations and job insecurity have previously been found in several settings (Ferrie et al. 1998, Iversen et al. 1989, Laszlo et al. 2010). In these data, a gender * ERI- interaction was not observed.

Table 4 gives a review of papers that use a work-related psychosocial factor as the exposure and ACR as the effect measure. In general, the design is cross-sectional. The early papers do not transform the cortisol data. The DC model is studied in 7 papers, and four studies report positive associations between demands or strain and ACR (Chandola et al. 2008, Kunz-Ebrecht et al. 2004b, Maina et al. 2008, Maina et al. 2009). The ERI model was examined in four papers, of which two report positive associations between ERI and ACR (Eller et al. 2006, Eller et al. 2011a). Only papers II and III evaluate several work-related psychosocial factors. This analysis is perceived as an advance.

WORK-RELATED PSYCHOSOCIAL FACTORS AND HRV

The use of HRV as a tool for the assessment of risk was developed in connection with clinical research revealing that HRV was a predictor of mortality (Malik, 1996). Just as salivary cortisol provided a non-invasive measure of the HPA axis, HRV is a non-invasive measure of the activity in the autonomic nervous system and thus is of interest as a research method in stress physiology. Table 5 presents studies that include work-related psychosocial factors as the exposure and HRV as the effect measure. The differences between design, size and analytical strategy are marked. Ten papers examine the DC model and HRV, and five of the six papers, which use the concept of job strain, are positive, i.e., report an association between job strain and HRV data in the expected direction (Chandola et al. 2008, Collins et al. 2010, Collins et al. 2005, Kang et al. 2004, van Amelsvoort et al. 2000). All four papers which that have included control as an explanatory factor have reported that increased control or decision latitude was associated with increased levels of HRV in at least part of the analyses (Collins et al. 2005, Eller et al. 2011b, Hemingway et al. 2005, Lindholm et al. 2009), whereas demands have scarcely been examined. The ERI model has been examined in 7 papers (Eller et al. 2011a, Eller et al. 2011b, Hanson et al. 2001, Hintsanen et al. 2007a, Loerbroks et al. 2010, Uusitalo et al. 2011, Vrijkotte et al. 2000). In general, all of the papers have reported significant associations between the ERI model and decreased levels of HRV, but for instance, Hintsanen et al. report that the association is present in women but not in men, whereas paper III reports an association present in men but not in women, and Loerbrok et al. only find an association in individuals who are 35-44 years old. The methodological differences reveal the challenges when handling the huge amount of data obtained by a HRV analysis.

Two studies have found low social status to be associated with low HRV measures (Hemingway et al. 2005, Lampert et al. 2005). Different indices of general work stress have been associated with low HRV (Clays et al. 2011, Kageyama et al. 1998, Sloan et al. 1994).

Two papers in this thesis, III and V, report on the association between psychosocial factors and HRV in a prospective manner. The large number of women in the OSH study is a strength, as only two studies have included such large numbers (Hintsanen et al. 2007a, Riese et al. 2004). Furthermore, the ECG recordings have been obtained during well-described conditions while the participants were lying down (III) or sitting (V). The data from the AW study were recorded during the clinical examination and included only 50 women and 20 men (III), but are still important as the study reports concomitant data on salivary cortisol and heart rate variability. There was only sparse evidence of an association between the DC model and HRV in paper III, but the ERI model was significantly associated with HRV, and, as expected negatively, i.e., high ERI was associated with low TP and HF. However, these associations were only observed in the men. An analysis of the interaction between gender and ERI emphasised this variability (appendix, table III-1). The analysis used in paper III was a repeated measures analysis of variance. However, as the data are inter-correlated repeated measures, the associations between the ERI model and HRV were examined in the OSH data using a mixed effects model (V). This study included HRV data collected during daily life, when the participating 170 women and 61 men under study were at rest at home. The analyses showed very significant inverse associations between the ERI model and TP as well as with HF. The factor 'year' was not associated with HRV. This lack of association was interpreted to suggest that HRV measurements reflect the present load. In this data set, a gender * ERI effect was not observed.

THE PHYSIOLOGICAL STRESS RESPONSE AND IMT

SALIVARY CORTISOL AND IMT

The association between cortisol levels and IMT had only been addressed in few studies until the publication of paper VI. As presumably the most early study on cortisol and atherosclerosis, Troxler et al. found correlations between morning serum cortisol and coronary atherosclerosis in 71 male patients designated for coronary angiography (Troxler et al. 1977). Three case-control studies have used IMT as the effect measurement when studying patients with Cushing's Syndrome: In a case control study of 28 patients with incidentally discovered adrenal adenoma and sub-clinical Cushing's syndrome, IMT was found to be increased in the patients, compared with 100 healthy control subjects (respectively, 0.81 vs. 0.62 mm) (Tauchmanova et al. 2002), and increased IMT values were found in patients treated for Cushing's syndrome, compared with the healthy control subjects after 1 year (Faggiano et al. 2003) and 5 years (Colao et al. 1999). Yener et al. compared 49 patients with non-functioning adrenal incidentaloma with 52 healthy controls and found that carotid IMT was increased in the patients (Yener et al. 2009). In these studies, other risk factors for atherosclerosis were increased, as these patients had hypertension, dyslipidaemia and insulin resistance.

Paper VI reported a possible association between hormonal risk factors (salivary cortisol, dehydroepiandrosterone-sulphate and testosterone), conventional coronary risk factors, and progression in IMT over 4 years. These sex-stratified analyses suggested that a large increase in cortisol in the morning (an increase during the first 20 or 60 minutes after awakening) was significantly and positively associated with IMT, but only in women. However, a supplementary analysis revealed no interaction between sex and cortisol. Thus, a supplement to paper VI's analyses of the associations between cortisol and IMT progression, including all of the participants and sex as covariates, was conducted. The analyses showed that ACR20min measured in 1998 was significantly associated with IMT progression (beta = 0.231, p = 0.02, appendix table VI-1), whereas the increase during the first 60 minutes, as well as the average value of the cortisol levels, was not associated with IMT progression. The AW paper was unique in finding a significant increase in morning cortisol associated with the progression of IMT in endocrinologically healthy people with a low prevalence of conventional risk factors (VI).

More recent research has supported the finding of an association between increased cortisol secretion and increased atherosclerotic changes. In a cohort of women < 65 years of age, Koertge et al. found that women with coronary stenosis had elevated cortisol levels compared with others and that women with vital exhaustion and high cortisol levels had a near 3-fold increased risk of having coronary artery disease (CAD) compared to women not having this combination (Koertge et al. 2002). In 2006, Matthew et al. reported a flatter diurnal cortisol curve (low diurnal decline) associated with coronary calcification in 718 individuals (Matthews et al. 2006), a finding that was supported by the study by Nijm et al., which included 30 patients examined 12-14 weeks after acute coronary syndrome. The patients had increased levels of cortisol measured in their urine over 24 hours and increased salivary cortisol levels at bedtime (Nijm et al. 2007). Bhattacharyya et al. found increased levels of cortisol in patients with CAD at awakening and at 15 and 30 minutes thereafter compared to patients with no signs of CAD (52 versus 32 patients) (Bhattacharyya et al. 2008), and Dekker et al. found increased cortisol measured as a total secretion, with the "area under the curve" associated with the carotid plaque score (Dekker et al. 2008). Hamer et al. examined cortisol and CAD in older participants in the Whitehall Study and found that heightened cortisol reactivity in connection with a stress test was associated with coronary calcification in individuals without a history of or objective signs of IHD (Hamer et al. 2010). Finally, high 24-hour urinary cortisol increased the risk of cardiovascular death but not all-cause mortality in a 6-year follow-up study (Vogelzangs et al. 2010).

Table 6 includes only 3 papers on salivary cortisol and atherosclerosis / IMT. The first paper is a cross-sectional study of the AW participants in 1998. High levels of cortisol in the morning were associated with low IMT measurements in women, whereas there was no association in men (Eller et al. 2001b). This finding is an example of why cross-sectional studies are less useful when exploring pathways from exposure to disease. Paper VI is the only published paper on salivary cortisol and IMT progression. Unfortunately, the data were only analysed in a sex-separated manner, although the supplementary analysis supported the finding that increased ACR is associated with increased progression of IMT in general. The paper by Dekker et al. reported a positive association between high cortisol secretion in the morning and a high plaque score, whereas there was no relationship between diurnal slope and plaque score (Dekker et al. 2008).

HRV AND IMT

Like the association between cortisol and IMT, the association between HRV and IMT has only been rarely examined. Gottsater et al. analysed HRV in 61 patients (22 women and 39 men) with type 2 diabetes and found that reduced HRV, in the form of reduced LF, might possibly be associated with increased IMT progression (Gottsater et al. 2006). In contrast to this study, the AW study included non-diabetic individuals. Reduced HRV tended to be associated with larger IMT measurements, although not significantly. Further analyses did not show any interactions between sex and HRV. It was suggested that the variability in HRV could be of interest, as the difference between LF / HF during the test and while sleeping was the variable, here called the LF/ $HF_{difference}$, which seemed most strongly associated with IMT. In an analysis including all of the participants, using sex as a covariate, this reactivity measurement was significantly associated with maximum IMT (appendix, table VII-1). Gautier et al. conducted analyses of IMT and HRV-data in two populations, The Pittsburgh Study including normotensives and hypertensive without medication, and KIDH suggesting that decreased LF was associated with IMT (Gautier et al. 2007). The three papers are reviewed in table 7.

Although the number of participants was limited, the AW study included in 2008 the measurement of IMT. In connection with this thesis, the possible association between the LF/ $HF_{difference}$ and progression in the mean and maximum IMT from 2002 to 2008 has been analysed. Initially, a correlation analysis was conducted including the IMT measurements as well as the logarithmically transformed LF/ $HF_{difference}$ and the conventional coronary risk factors: age, sex, waist-to-hip ratio, systolic blood pressure, tobacco use, alcohol use, physical activity, cholesterol and HbA_{1c} (table 8). Due to collinearity, the covariates included in the analysis of variance were: ln LF/ $HF_{difference}$, waist-to-hip ratio, systolic blood pressure, alcohol use, physical activity, cholesterol and HbA_{1c}. A backward elimination of the variables, which did not contribute significantly to the model, was the result in both cases, i.e., using progression in the mean or maximum IMT as the dependent variable in a model only including the ln LF/ $HF_{difference}$ (table 9). The R2 was 0.08 in connection with IMTmean progression and 0.09 in connection with IMTmax progression.

The association between HRV and IMT is still controversial, and future research on this issue is needed.

DISCUSSION

The aim of this study was to examine the influence of work-related psychosocial factors on the pathophysiology of atherosclerosis. Table 10 includes an overview of the results. Measurements of the activity in the HPA axis and in the autonomous nervous system were associated with the ERI model and with IMT in a group of ordinary, predominantly healthy people with generally normal values for conventional coronary risk factors. Gender differences were found in the physiological stress response to psychosocial load as measured by the ERI model (AW data only) but not in the physiological stress response or IMT. The ERI model was associated with increased levels of cortisol and decreased levels of HRV, and these changes were associated with increased IMT progression.

METHODOLOGICAL CHALLENGES

SIZE OF THE COHORT:

One of the reasons for the introduction of IMT as a surrogate measurement for IHD is that the number of individuals in the cohort and the follow-up time can be decreased. With regard to the use of IMT as a measurement of the effect on risk factors for IHD, the AW study was small, especially when a large number of the participants were younger women, who could be expected to have only low progression in IMT. However, when planning studies of treatment effects, the estimated sample sizes might be relatively small, with perhaps as few as 20 people (Bots et al. 2003). In the AW study, each participant was his/her own control, so the necessary population was smaller than if two different groups were compared. The population was unevenly distributed between the sexes, with 88 women and 42 men. As atherosclerosis develops later in women than in men, this inequality may be considered to be an advantage.

The exposure contrast in such a small cohort as the AW study makes it difficult to observe differences. This problem applies first and foremost to the conventional coronary risk factors and might be the reason why it was only possible to observe a few statistically significant differences (error of type II). In connection with the two stress models, only very few associations were observed in connection with the DC model. The participants' statements regarding greater levels of control and support confirm that the exposure contrast was small with regard to this model. There were also only a few individuals that belonged to the "strain" group. The scales were used as continual variables (control and demand). Accordingly, the results concerning the DC model and IMT changes were not very conclusive.

The more factors that are involved in the analyses, the larger the population should be. Thus, in the case of the AW study, it was an advantage that the variation in conventional coronary risk factors was small and within the normal range.

SELF-SELECTION:

The participants in the AW study established contact themselves after the announcement; thus, the population was self-selected. The reasons for enrolling in an investigation of psychological working environments and stress varied. Some participants expressed interest in the area of "working environment and stress", while others wanted information about their own health, either based on a general interest in their own health or on a history of heart disease in the family. The participants might, e.g., have had a special interest in living healthily or following advice about handling stress. Socially, the group was not representative of the general population either. A very large number were trained and employed in the health services; the population also had great control over their work and experienced a great deal of support. These conditions could make it difficult to generalise the results. However, the correlations observed were not influenced by the selection.

Only 502 of the 1117 eligible individuals in the OSH study volunteered for the study on biomarkers. However, the two groups did not differ significantly regarding age, sex, training, perceived stress or sick leave during 2005, and this lack of significance indicates that no major bias was introduced by the selection.

DROP-OUTS:

From the examination in 1998 until the follow-up in 2002, 35 participants dropped out of the AW study. Furthermore, between 2002 and 2008, another 18 participants dropped out. This process can be defined as a secondary self-selection. The analysis of differences between participants and non-participants showed that those participants who chose to leave the study were younger and healthier (however, without significant difference). Furthermore, the men who dropped out of the study experienced heavy demands at work in 1998. In both cases, the exposure contrast was narrowed, as well as, in consequence, the spread of material, for which reason any significance of the named factors would be more difficult to find.

QUESTIONNAIRES AS MEASUREMENTS OF PSYCHOSOCIAL STRAIN:

Questionnaires, as used, e.g., to identify strain according to the DC model, were originally expected to provide an objective assessment of the psychosocial strain inherent in a given job. However, people with the same work functions answer given questionnaires based on their personal job experiences and accordingly, provide different answers concerning the degrees of, e.g., demands and control in the workplace, despite having structurally similar jobs. This finding may be due to personal conditions (personality structure or family responsibilities), or people with the same job might, without it being intentional on the employer's part, actually have different degrees of demands and control (Kristensen, 1996). This might, e.g., be the case when a man is employed in a typically female job or when, in contrast, a woman is working in a man's job. In this way, the answers to such questionnaires, as well as the experienced reality, will depend on each person's background and interaction with his/her surroundings.

This situation is not only interesting with regard to whether a questionnaire is formulated or complete such that all respondents with a specific job will answer in the same way. The personal component of the answers to a questionnaire is presumably also reflected in the physiological changes connected to the challenges during a normal day.

The AW study included two stress models to estimate work-related stress: the DC model and the ERI model. The first model focuses on structural situations. There can be some expectation of being able to prove a dose-response relationship with the use of this model. However, in the case of psychosocial strain, the strength of this model depends on the subject being studied. A given strain will be meaningless to one individual, while another individual will feel significantly bothered by the same strain. A model such as the ERI model attempts to reflect this disparity, as both exposure and effect are incorporated into the questions. The subject must state the presence of a given situation and the degree to which it is a problem. This requirement highlights the relative and individual psychosocial strains and stresses. This may be part of the explanation for why more associations were found in connection with the ERI model than in connection with the DC model.

The AW study included an objective measurement of workload in the form of WWH. The measurement was not associated with the outcome measurements. This lack of association may have originated in the aforementioned problem of a lack of contrast in exposure. One may also argue that work in itself is not a risk. The exposure is how the working conditions are perceived: Are "demands" high or low? When aggregated data are used, an association between, e.g., "demands" and disease indicates that the working environment at that work place or in that job bears a risk. This risk may call for a regulation by the Danish Working Environment Authority. An association between individually assessed "demands" and disease is difficult to address by regulation, but the use of self-reported exposure is a necessity in research into stress physiology.

THE TIME FACTOR:

There is no time-related congruity among the score on a questionnaire, the physiological condition on clinical examination and measured IMT. When the participant answers a questionnaire, the subject will answer generally but presumably based on the reality of his/her current working environment. Thus, the participant may answer in a positive manner, whereas a few weeks earlier, he/she may have been experiencing difficult conditions that might, potentially, have existed for years. Such a change could easily be measured in the form of an improved symptom profile. The changes will presumably also appear relatively quickly in the form of normalised blood pressure, cortisol levels and HRV, but it would not be possible to observe changes in IMT over a short period of time. Use of the prospective examination, with the participant as his/her own control, and IMT progression used as an effect measurement, instead of maximum IMT, minimise measurement bias.

The results from papers II-V suggest that the two allostatic systems do not react in the same manner after psychosocial exposure. It is well known that the autonomous nervous system reacts within seconds and the HPA-axis within minutes in connection with an acute stressor (Eriksen et al. 1999). Such prolonged stressors as the re-organisation of the Danish administration did not influence HRV, although some changes in the HPA axis were observed.

IMT:

Although atherosclerosis is a universal change in the muscular and elastic arteries, the change also has a focal occurrence (Howard et al. 1994). Thus, an IMT variable based on a far larger number of measurements, preferably computer-based and over larger parts of the arterial tree, might have been preferable in our studies (Bots et al. 2003, Kanters et al. 1997, Schmidt C et al. 1999). Salonen and Salonen were among the first to use IMT, and in their first studies, they also used an equivalent, simple method including measurement of IMT three times on each of the CCAs (Salonen et al. 1993b). In this study, however, they chose to measure more proximally on the CCA. Several others have, in the same manner, used a limited number of measurements and in some studies, have also measured only the carotid (table 3). In connection with a study of very early changes, this design may work, but in connection with risk of manifest CVD, it may be preferable to examine the plaque score (Inaba et al. 2012).

In the AW study, it was demonstrated that IMT is an easily acquired and usable measurement. As IMT is measured completely distally on the CCA, larger IMT values were presumably obtained than if a computer-based method with, e.g., 100 measurements over 1 cm of the carotid artery had been used.

CORTISOL:

In connection with the use of salivary cortisol as a measurement of the physiological stress response, it is important to note that this measurement depends on the participants' compliance with the examination programme. It is especially important for the participants to take their first saliva sample as soon as they awaken. Research has shown that in groups of examination subjects, there can be problems with compliance (Clow et al. 2004, Kudielka et al. 2003a). It has been suggested that participants with small cortisol increases (or decreases) (e.g., a cortisol increase < 2.49 nmol/l) (Wüst et al. 2000) should be excluded. However, other examinations have shown that some people, generally or just on some days, do not react with a cortisol increase in the morning (Wüst et al. 2000). In order not to exclude true non-responders, all of the participants were included in the studies based on the AW data. As the OSH study included more individuals, a sub-sample was conducted of individuals who did not take medication and who had positive ACR. However, the results were not substantially different compared to the results from analyses that included the total population. Alderling et al. also found very few effects of medication (Alderling et al. 2006). The inclusion of medicated individuals in studies of salivary cortisol may not be a large confounder. Based on pulsatile cortisol secretion and problems with compliance, saliva measurements over several working days might have been included such that the saliva samples would be presented as an average over, e.g., two or three working days during the same week. However, several samples could actually reduce compliance, and as salivary cortisol has been shown to be relatively stable for days (Pruessner et al. 1997), the sampling was chosen to be conducted during one day only.

HRV:

With respect to the HRV analyses, there was the special problem that HRV was not carried out until 2002. In paper VII, two time windows were selected of 5 minutes each. This selection may be questionable. The first time window was selected during 15 minutes, when the stress test took place, and the period during which TP was smallest was chosen because during this period, the person was under the greatest pressure from the task. The participants spontaneously confirmed this observation by stating that they were less able to remember pictures than to add numbers. During sleep, a period with maximum TP was selected. Here, an EEG or similar measurement should have ensured that the participants were, in fact, sleeping. In addition, it would also have been possible to register the depth of sleep, a variable that must also be taken into consideration when assessing HRV during sleep (Vanoli et al. 1995).

The first method used, with which the contrast between HRV data under stress and during sleep was considered, had the advantage of a smaller amount of data being necessary for processing and analysing because the actual variability was presumably approximated.

STRESS TEST:

For ethical reasons, actual psychologically taxing tests, e.g., provocation of former traumas, were avoided. The chosen test series consisted of sufficient stressors, as we recorded raised pulse ($p < 0.01$ for both sexes) and blood pressure ($p = 0.12$) in connection with the test; similarly, TP and HF were significantly lower during the test, compared with 15 minutes later ($p < 0.01$) (paired samples test).

GENDER DIFFERENCES:

The analyses of interactions showed that there was a number of significant interactions but only in connection with the associations between ERI and the physiological stress response and only in the AW data. It seems plausible that the perception of stressors at the workplace is different between sexes. If the difference between sexes in the AW data is valid, the reason for the lack of interactions in connection with the OSH data may be the suggested influence of the Kommunal reform, i.e., the general increase in cortisol levels may have concealed a sex difference in connection with the effect of ERI. Additionally, as the participants in OSH were working under very similar working conditions, they may have perceived the work-related stressors in the same manner whether male or female.

It was not possible to reveal significant interactions between sex and the physiological stress response in connection with IMT progression. The limited size of the population included in these analyses may explain this inability, or, of course, a difference might not be present.

THE STRENGTHS OF THE STUDIES:

The AW study included younger and middle-aged working and generally healthy individuals. These age groups are relevant to the study, as targeted and relevant intervention in the form of stress prevention will have great significance over the long term and also presumably have a spin-off effect on these groups' children. At the same time, the inclusion of both men and women is a strength, as it provided the opportunity to identify the similarities and differences between the sexes' physiological and psychological reactions to psychosocial strain. The inclusion of several stressors in the AW study is likewise a strength. The studies were prospective and, therefore, provide more information about causal mechanisms than would cross-section examinations.

Data from the simultaneous measuring of activity on the HPA axis and the autonomic nervous system were reported in paper III. This is considered a strength, although the results from this paper were sparse. Additionally, papers IV and V should be read together and be compared, as these data are also simultaneous and provide more information when read together.

BIOLOGICAL PLAUSIBILITY

In this section, it is discussed how psychological strain via the activation of the HPA axis and the autonomous nervous system may induce and facilitate the progression of atherosclerosis. In particular, the proximal parts of the coronary arteries and the vessels' division sites are predisposed to atherosclerosis, which is an inflammatory condition. The earliest stage is endothelial dysfunction, which is found, among other results, to be due to the effects of cigarette toxins, in dyslipidaemia, insulin resistance and physical strain on the arterial wall (Hegele, 1996, Ross R, 1999). With endothelial dysfunction, the endothelial cells develop adhesion molecules, so monocytes can penetrate between the endothelial cells. In the sub-endothelial space, the monocytes are changed into macrophages and develop into foam cells by absorbing oxidised intimal lipoproteins. The condition is worsened with the growth of smooth muscle cells, the generation of a fibrous cap, formation of a necrotic core and, late in the process, calcification. Upon plaque rupture, thromboses are generated, and subsequently, ischemic damages may follow locally and cerebrally (Hegele, 1996). In the disease process, the macrophages produce cytokines, including interleukin 1 and platelet-derived growth factor, among others, which are also produced from blood platelets upon the generation of thromboses.

Psychological strains are potent stressors that stimulate both the HPA axis and the sympatico-adrenal system (SA system) (Kovács et al. 2005). The physiological stress response produced by such psychological stressors has a number of atherogenous effects. Corticoreleasing factor, which stimulates the pituitary gland into the secretion of adrenocorticotropt hormone, also increases the heart's contractility, the heart rhythm and the peripheral blood pressure (Hsu, 2005). Cortisol increases the blood pressure via a potentiation of angiotensin II's effects (Brem, 2001) and by inhibiting the effect of nitrous oxide, which causes vasodilatation (Kino et al. 2005). Cortisol also has a universal effect on glucose and lipid metabolism. Blood glucose is increased by stimulating gluconeogenesis and by inhibiting the effects of insulin, and the blood's free fatty acid and glycerol content is increased, based on both cortisol's own lipolytic effects in fatty tissue and the potentiation of other lipolytic hormones' effects (growth hormone and catecholamines) (Kino et al. 2005, Sapolsky et al. 1986). In connection with the stress response, the blood's proinflammatory substance content is increased. In this way, the sympathetic nerve endings can release interleukin 6 upon psychogenic stimulation (Black et al. 2002). Sub-sensitive glucocorticoid receptors can also contribute to the increased level of proinflammatory substances, which are observed in chronic stress and depression (O'Brien et al. 2004). Increased blood sugar, increased lipids in the blood and pro-inflammatory substances all increase endothelial dysfunction, which is the first stage in the development of atherosclerosis (Hegele, 1996, Ross R, 1999). It has also been shown that mental stress can induce passing endothelial dysfunction in people (Ghiadoni et al. 2000). As the inhibition of cortisol production prevented stress-induced endothelial dysfunction, cortisol may have a direct effect on the endothelium, giving rise to dysfunction (Broadley et al. 2005).

The SA system is a contributing cause of essential hypertension via renin-angiotensin and angiotensin II (Grassi, 1998). Finally, angiotensin II does not only work as a vasoconstrictor but also affects smooth muscle cells and can lead to the proliferation of these cells via transforming growth factor-beta, platelet-derived growth factor and fibroblast growth factor, just as the generation of foam cells is stimulated via an angiotensin II-mediated modification of low-density lipoprotein cholesterol (Black et al. 2002).

SUGGESTIONS FOR FUTURE RESEARCH

The field of work-related psychosocial factors might develop further, as the mentioned stress models may be further developed. The use of a comprehensive questionnaire, such as the COPSOQ, might be recommended, as this questionnaire covers a large number of stressors (Kristensen et al. 2005). Furthermore, the work-family interface is important to examine, as it is a clinical experience that stress often originates in double exposure and as intervention may prevent future disease (Beauregard et al. 2011, Low et al. 2010, Wang et al. 2007).

The association between IMT and incidence of CVD is rather small (Lorenz et al. 2007). This small association implies that the disease is more complicated than could be caused by a simple stenosis (Underhill et al. 2010). Vulnerable plaque is characterised by a thin fibrous cap and inflammation. These characteristics may be utilised in detection. By means of positron emission tomography (PET), the accumulation of flour-18-labelled 2-deoxy-D-glucose (FDG) in metabolically active plaques may be detected (Hiari et al. 2011). In the future, a study of the associations between psychosocial stressors and vulnerable coronary plaques detected by FDG PET imaging might be a new step forward in stress research. Additionally, gene * environment interactions may be examined, as Hintsanen et al. have demonstrated an effect of job strain in men who are homozygous for neuregulin-1 but not in other men (Hintsanen et al. 2007b).

The diurnal changes in physiology and a feasible sampling scheme may be investigated in connection with salivary cortisol, as well as with HRV. The use of dexamethasone tests may provide further information on reactivity in the HPA axis, as well as measurements of both systems in connection with stress tests. The interrelationship between the two allostatic systems is important to understand, as this understanding will presumably deepen our understanding of psycosocial stressors, physiological stress response, and physiological effects (Licht et al. 2010, Stalder et al. 2010). Additionally, the effect that each type of work-related psychosocial factor, that is, the duration and intensity of the stressors, has on the two allostatic systems and the possible dose-response-relationship should be examined. Furthermore, the effect that such physiological changes may have on early atherosclerosis and metabolism should be studied. The significance of the physiological stress response, in terms of it being a chronic physiological stress condition, is poorly understood, but increasing evidence suggests that physiological stress is important in the development of several conditions and diseases (Thayer et al. 2010, The Scancort Group, 2011). Finally, the effects of interventions (physical activity, meditation or medication) may constitute a large and poorly examined field of stress research.

CONCLUSION

The thesis outlines a possible pathway between work stress and atherosclerosis independently of conventional coronary risk factors. Paper I demonstrated that IMT progression can be used as an outcome in studies of the DC model and the ERI model and highlighted, as the first study, private stressors as possibly also being of significance for development of IMT. Papers II – V examined the partial hypothesis a, the link between work-related psychosocial factors and the physiological stress response. Paper II - IV showed that especially ERI was positively associated with levels of salivary cortisol and ACR. In the papers III and V, the ERI model was inversely associated with TP, HF, and LF / HF. Papers VI and VII examined the partial hypothesis b, the link between the physiological stress response and IMT, which had not been examined before. As the first paper studying the link between reactivity in the HPA-axis and IMT, paper VI reported increased ACR to be associated with IMT progression. Similarly, paper VII was the first to study the association between HRV and IMT in healthy individuals revealing non-significant associations between low HRV and high IMT measures.

The DC model was only sparsely associated with the measurements of the physiological stress response, whereas the ERI model, among both men and women and in two cohorts, was associated with a rise in salivary cortisol in the morning, and decreased HRV-measures. This finding may be important because the DC model, which highlights in particular structural conditions in relation to the working environment, has been dominant in stress research. In contrast, the more recent ERI model, which highlights person-related conditions, is supposed to be more sensitive for use on the future work market.

The measurements for quantity of work, i.e., the number of working hours in the home or in the workplace, were not associated with the physiological stress response, contrary to the experience of time pressure, which, especially in women, was associated with a large rise in salivary cortisol in the morning.

The physiological stress response in the form of increased ACR was associated with increased progression of early atherosclerosis, as well as increased activity of the autonomic nervous system, in the form of increased $LF/HF_{difference}$ was significantly associated with IMT progression from 2002 until 2008.

A significant gender difference was observed with regard to the effects of psychosocial factors on the physiological stress response and in connection with IMT progression in the AW study, i.e., the effects of the ERI model were most pronounced in the men (ACR and TP), and ERI only increased IMT progression in men. There was, however, not a difference between sexes with regard to the effect of the physiological stress response on IMTprogression.

The findings of the AW study, as well as those in the literature, indicate that atherosclerosis may, independent of other risk factors, be provoked by psychosocial strain. In a population in which conventional coronary risk factors have low prevalence, i.e. younger people, greater focus on psychosocial factors might be important for the prevention of atherosclerosis. The use of physiological measurements for stress will be of great significance in connection with understanding work stress and the long-term effects of this condition.

TABLE 1. THE AW STUDY:

PHYSIOLOGICAL VARIABLES, DEMOGRAPHIC CHARACTERISTICS AND WORK-RELATED PSYCHOSOCIAL FACTORS IN THE PARTICIPANTS, MEAN AND STANDARD DEVIATION (SD).

	1998				2002				2008			
	Women (N = 88)		**Men (N = 42)**		**Women (N = 63)**		**Men (N = 31)**		**Women (N = 58)**		**Men (N = 28)**	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	Mean	SD	
Age (years)	45	8	45	9	50	7	51	8	55	7	57	7
IMT mean (mm)	0.731	0.116	0.834	0.192	0.959	0.245	1.039	0.208	0.957	0.115	1.104	0.157
Body mass index (kg / m2)	23.7	2.9	25.9	2.7	24.9	3.2	27.0	2.8	24.9	3.5	27.4	3.0
Waist-hip ratio	0.79	0.06	0.90	0.06	0.80	0.06	0.91	0.09	0.81	0.06	0.95	0.07
Fibrinogen (micromol / l)	8.17	1.73	8.38	2.00	10.60	1.76	11.16	2.80	9.99	1.34	10.40	1.49
Cholesterol (mmol / l)	5.3	1.0	5.4	1.0	5.5	1.1	5.4	1.0	5.99	1.11	5.24	1.48
HDL cholesterol (mmol / l)	1.62	0.45	1.38	0.39	1.77	0.44	1.58	0.88	1.87	0.48	1.34	0.37
HbA_{1c} (%)	4.5	0.4	4.7	0.7	5.3	0.2	5.4	0.5	5.3	0.3	5.4	0.5
Systolic blood pressure (mmHg)	122	12	135	15	129	15	141	18	133	16	139	15
Alcohol (drinks / week)	7	7	10	10	2	4	6	10	7	6	11	10
Smoker, yes %	27.3		21.4		34.9		25.8		16.7		19.2	
Physical activity, 2-4 hours / week %	65.9		42.9		63.5		54.8		68.8		68.2	
Social status, % (N):												
1: Academics	8.0 (7)		28.6 (12)		12.6 (8)		27.3 (8)		10.4 (5)		28.6 (8)	
2: 3-4 years education, non-manual	50.0 (44)		21.4 (9)		43.8 (28)		18.2 (6)		41.7 (20)		10.7 (3)	
3: 1-2 years education, non-manual	22.7 (20)		11.9 (5)		22.9 (14)		9.1 (3)		27.1 (13)		21.4 (6)	
4: Skilled worker	14.8 (13)		38.1 (16)		14.6 (9)		45.4 (14)		14.6 (7)		39.3 (11)	
5: Unskilled worker	4.5 (4)		0 (0)		6.3 (4)		0 (0)		6.3 (3)		0 (0)	
Children at home, yes %	66.7		54.8		54.2		50.0		35.4		21.4	
Cohabitant, yes %	85.2		88.0		88.9		84.8		79.2		75.0	
Work hours per week	35.6	6.7	40.7	7.4	35.1	5.1	35.1	5.1	32.9	10.2	33	10
Lack of control	37	22	24	22	37	14	37	14	33	20	22	19
Demands	54	24	52	22	50	17	50	17	43	20	41	19
Lack of support	24	16	27	22	22	13	22	13	20	21	17	14
Effort	11	3	11	4	11	2	11	2	10	3	11	4
Reward	46	7	44	9	41	4	41	4	45	9	44	9
Effort reward imbalance	0.58	0.28	0.62	0.28	0.57	0.15	0.57	0.15	0.55	0.38	0.53	0.23
Overcommitment	15	3	15	3	14	3	14	3	13	3	13	3

TABLE 2. THE OSH STUDY:

PHYSIOLOGICAL VARIABLES, DEMOGRAPHIC CHARACTERISTICS AND WORK-RELATED PSYCHOSOCIAL FACTORS IN THE PARTICIPANTS, MEAN AND STANDARD DEVIATION (SD).

	Salivary cortisol-study				HRV-study			
	Women (N = 352)		**Men (N = 128)**		**Women (N = 170)**		**Men (N = 61)**	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Age (years)	49.1	8.7	51.7	9.5	49.3	8.8	51.2	9.7
Waist hip ratio	0.80	0.07	0.90	0.06	0.80	0.07	0.90	0.06
Blood pressure, systolic (mmHg)	136.0	20.5	144.1	19.5	136.1	21.1	144.6	18.6
Cholesterol (mmol /l)	5.3	1.0	5.3	1.0	5.3	1.0	5.3	1.0
HbA_{1c} (%)	5.4	0.4	5.5	0.6	5.4	0.4	5.5	0.6
Smoking, yes (%)	15.1		14.8		15.1		14.8	
Alcohol (drinks / week)	6.1	6.0	10.8	8.9	6.1	6.0	10.8	8.9
Physical activity ≤ 4 hours per week (%)	51.8		37.5		45.5		34.4	
Social status, % (N):								
1: Academics	26.1 (92)		52.3 (67)		25.9 (44)		47.5 (29)	
2: 3-4 years education, non-manual	31.3 (110)		28.1 (36)		28.2 (48)		27.9 (17)	
3: 1-2 years education, non-manual	19.3 (68)		8.6 (11)		19.4 (33)		13.1 (8)	
4: Skilled worker	9.7 (34)		5.4 (7)		24.1 (41)		9.8 (6)	
5: Unskilled worker	13.6 (48)		5.4 (7)		1.2 (2)		0.0 (0)	
The Effort Reward Model								
Effort	10.6	3.6	9.8	3.3	10.8	3.7	10.4	3.5
Reward	26.7	5.7	26.4	5.8	26.3	6.5	25.9	6.5
ERI	0.76	0.42	0.71	0.39	0.83	0.52	0.81	0.49

TABLE 3. *A short review of studies including work-related psychosocial factors as the exposure and IMT measurements as the effect measure.*

First author / year	Participants	IMT measure	Psychosocial factors
Studies, which use IMT progression as the effect measure			
(Lynch et al. 1997b)	940 men 42- 60 years	On average 100 estimates recorded over 1-1.5 cm on the far wall on both left and right carotid. Outcome was mean change in : mean max (average of maximum IMT on left and right), mean of all measures, and plaque-height).	Work place demands-index, Economic reward
(Everson et al. 1997)	994 or 2012 men 42- 60 years	Same as used in Lynch 1997a	Work place demands-index
Paper I, 2007	63 women 32 men 34-63 years	Progression in IMTmean: Difference between measures from 2002 and 1998: Average of three measures on CCA at the transition to the bulb on both left and right carotid.	Cohabitation, children, hours housework, time pressure, working hours, DC model, ERI model including overcommitment
(Krause et al. 2009)	621 men 42-60 years	Measurements as in Lynch 1997. Outcome defined as natural log of maximum IMT at 11 years (measured in 1998-2001) minus natural log of maximum IMT at baseline	Number of workdays per week and hours per day.
(Rosenstrom et al. 2011)	335 men 374 women 24-39 years	Difference between an average of at least 4 measurements approximately 1 cm proximal to the bifurcation on left CCA in 2001 and 2007	Job strain
Studies, which use baseline measures of exposure and IMT measures at follow-up as the outcome.			
(Gallo et al. 2003)	362 women 58 years (mean)	Plaques IMT mean and max Risk factors were measured pre-menopausally and IMT app. 11 years later.	Jobs groups: blue-collar, white-collar and clerical Job characteristics used as covariates.
(Kivimaki et al. 2007)	358 men 33-35 years	At least 4 measurements approximately 1 cm proximal to the bifurcation on CCA	Job strain. Childhood factors also included in analyses.
Cross-sectional studies			
(Muntaner et al. 1998)	5284 men 5517 women 45-64 years	Mean of measures at bifurcation, interna and CCA	Six job indicators, aggregated data.
(Nordstrom et al. 2001)	218 women 249 men 40-60 years	Plaques IMT averaged over 1 cm	Work stress-index
(Rosvall et al. 2002)	1550 women 1108 men 54 years (mean)	Plaques IMT mean and max	Demands and decision latitude
(Hintsanen et al. 2005)	542 women 478 men 32.5 years (mean)	At least 4 measurements 1 cm proximally to the bifurcation on left CCA	Demands and control
(Hintsanen et al. 2007b)	349 women and 357 men 32.5 years (mean)	At least 4 measurements 1 cm proximally to the bifurcation on left CCA	Job strain and social support
(Bugajska et al. 2008)	75 men 75 women 35-65 years	IMT and plaques	Perceived stress (short version of Occupational Stress Indicator)
(Xu et al. 2010)	508 men 226 women 41 years (mean)	At least 5 measurements 1 cm proximal to the bifurcation on CCA	ERI model
(Fujishiro et al. 2011)	6814 adults 45-84 years	Mean of the max IMT measured at both CCA, far and near wall 10 mm proximally, at the bulb and at the interna	Job control and demands

Analysis	Result
Analysis of variance, including adjustment for baseline IMT, age and covariates to compare IMTprogression in the different groups formed by high / low demands and high / low economic reward.	Increased progression in IMTmax and plaque height (but not IMTmean) in the group with high demands and low economic reward compared to low demands 7 high economic reward.
Grouping of individuals according to low/high demands and reactivity. Analysis of variance, adjustment for baseline IMT, age and covariates	Interaction between blood pressure-reactivity and demands: Increased reactivity in combination with demands was associated with increased progression in IMT-measures.
Simple and multiple linear regression analyses with IMTprogression as outcome and each psychosocial factor as the independent variable adjusting for age and baseline IMT.	Women: Effort associated with IMTprogression. Men: ERI ass with IMTprogression. Furthermore, effort and overcommitment was associated with IMTprogression in both genders.
Ln-transformed IMT-data. Multiple linear regression analysis. Results were adjusted for relevant covariates.	Men who worked 3 days per week experienced 23% increase in IMT while men who worked 7 days per week experienced 40 % increase in IMT. Those with IHD had increased IMTprogresion.
Partial correlation analysis. Blomquist's equation to obtain regression slope and bootstrapping for estimating standard error.	Two change patterns were seen: Large decrease in job strain was associated with slower progression in IMT. Small changes in the theoretically job strain axis involved a link between changes towards passive jobs and was associated with a faster IMT progression.
Analysis of variance and hierarchical regression analysis	Clerical workers had significant greater IMT than the other groups after adjustment for relevant risk factors.
Fitting of separate age-adjusted linear regression models to the data	Dose-response relationship between job strain and IMT with only little effect of other risk factors.
Multivariate age-adjusted regression analysis	Negative associations between complexity of work and skill discretion and IMT. Physical demands were associated with IMT in black men, and job insecurity with IMT in white women and black men.
Gender-separated analysis: Age-adjusted logistic regression and age, and height multiple regression analysis	Among men: Strong linear trend of more plaques if more stressed. The highest quintile compared to the lowest had greater IMT. Women- no associations.
Linear and logistic regression models. Adjustment for various combinations of variables.	Women with high demands and low decision latitude had greater IMT and more plaques than the group with low demands and high decision latitude. No associations in men. More complex than suspected.
Gender-separated analysis. Multiple linear regression analysis.	No association in women Men: Job control was not associated with IMT. Demands and strain was significantly associated with IMT also after adjustments.
Gender-separated analysis. Analysis of covariance.	Job strain was significantly associated with IMT in men with genotype T/T but not with heterozygote or C/C.
Student's t-test and bivariate correlation analysis.	Negative association between the stress-index and early atherosclerotic changes.
Chi-square-test or unpaired t-test to test differences between groups. Multiple linear regression analysis.	Effort and overcommitment was positively ass with IMT and reward was inversely associated with IMT in both genders. In men, but not in women, the association disappeared in the fully adjusted model.
Multiple linear regression analysis with adjustment for groups of variables. No interaction between sex and occupational factors.	Increased job control was associated with thinner IMT (1 SD increase in control ass with 0.007 mm thinner IMT). Suggestive evidence of multiplicative interaction between demands and control. No significant association between strain and IMT

TABLE 4. *A short review of studies including work-related psychosocial factors as the exposure and ACR expressed as change in cortisol level from the awakening level as the effect measure.*

Author / year	Participants	Cortisol measures	Psychosocial factor
(Steptoe et al. 2004b)	105 men 92 women 45-59 years	10 samples during a normal working day incl. at awakening and 30 minutes later.	ERI model including overcommitment
(Kunz-Ebrecht et al. 2004b)	97 men 84 women 45 – 58 years	Sampling as in Steptoe 2004b	DC model
Paper II, 2006	55 women 28 men 34 –63 years	At awakening, and after 20, 30 and 60 minutes, after 8 hour and at 18:00.	Cohabitation, children, hours housework, time pressure, working hours, DC model, ERI model including overcommitment
(Alderling et al. 2006)	348 women 181 men 20-64 years	At awakening, after 30 minutes, at lunch and immediately before going to bed on a working day.	DC model
(Chandola et al. 2008)	2811 men and women 37-60 years	At awakening and after 30 minutes	DC model
(Maina et al. 2008)	12 men 56 women, mean 30 years	At awakening, after 30 minutes and during the day.	DC model
(Wright, 2008)	43 men 55 women mean 37 years	At awakening and after 30 minutes	DC model
(Maina et al. 2009)	20 women 16 men mean 42 years	At awakening and after 30 minutes	DC model
Paper III, 2011	48 women 22 men 34-63 years	At awakening, after 30 min, and at 18:00 in 2002 and 2008.	Cohabitation, children, hours housework, time pressure, working hours, DC model, ERI model
Paper IV, 2012	352 women 128 men 27-67 years	At awakening, and after 30 min in 2006 and 2008.	ERI model

	Analysis	Result
	Repeated measures analysis of variance. Gender, occupational grade and overcommitment as between-subject factor.	No associations between ERI and cortisol. ACR was significantly greater in overcommitted men compared with non-overcommitted. Gender * overcommitment interaction, i.e. the overcommitment effect was present in the men but not in the women.
	Analysis of covariance.	ACR was greatest in the lower socioeconomic status-participants who reported high demands in both men and women. No effect of control, but over the remainder of the day, cortisol levels were inversely associated with control in men.
	Univariate and repeated measures analysis of variance, one (S1), two (S1 and S30), or all 6 measures of cortisol as the dependent variable.	Time pressure was associated with increased levels of cortisol. No association with demands or control but lack of support was associated with low ACR in men. ERI was positively associated with ACR in both genders. Also, overcommitment was significantly and positively associated with cortisol levels in men.
	Participants were grouped in the four strain categories. Log transformed cortisol data. Mixed model testing interaction between strain group and time.	No main effect of strain on cortisol in men or women. Half an hour after awakening low strain-women had significantly lower cortisol than the others. No differences were seen among the men. Very little effect of medication was seen.
	Cox proportional hazards regression models	Job strain was associated with increased ACR in cross sectional analysis but not in prospective analysis.
	Log-transformed cortisol data. Spearman's rank correlation	In women demands were associated with increased ACR, but there were no associations among men.
	Ln-transformed cortisol values, structural equation models	Job strain did not predict cortisol values.
	Square root transformated cortisol data. The generalized estimating equations method adjusting for covariates	Job strain significantly influenced the total amount of cortisol response to waking, but neither control, demands nor strain were assocaited with mean increase in cortisol.
	Ln-transformed cortisol –values, repeated measures analysis of covariance	Only association among men: High number of hours of housework was associated with low cortisol levels. Effort and ERI was associated with high levels of cortisol, but these disappeared when adjustment for hours of housework was included in the model.
	Log-transformed cortisol values. Mixed effects model including subject as a random factor	ERI was negatively and significantly associated with cortisol at awakening in women and positively (but not significantly) associated with ACR.

TABLE 5. *A short review of studies including work-related psychosocial factors as the exposure and HRV as the effect measure. Experimental studies not included.*

First author / year	Participants	HRV-measure	Psychosocial factor
(Sloan et al. 1994)	31 men 2 women mean 40 years	Spectral analysis	Perceived stressful periods
(Kageyama et al. 1998)	223 men 21-42 years	Time domain	Work-stress index
(Sasaki et al. 1999)	278 men, mean 37 years	HFnu	Working hours
(van Amelsvoort et al. 2000)	113 men 22 women 18-55 years	SDNN / LF	Job strain
(Vrijkotte et al. 2000)	109 men 25-55 years	Heart rate and RMSSD	ERI and over-commitment
(Hanson et al. 2001)	39 men 31 women mean 37 years	HF	ERI model
(Park et al. 2001)	238 men 22-46 years	Spectral analysis	Working hours
(Kang et al. 2004)	169 male, > 40 years old	SDNN	Job strain
(Riese et al. 2004)	159 women mean 36 years	RMSSD	Job strain
(Collins et al. 2005)	36 men 35-59 years	HF	Demand, decision latitude and social support, job strain
(Hemingway et al. 2005)	2197 men 45-68 years	Time domain and spectral analysis	Job control
(Hintsanen et al. 2007a)	406 men 457 women mean 32 years	Time domain and spectral analysis	ERI model
(Chandola et al. 2008)	3490 individuals 47-67 years	Spectral analysis measured in 2002-04	Job strain reported in 1985-88
(Lindholm et al. 2009)	132 individuals 25-62 years	RMSSD	DC model
(Collins et al. 2010)	36 men 35-59 years	HF	Job strain
(Loerbroks et al. 2010)	520 men 71 women 17-65 years	RMSSD	Job strain and ERI
(Clays et al. 2011)	653 men 40-55 years	Time domain and spectral analysis	Work stressor index
(Uusitalo et al. 2011)	10 men 18 women 24-57 years	Time domain and spectral analysis	ERI model
Paper III, 2011	48 women 22 men 34-63 years	Spectral analysis	Cohabitation, children, hours house-work, time pressure, working hours, DC model, ERI model
Paper V, 2011	170 women 61 men 27-67 years	Spectral analysis	ERI model

Analysis	Results
Log-transformed LF and HF power and the LF/HF ratio. Mixed effect regression model including subject as random effect. Stress entered as a continuous variable.	Psychological stress was significantly associated with an increase in the LF / HF ratio, suggesting increases in the relative predominance of sympathetic nervous system activity during stressful periods of the day.
Pearson correlation analysis	Job stressors were not correlated to HRV.
Analysis of variance	No association between working hours and HFnu
Log-transformed HRV-data. Least square means adjusted for covariates	The elevated %LF during work in employees exposed to high job strain indicated a direct shift in the autonomic cardiac balance towards sympathetic dominance.
Multivariate analysis of variance	High ERI was associated with high HR and there was a trend towards a lower RMSSD during work and leisure but not during sleep. Overcommitment was not associated with HRV-data.
Log-transformed data. Mixed effect model testing interaction between time and effort, reward and ERI, respectively one at a time.	The interaction between effort-reward imbalance and time of day, i.e. the cardiac vagal control of subjects with a high effort-reward imbalance increase as the day progresses.
Log-transformed data. Partial correlation and multiple linear regression inclusive adjustment for age and sleeping hours.	Long working hours (>52 hours / week) was associated with low LF.
Student's t-test	The SDNN of HRV was significantly lower in the high strain group than in the low strain group.
Analysis of variance, stepwise regression analysis.	High job strain among young female nurses is not associated with an unfavourable ambulatory cardiovascular profile.
Mixed effect model	Job strain and low decision latitude were associated with low vagus tonus persisting throughout the 48 hr. Job strain was associated with increased sympathetic control during working hours.
Ln-transformed HRV-data. Linear regression.	The age-adjusted mean low-frequency power was 319 ms2 among those participants in the bottom tertile of job control compared with 379 ms2 in the other participants (P=0.004).
Log-transformed HRV-measures. Gender-separated analysis, multiple linear regression.	In age-adjusted regression models, higher ERI was associated with lower HRV, and lower reward was associated with higher HR among women. No significant associations of the ERI- model with HR and HRV in men.
Cox proportional hazard regression models	Significant associations between number of work stress-reports (0, 1 or 2) and decreased LF, HF, and SDNN.
Log-transformed HRV-data. Non-parametric test, chi2-test.	High job control was associated with high levels of RMSSD during recovery time (in the 36-45 years old.
Graphically analysis and analysis of variance	Reduction in cardiac vagal activity in high strain subjects
Partial correlation analysis, multilevel analysis. Curvilinear modelling of effect of age	Job strain and HRV were unrelated. ERI was only related to HRV during work and leisure time in employees aged 35-44.
Log-transformed HRV-data, multiple regression analysis, analysis of covariance	The work stressor index was significantly associated with lower pNN50, lower high frequency power and a higher ratio of low frequency over high frequency power.
Ln-transformated HRV-data, two-way analysis of variance, partial correlation,	Effort at work had many HRV correlates: the higher the work effort the lower daytime HRV
Ln-transformed HRV-data. Repeated measures analysis of covariance	Reward was associated with increased levels of TP while ERI was associated with decreased levels of TP in men. No associations were seen in the women.
Ln-transformated HRV-data, mixed effect model	ERI was associated with decreased TP, decreased HF, and increased LF / HF during leisure time.

TABLE 6. *A short review of studies including levels of salivary cortisol / ACR as the explanatory variable and carotid atherosclerosis / IMT as the effect measure.*

First author / year	Participants	Cortisol-measure	Atherosclerosis / IMT-measure	Analysis	Results
(Eller et al. 2001b)	84 women 37 men 30-60 years	ACR (20 and 60 min) and single measures at awakening, and after 60 minutes	IMT	Gender-separated analysis. Linear regression.	Inverse association between high cortisol levels and low IMT, though only significant in women.
Paper VI, 2005	63 women 32 men 34-63 years	ACR (20 minutes)	IMTprogression during 4 years	Gender-separated analysis. Linear regression, analysis of variance	Increased ACR was associated with increased progression in women in gender-separated analysis.
(Dekker et al. 2008)	1866 individuals mean 74 years	At awakening, after 30 minutes, at 17:00 and at bedtime	Plaque-score: Both left and right internal carotid arteries, carotid bifurcation, and CCA were examined for the presence of plaques.	Persons with and without plaques were compared by means of Student's t test for continuous variables and Pearson's chi2 for the categorical variables. The associations of these risk factors with two summary measures of cortisol, area under curve and slope using linear regression models.	Participants with total cortisol excretion in the highest tertile had more atherosclerotic plaques compared with those in the lowest tertile in a fully adjusted model

TABLE 7. *A short review of studies including HRV as the explanatory variable and IMT as the effect measure.*

First author / year	Participants	HRV-measure	Atherosclerosis / IMT-measure	Analysis	Results
(Gottsater et al. 2006)	61 patients with type 2 diabetes (39 men) 45 – 69 years	HRV-analysis of 24 hour ECG, spectral analysis	IMT measured on the right CCA and the bulb of the carotid bifurcation.	Differences between groups were evaluated with the Mann–Whitney U-test and differences over time within groups with the Wilcoxon's signed rank test. Partial correlations were evaluated, controlled for age.	There was a correlation between HRV and IMT at baseline. LF was inversely associated with IMT progression in type 2 diabetic patients
Paper VII, 2006	50 women and 24 men 34 – 63 years	Spectral analysis during test and sleep	IMT measured on both CCAs	Student's t-test and linear regression analysis	Non-significant trends of an association between low HRV measures and greater IMT measures. LF/HF- difference between test and sleep inversely associated with IMT in men.
(Gautier et al. 2007)	Two samples: a) 274 men and 80 women, 40- 70 years old from The Pittsburgh Study b) 63 men from KIDH	Spectral analysis	IMT, measured on CCA, bulb and interna, mean of all measures (140 per segment)	Ln-transformed HRV measures. Hierarchial regression analysis.	Mean carotid IMT correlated inversely with LF and TP, whereas no significant relationship existed between HF or LF/HF and IMT.

TABLE 8. *Bivariate correlations between measures of IMT (2002 and progression in mean and max estimates) and physiological variables measured in 2002 in 86 participants, which were examined both in 2002 and 2008, AW-study.*

	1	2	3	4	5	6	7	8	9	10	11	12	13
1: $LF/HF_{difference}$	1	0.393**	0.349**	-0.272*	-0.251*	-0.224*	-0.146	-0.096	0.253*	-0.094	-0.015	-0.056	-0.155
2: IMTmax-prog	0.393**	1	0.831**	-0.485**	0.121	-0.354**	-0.045	-0.077	0.099	0.030	0.011	-0.072	-0.063
3: IMTmean-prog	0.349**	0.831**	1	-0.657**	0.112	-0.294**	0.060	-0.023	0.133	-0.005	-0.043	0.079	-0.148
4: IMTmean	-0.272*	-0.485**	-0.657**	1	0.116	0.297**	0.065	0.044	-0.102	0.053	-0.026	-0.087	0.135
5: Sex	-0.251*	0.121	0.112	0.116	1	-0.021	0.566**	0.223*	0.087	0.295**	0.079	-0.069	0.166
6:Age	-0.224*	-0.354**	-0.294**	0.297**	-0.021	1	0.054	0.160	0.023	0.085	0.193	0.311**	0.240*
7: WHR	-0.146	-0.045	0.060	0.065	0.566**	0.054	1	0.037	0.132	0.293**	0.026	0.074	0.288**
8: BPsys	-0.096	-0.077	-0.023	0.044	0.223*	0.160	0.037	1	-0.157	0.054	0.092	0.212	-0.166
9: Tobacco	0.253*	0.099	0.133	-0.102	0.087	0.023	0.132	-0.157	1	0.295**	-0.182	0.193	0.052
10: Alcohol	-0.094	0.030	-0.005	0.053	0.295**	0.085	0.293**	0.054	0.295**	1	0.071	0.080	0.090
11: Physical activity	-0.015	0.011	-0.043	-0.026	0.079	0.193	0.026	0.092	-0.182	0.071	1	0.091	0.025
12: Cholesterol	-0.056	-0.072	0.079	-0.087	-0.069	0.311**	0.074	0.212	0.193	0.080	0.091	1	-0.032
13: HbA_{1c}	-0.155	-0.063	-0.148	0.135	0.166	0.240*	0.288**	-0.166	0.052	0.090	0.025	-0.032	1

*** Correlation is significant at the 0.01 level (2-tailed).*
** Correlation is significant at the 0.05 level (2-tailed).*

1: LF / $HF_{difference}$ = LF/HF test – LF/HF sleep in 2002; 2: IMTmax-prog = Progression in IMTmax, 2002 until 2008; 3: IMTmean-prog = Progression in IMTmean, 2002 until 2008; 4: IMTmean, 2002;
5: Sex; 6: Age; 7: WHR = Waist hip ratio; 8: BPsys = Systolic blood pressure; 9: Tobacco; 10: Alcohol; 11: Physical activity; 12: Cholesterol; 13: HbA_{1c} = Glycated haemoglobin

TABLE 9. *Results from analysis of HRV-data from 2002 and progression from 2002 until 2008 in A: IMTmean and B: IMTmax. All participants included in same analysis of variance. $LF/HF_{difference}$ was ln-transformed. Enter model includes systolic blood pressure, waist hip ratio, cholesterol, HbA_{1c}, tobacco, physical activity in leisure time, and LF / $HF_{difference}$. All covariates were measured in 2002.*

IMTmean progression; Enter model R^2 = 0.13			Final model R^2 = 0.08	
	F	p	F	p
Blood pressure, systolic	0.083	0.775		
Waist hip ratio	0.063	0.803		
Cholesterol	0.347	0.559		
HbA_{1c}	0.270	0.606		
Alcohol	0.052	0.821		
Physical activity	1,294	0.261		
Ln (LF / $HF_{difference}$)	3,940	0.053	4.545	0.04
IMTmax-progression; Enter model R^2 = 0.17			Final model R^2 = 0.09	
	F	p	F	p
Blood pressure, systolic	0.172	0.681		
Waist hip ratio	1.234	0.272		
Cholesterol	0.430	0.515		
HbA_{1c}	0.260	0.613		
Alcohol	2.180	0.147		
Physical activity	0.914	0.344		
Ln (LF / $HF_{difference}$)	3.846	0.056	5.510	0.03

TABLE 10. *The table is an overview of the results of the thesis. The columns indicate the measures of the physiological stress response and IMTprogression, while the rows include the psychosocial factors examined.*

0	*The association was examined, but not present*		
+	*Positive association, $p < 0.05$*	–	*Negative association, $p < 0.05$*
(+)	*Positive association, $0.05 < p < 0.10$*	(–)	*Negative association, $0.05 < p < 0.10$*

* *Significant interaction between gender and psychosocial factor (analysis in appendix)*

	S1	**ACR**	**Cortisol levels during the day**	**TP**	**HF**	**LF/HF**	**IMTprogression**
Social status, high	0	0	+ [III, ♂]	+ [III, ♀] * – [III, ♂]	+ [III, ♀] – [III, ♂]	– [III, ♀]	0
Cohabitant, yes	0	(+) [II, ♀]	0	0	0	0	+ [I, ♀] (*)
Children, yes	0	0	– [III, ♂]	0	0	0	– [I, ♂] *
Housework, high	0	0	– [III, ♂]	0	0	– [III, ♂]	0
Time pressure, high	+ [II, ♂]	+ [II, ♀]	(+) [II, ♀]				0
Weekly work hours	0	0	0	0	+ [III, ♂]	– [III, ♂]	0
Lack of control	0	0	0	– [III, ♀] *	0	0	0
Demands	0	0	0	0	0	0	+ [I, ♀]
Lack of Support	0	– [II, ♂]	0	0	– [III, ♂]	+ [III, ♀]	0
Effort	(–) [IV, ♀s-p]	(+) [II, ♀] + [II, III, ♂] + [IV, s-p]	(+) [II, ♀] + [II, III ♂] *	– [V]	– [V]	0	+ [I, ♀]
Reward	+ [IV, ♀]	0	0	+ [III, ♂] * [V]	+ [V]	0	+ [I, ♀] * – [I, ♂]
ERI	– [IV, ♀] – [IV, ♀s-p]	+ [II, ♀♂ *; III ♂] (+) [IV, s-p]	+ [II, III, ♂] *	– [III, ♂] * – [V]	– [V]	+ [V, s-p]	+ [I, ♂] *
Overcommitment	0	+ [II, ♂] *	+ [II, ♂] *				+ [I, ♀]
ACR							+ [VI, ♀; both genders (appendix)]
HRV							0 [VII] + [table 9]

Abbreviations: S1 = cortisol on awakening; ACR = cortisol rise the first 30 minutes after awakening; TP = total power; HF = High frequency power; LF = Low frequency power; ERI = Effort reward imbalance; s-p = healthy sub-population.

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APPENDIX

PAPER I: The paper includes gender-separated results of a series of simple linear regression analyses using IMTprogression as the dependent variable. The scores of the DC model and the ERI model from 1998 and 2002 were averaged. Baseline levels of all other variables (psychosocial factors at home, physiological measures and behavioural measures) were used as independent variables, one at a time. IMTmean 1998 was included in all analyses. A one-way analysis of variance was used to evaluate gender difference in IMTprogression.

The supplementary analysis include two series of repeated measures analysis of variance including all participants, one including an interaction term, "gender * psychosocial factor", and one including gender as a factor.

Table I-1. Results from the analysis of possible interactions between psychosocial factors and gender in connection with IMTprogression.

A: Analysis of variance adjusting for IMTmean1998 and age, and including an interaction term gender * psychosocial factor.

B: Analysis of variance testing associations between IMTprogression and each psychosocial factor including gender as a factor without the interaction between gender and the psychosocial factor and adjusting for age and IMTmean98.

	A			B
	Psychosocial factor in question	Gender	Interaction term	Psychosocial factor
	F / p	F / p	F / p	F / p
Social status	1.12 / 0.29	0.001 / 00.98	0.77 / 0.38	0.83 / 0.36
Cohabitant	0.14 / 0.71	0.66 / 0.42	3.09 / 0.08	0.02 / 0.89
Children	0.70 / 0.40	7.73 / 0.007	6.68 / 0.01	(0.09 / 0.72)
Hours housework	0.13 / 0.72	5.14 / 0.03	3.63 / 0.06	0.13 / 0.72
Time pressure	0.001 / 0.98	7.20 / 0.01	5.20 / 0.03	(0.70 / 0.40)
WWH	0.05 / 0.83	0.25 / 0.62	0.53 / 0.47	0.05 / 0.82
Control	1.52 / 0.22	2.68 / 0.11	0.48 / 0.49	1.11 / 0.30
Demands	2.87 / 0.09	0.55 / 0.46	0.004 / 0.95	3.45 / 0.07
Support	0.90 / 0.35	1.66 / 0.20	0.14 / 0.71	1.09 / 0.30
Effort	6.17 / 0.02	0.06 / 0.80	0.05 / 0.82	7.83 / 0.006
Reward	0.18 / 0.67	11.32 / 0.001	8.82 / 0.004	(0.95 / 0.33)
ERI	12.91 / 0.001	5.43 / 0.02	9.23 / 0.003	(5.33 / 0.02)
Overcommitment	3.76 / 0.06	0.84 / 0.36	0.39 / 0.59	7.94 / 0.006

The brackets indicate that the result is of less interest as the gender * psychosocial factor term is significant, and therefore the gender-separated results from the paper are more correct.

PAPER II: The paper includes gender- separate results from three series of analyses. Levels of cortisol were used as the dependent variable in univariate and repeated measures analysis of variance. In all analyses age, physical activity, tobacco and time of the first sample were used as covariates. The univariate analysis was used to evaluate the associations of S1 and the psychosocial factors. The repeated measures analysis was used to evaluate associations of two repeated measures and the psychosocial factors. As the first repeated measure, ACR, had two levels: S1 and S30. The second repeated measure was cortisol levels throughout the day, i.e. all six measures of cortisol (at awakening, +20, +30, +60 min, +8 h, and 18:00).

The supplementary analysis includes 4 series of analyses: Table II-1 shows results of analyses parallel to those in the paper, but including ln-transformed cortisol values. Table II-2, a-c shows results from univariate (using lnS1 as the dependent variable) and repeated measures analysis of variance (using lnS1 and ln S30 as the dependent variable or all 6 cortisol measures, ln-transformed, as the dependent variable) including all participants, one including an interaction term, "gender * psychosocial factor", and one including gender as a factor.

Table II-1. Results from analyses parallel to those in the paper, but including ln-transformed cortisol values. The results are only reported in the form of p-values for:

a) awakening cortisol level (lnS1) by the use of analysis of variance.

b) the cortisol rise (lnS1 and LnS30) by the use of repeated measures analysis of variance.

c) cortisol during the day (lnS1, lnS20, lnS30, lnS60, lnS8hours, and lnS18:00) by the use of repeated measures analysis of variance.

All analyses are adjusted for time of measurement.

	a) Awakening level, lnS1 (p)		b) Cortisol rise 30 min (p)		c) Cortisol during the day, 6 measurements (p)	
	Women	Men	Women	Men	Women	Men
Social status	0.26	0.70	0.96	0.98	0.55	0.97
Cohabitant	0.73	0.11	0.04	0.74	0.72	0.52
Children	0.93	0.87	0.08	0.53	0.45	0.33
Hours housework	0.25	0.99	0.94	0.89	0.85	0.99
Time pressure	0.64	0.02	0.16	0.18	0.11	0.13
WWH	0.67	0.39	0.50	0.25	0.49	0.18
Control	0.58	0.99	0.74	0.75	0.76	0.43

Demands	0.63	0.31	0.93	0.22	0.84	0.19
Support	0.19	0.31	0.69	0.03	0.62	0.15
Effort	0.52	0.05	0.38	0.008	0.19	0.006
Reward	0.32	0.08	0.84	0.82	0.15	0.05
ERI	0.21	0.02	0.21	0.005	0.09	0.002
Overcom-mitment	0.96	0.20	0.47	0.06	0.67	0.005

Table II-2. A: Results from analyses including all participants in same analysis of variance with an interaction term (gender * psychosocial factor) and adjustment for time of measurement, age, tobacco and physical activity. B: Results from analyses including gender as a factor and adjustment for time of measurement, age, tobacco and physical activity without the interaction term between gender and the psychosocial factor.

a) Awakening level, lnS1				
	A			**B**
	Psychoso-cial factor	**Gender**	**Interac-tion term**	**Psychoso-cial factor**
	F / p	**F / p**	**F / p**	**F / p**
Social status	0.27 / 0.61	1.61 / 0.21	1.21 / 0.28	0.44 / 0.51
Cohabitant	0.69 / 0.41	1.84 / 0.18	2.55 / 0.12	0.17 / 0.69
Children	0.02 / 0.00	0.23 / 0.64	0.001 / 0.98	0.06 / 0.81
Hours house-work	0.59 / 0.45	0.61 / 0.44	0.37 / 0.55	0.54 / 0.47
Time pressure	6.73 / 0.01	2.88 / 0.09	2.98 / 0.09	4.56 / 0.04
WWH	0.92 / 0.34	0.34 / 0.56	0.23 / 0.63	1.41 / 0.24
Control	0.12 / 0.74	0.04 / 0.64	0.00 / 0.99	0.72 / 0.40
Demands	0.56 / 0.46	1.38 / 0.24	1.28 / 0.26	1.12 / 0.30
Support	0.00 / 0.98	1.42 / 0.24	2.93 / 0.09	0.31 / 0.58
Effort	6.45 / 0.01	2.82 / 0.09	2.42 / 0.12	5.54 / 0.02
Reward	5.37 / 0.02	1.41 / 0.24	1.60 / 0.21	4.09 / 0.05
ERI	11.06 / 0.001	4.15 / 0.05	4.02 / 0.05	(8.02 / 0.01)
Overcommit-ment	2.06 / 0.11	2.51 / 0.12	2.35 / 0.13	0.99 / 0.93

b) Cortisol rise 30 min (lnS1and lnS30)				
	A			**B**
	Psychoso-cial factor	**Gender**	**Interac-tion term**	**Psychoso-cial factor**
	F / p	**F / p**	**F / p**	**F / p**
Social status	0.07 / 0.79	0.36 / 0.55	0.28 / 0.60	1.23 / 0.27
Cohabitant	0.01 / 0.93	0.06 / 0.81	0.03 / 0.87	0.37 / 0.54
Children	0.23 / 0.64	0.39 / 0.54	1.55 / 0.22	0.27 / 0.60
Hours house-work	0.10 / 0.75	0.24 / 0.63	0.35 / 0.56	0.18 / 0.67
Time pressure	6.29 / 0.02	0.09 / 0.77	0.15 / 0.70	1.36 / 0.25
WWH	0.77 / 0.38	2.73 / 0.10	2.70 / 0.11	0.31 / 0.58
Control	0.22 / 0.64	0.67 / 0.42	1.41 / 0.24	0.01 / 0.94
Demands	1.24 / 0.27	1.59 / 0.21	1.75 / 0.19	0.08 / 0.77
Support	0.57 / 0.45	1.72 / 0.19	2.48 / 0.12	1.44 / 0.24
Effort	13.27 / 0.001	3.71 / 0.06	3.65 / 0.06	1.29 / 0.26
Reward	5.80 / 0.02	0.76 / 0.39	0.79 / 0.38	0.78 / 0.39
ERI	17.86 / 0.000	5.02 / 0.03	5.59 / 0.02	(0.98 / 0.33)
Overcommit-ment	11.66 / 0.001	8.83 / 0.004	8.96 / 0.004	(1.86 / 0.18)

The brackets indicate that the result is of less interest as the gender * psychosocial factor term is significant, and therefore the gender-separated results from the paper are more correct.

c) Cortisol during the day (6 measures)				
	A			**B**
	Psychoso-cial factor	**Gender**	**Interac-tion term**	**Psychoso-cial factor**
	F / p	**F / p**	**F / p**	**F / p**
Social status	0.10 / 0.76	0.005 / 0.94	0.12 / 0.73	0.09 / 0.76
Cohabitant	0.39 / 0.54	0.36 / 0.55	0.79 / 0.38	0.31 / 0.58
Children	0.001 / 0.97	3.38 / 0.07	2.08 / 0.16	0.42 / 0.52
Hours house-work	0.07 / 0.80	0.47 / 0.49	0.19 / 0.66	0.04 / 0.83
Time pressure	4.02 / 0.05	0.02 / 0.89	0.18 / 0.67	3.93 / 0.05
WWH	1.02 / 0.32	2.09 / 0.15	2.54 / 0.12	0.28 / 0.60
Control	0.002 / 0.96	1.07 / 0.31	0.57 / 0.45	0.00 / 0.99
Demands	0.96 / 0.33	0.23 / 0.63	0.87 / 0.36	0.70 / 0.34
Support	1.76 / 0.19	7.31 / 0.01	4.83 / 0.03	(3.26 / 0.08)
Effort	17.96 / 0.000	3.46 / 0.07	5.19 / 0.03	(12.52 / 0.001)
Reward	1.28 / 0.26	1.45 / 0.23	1.16 / 0.29	0.50 / 0.48
ERI	15.64 / 0.000	3.77 / 0.06	6.57 / 0.01	(8.44 / 0.005)
Overcommit-ment	8.03 / 0.007	3.43 / 0.07	4.44 / 0.04	(3.70 / 0.06)

The brackets indicate that the result is of less interest as the gender * psychosocial factor term is significant, and therefore the gender-separated results from the paper are more correct.

PAPER III: The initial analyses presented in the paper included gender separated results from four series of repeated measures analysis of covariance models using four dependent variables: LnTP, LnHF, LnLF/HF and LnCortisol. For each model, year of examination (2002 = 0; 2008 = 1) was included as a factor, and age and time of measurement (calculated as hours after midnight) were included as covariates. Furthermore, each of the psychosocial factors was included as either a factor (social status, cohabitant, and children) or as a covariate (housework, WWH, lack of control, demands, lack of social support, effort, reward, and ERI).

The supplementary analyses included 2 series of analyses using either lnTP or lnCortisol as the dependent variable. The analyses including lnCortisol as the dependent variable did not reveal any significant interactions between gender and psychosocial factors and are not shown. Both series of analyses include all participants, the first including an interaction term, "gender * psychosocial factor", and the other including gender as a factor.

Table III-1. A: Results from analyses including all participants in repeated measures analysis of covariance with an interaction term (gender * psychosocial factor), including gender and year of measurements factors and adjustment for time of measurement, and age, using lnTP as the dependent variable B: Results of the same series of analyses including gender as a factor without the interaction term between gender and the psychosocial factor. Men are used as the reference.

	A			B
	Psychosocial factor	Gender	Interaction term	Psychosocial factor
	Estimate / SE / p	Estimate / SE / p	Estimate / SE / p	Estimate / SE / p
Social status	0.11 / 0.05 / 0.03	0.28 / 0.29 / 0.15	-0.13 / 0.07 / 0.04	(0.03 / 0.03 / 0.32)
Children	- 0.20 / 0.15 / 0.19	-0.15 / 0.10 / 0.15	0.20 / 0.17 / 0.26	0.02 / 0.05 / 0.69
Hours housework	-0.05 / 0.11 / 0.64	-0.27 / 0.26 / 0.29	0.10 / 0.12 / 0.43	0.02 / 0.06 / 0.77
WWH	0.03 / 0.01 / 0.05	0.82 / 0.63 / 0.19	-0.02 / 0.02 7 0.16	0.01 / 0.007 / 0.12
Lack of control	0.001 / 0.004 / 0.75	0.49 / 0.16 / 0.002	-0.02 / 0.01 / 0.003	(-0.01 / 0.002 / 0.00)
Demands	0.001 / 0.003 / 0.70	-0.14 / 0.22 / 0.54	0.001 / 0.004 / 0.79	0.002 / 0.002 / 0.36
Lack of support	-0.01 / 0.003 / 0.07	-0.05 / 0.13 / 0.72	-0.002 / 0.004 / 0.53	-0.006 / 0.002 / 0.003
Effort	-0.01 / 0.01 / 0.43	-0.36 / 0.25 / 0.15	0.02 / 0.02 / 0.22	0.002 / 0.01 / 0.80
Reward	0.39 / 0.01 / 0.001	1.78 / 0.54 / 0.001	-0.05 / 0.01 / 0.001	(0.008 / 0.006 / 0.20)
ERI	-0.37 / 0.27 / 0.08	-0.46 / 0.20 / 0.02	0.56 / 0.27 / 0.04	(-0.03 / 0.13 / 0.84)

The brackets indicate that the result is of less interest as the gender * psychosocial factor term is significant, and therefore the gender-separated results from the paper are more correct.

Paper VI: The paper included gender separated results of a series of simple regression analyses using IMTprogression as the dependent variable and conventional coronary risk factors as well as hormonal measures as the independent variable.

The supplementary analyses included an analysis of interaction between sex and measures of cortisol, which was not present. Furthermore, linear regression analyses including all participants and including sex as a covariate.

Table VI-1: Results in the form of beta-coefficients and p-values from linear regression analyses including age, sex and either cortisol increase during the first 20 minutes in 1998 or change during the first hour in 1998 adjusting for IMTmean1998:

	IMTprogression	IMTprogression
	Beta / p	Beta / p
Age	0.231 / 0.03	0.259 / 0.02
Sex	0.293 / 0.01	0.300 / 0.004
IMTmean 1998	-0.220 / 0.05	-0.218 / 0.06
S1-20min 1998	0.231 / 0.02	
S1-60min		0.155 / 0.125
R2	0.18	0.15

PAPER VII: This paper presented results for the whole population and separately for each sex. IMT progression or IMT max (2002) were used as the dependent variables in a series of linear regression analyses including log-transformed values of TP, LF, HF, LF / HF, and LF / $HF_{difference}$, one at a time.

The supplementary analyses were conducted to reveal any sex * HRV interaction, and to examine the associations between HRV-measures and IMTmax or IMTprogression for all participants including sex as a factor.

Table VII-1. A: Results from analyses including all participants in analysis of covariance with an interaction term (sex * HRV measure), including sex as a factor and adjustment for time of measurement, and age when using IMTmax as the dependent variable. When using IMTprogression as the dependent variable also IMTmean98 was included. Men are used as the reference.

B: Results of the same series of analyses including sex as a factor without the interaction term between sex and the psychosocial factor.

		A			B
IMTmax		HRV-measure	Sex	Interaction term	HRV-measure
		F / p	F / p	F / p	F / p
Test:	logTP	1.30 / 0.26	2.23 / 0.14	1.47 / 0.23	1.46 / 0.23
	logLF	1.59 / 0.21	3.08 / 0.08	2.04 / 0.02	(1.51 / 0.22)
	logHF	3.87 / 0.05	0.78 / 0.38	0.19 / 0.66	5.17 / 0.03
	logLF / HF	4.34 / 0.04	3.59 / 0.06	1.23 / 0.27	3.03 / 0.08
Sleep:	logTP	0.57 / 0.45	0.86 / 0.36	0.58 / 0.45	1.31 / 0.26
	logLF	1.90 / 0.17	0.05 / 0.82	0.00 7 0.99	2.19 / 0.14
	logHF	0.45 / 0.50	0.71 / 0.40	0.27 / 0.60	1.22 / 0.27
	logLF / HF	0.93 / 0.34	0.01 / 0.91	1.20 / 0.28	0.38 / 0.58
LogLF / $HF_{difference}$		8.35 / 0.005	1.28 / 0.26	2.96 / 0.09	6.35 / 0.01
IMTprogression			A		B
Test:	logTP	0.004 / 0.95	1.06 / 0.31	0.59 / 0.45	0.00 / 0.99
	logLF	0.001 / 0.98	1.40 / 0.24	0.75 / 0.39	0.00 / 0.99
	logHF	0.008 / 0.93	0.37 / 0.55	0.05 / 0.82	0.00 /0.99
	logLF / HF	0.12 / 0.73	3.34 / 0.07	1.25 / 0.27	0.004 / 0.95
Sleep:	logTP	0.14 / 0.71	0.08 / 0.75	0.007 / 0.93	0.14 / 0.71
	logLF	0.21 / 0.65	0.21 / 0.65	0.53 / 0.47	0.05 / 0.83
	logHF	0.03 / 0.87	0.08 / 0.90	0.18 / 0.68	0.28 / 0.65
	logLF / HF	1.37 / 0.25	0.04 / 0.84	0.29 / 0.59	1.08 / 0.30
LogLF / $HF_{difference}$		3.37 / 0.07	2.05 / 0.15	2.78 / 0.10	2.01 / 0.16

The brackets indicate that the result is of less interest as the sex * psychosocial factor term is significant, and therefore the gender-separated results from the paper are more correct.

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Psychosocial Factors at Home and at Work and Four-Year Progression in Intima Media Thickness

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This study analyzed the relationship between psychosocial factors and progression in intima media thickness (IMT). In 1998 and 2002, 95 healthy participants underwent a clinical examination, including ultrasound of the arteria carotis communis (ACC). IMT progression in women was 0.033 mm/year (SD = 0.033) and in men 0.048 mm/year (SD = 0.033). For cohabiting women as opposed to single women, the means for total IMT progression over the 4 years were, respectively, 0.137 (SE = 0.019) and 0.016 (SE = 0.048) mm. For women with above average as opposed to below average mean scores of effort, IMT progression were 0.149 (SE = 0.026) and 0.098 (SE = 0.024) mm, respectively. For men without children as opposed to men with children, mean scores for IMT progression were 0.231 (SE = 0.029) and 0.137 (SE = 0.028) mm, respectively. For men above average as opposed to those below average, scores of effort–reward imbalance IMT progression were 0.216 (SE = 0.030) and 0.155 (SE = 0.027) mm, respectively. Adjustment for confounders did not change the results significantly. We found that psychosocial factors were independent significant predictors of IMT progression. The associations were different between the genders.

Key words: intima media thickness progression, work, demand–control model, effort–reward model, gender

Behavioral cardiology has made important progress during recent years (Rozanski, Blumenthal, Davidson, Saab, & Kubzansky, 2005). However, a central problem in the analysis of the connection between stress or other risk factors and ischemic heart disease (IHD) is that clinically manifested IHD (as in the case of acute myocardial infarction [AMI]) is preceded by the silent development of atherosclerosis over a number of years. The development of ultrasound techniques has made it easier to measure a person's degree of atherosclerosis. Because atherosclerosis is a condition that develops universally in the elastic arteries, it is possible to use ultrasound on the arteria carotis communis (ACC) to estimate the person's degree of atherosclerosis (Heiss et al., 1991; J. T. Salonen & Salonen, 1993). The vessel's intima media thickness (IMT) has been shown to be associated with the degree of atherosclerotic changes in the coronary arteries (Burke et al., 1995; Craven et al., 1990; Crouse, Craven, Hagaman, & Bond, 1995; Geroulakos, O'Gorman, Kalodiki, Sheridan, & Nicolaides, 1994; Heiss et al.; J. T. Salonen & Salonen, 1993), with risk factors for IHD (Crouse, et al., 2002; Gnasso et al., 1996; Heiss et al.; Lynch, Kaplan, Salonen, Cohen, & Salonen, 1995; Prati et al., 1992; Rosvall et al., 2002; J. T. Salonen, Seppanen, Rauramaa, & Salonen,1989; R. Salonen & Salonen, 1991), and with incidence of future IHD (Hodis et al.,1998; J. T. Salonen & Salonen, 1993).

The progression in IMT might give a more precise estimate of the importance of a given risk factor during a certain period than the IMT itself (Crouse, 2001). As with the connection between manifest IHD and IMT, a connection has been shown between progression in IMT and the risk factors for IHD (Agewall, Wikstrand, Dalö, & Fagerberg, 1996; Barnett, Spencec, Manuck, & Jennings, 1997; Everson, Kaplan, Goldberg, Salonen, & Salonen, 1997; Lynch, Kaplan, Salonen, & Salonen, 1997; Lynch, Krause, Kaplan, Salonen, & Salonen, 1997), as well as between progression in IMT and the incidence of IHD (Crouse, 2001; Hodis et al., 1998; Terry et al., 2000).

Research into the significance of psychosocial work environment factors has largely been based on Karasek's demand–control model, which was introduced in 1979 (Karasek, Russell, & Theorell, 1982; Karasek et al., 1988). According to this model, people with a job situation characterized as highly demanding and who have little control over their work situation are at particular risk of developing stress-related illnesses. This particular working condition is called *strain*. In this stress model, the feeling of having social support is a modifying factor (Johnson & Hall, 1988). The worst

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possible working condition is, therefore, one with strain and with no feeling of social support.

In the early 1990s, Siegrist introduced a new stress model called the effort–reward imbalance model, which has attracted quite a lot of interest (Siegrist, 1996). In this model, people who experience imbalance between effort and reward at work are at risk for stress-related disease. Furthermore, if a person has a behavior pattern or personality characterized as *overcommitted*, he or she will have a particularly high risk of developing a stress-related illness (Peter & Siegrist, 2000; Siegrist, 1996; Siegrist & Peter, 1996; Siegrist, Peter, Junge, Cremer, & Seidel, 1990).

Both models have been confirmed in studies based on different designs and have shown to be independent predictors of IHD (Bosma, Peter, Siegrist, & Marmot, 1998). However, research has often been based on cross-sectional analyses. Furthermore, only very few studies have included both the demand–control model and the effort–reward imbalance model (Kivimaki et al., 2002; Peter, Siegrist, Hallqvist, Reuterwall, & Theorell, 2002).

The aim of the present study was to analyze the connections between progression in IMT over a period of 4 years and some psychosocial factors at home and the two previously mentioned stress models.

Method and Participants

In 1998, 130 participants were enrolled in a prospective study of IMT and stress, including the significance of conventional coronary risk factors (Eller & Netterstrom, 2001) and measurement of the physiological stress response (Eller, Netterstrom, & Hansen, 2001; Eller, Malmberg, & Bruhn, 2005; Eller, Netterstrom, & Allerup, 2005). All the participants were in good health, were employed, and had volunteered in response to a public notification. In 2002, 95 of the participants agreed to be part of a second study. This group consisted of 63 women and 32 men. However, 1 man did not fill out the questionnaire about psychosocial factors at work and was excluded. The average follow-up time was 4 years (range = 207–224 weeks). The study was approved by the local scientific ethical committee.

The Effect Measurement of IMT Progression

The effect measurement of the study was the progression in IMT from 1998 to 2002. There are no generally agreed guidelines for measuring IMT (Aminbakhsh & Mancini, 1999; Simon, Gariepy, Chironi, Megnien, & Levenson, 2002). In this study, IMT was measured as follows: The participant was placed in a supine position, with the head turned slightly to the side that was not being examined. In this position, at the point where the transition between the ACC and the bulbus was defined, the IMT was measured at a right angle to a tangent in the longitudinal direction of the artery. Six measurements were taken, three on the right and three on the left (Eller & Netterstrom, 2001). Each measurement was taken at the point where the transition between the ACC and the bulbus was defined, although, after each measurement, the participant was allowed to change position, that is, the measurements were taken from different angles. In each measurement, the maximum thickness was quantified.

In 1998, ultrasound examinations were recorded on video. These video recordings were studied in 2002, and the measurements were repeated to ensure that they were carried out according to exactly the same criteria. In 2002, measurements were carried out during the examination. The same person performed all examinations and measurements. All measurements were performed excluding reference to all independent variables, and in 2002, excluding reference to the 1998 measurements.

Subsequently, averages were calculated for the six measurements from 1998 (IMT98) and from 2002 (IMT02), respectively. IMT progression was defined as the difference between these two averages (IMT02 – IMT98). Thus, the IMT progression reported is the millimeters of progression in IMT over 4 years.

Measurement of Exposure

Psychosocial factors at home. We included several home-related psychosocial factors in the study: Social Status, Cohabitation, Children, Housework, and Time Pressure.

Social Status was coded as follows: 1 (*academic or manager of more than 50 people*), 2 (*theoretical education of 3–4 years' duration or manager of up to 50 people*), 3 (*theoretical education of 1–2 years' duration or manager of up to 10 people*), 4 (*skilled labor*), and 5 (*unskilled labor*; Netterstrøm, Nielsen, Kristensen, Back, & Møller, 1999).

Each participant's Cohabitation was coded as follows: *no* indicated the participant was not cohabiting with another adult, and *yes* indicated that the participant was cohabiting with another adult.

We quantified the Children factor simply as the number of children in the home.

To establish to what extent each participant was responsible for running the home and looking after the children—the Housework factor—we asked the participants to indicate the time spent every week cleaning, shopping, cooking, looking after children, or caring for other adults. The options were 1 (*less than 10 hr/week*), 2 (*10 to 20 hr/week*), or 3 (*more than 20 hr/week*).

Time Pressure—the feeling of being busy and under pressure in one's daily life—was assessed using five statements about stress in connection with family-related tasks (shopping, taking children to nursery school or picking them up, being together with family and friends). If the statement was not relevant, we coded it as 0. Otherwise, participants had to state how often they felt time pressure in the specific situation; we scored their answers as follows: 1 (*never*), 2 (*seldom*), 3 (*sometimes*), 4 (*often*), or 5 (*always*). The five scores were put together, so that those with no or little time pressure received a low score (0–5), whereas those experiencing greater and intensive time pressure in all the previously mentioned situations received the maximum score (25).

Psychosocial factors at work. Weekly Working Hours (WWH) referred to the number of working hours participants engaged in per week. In Denmark, the typical work week is 37 hr. We asked participants to state how many hours they actually worked, including work at home in the evenings or on weekends preparing for meetings and so forth.

We analyzed Psychosocial Work Environment using the two previously mentioned stress models, for which we asked participants identical questions in 1998 and 2002.

For the demand–control model, we used a series of questions, the majority of which came from the Whitehall study, translated and validated for use in the Copenhagen City Heart Study (Netterstrøm, Kristensen, Damsgaard, Olsen, & Sjol, 1991). Of the items, 12 evaluated the degree of control (Chronbach's $\alpha = .71$), only 2 evaluated demands (Chronbach's $\alpha = .40$), and 4 evaluated the degree of support (Chronbach's $\alpha = .81$). The scale for each dimension ranged from 0 to 100 with a higher score indicating a higher degree of stress caused by the respective dimension. We used the questionnaire developed by Siegrist et al. (2004)—with their approval—to study the participants' degree of effort, reward, and overcommitment. Only 5 items assessed the effort dimension (Chronbach's $\alpha = .77$), as we omitted the question regarding physical demands at work. Only 10 items evaluated the reward dimension (Chronbach's $\alpha = .72$), as we had to omit the question regarding respect from a superior due to errors in the questionnaire. In this way, we calculated the effort–reward imbalance ratio as effort/reward $\times$ (5/10). Originally, effort–reward imbalance was considered a risk factor only if it was less than 1. However, it has been found that effort–reward imbalance can also be used as a continuous variable (Pikhart et al., 2001). We used this latter method in the present study. Six items evaluated the degree of overcommitment (Chronbach's $\alpha = .82$).

Confounders. We used baseline levels of the following as possible confounders: blood pressure; waist–hip ratio (WHR); body mass index (BMI); fibrinogen; cholesterol; high density lipoprotein (HDL) cholesterol; tobacco use (g/day); alcohol intake (drinks/week); and degree of physical activity, rated as 1 (*passive or almost passive*), 2 (*light physical activity for 2–4 hr/week*), 3 (*more intensive physical activity for more than 4 hr/week*), or 4 (*heavy physical activity for more than 4 hr/week*).

Strategy for the Analysis

The data was processed using SPSS version 11.0. All analyses were performed separately for the two genders. This method was chosen because it is a well known fact that the onset of manifest IHD comes several years later for women than it does for men. Furthermore, both the physiological stress response and the perception of the stress test might be different in the two genders (Rhodes & Rubin, 1999; Sandanger, Nygard, Sorensen, & Mourn, 2004; Ursin & Eriksen, 2004).

A one-way analysis of variance (ANOVA) test was used to evaluate gender difference in IMT progression.

The scores of the demand–control model and the ERI model from 1998 and 2002 were averaged. In the case of missing values, the average was calculated taking this into consideration, to keep the sample intact. Baseline levels of all other variables (psychosocial factors at home, physiological measures, and behavioral measures) were used as independent variables. Those psychosocial variables that were significant for IMT progression were dichotomized at mean. Subsequently, the IMT progression in women and men experiencing above-average scores as opposed to below-average scores in the psychosocial factors significant for IMT progression was estimated by the use of general linear models (GLM) (univariate analyses) with adjustment for the other variables.

Results

Participants

The participants were between 34 and 63 years of age at the time of the follow-up study. The physiological characteristics of the participants are presented in Table 1. Those participants from 1998 who did not take part in the 2002 follow-up study (the nonparticipants) were significantly younger and had a better coronary risk profile (blood pressure, BMI, WHR, cholesterol, HDL cholesterol, fibrinogen, tobacco) than did the participants. The differences in the coronary risk factors between the participants and nonparticipants were not significant, apart from the degree of smoking among

Table 1. *Characteristics of the Study Participants*

	Women[a]			Men[b]		
	%	*M*	*SD*	%	*M*	*SD*
Social status						
1: Academic	7.9			31.3		
2: 3–4 years of education	46.0			15.6		
3: 1–2 years of education	25.4			6.3		
4: Skilled worker	14.3			46.9		
5: Unskilled worker	6.3			0		
Children at home: Yes	54.0			51.7		
Cohabitant: Yes	85.7			83.4		
Housework: Less than 20 hr/week	19.0			16.1		
Smoker: Yes	25.3			25.0		
Physical activity: 2–4 hr/week	62.9			40.6		
Time pressure score		9.1	4.2		7.1	3.8
Weekly working hrs		35.1	5.1		38.8	5.9
Control score		37	14		30	17
Demands score		50	17		51	15
Support score		22	13		26	16
Effort score		11	2		11	3
Reward score		41	4		41	4
Effort– reward imbalance		0.57	0.15		0.58	0.17
Overcommitment score		14	3		14	3
Age (years)		46	7		47	8
IMT98 (mm)		0.748	0.120		0.846	0.207
BMI (kg/m^2)		24.1	3.1		26.1	2.6
WHR		0.79	0.06		0.90	0.05
Fibrinogen (micromol/l)		8.11	1.54		8.70	2.02
Cholesterol (mmol/l)		5.5	1.1		5.0	1.1
HDL cholesterol (mmol/l)		1.65	0.46		1.38	0.42
Systolic blood pressure (mmHg)		126	11		135	11
Alcohol (drinks/week)		4	4		9	10

Note. IMT98 = average of six measurements of intima media thickness taken in 1998; BMI = body mass index; WHR = waist–hip ratio; HDL = high density lipoprotein.
[a]*N*=63. [b]*N*=31.

the men: Nonparticipants smoked significantly less than did the participants.

For the women, there were no differences in relation to the two stress models. For the men, there was no difference between the participants and the nonparticipants with regard to the effort–reward imbalance model, but in 1998 the nonparticipating men had reported greater demands than did the participants (t(x) = y, $p = 0.09$).

Of the 94 participants in the follow-up study, 2 women and 3 men were receiving medical treatment for hypertension or heart conditions in 1998. In addition, 4 women and 4 men had started treatment with antihypertensive medicine at the time of the follow-up study in 2002, and 2 men had suffered an AMI between 1998 and 2002. A total of 6 women and 6 men were suffering from hypertension or IHD in 2002. No one was on lipid-lowering medication.

Among the women, 90% were working, and 97% of these were employed within the health sector, primarily as secretaries, midwives, physiotherapists, or nurses. Nearly 86% were cohabiting. Sixteen women (25%) were menopausal.

The employment rate among the men was 84%, and 41% of these were employed in the health sector as doctors, nurses, or technical personnel. Other professions among the men were teachers, policemen, engineers, and salesmen. Of the men, 78% were cohabiting.

For the demand dimension, the mean score was approximately 50, whereas the control and support dimensions were asymmetrical. The mean values for control and support were, respectively, 37 and 22 for women and 30 and 26 for men. This shows that the population experienced a high level of subjective control and social support at work. Furthermore, there were only 3 women and no men in the strain group (high demands, low control).

During the 4 years, the 63 women had an average IMT progression of 0.121 mm (SE = .017) and the 31 men had an average IMT progression of 0.180 mm (SE = .024) after adjustment for age. The difference between the two genders was significant, $F(x, y) = 4.35$, p = .04. For women, the annual IMT progression was 0.033 (SD = .033) mm/year and for men, 0.048 (SD = .033) mm/year.

Table 2 shows beta coefficients and *p* values from the simple linear regression analysis with IMT progression as the dependent variable and each of the psychosocial factors at home and at work and the remaining variables as the independent variable one at a time.

Among the women, demands, effort, reward, and overcommitment were significantly associated with IMT progression. However, higher reward was associated with higher IMT progression, the opposite direction of association to what was expected. Furthermore, cohabitation was associated with IMT progression, that is, the women living alone had lower IMT progression than did those who were cohabiting.

Among the men, reward (negatively), effort–reward imbalance, children (negatively), and age were significantly associated with IMT progression.

Results of the multiple regression analyses are shown in Table 3 (women) and Table 4 (men). For the women, the stepwise regression analysis included demands, effort, reward, and overcommitment (Model 1). The result of this analysis was that only overcommitment was included in the final model (R^2 = 12.0, β = .346, and $p < .01$). In Model 2, cohabitation was included along with all variables from Model 1. In this case, overcommitment (β = .399, $p < 0.01$) and cohabitation (β = –.353, $p < .01$) were included in the final model (R^2 = 24.5). Age was then introduced (Model 3), although the result did not change. Although overcommitment describes coping style and personality, this dimension was excluded from the last model (Model 4). The final model (R^2 = 19.7) subsequently included effort (β = .337, $p < .01$) and cohabitation (β = .348, $p < .01$).

Reward and effort–reward imbalance are strongly correlated. Therefore, among the men, the first step included only effort–reward imbalance (Model 1). Model 2 included children (β = – .449, $p < .01$) and effort–reward imbalance (β = .498, $p < .01$). The last model (Model 3) included age and systolic blood pressure as well as children and effort–reward imbalance. The final model (R^2 = 57.3) included only age (β = .297, p = .05), children (β = –.338, p = .03) and effort–reward imbalance (β = .509, $p < .01$).

For cohabiting women versus single women, the means for IMT progression were .137 (*SE* =.019) and .016 (*SE* = .048) mm, respectively. For women with above average as opposed to below average mean scores of effort, IMT progression were .149 (*SE* = .026) and .098 (*SE* = .024) mm, respectively. For men without children as opposed to men with children, IMT progression was .231 (*SE* = .029) compared to .137 (*SE* = .028) mm. For men with above average as opposed to below average scores of effort–reward imbalance, IMT progression were .216 (*SE* = .030) and .155 (*SE* = .027) mm, respectively. Mean IMT, progression in men with children but low effort–reward imbalance (n = 8) was .120 (*SE* = .038) mm compared to a mean IMT pro-

Table 2. *Results of Simple Linear Regression Analyses Using Each of the Psychosocial Factors As Independent Variables One at a Time and IMT Progression as Dependent Variable*

	Women[a]			Men[b]		
	R^2	β	p	R^2	β	p
Social status	0.9	.097	.46	7.9	.280	.13
Cohabitation (no/yes)	8.6	.293	.02	3.9	–.198	.29
Children	0.4	.064	.62	33.6	–.580	<.01
Housework hours	0.0	.008	.95	9.1	–.302	.10
Time pressure	3.9	.197	.17	8.2	–.287	.12
WWH	0.2	–.041	.77	0.2	–.048	.80
Control	1.2	–.110	.40	1.2	–.108	.56
Demands	7.0	.264	.04	0.5	–.069	.71
Support	0.1	.031	.81	2.3	.152	.41
Effort	7.8	.280	.03	3.1	.177	.34
Reward	6.4	.253	.05	12.5	–.353	.05
Effort–reward imbalance	2.3	.150	.25	29.7	.545	< .01
Overcommitment	12.0	.346	< .01	1.6	.127	.50
Age	0.0	–.004	.97	20.5	.453	.01
BMI	0.0	–.020	.88	0.6	–.78	.68
WHR	0.0	.84	.52	0.1	.100	.59
Fibrinogen	2.0	–.146	.26	1.4	.120	.53
Cholesterol	2.5	.158	.23	3.8	.194	.30
HDL cholesterol	0.6	.079	.55	0.0	–.006	.98
Systolic blood pressure	0.4	.067	.61	11.0	.331	.09
Alcohol	3.1	–.176	.17	0.1	.026	.89
Smoking	1.5	–.124	.34	0.4	–.063	.74
Physical activity	2.7	.123	.21	1.0	–.102	.58

Note. WWH = weekly working hours; BMI = body mass index; WHR = waist–hip ration; HDL = high density lipoprotein.
[a]N = 63. [b]N = 31.

Table 3. *Results of Enter Models and Stepwise Regression Analyses for Women Using IMT Progression as the Dependent Variable*

	Enter Model		Final Model		
	β	*p*	R^2	β	*p*
Model 1			12.0		
Demands	.065	.75			
Effort	.007	.98			
Reward	.074	.66			
Overcommitment	.257	.18		.346	< .01
Model 2			24.5		
Cohabitation	-.366	<.01		–.353	< .01
Demands	–.067	.74			
Effort	.130	.56			
Reward	.063	.69			
Overcommitment	.322	.08		.399	< .01
Model 3			24.5		
Age	.143	.30			
Cohabitation	.402	< .01		.353	< .01
Demands	–.041	.84			
Effort	.120	.59			
Reward	.135	.43			
Overcommitment	.277	.14		.399	< .01
Model 4			19.7		
Age	.191	.16			
Cohabitation	.398	< .01		.348	< .01
Demands	.047	.81			
Effort	.196	.37		.337	< .01
Reward	.235	.16			

Note. $N = 63$.

Table 4. *Results of Enter Models and Stepwise Regression Analyses for Men Using IMT Progression as the Dependent Variable*

	Enter model			Final model	
	β	*p*	R^2	β	*p*
Model 1			29.7		
Effort-reward imbalance	.545	< .01		.545	< .01
Model 2			49.7		
Children	–.449	< .01		–.449	< .01
Effort-reward imbalance	.498	< .01		.498	< .01
Model 3			57.3		
Age	.282	.08		.297	.05
Systolic blood pressure	.040	.80			
Children	–.343	.03		–.338	.03
Effort-reward imbalance	.498	< .01		.509	< .01

Note. $N = 31$.

gression of .304 (*SE* = .042) mm in men without children and high effort–reward imbalance ($n = 6$). Adjustment for confounders did not change the results significantly.

Discussion

The study showed that men had a significantly higher IMT progression than did women, and that the significance of the independent variables of IMT progression was different for the two genders. Among women, overcommitment, effort, and cohabitation proved to be of significant importance to IMT progression, whereas effort–reward imbalance, children, and age were significant for the IMT progression among men.

For women, the annual IMT progression was .033 (*SD* = .033) mm/year and for men, .048 (*SD* = .033) mm/year. This progression is equivalent to that found in other studies (Aminbakhsh & Mancini, 1999; Chambless et al., 2002; Fujii et al., 2003; Lakka, Lakka, Salonen, Kaplan, & Salonen, 2001; Nordstrom, Dwyer, Merz, Shircore, & Dwyer, 2003; Sander, Kukla, Klingenhöfer, Winbeck, & Conrad, 2000), when one takes the methodological differences into account. As the initial ages for men and women to have clinically manifested IHD are staggered by several years, that is, men experience IHD before women do, we had expected that the women's IMT progression would be lower than that of the men. By comparing different study results, Aminbakhsh and Mancini (1999) found that a progression of 0.03 mm/year is associated with increased risk of AMI. Among the 6 men suffering from either IHD or hypertension, the average IMT progression was 0.058 mm/year, which seems to confirm this finding.

Based on the extensive literature on the importance of various conventional coronary risk factors for the development of atherosclerosis and IHD, it is remarkable that the conventional coronary risk factors appear to play such a limited role in connection with IMT progression in this study. It should be noted that, with respect to the conventional coronary risk factors, the participants had predominantly normal values, and that there was very little variation within the material. With a small variation in exposure, it is difficult to show the significance of a risk factor, and it would, in this case, have required a larger population.

The importance of the psychosocial factors for progression of IMT has been shown in several studies. Part of the literature deals with individual psychological conditions (Agewall et al., 1996; Barnett, Spence, Manuck, & Jennings, 1997; Julkunen, Salonen, Kaplan, Chesney, & Salonen, 1994; Paterniti et al., 2001) and social status (Lynch, Kaplan, et al., 1997), but the attempt has also been made to show a connection between work environment factors and IMT. A large-scale Swedish cross-sectional study of middle-aged people could not show any difference between IMT among 1,108 men, but showed a difference in IMT among women in the relaxed group versus those in the active and strain groups, insofar as the relaxed group (n = 320) had an IMT that was .15 mm thinner than that of the strain group (n = 400) and .10 mm thinner than that of the active group (n = 339; Rosvall et al., 2002). The few associations in a cross-sectional study point toward the use of prospective studies. In a large Finnish population study, Lynch, Krause, et al. (1997) found that progression in IMT was significantly associated with high demands and low financial reward. The progression was greatest in the subgroup of men with advanced atherosclerosis at baseline. In the same population it was also shown that cardiovascular reactivity and demands at work were interrelated in such a way that high cardiovascular reactivity in the form of higher blood pressure, increase in physical activity, and high demands resulted in the greatest IMT progression (Everson, Lynch, et al., 1997).

In the present study, an association was found between psychosocial factors at home and at work and IMT progression. The effort–reward model proved to be of importance to IMT progression in both women and men. The gender-specific associations, in which the IMT progression in women was dependent on overcommitment and in men on effort–reward imbalance, are in line with the study by Peter et al. (2002) dealing with AMI and work stress. However, when overcommitment was kept out of the analysis, effort was also of significance to IMT progression in women.

The reason that the demand–control model did not show any association with IMT progression in men might be that the exposure generated by this model was minor and the variation small. However, for the women, demands showed significant association with IMT progression. Also, demands were correlated with effort in a bivariate correlation analysis.

The study has some important limitations. First of all, the study includes only a small population, of which especially the women have only limited IMT progression in 4 years, due to their age and gender. A study of early nonsymptomatic atherosclerosis should include a larger population. Furthermore, the variation in psychosocial exposure was limited, especially with respect to the demand–control model. These results should not, therefore, be interpreted to mean that the demand–control model does not describe a stressing work environment. This may also mean that the findings underestimate the significance of the psychosocial factors. The limited variation in the confounders may also explain why conventional risk factors did not seem to have an impact in this study.

Despite these flaws, the findings could be seen as an indication that psychosocial factors have a causal effect on IMT progression. Finally, the findings indicate that the questionnaires used in the study probably reveal only part of the strain on the participants. The protection effect on the men of having children at home was remarkable. Future research should look into gender differences in psychosocial strain at home as well as at work, as gender is probably of great importance with respect to the interpretation of psychosocial factors.

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PAPER II

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Psychosocial factors at home and at work and levels of salivary cortisol

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Abstract

Background: Salivary cortisol as a physiological measure of stress has attracted great interest in recent years.
Method: A 55 women and 28 men, all healthy volunteers, were included in a study on psychosocial factors at work and at home and salivary cortisol. General linear models, univariate and repeated measures, respectively, were used to evaluate the associations between psychosocial factors and cortisol excretion measured six times during a working day. Age, physical activity, tobacco use and the time of the first saliva sample were used as covariates.
Results: In the women, high degrees of time pressure, effort and effort reward imbalance were significantly associated with higher levels of cortisol. In the men, high degrees of effort, effort reward imbalance and overcommitment were significantly associated with higher levels of cortisol.
Conclusion: Psychosocial factors are of significance to the level of salivary cortisol. The study emphasises the benefits of taking physiological measurements of stress in epidemiological studies.

Keywords: Awakening cortisol response; Salivary cortisol; Psychosocial factors; Work stress; The demand control model; The effort reward imbalance model

1. Introduction

Cortisol in saliva deteriorates very slowly, even at room temperature, and therefore this method enables repeated cortisol measures during the day at the work place and makes the method an obvious choice for measuring a part of the physiological stress reaction (Kirschbaum and Hellhammer, 1994).

Since the introduction of the method, researchers have used a number of different strategies for measuring and analysing (Hjortskov et al., 2004). The awakening cortisol response (ACR) has been defined as the increase in saliva cortisol during the first 30 min after awakening (Clow et al., 2004). ACR shows a high degree of intra-individual stability when measured for several days or weeks (Pruessner et al., 1997) and therefore it can presumably be used to measure the level of psychological strain in daily life. Increased ACR has been found associated with perceived stress (Pruessner et al., 1997, 1999; Steptoe et al., 2000), whereas low ACR was correlated with burn-out (Pruessner et al., 1999). The rise in cortisol excretion in the morning may reflect anticipatory stress, which has been found to be an important determinant of cortisol excretion in relation to a stress test (Gaab et al., 2005). The decline of cortisol excretion throughout the day might as well be related to the strains which the individual encounters, while stressors during the day have been found to be associated with higher salivary cortisol (Smyth et al., 1998). Further, a steep decline was associated with positive maternal relationship (Adam and Gunnar, 2001).

ACR or other measurements of increased cortisol excretion in the morning were found to be associated with several aspects of health. Kudielka and Kirschbaum (2003) found an association between health complaints and ACR. Brooke-Wavell et al. (2002) showed an association between ACR and bone density, and Eller et al. (2001, 2005) demonstrated that the cortisol rise in the morning and the intima media thickness in arteria carotis communis were related. Based on the available literature, Clow concluded in 2004 that ACR is a "discreet and dynamic part of the circadian cortisol secretory cycle. Its main role and importance for health has yet to be fully elucidated, however early evidence suggests that it may be physiologically significant and be affected by stress" (Clow et al., 2004).

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Salivary cortisol has not yet been used as a measure of strain due to family life and only few epidemiological studies evaluate levels of salivary cortisol, ACR and psychosocial factors. In women who worked overtime, morning salivary cortisol was significantly increased (Lundberg and Hellström, 2002). Based on the Whitehall study, Kunz-Ebrecht has reported gender differences in ACR on working days, but not on days off. Women had greater ACR than men on working days, whereas ACR in the two sexes was similar on days off (Kunz-Ebrecht et al., 2004a,b). In addition, lower social class was associated with greater ACR on working days and on days off. Similarly, ACR was positively correlated with job demands, but the association was less pronounced in the higher social classes. Considering the demand control model (Karasek et al., 1981), job control was not correlated with ACR (Kunz-Ebrecht et al., 2004b). Steptoe et al. have evaluated the effort reward model (Siegrist, 1996) and associations to ACR in data from Whitehall. The authors found that over-commitment was associated with ACR in men, but not in women. Contrary to this, effort and reward were not associated with ACR (Steptoe et al., 2004).

It is characteristic of the above-mentioned studies, that they did not use concurrent data on all aspects of the subjective psychosocial strain at work, and at home. Therefore, the relative importance of the different factors associated with cortisol excretion cannot be estimated. At the same time, it is becoming increasingly apparent that stress research cannot concentrate solely on working conditions. Furthermore, though ACR may be the relevant part of the diurnal cortisol excretion to focus on, there is still the question of whether changes in cortisol excretion due solely to psychosocial factors are seen in this part of the 24 h.

The following is a description of results regarding levels of salivary cortisol on a working day, seen in relation to a number of psychosocial conditions at home and at work. The study had four hypotheses:

1. Participants of lower social class will have higher cortisol excretion than participants of higher social classes, as found by Kunz-Ebrecht et al. (2004a).
2. Participants with an increased degree of pressure in daily life, measured as hours spent on housework, and as a feeling of time pressure in connection with daily activities, will have higher cortisol excretion than others.
3. Cortisol excretion will be positively associated with a number of weekly working hours.
4. Participants who perceive greater subjective strain at work will have higher cortisol excretion than others. The demand control model and the effort reward imbalance model were used to determine subjective levels of workload as Kunz-Ebrecht et al. (2004b) and Steptoe et al. (2004) have evaluated these stress models earlier in connection with ACR.

2. Methods

2.1. Participants

A total of 95 participants (63 women and 32 men) agreed to be part of a study in 2002 on occupational health and salivary cortisol. All participants were in good health, had employment and volunteered, in response to a public notification, to participate in a follow-up study started in 1998 on intima media thickness and cortisol (Eller et al., 2001, 2005; Eller and Netterstrom, 2001). The local scientific ethical committee approved the study. All participants gave informed consent before the examination.

Among the participants, four women had to be excluded because they did not deliver saliva samples. The reasons for this were problems with taking the samples, i.e. nausea when chewing the cotton, forgetfulness and lack of time. Eight saliva samples (four from women and four from men) could not be analysed, due to too small quantities of saliva sampled. Thus, complete data on salivary cortisol was available from 55 women and 28 men. There were not significant differences in the independent variables between those who delivered data on all saliva samples and those who did not. The study took place between mid October 2001 and late January 2002. At this time of year, it is dark in the mornings, which means that the participants had to switch on the light when performing the tests.

The participants were given written and verbal instructions on how to perform the saliva tests, although it is understood that this is not sufficient to ensure correct testing (Broderick et al., 2004; Kudielka et al., 2003) the problem of non-compliance among the participants could not be dealt with in this study design. A possibility was to exclude those participants with a fall in cortisol level during the first 30 min, which could imply a lack of compliance. However, we included all participants, in order not to exclude true non-responders. Table 1 presents the characteristics of the participants.

2.2. Cortisol

The necessary equipment for the saliva samples (six Salivette® saliva collection tubes, polyester tampons) was sent to the participants, with instructions telling the participants to fast, to refrain from smoking and not to brush their teeth before taking the samples. Furthermore, the participants were instructed to take the first sample immediately after awakening, while they were still in bed. Further samples were to be taken 20, 30 and 60 min after awakening, after 8 h and at 18:00 p.m. The participants were instructed to write down the time at which each sample was taken, to ensure that the samples were taken at the correct times.

Table 1
Characteristics of the participants

	Women ($N = 55$)		Men ($N = 28$)	
	Mean	S.D.	Mean	S.D.
Age (years)	46.7	7.1	49.4	8.1
Awakening time	6:08	0:41	6:29	1:14
Smoking, yes (%)	11.0		14.3	
Social status (%)				
Academic	10.2		33.3	
Higher non-manual education	42.8		16.7	
Lower non-manual education	24.5		8.3	
Skilled	16.3		41.7	
Unskilled	6.1		0	
Cohabitant, yes (%)	85.7		83.4	
Children at home, yes (%)	55.1		45.8	
Hours house work >20	20.0		14.3	
Time pressure, score	9.1	4.2	7.1*	3.8
Weekly working hours	35.1	5.1	38.8*	5.9
Control, score	37	14	30*	17
Demands, score	50	17	51	15
Support, score	22	13	26*	16
Effort, score	11	2	11	3
Reward, score	41	4	41	4
Effort reward imbalance	0.57	0.15	0.58	0.17
Over-commitment, score	14	3	14	3

An asterisk indicates that the difference between the two genders is significant in a Student's t-test.

2.2.1. Method performance of measurement of cortisol in saliva

The assay used for the determination of cortisol in saliva was a competitive RIA (Spectria Cortisol Coated Tube RIA) purchased from Orion Diagnostica, Espoo, Finland. The analysis was carried out according to the manufacturer's instructions. Cross-reactivity to cortisone was <0.2%. A 1470 Wizard gamma counter (Wallac, Turku, Finland) was used for measuring radioactivity. A method evaluation of certified reference material in water performed by our laboratory showed no bias in the method, i.e. recovery was 97% [CI: 94%; 100.9%]. The limit of detection (LOD) was 1.59 nmol/l. Between run co-efficient of variations were 19% at 11.5 nmol/l and 16% at 49.2 nmol/l (Hansen et al., 2003).

2.3. Psychosocial factors at home

Social status was coded as follows: 1 = academic or manager of more than 50 people, 2 = higher non-manual education or manager of up to 50 people, 3 = lower non-manual education or manager of up to 10 persons, 4 = skilled labour, 5 = unskilled labour (Netterstrøm et al., 1999). The variable was dichotomised into *high social status* (status group 1 and 2) and *low social status* (status group 3–5).

2.3.1. Cohabitation

The participant's cohabitation was coded as follows: yes = cohabiting with other adults, or no = not cohabiting with other adults.

2.3.2. Children in the home

The number of children in the home. The variable was dichotomised into children yes/no.

2.3.3. Housework

In order to establish the degree to which the participant was responsible for running the house and looking after the children, the participants were asked to indicate the time spent every week cleaning, shopping, cooking, and looking after children or other adults. The options were: 1 = <10 h/week, 2 = 10–20 h/week, 3 = >20 h/week. The variable was dichotomised into housework less or more than 20 h/week.

2.3.4. Time pressure

The feeling of being busy and under pressure in one's daily life was highlighted by five statements about stress, in connection with family related tasks (shopping, taking children to nursery school or picking them up, being together with family and friends). If the statement was not relevant, code 0 was used. Otherwise, the participant had to quote how often he/she felt time pressure in the specific situation: never = 1, seldom = 2, now and then = 3, often = 4 and always = 5. The five scores were amalgamated, so that no or little time pressure received a low score (0–5) and intensive time pressure in the entire above-mentioned situations received maximum score (25). The variable was dichotomised at mean.

2.3.5. Weekly working hours (WWH)

The number of working hours per week. In Denmark the normal working hours are 37 h/week. The participants were asked to state how many hours they actually worked, including work at home in the evenings or weekends preparing for meetings, etc. The variable was dichotomised in 1 = WWH less or equal to 37 h/week and 2 = WWH more than 37 h/week.

2.3.6. Physical activity

The participants were asked to estimate their degree of physical activity rated as 1 = passive or almost passive, 2 = light physical activity for 2–4 h/week, 3 = more intensive physical activity for more than 4 h/week, and 4 = heavy physical activity for more than 4 h/week.

2.3.7. Tobacco

Use of tobacco in g/day.

2.4. Psychosocial work environment

The demand control model was analysed through questions, the majority of which came from the Whitehall study, translated and validated for use in the Copenhagen City Heart Study (Netterstrøm et al., 1998). Twelve items were used to evaluate the degree of control. The items were statements or questions to which the participant should indicate how often the statement was true (often, occasionally, seldom, never). A statement could be: "Others make decisions about my work" or "Do you have influence on how you work?" Only two items evaluated demands ("Is it necessary to work fast?" and "Do you have time enough to do your work?"), and four items evaluated degree of support ("How often do you get support from your superiors (/colleagues)?" and "How often are your superiors (/colleagues) willing to listen to your problems?"). The scale for each dimension ranged from 0 to 100, with a higher score indicating a higher degree of stress caused by the respective dimension. Control, demands and support were dichotomised at mean.

2.4.1. The effort reward imbalance model

The questionnaire developed by Siegrist et al. was used – with their approval – to study the participants' degree of effort, reward and over-commitment. Five out of six items were used for the effort dimension, as the question regarding physical demands at work was omitted. The reward dimension was evaluated by 10 out of the 11 original items, while the question regarding respect from a superior had to be left out due to errors in the questionnaire. In this way, the effort reward imbalance ratio (ERI) was calculated as effort/(reward × (5/10)). Originally, ERI was only considered a risk factor if it was >1. However, it has been found that ERI can also be used as a continuous variable (Pikhart et al., 2001). This was done in this study. Six items evaluated the degree of over-commitment. Effort, reward, ERI and overcommitment were dichotomised at mean.

3. Analytical strategy

The statistical analysis was carried out by the use of SPSS, Version 13. *p*-Values below 0.05 were considered statistically significant. As gender differences were expected in connection with the psychosocial factors (Eriksen and Ursin, 2004; Sandanger et al., 2004), all analyses were carried out separately for each gender, despite the fact that salivary cortisol was unrelated to gender (Hansen et al., 2003) and menstrual cycle phase has been shown not to affect the awakening cortisol rise (Kudielka and Kirschbaum, 2003). Initially, analyses were conducted to evaluate the reliability of the scales used in the study, as expressed as the cronbach's alpha. Differences in the psychosocial variables between the two genders were evaluated with Student's *t*-test.

The psychosocial factors were dichotomised in order to present the results as levels of cortisol in the group with psychosocial factor score above the mean compared to scores below the mean. Differences in levels of cortisol between the group with below mean scores and the group with above mean scores were also evaluated by the use of Student's *t*-test. However, in the more advanced analyses continuous variables were used.

Concentration of cortisol was analysed as the dependent variable in GLM, univariate and repeated measures methods. In all analyses age, physical activity, tobacco and time of the first sample were used as covariates. GLM, univariate was used to evaluate the associations of saliva cortisol at awakening and the psychosocial factors; In other words, salivary cortisol at awakening was used as the dependent variable and each of the psychosocial factors as the independent variable, one at a time. GLM, repeated measures was used to evaluate associations of two repeated measures and the psychosocial factors. At the first repeated measure, ACR had two levels: S1 and S3. The second repeated measure was cortisol levels throughout the day, i.e. all

Table 2
Cortisol excretion at awakening, and the cortisol rise in the morning in women and men shown for each dichotomised psychosocial factor (raw values, mean (S.D.) nmol/l)

	Women							Men						
	N	Awakening level			Cortisol rise			*N*	Awakening level			Cortisol rise		
		Mean	S.D.	*p*	Mean	S.D.	*p*		Mean	S.D.	*p*	Mean	S.D.	*p*
Social status														
High	29	14.82	7.04	0.41	11.63	12.97	0.38	14	12.89	7.72	0.89	13.44	12.32	0.51
Low	26	13.29	5.93		15.12	13.31		14	13.26	7.03		17.38	15.83	
Cohabitant														
Yes	50	14.20	6.94	0.79	13.98	13.33	0.17	24	13.60	7.34	0.37	12.46	12.22	0.01
No	5	13.73	3.61		6.98	10.17		4	9.93	6.67		29.90	14.94	
Children														
Yes	30	14.83	7.09	0.34	14.47	13.71	0.07	13	13.30	6.40	0.51	9.06	9.54	0.34
No	25	12.38	4.71		9.11	10.64		15	12.88	8.13		19.63	15.25	
Hours housework														
<20	43	14.32	6.13	0.59	12.07	11.63	0.28	24	12.78	6.68	0.74	16.48	15.16	0.25
>20	11	12.76	8.28		18.84	17.54		4	14.85	1121		10.78	6.34	
Time pressure														
Low	26	12.36	4.29	0.08	8.41	9.28	0.01	17	10.92	6.37	0.06	16.62	13.01	0.66
High	29	15.74	7.90		18.37	14.75		11	16.40	7.55		13.73	16.27	
Weekly work hours														
<37	42	13.25	5.99	0.26	16.77	11.87	0.01	16	10.88	5.11	0.08	14.79	13.40	0.71
>37	12	16.64	8.39		3.34	13.15		12	16.47	9.04		17.31	16.28	
Control														
High	22	14.34	6.87	0.40	13.22	14.25	0.48	18	13.17	7.19	0.92	16.49	15.41	0.63
Low	25	12.54	5.43		16.56	12.75		10	12.87	7.81		13.63	11.93	
Demands														
Low	31	13.30	5.58	0.67	14.87	13.08	0.59	18	13.22	7.73	0.83	17.11	13.31	0.46
High	21	14.19	7.42		12.55	14.07		10	12.77	6.55		11.80	16.17	
Support														
High	25	12.82	6.54	0.44	16.51	15.11	0.31	16	14.14	8.60	0.34	18.38	16.20	0.31
Low	24	14.42	6.54		11.98	12.11		12	11.65	4.93		12.34	11.26	
Effort														
Low	36	13.99	6.67	0.90	12.79	12.87	0.60	14	11.66	7.23	0.31	12.54	9.87	0.32
High	18	13.96	6.66		15.08	14.36		14	14.49	7.25		18.71	17.54	
Reward														
Low	27	13.29	5.90	0.48	14.66	14.99	0.81	17	12.65	7.13	0.47	16.85	14.40	0.45
High	19	14.87	7.90		15.72	11.62		10	14.90	7.43		12.21	11.79	
Effort reward imbalance														
Low	32	13.39	6.66	0.49	11.06	13.11	0.16	18	10.84	6.53	0.05	15.26	12.97	0.61
High	21	14.78	6.59		16.91	13.19		10	17.07	7.45		18.89	16.73	
Overcommitment														
Low	28	14.57	7.45	0.54	14.56	13.37	0.86	13	11.17	7.38	0.24	12.36	13.75	0.45
High	22	13.29	5.88		13.79	14.27		15	14.51	7.04		17.16	14.44	

p-Values by the use of Student's *t*-test.

six measures of cortisol (at awakening, +20, +30, +60 min, +8 h, and 18:00 p.m.). For each of the two repeated measures a series of analyses were carried out: The repeated measure was used as the dependent variable and each of the psychosocial factors was used as the independent variable one at a time, with age, physical activity, tobacco and time of the first saliva sample included as covariates.

Finally, levels of salivary cortisol throughout the day were depicted according to the feeling of time pressure and effort reward imbalance.

4. Results

In Table 1, characteristics of the participants are shown. The women were a little younger than the men, though not significantly. Only a few were smokers, 11% of the women and 14% of the men. Among the women, 66% had non-manual educations lasting up to 4 years, i.e. secretary, laboratory worker, nurse or midwife. Among the men, 50% were academics, i.e. physicians, or had higher non-manual educations lasting more than 3 years, i.e. policemen, engineer, teacher or working with computers. Furthermore, a large group was only skilled (42%, i.e. carpenter or electrician) but no men were unskilled. The frequency of cohabitation, and number of children were equal in the two genders. Men worked significantly more hours per week but women felt significantly higher degrees of time pressure. The subjective work environment was good, with only low scores on the scales of the two stress models. The women felt a significantly lower degree of control and higher degree of support compared to the men.

For the scales used, the levels of cronbachs alpha were the following: control 0.71, demands 0.40, support 0.81, effort 0.77, reward 0.72, and over-commitment 0.82. Thus, the cronbachs alpha of the demand-scale showed a less reliable scale.

In Table 2, the cortisol levels for each dichotomised psychosocial factor are shown as awakening cortisol, and cortisol rise during the first 30 min (mean (S.D.) nmol/l).

Social status had no significant influence on cortisol excretion in this population. Among the women, the cohabiting had higher cortisol levels during the day the single women (F, 3.22; p, 0.08). In both genders feeling of time pressure considering family related duties was positively and significantly associated with cortisol excretion, though in a different manner. Women feeling time pressure showed significantly increased awakening cortisol rise (mean/S.D.) 18.37/14.75 mmol/l versus 8.41/9.28 mmol/l in women not feeling time pressure (F, 5.16; p, 0.03). Also, levels of salivary cortisol throughout the day tended to be significantly elevated in women with high level of time pressure (F, 3.74; p, 0.06). The men feeling time pressure showed increased level of cortisol at awakening (mean/S.D.) 16.40/7.55 mmol/l versus 10.92/6.37 mmol/l in men not feeling time pressure (F, 4.98; p, 0.04).

The demand control model did only show limited associations to cortisol levels. In men, social support was negatively and significantly associated with cortisol excretion during the day (F, 4.76; p, 0.05), i.e. in men with a higher degree of social support level of awakening cortisol rise was (mean/S.D.) 18.38/16.20 mmol/l versus 12.34/11.26 mmol/l in men with lower degree of social support.

In women, effort was positively but only nearly significantly associated with cortisol rise in the morning (F, 3.31; p, 0.08) and during the day (F, 3.56; p, 0.07). However, in women having effort reward imbalance awakening cortisol rise was (mean/S.D.) 16.91/13.19 mmol/l contrary to cortisol rise in women not having effort reward imbalance whose cortisol rise was 11.06/13.11 mmol/l (F, 4.16; p, 0.05). In men, effort, effort reward imbalance and overcommitment was positively and significantly associated with cortisol rise in the morning and during the day. In men scoring high versus low on the effort

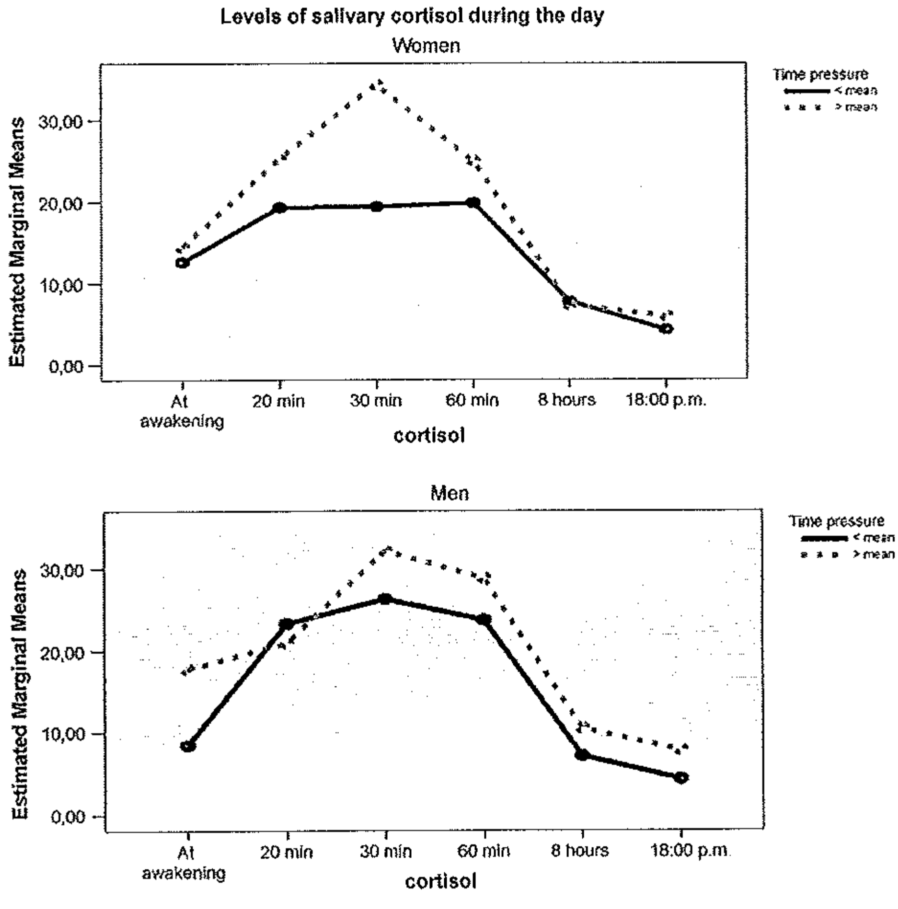

Fig. 1. Salivary cortisol adjusted for age, physical activity, tobacco, and time of first sample, according to time pressure.

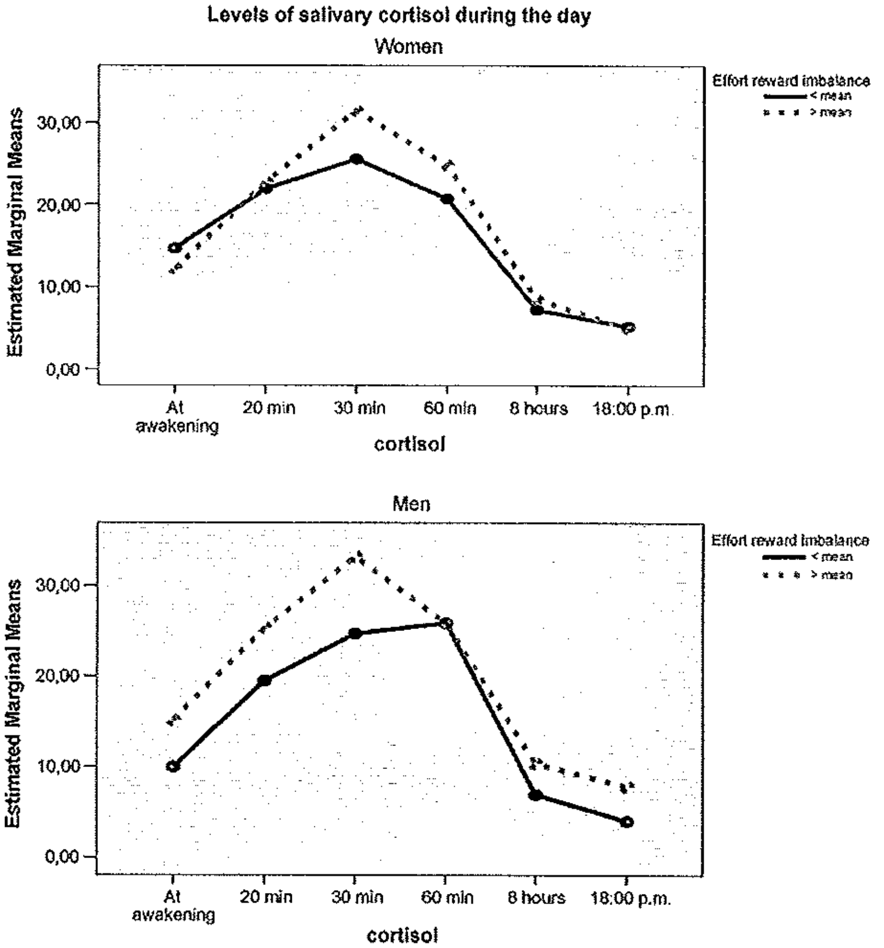

Fig. 2. Salivary cortisol adjusted for age, physical activity, tobacco, and time of the first sample, according to effort reward imbalance.

scale cortisol awakening rise was (mean/S.D.) 18.71/17.54 and 12.54/9.87 mmol/l (F, 5.52; p, 0.03). In men who experienced high versus low effort reward imbalance cortisol awakening rise was (mean/S.D.) 18.89/16.73 and 15.26/12.97 mmol/l (F, 7.64; p, 0.01). In men, who were overcommitted contrary to those who were not overcommitted cortisol awakening rise was (mean/S.D.) 17.16/14.44 and 12.33/13.75 mmol/l (F, 6.98; p, 0.02). Also, analyses including all 6 measures of cortisol showed significant associations to effort, effort reward imbalance and overcommitment in men. The F and p-values were: effort (F, 5.99; p, 0.03), effort reward imbalance (F, 7.60; p, 0.02) and overcommitment (F, 5.44; p, 0.03).

Finally, Figs. 1 and 2 show levels of cortisol in women and men, respectively, according to feeling of time pressure (Fig. 1) and effort reward imbalance (Fig. 2). It is seen that especially levels of cortisol are higher in the morning in people experiencing adverse circumstances.

5. Discussion

In this study, associations between the levels of salivary cortisol on a working day and psychosocial factors in both women and men were analysed. Among the women, the feeling of time pressure combined with high effort and effort reward imbalance were significantly and positively associated with levels of salivary cortisol. Also, cohabiting women had significantly higher levels of cortisol than single women. Furthermore, among the men, time pressure, support (negatively), effort, effort reward imbalance and over-commitment were significantly associated with cortisol excretion.

In data from Whitehall, cortisol has previously been found to be high in men with low social status and in women with high social status (Steptoe et al., 2003). In the present study, social status did not appear to be of significance for ACR. The finding might be due partly to the small scale of the study, partly to the fact that in Denmark social status may be of limited importance for people working outside the home. If the population had been larger or if unemployed people had been included in this group, increased cortisol excretion in these participants would have been expected.

In this study, cohabiting women and women feeling time pressure had significantly higher levels of cortisol than the other women. This finding may express the same association as found by Kunz-Ebrecht et al. (2004a) where ACR in women were larger than ACR in men on working days, i.e. that women experience a high level of strain from family responsibilities on workdays. Feeling of time pressure was negatively associated with age, i.e. younger women with small children felt more time pressure than older women. In connection with stress, tests, especially tests which include uncontrollable or social-evaluative threats, elicit increased activity in the HPA-axis (Dickerson and Kemeny, 2004). It may be that women feeling time pressure have increased levels of cortisol because of the uncontrollable and social-evaluative threat experienced when not doing well as a wife and mother. Similarly, men who have experienced time pressure had significantly higher awakening cortisol. Furthermore, the single women in this study had fewer children and did not experience the same high degree of time pressure as the cohabiting women. The results indicate that it is a strain for women to have a family and be in paid employment. This has previously been documented by Frankenhauser et al. by measurement of catecholamines (Frankenhaeuser et al., 1989) and by Brisson through measurements of blood pressure (Brisson et al., 1999). At present, no other studies of salivary cortisol and contemporary measures of psychosocial factors at work and at home have been published.

The psychosocial work environment was described by means of the demand control model (DC) and the effort reward imbalance model (ERI). Among the women, no associations between cortisol and the demand control model were found. Also, among men, the only dimension of the demand control model that showed significant association to cortisol excretion was support. This association, however, was contrary to expectations. Evaluation of the data showed that the men with high degrees of support were predominantly middle-aged (40–55 years of age) and employed in the public health service. They experience great support from their subordinates. However, the public health sector has faced large reductions during the years and this is a continuous challenge to the employees in higher positions. Furthermore, the lack of associations between the demand control model and the levels of cortisol may originate in the fact that the population in this study in general reported only a low degree of work related strain, according to the scores in the questionnaires. Finally, the demand-scale can be questioned, as it only consisted of two items and the chronbachs alpha was low. In a study by Steptoe and al. associations between job strain (high demands and low control) and morning cortisol has been demonstrated (Steptoe et al., 2000) and demands have been found to be important for ACR in women from Whitehall (Kunz-Ebrecht et al., 2004b).

Both effort and ERI were associated with cortisol excretion in both genders in the present study, and over-commitment was significantly associated with cortisol levels in men. Previously, cortisol excretion has been shown to be associated with over-commitment in men in data from Whitehall (Steptoe et al., 2004), whereas effort and reward was not associated with cortisol. The different findings may be explained as variations in work environment, culture and perception of the stressors described in the model, and is not seen as inconsistent.

The compliance of the participants when taking the saliva samples has to be mentioned, as a major concern about the methodology used. It is well known that some people do not take saliva samples as they are instructed (Clow et al., 2004). This may be managed by excluding those who have a small cortisol rise (for instance <2.49 nmol/l (Wüst et al., 2000)). However, as it is known that some people are non-responders, this may be questionable. Therefore this study included all participants who delivered all saliva samples as they were instructed. Furthermore, it is a limitation that salivary cortisol was only measured on a single day, as cortisol levels may vary from day to day (Clow et al., 2004; Pruessner et al., 1997).

In the discussion of the results it is also important to notice that the finding of significant versus non-significant correlations of measures is not enough to conclude that there are differences between the two genders. Furthermore, about 1/3 of the women worked less than 32 h per week, whereas most of the men worked 37 h or more per week. The life conditions among the women and the men were different, as they may generally be presumed to be.

6. Conclusion

Techniques for measuring cortisol in saliva provide a major advancement in physiological stress research. In recent years, there has been much interest in the awakening cortisol rise, i.e. the rise in cortisol excretion during the first 30 min after awakening. The data from the present study shows that cortisol excretion throughout the day might be useful in opening up new possibilities for understanding the relationship between illness and social and psychological factors. When studying the effects on health of the psychological work environment factors in large populations, it might be relevant to use only awakening cortisol response, as this is more feasible than taking a large number of saliva samples during the day. However, more information may be available if the diurnal variation in cortisol excretion is highlighted. This can especially be used to evaluate gender differences in connection with the work-family interface, an area that is likely to become a focus area in future research. Finally, this study highlights the benefits of including physiological measures in epidemiological studies of occupational stress to improve existing stress models.

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Long-term effects of psychosocial factors of home and work on biomarkers of stress

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ABSTRACT

Introduction: The current study analyzed the relationship between psychosocial factors measured at baseline and heart rate variability (HRV) and salivary cortisol measured at baseline and again, six years later.
Methods: In 2002 and 2008, measurements of HRV and salivary cortisol at three time points were obtained from 70 healthy participants (48 women and 22 men). The associations between the psychosocial factors measured in 2002 and the dependent variables, HRV and salivary cortisol measured in 2002 and 2008, were examined using a series of repeated measures ANCOVAs. The dependent variables were as follows: the logarithmically transformed levels of total power (LnTP), high frequency power (LnHF), the ratio between low and high frequency power (LnLF/HF) and salivary cortisol (LnCortisol).
Results: For women, high social status was associated with high LnTP, high LnHF, and low LnLF/HF. In work, lack of control was associated with low LnTP, and lack of support was associated with an increased LnLF/HF ratio. For men, high social status was associated with low LnTP, low LnHF and high LnCortisol. Greater number of hours spent doing housework was associated with both low LnLF/HF and low LnCortisol, whereas a large imbalance between effort and reward was associated with low LnTP and high LnCortisol.
Conclusion: Despite the small sample size, this study demonstrated that psychosocial factors impact levels of activity in the allostatic systems.

1. Introduction

Recent research suggests that psychosocial factors (e.g., psychosocial working environment) are independent risk factors for ischemic heart disease (IHD) (Kunz-Ebrecht et al., 2004a; Schlotz et al., 2004). To date, the majority of research examining the significance of psychosocial work environment has been based on the Demand Control Model (Karasek et al., 1982). This model proposes that people in high-demanding jobs with little control over their work are at particular risk of developing stress-related illnesses, although social support can be a moderator (Johnson and Hall, 1988). There is also a newer stress model called the Effort Reward Model that proposes that people experiencing an imbalance between effort and reward (ERI) at work are also at risk for stress-related diseases (Siegrist et al., 1990, 2004; Siegrist and Peter, 1996). These two models describe different dimensions of the psychosocial work environment, and studies based on different designs have demonstrated that both models are independent predictors of IHD (Bosma et al., 1998; Kivimaki et al., 2002; Peter et al., 2002).

Besides psychosocial factors at work, psychosocial factors at home are also important. For example, one's perception of work load can be influenced by his/her social status and family demands (Artazcoz et al., 2007; Barnett et al., 2005; Barrett-Connor, 1997).

Psychosocial factors may increase the risk of IHD through repeated or chronic activation of the autonomous nervous system (ANS) and the hypothalamic-pituitary-adrenal axis (HPA-axis; the two allostatic systems), including increased activity of the sympathetic nervous system and withdrawal of vagal activity and hyper (or hypo) secretion of cortisol (McEwen and Seeman, 1999) or, in other words, chronic stress.

1.1. The autonomous nervous system and psychosocial factors

Due to changes in sympathetic and parasympathetic activity, the heart rate constantly changes, and brief electrocardiograms (ECGs) of 2–5 min can be used to estimate activity in the autonomic nervous system. Total power (TP, ms^2) is defined as the variance of all NN intervals (NN = adjacent sinus rhythm heart beats) (Malik, 1996). In several studies, low TP predicted morbidity and mortality (Malik, 1996; Dekker et al., 2000; Thayer and Lane, 2007). Furthermore, elevated sympathetic tonus increased the risk of hypertension (Charkoudian and Rabbitts, 2009), and dysfunction of the autonomic nervous system may be involved in several diseases related to chronic stress (Charkoudian and Rabbitts, 2009; Esler, 2010; Thayer et al., 2010).

Low social position has been associated with low HRV (Hemingway et al., 2005). Long working hours and night work have been

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found to be associated with sympathetic dominance (Rauchenzauner et al., 2009; Su et al., 2008).

Most cross-sectional studies have examined associations between the Demand Control Model and HRV. Riese et al. found that job strain was not associated with HRV in female nurses (Riese et al., 2004), while low job control or job strain was associated with low HRV in a number of studies, which included mostly men (Collins et al., 2005; Hemingway and Marmot, 1999; Kang et al., 2004; Vrijkotte et al., 2000). Vrijkotte et al. found that ERI was associated with low HRV in men (Vrijkotte et al., 2000), whereas Hintsanen et al. demonstrated an association between ERI and low HRV in women but not in men (Hintsanen et al., 2007). Finally, Clays et al. found that two indices of work stress were associated with low parasympathetic activity (Clays et al., 2010). In a recent review on the autonomic nervous system and cardiovascular risk factors, Thayer et al. concluded that decreased vagal function is a risk factor for all-cause mortality and that work stress is associated with decreased HRV (Thayer et al., 2010).

1.2. The HPA-axis and psychosocial factors

Cortisol levels change over a 24-hour period and have large intra-individual differences. It is difficult to interpret a single measurement of cortisol; therefore, multiple measurements are needed to interpret measurements of cortisol in saliva. To date, researchers have used a number of different strategies for measuring and analyzing salivary cortisol (Hjortskov et al., 2004). For example, the awakening cortisol response (ACR; the difference in cortisol from awakening to some period after awakening) has been introduced as a measure of cortisol reactivity. Increased cortisol secretion in the morning has been linked to intima media thickness (Eller et al., 2001; Eller et al., 2005), and changes in diurnal levels of cortisol in the form of a "flattened" curve have been associated with increased risk of abdominal obesity and type-2 diabetes (Bjorntorp and Rosmond, 2000; Rosmond and Bjorntorp, 2000; Rosmond et al., 2003) and possibly increased risk for IHD.

Research examining associations between levels of cortisol and workplace stress have produced mixed results (Chandola et al., 2009). Increased levels of cortisol during workdays compared to weekends suggest that work-related factors increase the levels of cortisol (Kunz-Ebrecht et al., 2004a; Schlotz et al., 2004). Chida et al. found that psychosocial factors and work stress were associated with ACR suggesting that ACR could be used to measure the level of psychological strain in daily life (Chida and Steptoe, 2009). In some studies, low job control has been linked to increased levels of cortisol in the morning in women (Harris et al., 2007) and in men (Kunz-Ebrecht et al., 2004b). However, Kunz-Ebrecht et al. did not find an association between low job control and high level of cortisol in women. Similarly, Eller et al. found that ERI in men, but not in women, was associated with increased cortisol levels in the morning (Eller et al., 2006a), whereas Bellingrath et al. did not find ERI associated with cortisol levels (Bellingrath et al., 2008). These discrepancies may be explained by differences in study design or subtle changes in cortisol due to a variety of conditions, such as genetics, prenatal programming, or appraisal of stressors (Bellingrath et al., 2008; Entringer et al., 2009; Kudielka et al., 2009).

1.3. Psychosocial factors, stress and gender

The stress response depends on the stressor (Kovács et al., 2005), on the individual (e.g., genetics (44)), and on the perception of the stressor (Ursin and Eriksen, 2004).

Individuals perceive a psychosocial factor differently. Mothers tend to feel a larger responsibility for the home and children than fathers do, and this may influence perceptions of stressors at work and home (Brisson et al., 1999; Lundberg and Frankenhaeuser, 1999). Furthermore, at work, job responsibilities and job climate may differ systematically by gender even for individuals working in the same place (Artazcoz et al., 2007; Ursin and Eriksen, 2004; Messing et al., 2003). In addition, risk factors for ischemic heart disease differ across gender (Barrett-Connor, 1997; Brezinka and Padmos, 1994). Agelink et al. concluded that women up to the age of 55 years have a higher resting heart rate than men. Young and middle-aged women showed a significantly lower LF power and LF/HF ratio compared to similarly-aged men, whereas the study did not reveal significant gender differences in the HF power (Agelink et al., 2001).

Several studies have investigated differences in cortisol levels by gender. A thorough review by Kudielka and Kirschbaum (2005) concluded that adult men respond to a stressor with larger increases in cortisol levels than women and that this may be associated with a larger cardiovascular risk. On the other hand, the relative hypo-reactivity in cortisol levels in women might be associated with a higher risk for autoimmune diseases.

1.4. Scope of the study

Most studies include information on psychosocial factors from the home or work and use only data describing one of the two allostatic systems. The aim of the present study was to examine the relationship between psychosocial factors at home and work and levels of salivary cortisol and HRV over a 6-year period in a diverse Danish population. The hypotheses were the following:

Main hypothesis: Adverse working conditions, i.e. low control, high demands, low social support, high effort, low reward and high ERI are associated with high levels of cortisol and low levels of HRV.

Sub hypothesis: Social status and family circumstances moderate the association between work-related factors, HRV, and cortisol levels in the following way: social status moderates adverse working conditions leading to higher cortisol and lower HRV in low status individuals in adverse working conditions compared to levels of cortisol and HRV in high status individuals in adverse working conditions.

2. Methods and participants

In 1998, 130 participants were enrolled in a prospective study on work stress measuring physiological stress response and intima media thickness (Eller et al., 2005; Eller et al., 2006a,b). The participants volunteered in response to a poster posted near the canteen at Hillerød Hospital or to a notice in a local newspaper. No payment or gifts were given in return for participation. The majority of the participants were married or cohabiting with another adult (79.2% of the women and 90.9% of the men). All participants were employed outside the home, and the majority was working full time (37 h/week). The women were working within the health sector, primarily as secretaries, midwives, physiotherapists or nurses while the men were nursery school teachers, teachers, policemen, engineers, technical personnel and salesmen. In 2002, 95 of these participants agreed to participate in a second study. This group consisted of 63 women and 32 men. Finally, 86 participants (58 women and 28 men) were re-examined in a third study. In total, 48 women and 22 men participated in both studies of salivary cortisol and HRV in 2002 and 2008. In 2008, three women and seven men were not working, and eight women and six men were medicated with antihypertensive medicine. No one suffered from diabetes or depression. Basic demographic, physiological measures and psychosocial factors are shown in Table 1. The municipal scientific ethics committee approved the study.

Table 1
Demographic, physiological and psychosocial variables measured in 2002. *p<0.05; **p<0.01 by Students t-test in connection with continuous variables and Chi^2-test in connection with the dichotomized variables.

	Women (N = 48)		Men (N = 22)	
	Mean	SD	Mean	SD
Age (years)	50	7	51	7
Waist–hip ratio	0.79**	0.06	0.90	0.10
Blood pressure, systolic (mm Hg)	130	14	137	20
Cholesterol (mmol/L)	5.4	1.0	5.3	0.9
HbA_{1c} (%)	5.3	0.3	5.4	0.5
Smoking, yes (%)	16.7		27.3	
Alcohol (drinks/week)	6.6	5.9	12.7	12.6
Physical activity, 2–4 h/week (%)	68.8		68.2	
Social status:				
1: Academic	12.5		27.3	
2: 3–4 years of education	43.8		18.2	
3: 1–2 years of education	22.9		9.1	
4: Skilled worker	14.6		45.4	
5: Unskilled worker	6.3		0	
Social status, high (%)	56.3		45.5	
Children at home, yes (%)	54.2		50.0	
Housework, >20 h/week (%)	22.8		13.6	
WWH (h)	35*	6	38	5
The Demand Control Model				
Lack of control	39**	16	20	16
Demands	54	19	53	23
Lack of support	23	18	31	27
The Effort Reward Model				
Effort	12	4	13	5
Reward	40	6	41	6
ERI	0.64	0.29	0.68	0.32

Abbreviations: HbA_{1c}: glycated haemoglobin; WWH: weekly working hours; ERI: effort reward imbalance.

2.1. Dependent variables

HRV: Ambulatory ECGs were collected as part of the larger clinical examinations that took place each study year between 12:00 and 17:00. ECGs were collected during a 15-minute period while participants were lying down.

The ECG data were collected at the two testing points using different equipment. In 2002, a 7-lead DXP1000 Holter-recorder (Aspect Holter monitoring systems, Danica Biomedical A/S, Sweden) was used for ECG-recording; in 2008, the ECGs were recorded by a 3-lead LifeCard CF Holter monitor (Delmar Reynolds Medical Inc., Irvine, CA, USA). Only recordings of sinus rhythms were included in the study. Artefacts and non-normal beats in the ECG segments were autodetected by commercial software and verified by visual inspection. Before calculating HRV metrics, the RR-intervals were filtered for possible outliers (ectopic beats, falsely detected beats, and missed beats) using the algorithm published by Moody (Moody, 1993), resampled with a frequency of 4 Hz and linearly detrended.

The spectral power band between 0.18 and 0.4 Hz, i.e., the high frequency band (HF), is caused by changes in the parasympathetic activity, whereas the power spectral band between 0.04 and 0.18 Hz, i.e., the low frequency band (LF), includes both sympathetic and parasympathetic activity. The ratio between LF and HF is thought to be a proxy for the balance between the sympathetic and the parasympathetic tonus. An increased LF/HF ratio has been taken as an indication of increased sympathetic tone (Malik, 1996), but it has also been argued that an increased LF/HF ratio can be the result of increased parasympathetic activity (McCraty et al., 2009). The origin of very low frequency changes (VLF: 0–0.04 Hz) is unclear, but they are thought to reflect slow thermoregulatory oscillations and should not be interpreted in analyses of brief ECGs (Malik, 1996).

TP (frequency band 0–0.4 Hz), HF and the ratio between low frequency and high frequency power (LF/HF) were used as the dependent variables.

Cortisol: The equipment for the saliva samples (3 Salivette®, Saarsted, polyester tampons) was sent to the participants with instructions to fast, to refrain from smoking and not to brush their teeth 1 h before collecting the samples. Participants were further instructed to collect the first sample immediately after awakening while still in bed, another sample 30 min after awakening and a final sample at 18:00. The participants were asked to write down the exact time at which each sample was collected.

The collection of data took place between mid-October and late-January (2002/03 and 2008/09). The samples were collected in connection with the clinical examination at Hillerød Hospital and were analyzed at Denmark's National Research Centre for the Working Environment. The samples were kept frozen at −80 °C until analysis.

The same method for measuring cortisol in saliva was used both years; the assay used for the determination of cortisol in saliva was a competitive RIA (Spectria Cortisol Coated Tube RIA) purchased from Orion Diagnostica, Espoo, Finland. The analysis was carried out according to the manufacturer's instructions. The cross-reactivity to cortisone was <0.2%. A 1470 Wizard gamma counter (Wallac, Turku, Finland) was used for measuring radioactivity. A method evaluation of certified reference material in water showed no bias in the method, i.e., recovery was 97% [CI: 94%; 10.9%]. The limit of detection (LOD) was 1.59 nmol/l. Between run coefficients of variation were 19% at 11.5 nmol/l and 16% at 49.2 nmol/l (Hansen et al., 2003).

It has been demonstrated that verbal and written instruction is not sufficient to ensure correct sampling (Broderick et al., 2004; Kudielka et al., 2003). One possibility is to exclude participants with a level of cortisol that decreases during the first 30 min after awakening; this decrease could imply a lack of compliance (Wüst et al., 2000). Instead, we chose to underline the importance of reporting the exact time of sampling in the written instruction to the participants and proposed to adjust for this in the analyses. By including all participants, we avoided excluding true non-responders.

The dependent variable was cortisol.

2.2. Measurement of exposure in 2002

2.2.1. Psychosocial factors at home

'Social status' was coded as follows: 1 = university education or manager of more than 50 people; 2 = academic training for 3 to 4 years or manager of up to 50 people; 3 = academic training for 1 to 2 years or manager of up to 10 people; 4 = skilled labor; 5 = unskilled labor (Netterstrom et al., 1991, 1999). In the analyses, social status was included as a dichotomized variable: high social status = 0 (collapsing across 1 and 2); and low social status = 1 (collapsing across 3, 4 and 5).

'Children': The number of children at home as reported by the participants was coded as follows: no children at home = 0, living with one or more children = 1.

'Housework': To establish the extent to which participants were responsible for running the home and looking after children, the participants were asked to indicate the time spent every week cleaning, shopping, cooking, looking after children or caring for other adults (Eller et al., 2006a). A continuous variable was created using the following categories: <10 h/week = 1; 10–20 h/week = 2; and >20 h/week = 3.

2.2.2. Psychosocial factors at work

'Weekly working hours' (WWH) refers to the number of hours spent at work each week. In Denmark, the normal working hours are 37 h/week. Participants stated how many hours they actually worked each week, including work at home in the evenings or on weekends.

'Lack of control', 'Demands', and 'Lack of support' were assessed using items from the Whitehall study in the Copenhagen City Heart Study (Netterstrom et al., 1991). Twelve items were used to evaluate the degree of control (Cronbach's alpha = 0.71); two items evaluated demands (Cronbach's alpha = 0.40); and four items evaluated the degree of support (Cronbach's alpha = 0.81). The scale for each dimension ranged from 0 to 100, with a higher score indicating a higher degree of stress caused by the respective dimension. The demands-scale should be interpreted with caution because the Cronbach's alpha is low.

A questionnaire developed by Siegrist et al. was used to study the participant's degree of effort, reward and effort–reward imbalance at work (Siegrist et al., 2004). Only five items were used for the effort-dimension (Cronbach's alpha = 0.77) because the question regarding physical demands at work was omitted. The reward dimension only included 10 items (Cronbach's alpha = 0.72) because the question regarding respect from a superior had to be left out due to errors in the questionnaire. Thus, the effort–reward imbalance ratio (ERI) was calculated as effort/reward × (5/10).

2.3. Physiological variables

Several additional physiological variables were measured in 2002: waist–hip ratio (WHR, waist width /hip width), systolic blood pressure, alcohol consumption (units/week), tobacco (g/day) and degree of physical activity (0 = passive or light physical activity for up to 4 h/week and 1 = more intensive physical activity for more than 4 h/week). Levels of total-cholesterol and HbA_{1c} in the blood were obtained from routine analyses conducted at the Hillerød Hospital.

2.4. Strategy for the analysis

All analyses were carried out separately for women and men because of the known gender differences in physiology, daily life and perceptions of stressors (Artazcoz et al., 2007; Barrett-Connor, 1997; Messing et al., 2003; Brezinka and Padmos, 1994). The data were analyzed using SPSS version 11. Because the dependent variables (TP, HF, LF/HF, and Cortisol) were not normally distributed, these variables were transformed logarithmically before analysis and labeled LnTP, LnHF, LnLF/HF and LnCortisol. After logarithmic transformation, the data were normally distributed. A level of significance of $p < 0.05$ was chosen.

Analysis part 1: Four series of repeated measures ANCOVA models were conducted for each gender separately, using four dependent variables: LnTP, LnHF, LnLF/HF and LnCortisol. For each model, year of examination (2002 = 0; 2008 = 1) was included as a factor, and age and time of measurement (calculated as hours after midnight) were included as covariates. Furthermore, each of the psychosocial factors was included as either a factor (social status, cohabitant, and children) or as a covariate (housework, WWH, lack of control, demands, lack of social support, effort, reward, and ERI).

Analysis part 2: As in analysis part 1, repeated measures ANCOVA models were conducted for each gender separately using two dependent variables: LnTP and LnCortisol. For each model, year of examination was included as a factor, and age and time of measurement were covariates. Each of the variables from the two work stress models discussed in the introduction was included as a covariate. Variables that were significantly correlated with 'lack of control', 'demands', 'lack of support', 'effort', 'reward', and 'ERI' were included in the models in a hierarchical manner: first step, psychosocial factors; second step, behavioral variables; and last step, physiological variables.

For women, the models included the following variables: Model A: age, year and time of measurement; Model B: the variables from model A, 'social status', and 'WWH'; Model C: the variables from model B and tobacco; Model D: the variables from model C, WHR and cholesterol.

For men, the models included the following variables: Model A: age, year and time of measurement; Model B: the variables from model A, 'social status', and 'housework'; Model C: the variables from model B and alcohol; Model D: the variables from model C and HbA_{1c}.

Analysis part 3: Next, we examined the interactions between home and work-related psychosocial factors for men using a repeated measures ANCOVA. One model examined the effects and interaction of social status and ERI on LnTP, and the other model examined the effects and interaction of ERI and children at home on LnCortisol. These analyses were also carried out with adjustments as in models A–D.

3. Results

The sample included 48 women and 22 men for whom data on salivary cortisol and HRV were available from both testing points (2002, 2008). This sample was not significantly different from those excluded (the drop-outs) in physiological or psychosocial factors. Most of the participants were employed in the same job across the two testing points; however, by the second testing point, 13 participants (8 women and 5 men) had either retired or were working significantly fewer hours per week. There were no gender differences in the sample other than higher heart rates for women ($t = 2.2$, $p < 0.05$). TP decreased significantly across the testing points for both men and women. Increasing age was associated in both genders with not having children at home, spending less time doing housework and a lack of social support at work.

Tables 2 (women) and 3 (men) show the correlations between psychosocial factors and physiological variables. In women, WHR was correlated with effort and ERI, cholesterol was correlated with lack of support, and tobacco was correlated with demands. In men, HbA_{1c} was inversely correlated with demands, effort and ERI, and alcohol was correlated with reward. Cortisol was positively correlated with effort and ERI.

Tables 4 and 5 present results from the repeated measures ANCOVAs for the women and men, respectively. Results for the initial model (including only year and age as explanatory variables) are shown in the first lines. The year of examination was significantly associated with LnTP for men, and a similar tendency was apparent for the women. This

Table 2
Women, correlations between psychosocial factors and physiological factors.

	Age	Waist–hip ratio	Blood pressure	Cholesterol	HbA_{1c}	Tobacco	Alcohol	Physical activity	TP	Cortisol
Age	1	0.21	0.11	0.25	0.39**	0.08	−0.10	0.41**	−0.49**	−0.18
Social status	0.20	−0.10	0.19	0.24	−0.05	0.07	−0.01	−0.08	−0.41**	−0.09
Children	−0.44**	−0.03	0.13	−0.16	−0.13	−0.08	−0.13	−0.25	0.36*	0.25
Housework	−0.33*	0.05	0.22	−0.31*	−0.09	−0.17	0.10	−0.26	0.40*	0.06
WWH	0.11	−0.14	−0.09	−0.09	0.27	−0.04	0.10	0.29	−0.08	−0.02
Lack of control	0.14	−0.08	−0.14	0.09	−0.13	0.22	−0.05	0.05	−0.30	−0.09
Demands	0.17	0.11	0.09	0.03	−0.14	0.32*	−0.09	0.22	−0.15	−0.08
Lack of support	0.42**	0.14	0.25	0.46**	0.22	0.06	−0.08	0.17	−0.22	0.17
Effort	0.17	0.29*	−0.02	0.14	−0.22	0.10	−0.17	0.23	−0.27	0.03
Reward	0.01	−0.11	−0.14	−0.23	0.16	−0.16	−0.08	0.03	−0.00	−0.11
ERI	0.12	0.30*	0.05	0.26	−0.21	0.16	−0.09	0.10	−0.23	0.12

*$p < 0.05$; **$p < 0.01$.

Table 3
Men, correlations between psychosocial factors and physiological factors.

	Age	Waist–hip ratio	Blood pressure	Cholesterol	HbA_{1c}	Tobacco	Alcohol	Physical activity	TP	Cortisol
Age	1	0.28	0.30	0.24	−0.05	0.10	0.22	−0.31	−0.59**	−0.27
Social status	0.27	0.08	0.08	−0.02	0.04	−0.06	0.09	−0.01	0.03	−0.23
Children	−0.54**	−0.24	0.04	−0.23	0.00	−0.19	−0.32	0.18	0.30	−0.18
Housework	−0.17	−0.26	0.25	0.27	−0.03	−0.03	−0.22	0.19	0.15	0.17
WWH	−0.38	0.10	−0.14	−0.24	0.33	0.01	0.03	0.03	0.20	0.65**
Lack of control	0.01	−0.35	−0.19	0.01	0.12	0.16	−0.05	0.04	0.07	0.04
Demands	0.09	0.04	−0.05	−0.22	−0.46*	−0.27	0.12	−0.02	−0.08	0.10
Lack of support	0.16	0.35	0.12	−0.18	0.40	−0.07	0.14	0.00	−0.30	−0.19
Effort	−0.14	−0.29	−0.06	−0.14	−0.57**	0.03	−0.02	−0.10	0.03	0.56*
Reward	0.22	0.32	0.09	0.39	0.02	0.23	0.41*	−0.24	0.01	−0.34
ERI	−0.25	−0.36	−0.16	−0.32	−0.55**	−0.01	−0.15	−0.01	0.08	0.59**

*p<0.05; **p<0.01.

result may be partly due to the change in equipment used in the analysis of the ECG recordings between 2002 and 2008. The year of examination was also associated with LnCortisol for the women but not for men. LnTP was negatively and significantly associated with age for both genders, whereas LnLF/HF and LnCortisol were not. For the psychosocial variables, the associations were as follows:

Women (Table 4): High social status was significantly associated with high LnTP and low LnLF/HF. Lack of control was significantly associated with low LnTP. LnLF/HF tended to increase with loss of control, but this association did not reach significance ($p = 0.08$). Lack of support was significantly associated with increased LnLF/HF. LnCortisol was not associated with any psychosocial variable. Because there were so few associations in women, the results from the analyses including further adjustments are not shown. The significant associations between social status, lack of control and LnTP stayed significant in model D when all variables were included.

Men (Table 5): High social status was significantly associated with low LnTP, low LnHF, and high LnCortisol. Having children tended to be associated with high LnLF/HF and was significantly associated with low LnCortisol. 'Hours of housework' was significantly associated with increased LnLF/HF and decreased LnCortisol. WWH was significantly associated with high LnHF and low LnLF/HF. Effort was significantly associated with high LnCortisol. Reward was significantly associated with high LnTP. Finally, ERI was significantly associated with low LnTP and high LnCortisol.

Table 6 shows the results of adjustment in the four models A–D (men only) using LnTP and LnCortisol as the dependent variable, respectively. The associations between the Effort Reward Model and LnTP were significant in all models.

There appeared an interesting result in the final set of analyses examining the interaction models in men (Table 7). In the first model with LnTP as the dependent variable, there was a main effect of status, LnTP was lower in high social status men compared to low status men, and a main effect of ERI, LnTP was lower in men experiencing high ERI than low ERI. However, there was a status by ERI interaction. High status men experiencing high ERI had high LnTP compared to low status men with high ERI. Similarly, low status men with low ERI had higher LnTP than high status men with low ERI. This result stayed significant after adjusting for housework, alcohol, and HbA_{1c}.

In the interaction analysis including LnCortisol as the dependent variable, there were main effects of the number of children and the level of ERI and an interaction. Men with children experiencing high ERI at work had higher LnCortisol than did the other men. However, this result became insignificant when housework was included in the equation.

4. Discussion

This study examined physiological markers of stress in a healthy population as a function of psychosocial factors at home and at work. For the women in the study, few associations were found, whereas for the men, the associations were seen in support of the ERI model. However, the interpretation of these results must be done with caution, as the sample was small.

Social status was significant in regards to the dependent variables for men and women. For women, the associations were as expected (i.e., high social status was associated with high LnTP and low LnLF/HF). These associations indicate that women of high social status were positively related to high variability in the autonomic nervous system and relatively low sympathetic nervous system activity. For men, high

Table 4
Women. Estimates, standard errors (SE) and p-values from repeated measures ANCOVAs using LnTP, LnHF, LnLF/HF, and LnCortisol as the dependent variables, and each psychosocial factor (measured in 2002) one at a time as the independent variable. Age, year (Factor 0 or 1), and time of measurement included in all analyses. *p<0.05; **p<0.01.

	Ln TP		LnHF		Ln LF/HF		LnCortisol	
2002	Estimate ($\times10^{-2}$)	SE ($\times10^{-2}$)	Estimate ($\times10^{-2}$)	SE ($\times10^{-2}$)	Estimate ($\times10^{-2}$)	SE ($\times10^{-2}$)	Estimate ($\times10^{-2}$)	SE ($\times10^{-2}$)
Year	30.2	17.1	18.4	20.8	19.9	17.5	34.3**	8.4
Age	−3.1*	1.4	−4.1**	1.6	−0.5	0.1	−0.9	0.5
Social status	32.3*	24.9	46.3*	22.2	−27.3*	12.6	−3.2	7.4
Children	20.0	17.7	−9.0	25.1	17.1	15.0	−6.0	8.2
Hours housework	9.0	12.4	18.2	16.0	−13.1	10.3	−0.07	4.9
Weekly work hours	−0.3	1.4	−3.8	1.8	−1.2	1.2	−0.2	0.6
Lack of control	−1.5**	0.4	−0.7	0.7	0.7	0.4	−0.2	0.2
Demands	0.01	0.4	−0.3	0.6	−0.04	0.3	−0.2	0.2
Lack of support	−0.5	0.5	−0.9	0.6	0.8*	0.4	0.1	0.2
Effort	−0.06	0.2	0.4	2.7	−1.6	1.6	−1.0	0.9
Reward	−0.7	1.2	−0.3	1.8	−1.6	1.1	−0.4	0.6
ERI	4.6	28.0	1.1	41.2	23.2	23.3	−7.4	16.2

Table 5
Men. Estimates, standard errors (SE) and p-values from repeated measures ANCOVAs using LnTP, LnHF, LnLF/HF, and LnCortisol as the dependent variables, and each psychosocial factor (measured in 2002) one at a time as the independent variable. Age, year (Factor 0 or 1), and time of measurement included in all analyses. *p<0.05; **p<0.01.

	Ln TP		LnHF		Ln LF/HF		LnCortisol	
2002	Estimate ($\times10^{-2}$)	SE ($\times10^{-2}$)	Estimate ($\times10^{-2}$)	SE ($\times10^{-2}$)	Estimate ($\times10^{-2}$)	SE ($\times10^{-2}$)	Estimate ($\times10^{-2}$)	SE ($\times10^{-2}$)
Year	82.9**	19.9	121.0**	34.8	13.7	14.0	5.4	11.5
Age	−5.9**	1.3	−4.1*	1.8	1.1	0.8	−5.4	0.6
Social status	−54.0*	2.0	−71.3**	22.6	4.1	14.6	29.3**	7.6
Children	−39.3	25.8	52.5	30.9	33.7	16.3	−28.3**	1.2
Hours housework	−6.1	18.1	33.9	18.6	−27.7**	9.8	−26.3**	4.3
Weekly work hours	3.6	2.2	4.2**	1.1	−4.8**	1.0	−0.5	0.9
Lack of control	0.3	0.7	0.9	0.8	−0.2	0.5	−0.1	0.4
Demands	−1.8	0.5	−0.05	0.6	0.3	0.3	0.4	0.2
Lack of support	−0.7	0.4	−1.2*	0.4	0.08	0.3	−0.1	0.3
Effort	−20.7	20.3	4.2	2.8	−0. 5	10.5	2.9*	1.2
Reward	4.5**	1.6	2.2	2.0	0.6	10.2	−0.1	0.9
ERI	−72.5*	33.8	−53.5	51.2	−13.7	23.4	40.5*	19.4

social status was associated with low LnTP (low variability) and high levels of cortisol. In this particular sample of 22 men, of whom none had unskilled jobs, the high social status was significantly related to "chronic stress" relative to low social status. These findings illustrate that the simultaneous measurement of HRV and cortisol may be important for understanding models of psychosocial illness.

As most of the participants who were still working in 2008 held the same jobs as they had in 2002, their exposure may not have changed. The answers to questionnaires administered at baseline may mirror daily life circumstances at later times.

Few studies have examined the associations between psychosocial factors and spectral analysis. A study by Collins et al. of the ECGs of 36 male workers during a 48-hour period showed significant associations between job strain, low decision latitude and low vagal tone (Collins et al., 2005). Among women, lack of control was associated with low LnTP and tended to be associated with increased LF/HF. This finding suggests that using only 15-minute ECG recordings (as done in the current study) should be sufficient to capture meaningful differences. We suggest that the reason why the Demand Control Model was not associated with HRV in the men is due to the homogeneity of the sample, instead of reflecting a true lack of association between the Demand Control Model and HRV.

Two studies have examined HRV and the ERI model. Hintsanen et al. found that high ERI was associated with low HRV for women, while no associations were found for men (Hintsanen et al., 2007). On the other hand, Vrijkotte et al. found ERI was associated with high heart rate and low vagal tone in men (Vrijkotte et al., 2000). In the present study, reward was associated with high LnTP and effort, and ERI was associated with the expected low LnTP for men but not for women and is consistent with the findings of Vrijkotte et al. Although the findings by Hintsanen et al. appear contradictory, it may be due to differences in samples. An association is only found if there is a sufficient variation in exposure and, of course, if the psychosocial factor in question is, in fact, seen as a stressor. Therefore, conflicting findings are frequent.

This study revealed several associations between psychosocial factors and levels of salivary cortisol in men. Having children and doing housework were associated with significantly lower levels of salivary cortisol, while ERI, in particular, was associated with significantly higher levels of salivary cortisol. Low levels of cortisol have been found in individuals suffering from posttraumatic stress syndrome (PTSD) (Yehuda et al., 1995). In this particular sample, no one suffered from PTSD and, therefore, the low levels of salivary cortisol in connection with family matters for the men seem to originate in a "high quality" of life, whereas the high levels of salivary cortisol in connection with ERI at work were interpreted as a result of a state of chronic stress.

Because the sample included more than twice the number of women than men, we expected to see more significant associations for the women. However, we found the opposite, as associations were

Table 6
Men. Results of repeated measures ANCOVAs including adjustment for covariates as mentioned below for each model and using LnTP and LnCortisol as the dependent variable.

LnTP	A : Adjustment for age, year of measurement and time of measurement		B: A + housework		C : B + alcohol		D: C + HbA_{1c}	
2002	Estimate ($\times10^{-2}$)	St. E ($\times10^{-2}$)	Estimate ($\times10^{-2}$)	St. E ($\times10^{-2}$)	Estimate ($\times10^{-2}$)	St. E ($\times10^{-2}$)	Estimate ($\times10^{-2}$)	St. E ($\times10^{-2}$)
Lack of control	0.3	0.7	0.3	0.7	−0.4	0.7	−0.9	0.8
Demands	−1.8	0.5	−0.3	0.6	−0.4	0.6	1.0	0.7
Lack of support	−0.7	0.4	−0.7	0.5	−1.2*	0.5	−1.3	0.5
Effort	−2.7	2.3	−3.2	2.4	−4.1	2.4	−1.7*	3.9
Reward	4.5**	1.6	4.3**	1.6	4.6*	1.8	7.4**	1.3
ERI	−72.5*	33.8	−76.0*	34.9	−81.5*	34.4	−173.6**	54.0
LnCortisol	**A**		**B**		**C**		**D**	
2002	Estimate ($\times10^{-2}$)	St. E ($\times10^{-2}$)	Estimate ($\times10^{-2}$)	St. E ($\times10^{-2}$)	Estimate ($\times10^{-2}$)	St. E ($\times10^{-2}$)	Estimate ($\times10^{-2}$)	St. E ($\times10^{-2}$)
Lack of control	−0.1	0.4	0.1	0.2	−0.07	0.4	−0.07	0.4
Demands	0.4	0.2	0.3*	0.3	0.6	0.4	0.6	0.5
Lack of support	−0.1	0.3	−0.4	0.3	−0.3	0.4	−0.3	0.4
Effort	2.9**	0.6	2.1*	0.7	3.3	2.1	4.2	3.8
Reward	0.5	0.7	−0.3	1.0	−0.3	1.1	−0.7	1.1
ERI	34.4*	13.7	34.0	20.6	37.0	27.4	29.4	31.2

*p<.05 **p<.01.

Table 7
Men. Estimates, standard errors (SE) and p-values from repeated measures ANCOVAs using LnTP as the dependent variable, and both social status and ERI (measured in 2002) and their interaction as independent variables. A: Age, year (Factor 0 or 1) and time of measurement included as covariates in all analyses; B: A + housework; C: B + alcohol; D: C + HbA_{1c}.

LnTP	A		B:A + housework		C:B + alcohol		D: C + HbA_{1c}	
2002	Estimate ($\times10^{-2}$)	SE ($\times10^{-2}$)	Estimate ($\times10^{-2}$)	SE ($\times10^{-2}$)	Estimate ($\times10^{-2}$)	SE ($\times10^{-2}$)	Estimate ($\times10^{-2}$)	SE ($\times10^{-2}$)
Social status	−229.2**	37.7	−230.2**	38.1	−222.3**	35.0	−192.2**	27.8
ERI	−340.0**	53.6	−353.2**	54.3	−347.9**	49.8	−386.1**	40.2
ERI* social status	305.6**	58.2	313.9**	58.7	300.2**	54.1	255.0**	42.4

* $p<0.05$ and ** = $p<0.01$.

fewer and more blunted for women compared to those for the men. This finding may be due to statistical chance, but it may also be caused by "the tend and befriend" response (Motzer and Hertig, 2004). The prominent stress theories used to explain the findings in this study were constructed by men and grounded in research on men. The current results indicate a need for studies on stress in women.

4.1. Strengths and limitations

The present study has several limitations, most importantly, the limited size of the sample. As only a small number of individuals were included, a sufficient difference in exposure may be lacking. A finding of no association in this study is not evidence that the factors included are not associated at all but may be a result of insufficient power. Furthermore, changes in the allostatic systems may be so subtle that they are missed if only small time windows are measured (HRV) or if only a few measurements are collected (cortisol) as in the present study. In addition, it is known that non-compliance with the research protocol for collecting salivary cortisol may be a problem. It is important that participants collect the first saliva sample precisely at awakening (Broderick et al., 2004; Kudielka et al., 2003). In our study, participants were instructed to write down the exact time of measurement and also to collect the sample at awakening while still lying in bed. We believe that this ensured a high level of compliance.

Lastly, the analysis strategy used in this study includes a risk of type 1 error by running 10 ANCOVAs (one for each psychosocial factor) for each gender. However, the concomitant finding of significant associations in the two dependent variables (LnTP and LnCortisol) implies that at least social status and the Effort Reward Model are of importance for development of chronic stress in men.

The strength of this study was the concomitant sampling of data from several kinds of psychosocial stressors and repeated data from the two allostatic systems.

4.2. Conclusion

The two hypotheses of the study were only partly confirmed. However, despite the small sample size, this study demonstrated that psychosocial factors impact levels of activity in the allostatic systems.

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PAPER IV

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Effort reward imbalance, and salivary cortisol in the morning

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ABSTRACT

Background: Effort reward imbalance (ERI) is suggested to increase risk for stress and is hypothesized to increase cortisol levels, especially the awakening cortisol response, ACR.
Methods: In 2006 and 2008, 480 individuals collected saliva samples at awakening and 30 min post-awakening. Mixed effects models with subject as a random effect and appropriate covariates were used to evaluate associations between the Effort Reward Model, and salivary cortisol at awakening (S0), and ACR.
Results: ERI was negatively and significantly associated with S0 for women and positively associated with ACR. S0 and ACR increased significantly from 2006 to 2008.
Conclusion: ERI was significantly associated with cortisol levels at awakening (inverse association) for women, and positively associated with ACR. The population experienced a significant increase in morning cortisol levels and ACR from 2006 to 2008, which may originate in a re-organization of the included work places.

1. Background

The Effort Reward Model (ERI-model), put forward by Siegrist in 1996, focuses on the social reciprocity in connection with work (Siegrist, 1996). The key issue is, that the effort spent in the job is rewarded not only by monetary reward but also by job security, promotion prospects and prestige. If this exchange is balanced, no harm will occur. However, if the individual experiences an imbalance between effort and reward at work (ERI; effort-reward-imbalance), he or she will be at risk of stress (Siegrist et al., 1990, 2004). ERI has been associated with self-rated health in a study in four post-communist countries (Pikhart et al., 2001), with employee well-being in ancillary health care workers (van Vegchel et al., 2001), with risk factors for ischemic heart disease like hypertension and total-cholesterol in men (Peter et al., 1998), and with increase in body mass index (Kivimaki et al., 2002). ERI has also been an independent predictor of IHD in different populations (Bosma et al., 1998; Kivimaki et al., 2002).

Cortisol levels change over a 24-h period and show large intra-individual differences. To date, researchers have used a number of different strategies for measuring and analysing salivary cortisol (Hansen et al., 2003, 2009; Hjortskov et al., 2004). For example, the awakening cortisol response (ACR; the difference in cortisol from awakening to some period after awakening) has been introduced as a measure of cortisol reactivity. The increase in cortisol levels in the morning is different from the circadian changes in cortisol (Wilhelm et al., 2007), and the size may be dependent on the anticipation of the new day (Kunz-Ebrecht et al., 2004; Wilhelm et al., 2007). Increased levels of cortisol during workdays compared to weekends suggest that work related factors increase the levels of cortisol (Kunz-Ebrecht et al., 2004; Schlotz et al., 2004). The significance of changes in cortisol secretion in the morning and during the 24 h is only partly described, and the results are mixed. Part of the literature suggests that having a “flat curve” including low ACR and high level of cortisol in the evening is associated with disease such as metabolic syndrome and cardiovascular disease (Bjorntorp et al., 1999; Bjorntorp and Rosmond, 2000; Kumari et al., 2011; Rosmond et al., 2003; Rosmond and Bjorntorp, 2000). Other studies suggest that a high ACR is associated with risk of atherosclerosis (Dekker et al., 2008; Eller et al., 2005), impaired physical functioning (Kumari et al., 2010), and incident depression (Adam et al., 2010). It has been suggested that while acute stress is associated with increased cortisol secretion, chronic stress during years might result in an “exhausted HPA-axis” and low cortisol secretion (Rosmond and Bjorntorp, 2000; Yehuda et al., 1996).

As both the ERI-model and salivary cortisol have been associated with different health measures, it is of interest to examine whether the ERI-model is also associated with salivary cortisol. If

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an association between the ERI-model and changes in the cortisol secretion is revealed, changes in cortisol secretion could be part of the patho-physiological pathway between the ERI-model and IHD. However, the results from research on the ERI-model and salivary cortisol have been mixed. Most studies have failed to reveal an association between ERI and different measures of salivary cortisol (Bellingrath et al., 2008; Hanson et al., 2000; Harris et al., 2007; Steptoe et al., 2004). Bellingrath et al. examined cortisol in blood and saliva in connection with the Trier Social Stress Test and found cortisol secretion associated with overcommitment but not with effort, reward or ERI (Bellingrath et al., 2008). Hanson et al. used a semi-random sampling method according to which the saliva samples were collected when a palm-top computer beeped (between 8:00 a.m. and 10:30 p.m.) (Hanson et al., 2000). As cortisol secretion has the described large diurnal variation it seems not surprising that possible subtle changes in connection with ERI-condition were not revealed. The two studies by Harris et al. (2007) and Steptoe et al. (2004) included "state-of-the-art" ACR-measurements, and still no associations between ERI and ACR were revealed. Eller et al. (2006) found that ERI was associated with increased cortisol levels in the morning in both sexes in a cross sectional as well as in a prospective design, whereas Maina et al. (2009) found an inverse association between ERI and ACR.

2. Aim of the study

The aim of the present study was to examine the relationship between the ERI-model, and levels of salivary cortisol in the morning over a 2-year period in a Danish population of public sector employees. The hypothesis was that high effort, low reward, and high ERI are associated with high ACR.

3. Methods and participants

The participants were a sub-sample of the study-population in a study on possible changes in health in relation to a huge re-organization of the public administration in the Danish counties and municipalities that took place on January 1st 2007 (Netterstrom et al., 2010). In November 2004, Statistics Denmark identified 2030 potential participants through an extraction of data based on places of employment and salary code. The identified participants were employed in the public administration in one of the five municipalities or the two counties included in the study. Four municipalities and one of the counties were merging with others, while one municipality and one county remained unmerged. In spring 2006, the identified employees were invited to participate in the questionnaire study, and 1379 individuals returned the questionnaire (response rate 68%). Of these, 262 had left the work place of interest, i.e. had retired or taken a job in the private sector, and were therefore excluded from the study. Along with the invitation to participate in the questionnaire study, the individuals also received an invitation to participate in the sub-study including biological measures. Of the remaining 1117 individuals, 502 individuals volunteered to participate in the sub-study on biomarkers. The 616 non-participants and the 502 participants did not differ significantly in age, sex, training, perceived stress or sick leave in 2005.

The participants were clinically examined between August and November in 2006. Exclusion criteria were not being able to understand and speak Danish and not being motivated to comply with the sampling instructions, but did not include any medical conditions or health criteria. At the examination, the participants were instructed to collect two saliva samples: at awakening and 30 min hereafter on a working day. Thirty-one participants did not deliver saliva samples at all. In 2008, the procedure was repeated including 391 individuals of whom 369 delivered two saliva samples. The participants were examined in the same order and in the same months as in 2006 and the follow-up time was approximately 2 years for every one. As in 2006, the sampling of saliva in 2008 took place on a working day at awakening and 30 min hereafter. Three hundred fifty-six participants (255 women and 101 men) delivered all four saliva samples and information on sampling time, and in total 480 participants were represented with at least 2 saliva samples.

The regional Committee on Biomedical Research Ethics approved the study and all procedures were carried out with adequate understanding and written consent of the subjects.

3.1. Cortisol

The equipment for the saliva samples (2 Salivette®, Saarsted, cotton tampons) was given to the participants at the clinical examination together with written instructions about sampling. The participants were asked to write down the exact time at which each sample was collected. The samples were kept frozen at −80 °C until analysis at Denmark's National Research Centre for the Working Environment. The same method for measuring cortisol in saliva was used in both years. The assay used for the determination of cortisol in saliva was a competitive RIA (Spectria Cortisol Coated Tube RIA) purchased from Orion Diagnostica, Espoo, Finland. The analysis was carried out according to the manufacturer's instructions. The cross-reactivity to cortisone was <0.2%. A 1470 Wizard gamma counter (Wallac, Turku, Finland) was used for measuring radioactivity. A method evaluation of certified reference material in water showed no bias in the method, i.e. recovery was 97% [CI: 94%; 100.9%]. The limit of detection (LOD) was 1.59 nmol/l. Between run coefficients of variation were 19% at 11.5 nmol/l and 16% at 49.2 nmol/l (Hansen et al., 2003).

Salivary cortisol at awakening was labelled S0. The difference between salivary cortisol 30 min after awakening and S0 was labelled ACR.

3.2. The ERI-model

The ERI-model was measured by a part of a Danish version of the original questionnaire constructed by Siegrist et al. (Weyers et al., 2006). The participant's degree of effort was evaluated by four questions (time pressure due to a heavy work load, interruptions, experiencing a more demanding work over years, and pressure to work overtime) (Cronbach's alpha = 0.75). Reward was evaluated by 7 questions: Two statements considering esteem (respect from superiors and at work in general), 3 statements considering job promotion (promotion and work prospects, wage) and 2 statements considering job security (experiencing an undesirable change and risk of being laid off) (Cronbach's alpha = 0.82). Answers were given on a five point scale: 'disagree', 'agree and I am not at all distressed', 'agree and I am somewhat distressed', agree and I am distressed' or 'agree and I am very distressed'. The answers yielded from 1 to 5 points, 5 points corresponding to the highest level of effort or reward. The points were summed up for the two dimensions; i.e. effort and reward, and ERI was calculated as effort/(reward × 4/7). The correction factor 4/7 is included, as the two dimensions do not include the same number of questions (Siegrist et al., 2004).

3.2.1. Covariates

All variables were measured in both 2006 and 2008. The variable 'year' was coded as 0 = 2006, and 1 = 2008. 'Time of awakening' (which is the time of measurement for S0) was measured as hours after midnight. According to answers on use of medicine, the variable 'Medicine use' was coded as 'healthy' = no medicine or occasionally use of painkillers and 'medicated' = daily use of medicine. The following physiological and lifestyle variables were used as covariates: age, waist–hip ratio (waist width/hip width), total-cholesterol and HbA_{1c} in the blood, alcohol consumption (drinks/week), tobacco (g/day) and degree of physical activity (0 = passive or light physical activity for up to 4 h/week and 1 = more intensive physical activity for more than 4 h/week).

3.2.2. Further variables of interest

Perceived stress was measured in the following manner. The participants were presented for the following sentence in the questionnaire: "Stress" is a condition characterised by unrest, agitation or anxiety and/or sleeping problems. Do you experience stress at the moment? The answers were dichotomized in "no" ("not at all" – "a little") and "yes" ("to some extent" – "very much").

Sleeping quality was covered by four questions about frequency (daily, sometimes, once in a while, never) of a sleeping problem (problems falling asleep, interrupted sleep, troubled sleep and early awakening) during the recent 4 weeks.

3.3. Strategy for the analysis

The data were analysed using SPSS version 11 and R (R Development Core Team, 2009) using the nlme-package (Pinheiro et al., 2010; Pinheiro and Bates, 2000). A significance level of 0.05 was used. Due to the nature of the data (repeated intercorrelated data), a random intercept model, i.e. a mixed effects model with subject as the random effect, was used. We chose to conduct all analyses (1) for the total population including sex as a factor, and (2) separately for women and men because of the known sex differences in physiology, daily life and perceptions of stressors (Artazcoz et al., 2004; Barrett-Connor, 1997; Brezinka and Padmos, 1994). Furthermore, tests for interactions between sex and the ERI-model were performed in each analysis.

A negative ACR has been suggested as a sign of non-compliance or to be the result of being an "early awakener" (Wilhelm et al., 2007; Wüst et al., 2000). The percentage of individuals showing negative ACR was similar in the two years, 15.5% in 2006 and 15.8% in 2008 (women 14.3% and men 19.2%), and 17 individuals showed a negative ACR in both years (5.1%). Also, medication may influence salivary cortisol (Granger et al., 2009). The analyses were conducted for (A) 'the total population', and (B) 'the healthy sub-sample': the individuals without daily medicine use for whom ACR >0 nmol/l. This sub-sample included 237 women and 85 men of the 480 participants (67.0%).

Analysis part 1: Sex differences were examined by the use of Student's *t*-test for continuous variables and chi-square tests for dichotomous variables. Changes between years were examined by means of paired *t*-test for continuous variables, while McNemar's test was used for dichotomous variables.

Table 1
Demographic, physiological and psychosocial variables.

	Women (N = 352)				Men (N = 128)			
	2006		2008		2006		2008	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
The Effort Reward Model								
Effort	10.6*	3.6	10.1	3.2	9.9	3.3	9.7	3.5
Reward	26.7	5.7	26.5	6.1	26.4	5.8	26.8	5.7
ERI	0.76	0.41	0.75	0.44	0.72	0.39	0.71	0.44
Age	49.1*	8.7			51.7	9.5		
Waist–hip ratio	0.80*	0.07	0.81*	0.07	0.90	0.06	0.90	0.05
Cholesterol (mmol/l)§	5.3	1.0	5.6	1.0	5.3	1.0	5.5	0.9
HbA_{1c} (%)§	5.4	0.4	5.5	0.4	5.5	0.6	5.6	0.6
Smoking, yes (%)§	15.1	35.8	4.3	20.2	15.1	35.9	3.2	17.6
Cigarettes per day§	1.97	5.28	0.47	2.47	1.82	5.28	0.49	2.94
Alcohol (drinks/week)	6.1*	6.0	6.0*	6.2	10.8	8.9	11.1	9.6
Physical activity ≤4 h/week (%)§	51.8*	50.0	54.9*	49.9	38.1	48.8	38.1	48.8
Perceived stress, yes (%)	26.4*	44.1	30.4*	46.0	18.3	38.7	20.7	40.5
Early awakening, yes (%)	24.8*	43.2	22.4*	41.7	14.9	35.6	14.9	35.6

Abbreviations: HbA_{1c}: glycated haemoglobin; ERI: effort reward imbalance.
* $p < 0.05$ sex difference by Student's *t*-test (Chi-square test for dichotomous variables).
§ $p < 0.05$ change between years by paired *t*-test (McNemar's test for dichotomous variables)

Analysis part 2: Bivariate correlation analyses were conducted to examine correlation between ERI, sleep quality, frequency of early awakening and sampling time in 2006 and 2008.

Analysis part 3: A series of mixed effects analyses with individual as a random factor were conducted. All analyses were done with ln S0 and ACR as dependent variables, for the total population and for the healthy subpopulation, for both sexes jointly as well as each sex separately. In connection with the analysis using only the subpopulation, ACR was not normally distributed, and analysis showed that square root transformed ACR (sqrtACR) was to be preferred as the dependent variable. As a first step, groups of covariates were included sequentially as in a hierarchical regression in order to identify which variables to correct for when estimating the effect of the psychosocial variables. The first group of covariates included age, time of measurement, sex (when both sexes were analysed jointly) and ln S0 when analysing ACR/sqrtACR. The second group included lifestyle variables (tobacco, alcohol, and physical activity) whereas the third group included physiological variables (waist–hip ratio, cholesterol, and glycated haemoglobin). After identifying the relevant covariates, year was included as a factor and then each of the three psychosocial variables were included in three separate analyses to avoid multicollinearity.

Analysis part 4: As the estimates from the mixed effects models are difficult to interpret due to the transformations, fitted values of S0 (nmol/l) and ACR (nmol/l) were derived from the sex-specific models for each year and for two specified values of ERI (Tables 4A and 4B). We used ERI = 0.5 and ERI = 2.0. Physical activity was fixed at the low value (inactive/less than 4 h/week) and all other covariates were fixed at their median values (age = 52, time of measurement = 06:20, tobacco = 0, alcohol = 4 drinks/week for women, 9 drinks/week for men).

4. Results

All together, the population under examination included 352 women and 128 men working in the non-state administration of the public sector in Denmark. Approximately 90% were white-collar workers. Table 1 shows mean and standard deviations of demographic, physiological and psychosocial variables in the population for each sex and each year. Women reported significantly more effort in 2006 than the men did, but besides this difference the levels of the effort, reward, and ERI, were similar between sexes and did not change significantly between years. Mean ERI was 0.76 in women and 0.71 in men. The men were significantly older and more obese than the women, and men had larger alcohol consumption than the women. The proportion of smokers decreased significantly from 15% in 2006 to 3–4% in 2008. The level of physical activity did not change between the two years but was different between the two sexes as 53.1% of the women and 38.1% of the men reported a level of physical activity of up to 4 h/week. Mean levels of total-cholesterol and HbA_{1c} were within the reference values, but increased between the two years in both sexes. In both years and sexes the mean awakening time was approximately 06:20 h. Level of cortisol at awakening, S0, was (mean/SD) 9.42/5.78 and 10.00/6.15 nmol/l in women and men, respectively, in 2006. The percentages of individuals reporting perceived stress and bad sleeping quality were not significantly different from 2006 to 2008. The list of medication taken included medicine for hypertension and heart conditions, hormonal treatment (anticonception and thyreotoxin), sedatives, antidepressive medicine and sleeping pills.

The bivariate correlation analyses revealed that ERI in both years (and in both sexes) were significantly associated with bad sleeping quality and early awakening. However, ERI was not associated with sampling time.

The results of the hierarchical regressions are shown in Table 2A (total population) and Table 2B (healthy subpopulation). The basic covariates were significant in all cases except for sqrtACR for women in the healthy subpopulation. The addition of the lifestyle variables significantly improved the fit for ln S0 in all cases except for women in the healthy subpopulation, whereas the physiological variables were insignificant in all models. The changes in Akaike Information Criteria (AIC) support these findings. AIC is a measure of goodness of fit with better models having smaller AIC (Akaike, 1974). Thus a negative change in AIC in Tables 2A and 2B indicates an improvement. Based on this, we chose to include the basic variables and the lifestyle variables in all models; the conclusions based on ACR/sqrtACR do not change qualitatively if the lifestyle variables are excluded from the modelling.

Table 3A shows results (estimates, standard errors (SE) and significance) of the mixed effects analyses for the total population using ln S0 and ACR as the dependent variables. Results are given for both sexes jointly and for women and men separately. Table 3B shows corresponding results for the healthy subpopulation. In all cases, the outcome variable increases from 2006 to 2008 and in most cases the increase is significant. Effort is positively associated with ACR/sqrtACR in all models and the association is significant for the healthy subpopulation when both sexes are analysed jointly. Effort is negatively associated with ln S0 except for men where the effect is reversed. Reward is significantly positively associated with ln S0 for women in the total population. ERI is negatively associated with ln S0 except for men in the total population were the association is positive. The association is significant for women. ERI is positively associated with ACR/sqrtACR but not significant. The intrapersonal-correlation coefficients regarding ln S0 and ACR were 0.24 and 0.25, respectively, underlining the need for mixed effects modelling of these data. There were no significant

Table 2A
p-values and changes in AIC from successive tests of covariates for the total population. Basic covariates are age, time of measurement, sex (when analysing both sexes jointly) and in the case of ACR also ln S0. Basic covariates are tested against a null model with no covariates. Lifestyle variables are physical activity, tobacco and alcohol and the *p*-value/AIC-change is for addition of these to the basic covariates. Physiological variables are HbA_{1c}, waist–hip ratio and cholesterol and the *p*-value/AIC-change is for addition of these after the addition of the lifestyle variables.

	All (*N* = 480)		Women		Men	
	Change in AIC#	*p*	Change in AIC	*p*	Change in AIC	*p*
ln (S0)						
Basic covariates	−21.8	<0.001	−9.3	0.002	−8.3	0.003
Lifestyle variables	−14.4	0.001	−11.3	<0.001	−0.1	0.106
Physiological variables	2.3	0.296	2.1	0.276	−0.9	0.076
ACR						
Basic covariates	−108.4	<0.001	−67.8	<0.001	−33.8	<0.001
Lifestyle variables	3.6	0.486	1.8	0.239	2.5	0.321
Physiological variables	7.4	0.958	5.2	0.850	4.4	0.650

AIC: Akaike Information Criteria, a measure of goodness of fit with better models having smaller AIC

Table 2B
p-values and changes in AIC from successive tests of covariates for the healthy subpopulation. Basic covariates are age, time of measurement, sex (when analysing both sexes jointly) and in the case of sqrtACR also ln S0. Basic covariates are tested against a null model with no covariates. Lifestyle variables are physical activity, tobacco and alcohol and the *p*-value/AIC-change is for addition of these to the basic covariates. Physiological variables are HbA_{1c}, waist–hip ratio and cholesterol and the *p*-value/AIC-change is for addition of these after the addition of the lifestyle variables.

	All (*N* = 331)		Women		Men	
	Change in AIC#	*p*	Change in AIC	*p*	Change in AIC	*p*
ln (S0)						
Basic covariates	−9.2	0.002	−3.5	0.023	−4.5	0.015
Lifestyle variables	−8.4	0.002	−9.6	0.001	2.3	0.294
Physiological variables	4.8	0.771	2.3	0.290	0.0	0.109
sqrtACR						
Basic covariates	−3.0	0.027	4.6	0.494	−1.6	0.047
Lifestyle variables	5.8	0.976	5.8	0.894	4.2	0.604
Physiological variables	4.9	0.544	4.9	0.139	4.6	0.712

AIC: Akaike Information Criteria, a measure of goodness of fit with better models having smaller AIC

interactions between sex and the psychosocial variables in any of the models.

Tables 4A and 4B show estimated levels of S0 and ACR in women and men in the total population and the healthy sub sample, respectively. ACR increases by approximately 2 nmol/l from 2006 to 2008. Also S0 increases from 2006 to 2008 but only by 1–1.5 nmol/l or even less for the men in the healthy sub-sample. ACR increases with ERI; for men the increase is approximately 1 nmol/l whereas the increase for women is 0.5 nmol/l in the total population but almost 2 nmol/l in the healthy sub-sample. For women, S0 decreases by

Table 3A
Results of analysis part 1 using ln S0 and ACR, respectively, as the dependent variable for the total population including both sexes at the same time and with stratification on sex. Estimates, standard errors (SE) and level of significance of year and each of the psychosocial factors from mixed effects models correcting for age, time of measurement, sex (when analysing both sexes jointly) physical activity, alcohol, tobacco, ln S0 (when analysing ACR), and year (for the psychosocial factors).

	ln S0		ACR	
	Estimate ($\times 10^{-2}$)	SE ($\times 10^{-2}$)	Estimate ($\times 10^{-2}$)	SE ($\times 10^{-2}$)
Total population (*N* = 480)				
Year	10.79**	3.49	202.99***	42.75
ln Effort	−3.99	5.77	100.57	70.12
Reward	0.60#	0.34	−1.43	4.15
ln ERI	−6.32	4.04	51.54	49.14
Women (*N* = 352)				
Year	9.29*	3.97	205.85***	50.87
ln Effort	−9.97	6.65	66.25	83.21
Reward	0.99*	0.39	−1.88	4.94
ln ERI	−11.72*	4.68	38.28	58.87
Men (*N* = 128)				
Year	15.51*	7.16	196.27*	79.27
ln Effort	13.19	11.72	171.76	134.06
Reward	−0.34	0.71	0.08	7.79
ln ERI	7.60	8.16	75.36	92.11

# $p < 0.10$
* $p \le 0.05$.
** $p \le 0.01$.
*** $p \le 0.001$.

Table 3B
Results of analysis part 1 using ln S0 and sqrtACR, respectively, as the dependent variable for the sub population including both sexes at the same time and with stratification on sex. Estimates, standard errors (SE) and level of significance of year and each of the psychosocial factors from mixed effects models correcting for age, time of measurement, sex (when analysing both sexes jointly) physical activity, alcohol, tobacco, ln S0 (when analysing sqrtACR), and year (for the psychosocial factors).

	ln S0		sqrtACR	
	Estimate ($\times 10^{-2}$)	SE ($\times 10^{-2}$)	Estimate ($\times 10^{-2}$)	SE ($\times 10^{-2}$)
Total population (*N* = 331)				
Year	9.47*	4.65	34.37***	9.23
ln Effort	−11.27	7.26	30.22*	14.67
Reward	0.50	0.43	−0.58	0.88
ln ERI	−8.61#	5.06	17.22#	10.24
Women (*N* = 242)				
Year	9.63#	5.16	38.59***	10.68
ln Effort	−16.68#	8.44	29.56#	17.60
Reward	0.79	0.51	−1.36	1.06
ln ERI	−13.21*	5.99	22.77#	12.48
Men (*N* = 89)				
Year	10.49	9.83	25.75	17.89
ln Effort	0.54	14.71	40.17	28.45
Reward	−0.003	0.87	1.05	1.66
ln ERI	−0.33	9.98	8.92	19.26

# $p < 0.10$.
* $p \le 0.05$
** $p \le 0.01$
*** $p \le 0.001$.

Table 4A
Estimated values of S0 and ACR (mean (95% confidence intervals)) in the total population in 2006 and 2008 fitted by means of the mixed effect models used to estimate the associations in Table 3A.

	2006		2008	
	Mean	CI	Mean	CI
Women				
S0 (nmol/l)				
ERI = 0.5	8.76	(8.00–9.57)	9.72	(8.84–10.67)
ERI = 2.0	7.45	(6.50–8.51)	8.28	(7.18–9.49)
ACR (nmol/l)				
ERI = 0.5	7.25	(6.11–8.38)	9.16	(7.94–10.38)
ERI = 2.0	7.78	(6.06–9.50)	9.69	(7.91–11.47)
Men				
S0 (nmol/l)				
ERI = 0.5	7.56	(6.49–8.76)	8.74	(7.46–10.17)
ERI = 2.0	8.44	(6.59–10.66)	9.75	(7.58–12.35)
ACR (nmol/l)				
ERI = 0.5	7.81	(5.98–9.63)	10.03	(8.09–11.97)
ERI = 2.0	8.88	(6.16–11.60)	11.10	(8.27–13.93)

Table 4B
Estimated values of S0 and ACR (mean (95% confidence intervals)) in the subpopulation in 2006 and 2008 fitted by means of the mixed effect models used to estimate the associations in Table 3B.

	2006		2008	
	Mean	CI	Mean	CI
Women				
S0 (nmol/l)				
ERI = 0.5	8.31	(7.47–9.26)	9.27	(8.25–10.43)
ERI = 2.0	6.92	(5.85–8.19)	7.72	(6.49–9.19)
ACR (nmol/l)				
ERI = 0.5	6.59	(5.48–7.81)	8.62	(7.20–10.15)
ERI = 2.0	8.32	(6.39–10.49)	10.57	(8.31–13.10)
Men				
S0 (nmol/l)				
ERI = 0.5	7.21	(6.03–8.61)	7.98	(6.63–9.61)
ERI = 2.0	7.17	(5.33–9.65)	7.95	(5.83–10.82)
ACR (nmol/l)				
ERI = 0.5	8.19	(6.32–10.30)	9.82	(7.57–12.37)
ERI = 2.0	8.91	(5.86–12.60)	10.61	(7.02–14.95)

approximately 1.5 nmol/l when ERI increases. For men in the total population increasing ERI increases S0 by 1 nmol/l, whereas the effect in the healthy subpopulation is negligible.

5. Discussion

This study examined the significance of the ERI-model for salivary cortisol in the morning measured with 2 years between. The analyses were conducted for the total population and for a sub-sample consisting of healthy individuals with morning-rise in cortisol.

Studies of the significance of a psychosocial factor for the physiological stress response demand a large variation in the size of the psychosocial factor in question (Eller et al., 2009). Effort and reward were measured by use of 4 and 7 items respectively. However, the original questionnaire developed by Siegrist et al. (2004) includes 5 and 11 items respectively. This means, that the scores for effort and reward cannot be compared between studies, but it may be possible to compare measures of ERI. In four of the five population studies included in the paper by Siegrist et al., ERI was 0.53 to 0.67, while ERI in the fifth study was 1.11 (Siegrist et al., 2004). Therefore, ERI in this study is believed to be high (0.76 in women and 0.71 in men). Also, there was a large variation in ERI (0.20–3.72 in women and 0.21–2.70 in men). The population included individuals, who had been stressed for a long time, but also individuals with only a minor load.

Only a couple of studies have examined the association between the ERI-model and salivary cortisol (Bellingrath et al., 2008; Eller et al., 2006, 2011; Hanson et al., 2000; Harris et al., 2007; Steptoe et al., 2004) and only in one study a positive association between ERI and ACR was found (Eller et al., 2006, 2011). Cortisol level at awakening was not associated with effort, reward or ERI in the two studies, which examined this (Eller et al., 2006; Harris et al., 2007). The association between cortisol-rise in the morning and ERI was studied by Steptoe et al. but was not demonstrated (Steptoe et al., 2004). Bellingrath et al. did not find an association between the ERI-model and cortisol-secretion during the day. However, in individuals experiencing low reward the reactivity in the HPA-axis after taking dexamethasone was decreased (Bellingrath et al., 2008). We found a positive but non-significant association between ERI and ACR.

Analyses similar to the ones reported for the total population without medicated individuals lead to similar results as those reported for the total population. Hence, we were not able to show that medication influences levels of cortisol. We conjecture that this is due to the amount and type of medication in this population. Although several medications may affect saliva cortisol (Granger et al., 2009), we suggest to conduct analyses with and without medicated participants as we have done in this study as exclusion of medicated participants will reduce power.

Our sub-sample included only participants with a cortisol-rise (ACR > 0), as it has been suggested that negative ACR may indicate non-compliance (Kudielka et al., 2003). In the current study, the percentage of negative ACR was approx. 15.7%, and similar but slightly smaller than reported previously (Hansen et al., 2003; Wüst et al., 2000). The fact that this number was unchanged between the 2 years suggests that the population was instructed and complied in the same manner each year. Verbal and written instruction may not ensure correct saliva sampling (Broderick et al., 2004; Kudielka et al., 2003).

The variable 'year' was of huge significance to cortisol levels as the measurements were significantly larger in 2008 than in 2006. Several factors may be considered when interpreting this result. First of all, the significance of 'year' is on the population level. This means that individual challenges such as divorce or death of a family member, which could cause physiological stress and may increase cortisol levels, would be unlikely as the main cause of our finding as these events do not occur at the population level. A change in season for the saliva sampling might influence ACR due to different light exposure in the morning (Persson et al., 2008). To eliminate such seasonal change in cortisol levels, the participants were examined during the same months of the year in 2006 and 2008. The difference could also have been the result of changes in methods, but the methods for sampling and analysing the saliva were unchanged between the years. Also, 'year' could be a different manner to measure 'age', but both variables were included in the analyses and an effect of age was not revealed. The world wide financial crisis hit Denmark in the spring 2008 and may have had implications for the economy of the participants. Financial strain has been associated with risk of recurrent IHD in women (Georgiades et al., 2009), and with increased ACR in men (Steptoe et al., 2005). In line with such effects is a possible effect of job-insecurity, which has been shown to increase risk of hospitalization and heart disease (Ferrie et al., 1998; Iversen et al., 1989). The most prominent change in life circumstances shared by the participants in the present study was the major reorganization of non-state public offices implemented on January 1st 2007. Before the reorganization the non-state public sector in Denmark had two levels of administration: the counties and the municipalities. After reorganization most of the 14 counties and 275 municipalities merged into larger units; the 14 counties were merged into 5 regions and the 275 municipalities were merged into 98 municipalities. The administration of a number of tasks was moved from the counties to the municipalities. Several problems originating in the changes were to be taken care of during 2007 and 2008, but of course, the changes were prepared during 2006 in the form of a large number of meetings and discussions. The perceived stress score did, however, not increase significantly between the 2 years and the ERI scores were unchanged. On the other hand, the organizational changes may have led to changes in other psychosocial work-stressors than those measured by the ERI-model, such as increasing demands or lack of control, feeling of injustice, lack of predictability or other factors in the psychosocial work environment (Eller et al., 2009; Kristensen et al., 2005). This could have caused the increase in cortisol levels between the 2 years. Under any circumstances, an organizational change like the one described must be characterised as including novelty, taxing social interactions and limited predictability, all being outlined as being able to evoke HPA activation (Dickerson and Kemeny, 2004; Hanson et al., 2000). It is tempting to interpret the findings as demonstrating the importance of a stable work environment including predictability and social support.

The clinical importance of the findings may be discussed as the changes in cortisol and ACR are small and within the normal range of cortisol. On the individual level, the changes are not of importance. However, the changes are changes in the mean cortisol levels of the cohort and may therefore be relevant to aspects of public health (Bjorntorp et al., 1999; Dallman et al., 2005; Rose, 1992). Only one study (Eller et al., 2005) has examined a direct association between ACR and intima media thickness (IMT), a risk marker of myocardial infarction and stroke (Lorenz et al., 2007). In the study by Eller et al., ACR measured as the increase in cortisol during the first 20 min after awakening was positively associated with progression of IMT over 4 years in women but not with progression of IMT in men (Eller et al., 2005). In that particular study, IMT increased 0.09 mm/year per nmol/l increase in ACR in women. In the current study ACR increased about 2 nmol/l in connection with the psychosocial stressors (i.e. organizational changes). According to the meta-analysis by Lorenz et al. the age- and sex-adjusted relative risks were 1.15 (95% CI, 1.12–1.17) for myocardial infarction and 1.18 (95% CI, 1.16–1.21) for stroke per 0.10 mm common carotid artery IMT difference (Lorenz et al., 2007). The increased values of ACR may therefore be of clinical relevance.

The associations between ERI and S0 versus between ERI and ACR were inverse, i.e. increased ERI was associated with low S0 and high ACR. It is possible that the experience of ERI leads to poor sleep quality and a too early saliva sampling. Increasing ERI was not significantly associated with a more early sampling time, but in both years (and both genders) increasing ERI was associated with decreasing sleep quality and frequency of early awakening during the recent 4 weeks.

5.1. *Strengths and limitations*

The study is relatively large even if only 101 men were examined twice. Also, the large variation in ERI, as well as the repeated measurement of all variables, is a strength. As the data were intercorrelated (repeated measurements on the same individuals) the mixed effects models were including "subject" as a random effect. This is important as this incorporates individual specific cortisol levels and allows generalization of the results to other samples comparable to this sample (Zuur et al., 2009).

We have only measured ACR on a single working day in the 2 years. The reproducibility of ACR has been reported as being high (Pruessner et al., 1997), but a recent case study reveals large differences in the same individual (Stalder et al., 2009). Therefore, measurement on at least two days should be preferred in future studies; also more measurements on the same day, i.e. at awakening, after 30 min, 45 min and 60 min, are preferable (Adam and Kumari, 2009). A drawback of this study is the lack of validation of the time of awakening and the time of sampling. Compliance with sampling procedure, i.e. the first sampling immediately after awakening, is decisive for ACR (Kudielka et al., 2003). Approximately 15% of the population in this study demonstrated a negative ACR. Since this may be a sign of non-compliance, we have chosen to present results from analyses based on the total population as well as results based only on the sub-sample with positive ACR. The results are qualitatively similar, and the present results should therefore not be understood as suggesting that a negative ACR is simply reflecting non-compliance with the sampling protocol. In this study, we have focused on the ERI-model and its connection to ACR and awakening cortisol. However, several other variables may be of importance for cortisol levels. Among others, alcohol use the night before saliva sampling, sleep length and quality may be of significance to levels of salivary cortisol in the morning (Dahlgren et al., 2009; Kumari et al., 2009; Randler and Schaal, 2010). This study informs only on salivary cortisol in the morning.

6. Conclusion

ERI was significantly associated with cortisol levels at awakening (inverse association) for women, and positively associated with ACR. The population experienced a significant increase in morning cortisol levels and ACR from 2006 to 2008, which may originate in a re-organization of the included work places.

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Effort reward imbalance is associated with vagal withdrawal in Danish public sector employees

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ABSTRACT

Objectives: The current study analyzed the relationship between psychosocial work environment assessed by the Effort Reward Imbalance Model (ERI-model) and heart rate variability (HRV) measured at baseline and again, two years later, as this relationship is scarcely covered by the literature.
Methods: Measurements of HRV during seated rest were obtained from 231 public sector employees. The associations between the ERI-model, and HRV were examined using a series of mixed effects models. The dependent variables were the logarithmically transformed levels of HRV-measures. Gender and year of measurement were included as factors, whereas age, and time of measurement were included as covariates. Subject was included as a random effect.
Results: Effort and effort reward imbalance were positively associated with heart rate and the ratio between low frequency (LF) and high frequency power (HF) and negatively associated with total power (TP) and HF. Reward was positively associated with TP.
Conclusion: Adverse psychosocial work environment according to the ERI-model was associated with HRV, especially in the form of vagal withdrawal and most pronounced in women.

1. Background

1.1. The Effort Reward Imbalance Model (ERI-model)

In connection with research on work stress and possible health effects a key issue is how to define and measure the exposure. The ERI-model, put forward by Siegrist in 1996, focuses on the social reciprocity in connection with work. Social reciprocity is seen as a fundamental principle of interpersonal behavior characterized by mutual cooperative investments based on the norm of return expectancy where efforts are equalized by respective rewards (Siegrist and Peter, 1994). For the modern human being, work life plays a major role in life and for identity. According to the ERI-model, work-stress occurs if you experience an imbalance between the effort spent at work and the reward gained (ERI). 'Effort' is mostly seen as the psychological efforts in doing the job tasks well and delivering a good result, but sometimes physical efforts at work are included. The dimension 'reward' is broadly defined as not only monetary reward (wage) but also by job security, promotion prospects and prestige. If you are working at a low wage but in a prestigious job with great promotion prospects this is not supposed to increase your risk of stress. On the other hand, if the promotion prospects fail you may experience ERI and develop stress (Siegrist et al., 1990; Siegrist et al., 2004; Siegrist and Peter, 1996).

ERI has been associated with several measures of health: with self-rated health (Pikhart et al., 2001), with employee wellbeing (van Vegchel et al., 2001), and with risk factors for ischemic heart disease like hypertension, total-cholesterol, and increase in body mass index (Kivimaki et al., 2002; Peter et al., 1998). Furthermore, ERI has been an independent predictor of ischemic heart disease in different populations (Bosma et al., 1998; Kivimaki et al., 2002).

1.2. The autonomic nervous system and health

The patho-physiological pathway between psychosocial stressors like ERI and stress-related disease is suggested to include a repeated or chronic activation of the autonomous nervous system (ANS), leading to increased activity of the sympathetic nervous system and withdrawal of vagal activity (McEwen and Seeman, 1999). The joint influences on the heart of the sympathetic and parasympathetic nervous systems may be measured by recording over several minutes the temporal variation in the time between successive heart beats using various measures in the time domain (variation per unit time) or in the frequency domain (power in specific frequency bands)

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as described by (Kristal-Boneh et al., 1995). A recent review on the autonomic nervous system and cardiovascular risk factors concluded that decreased vagal activity (vagal withdrawal) is a risk factor for all-cause mortality and that work stress is associated with decreased variability in heart rate (HRV) (Thayer et al., 2010). In several studies, low TP predicted morbidity and mortality (Dekker et al., 2000; Malik, 1996; Thayer and Lane, 2007). Elevated sympathetic tonus increased the risk of hypertension (Charkoudian and Rabbitts, 2009), and dysfunction of the autonomic nervous system may be involved in several diseases related to chronic stress (Charkoudian and Rabbitts, 2009; Esler, 2010; Thayer et al., 2010).

1.3. The research project

January 1st 2007, a major organizational change in the administration of the municipalities and counties in Denmark was implemented. A questionnaire study on possible changes in health and well being in relation to the organizational change was conducted among employees in the public administration in the five municipalities and the two counties included in the study (Netterstrom et al., 2010). The working conditions during and after the re-organization were straining including job insecurity and large demands. Therefore, we found this population especially useful in connection with research on the ERI-model.

1.4. Aim and hypothesis of the study

The aim of the present study was to examine the relationship between the ERI-model, and HRV over a 2-year period in a Danish population of public sector employees. The hypothesis was that self reported high effort, low reward, and ERI is associated with low levels of TP and HF, as well as with high LF/HF.

2. Participants and methods

The participants were a sub-sample of the study-population in a study on possible changes in health in relation to the reform of the public administration in the Danish counties and municipalities taking place January 1st 2007 (Netterstrom et al., 2010). The regional Committee on Biomedical Research Ethics approved the study and all procedures were carried out with adequate understanding and written consent of the subjects. In spring 2006, employees in the public administration in 5 municipalities and 2 counties in Denmark were invited to participate in the questionnaire study ($N=1379$). Statistics Denmark located the eligible individuals using information on employment in 2004 and took care of the first contact as to keep confidentiality. Along with the invitation to participate in the questionnaire study, the individuals also received an invitation to participate in the sub-study including biological measures. At the time of contact, a number of individuals had left the work place of interest and they were excluded of the study ($N=262$). However, 502 individuals volunteered to participate in the sub-study on biomarkers (44.9%). The 616 non-participants and the 502 participants did not differ significantly in age, gender (69.8 versus 72.7% women), training, perceived stress or sick leave in 2005.

The participants were clinically examined between August and November in 2006. A total of 236 individuals had their electro cardiogram (ECG) taken during approximately 18 h starting during the clinical examination and ending the next morning. The clinical examinations took place between 9:00 and 12:00 at the work place. At the end of the examination, the ECG-logger was attached and the participant instructed to avoid bathing but otherwise behave as usual. The following morning, the participant was allowed to remove the logger and return the logger at the work place along with a short diary. In 2008, the procedure was repeated including 195 individuals. Each year, the participants were asked to keep a short diary telling when they were seated reading or watching television at home after work. In 2006, 205 individuals delivered adequate diary information (seated rest) concomitant with technically satisfying ECGs during the relevant 15 min period, and were included in the study. In 2008, 170 were included by the same criteria. Finally, 170 women and 61 men delivered contemporary data on seated-rest – HRV and psychosocial work environment in either 2006 ($N=77$) or 2008 ($N=28$) or both years ($N=126$).

Basic demographic, physiological and psychosocial measures are shown in Table 1.

2.1. Heart rate variability (HRV)

TP (ms^2) is defined as the variance of all NN intervals (NN = adjacent sinus rhythm heart beats) (Malik, 1996). A higher heart rate is associated with lower TP, i.e. short NN intervals leaves little space to variation. The spectral power band between 0.15 and 0.4 Hz, the high frequency band (HF), is caused by changes in the parasympathetic activity, whereas the power spectral band between 0.04 and 0.15 Hz, i.e., the low frequency band (LF), includes both sympathetic and parasympathetic activity. The ratio between LF and HF is thought to be a proxy for the balance between the sympathetic and the parasympathetic tonus. An increased LF/HF ratio has been considered as an indication of increased sympathetic tone (Malik, 1996). The origin of very low frequency changes (VLF: 0–0.04 Hz) is unclear, but they are thought to reflect slow thermoregulatory oscillations and should not be interpreted in analyses of brief ECGs (Malik, 1996).ECGs were collected during approximately 18 h. However, this study included only averaged values of spectral analysis during 3 times 5 min (in total 15 min) of sitting while reading or watching television. Starting time of measurement [mean (SD)] was 20:32 (1:48) and 20:40 (1:31) in 2006 and 2008, respectively.

The ECG data were recorded by a 3-lead LifeCard CF Holter monitor (Delmar Reynolds Medical Inc., Irvine, CA, USA). Only recordings of sinus rhythm were included in the study. Artefacts and non-normal beats in the ECG segments were autodetected by commercial software and verified by visual inspection. Before calculating HRV metrics, the RR-intervals were filtered for possible outliers (ectopic beats, falsely detected beats, and missed beats) using the algorithm published by Moody (Moody, 1993), resampled with a frequency of 4 Hz and linearly detrended.

Table 1
Demographic, physiological and psychosocial variables in participants measured in 2006 or 2008. Women: $N=140$ in 2006 and $N=123$ in 2008. Men: $N=52$ in 2006 and $N=41$ in 2008. * $p<0.05$: gender difference by Students *t*-test.

		Women		Men	
		Mean	SD	Mean	SD
The Effort Reward Imbalance Model					
Effort	2006	10.7	3.7	10.4	3.5
	2008	10.2	3.2	10.4	4.1
Reward	2006	26.3	6.5	25.9	6.5
	2008	26.3	6.3	25.8	6.5
ERI	2006	0.82	0.52	0.81	0.49
	2008	0.78	0.50	0.84	0.57
Training:					
Manual (%)		25.5		10.0	
Short non-manual (%)		19.6		13.3	
Advanced non-manual (%)		54.8		76.6	
Age (years)		49.3*	8.8	51.2	9.7
Percent smokers		15.1		14.8	
Alcohol (drinks/week)		6.1*	6.0	10.8	8.9
Percent physical activity 2–4 h per week		45.5		34.4	
Waist hip ratio		0.80*	0.07	0.90	0.06
Blood pressure, systolic (mmHg)		136.1*	21.1	144.6	18.6
Total cholesterol (mmol/l)		5.3	1.0	5.5	0.6
HbA_{1c} (%)		5.4	0.4	5.5	0.6

Abbreviations: ERI: effort reward imbalance; HbA_{1c}: glycated hemoglobin.

As the data were not normally distributed, all dependent variables were logarithmically transformed before analysis. The four transformed dependent variables were: Heart rate (HR), TP, HF, and LF/HF labeled lnHR, lnTP, lnHF, and ln(LF/HF), respectively.

2.2. Measurement of exposure in 2006 and 2008

The ERI-model was measured according to the questionnaire by Siegrist et al. (Siegrist et al., 2004). The participant's degree of effort was evaluated by four questions (time pressure due to a heavy work load, interruptions, experiencing a more demanding work over years, and pressure to work overtime) (Cronbach's alpha = 0.75). Reward was evaluated by 7 questions: Two statements considering esteem (respect form superiors and at work in general), 3 statements considering job promotion (promotion and work prospects, wage) and 2 statements considering job security (experiencing an undesirable change and risk of being laid off) (Cronbach's alpha = 0.82). Answers were given on a five point scale: 'disagree', 'agree and I am not at all distressed', 'agree and I am somewhat distressed', agree and I am distressed' or 'agree and I am very distressed'. The answers yielded from 1–5 points, 5 points corresponding to the highest level of effort or reward. The points were summed up for the two dimensions; i.e. effort and reward, and ERI calculated as effort/(reward × 4/7). The correction factor 4/7 is included, as the two dimensions do not include the same number of questions.

2.3. Covariates

Data on alcohol consumption (drinks/week), tobacco (g/day) and degree of physical activity (four levels from very low activity: 1 = almost physical passive to high activity: 4 = intensive physical activity for more than 4 h/week) were measured in 2006 and 2008 and included as covariates. According to answers on use of medicine, the variable 'Medicine use' was coded as 'non-medicated' = no medicine or occasionally use of painkillers, and 'medicated' = daily use of medicine. Physiological covariates included Waist hip ratio (WHR) calculated as waist width divided by hip width, systolic blood pressure (Bpsys, mmHg), total-cholesterol (mmol/l) and glycated hemoglobin (HbA_{1c}, %).

2.4. Strategy for the analysis

The data were analyzed using SPSS version 11. A level of significance of $p<0.05$ was chosen. Due to the nature of the data, i.e. repeated inter-correlated data, we used mixed effects model including subject as the random effect.

All analyses were conducted for 1) the total population including gender as a factor and 2) separately for women and men because of the known gender differences in physiology, daily life and perceptions of stressors (Artazcoz et al., 2004; Barrett-Connor, 1997; Brezinka and Padmos, 1994; Messing et al., 2003). Furthermore, the population was stratified and the analyses conducted for the sub-population 'non-medicated'.

Analysis part 1: Four series of mixed effects models were conducted, using four dependent variables: lnHR, lnTP, lnHF, and ln (LF/HF). For each model, year of examination (2006 = 0; 2008 = 1) was included as a factor, and age and time of measurement (calculated as hours after midnight) were included as covariates. Furthermore, each of the psychosocial factors was included as a covariate (effort, reward, and ERI) (Table 2).

Analysis part 2: Rerunning the analyses using the same dependent variables as in part 1, selecting the non-medicated individuals. The mixed effects models were used including for each model, year of examination as a factor, and age and time of measurement as covariates (Table 3).

Analysis part 3: For the non-medicated sub-population three models were conducted using lnTP, lnHF and Ln(LF/HF) as the dependent variable, ERI as the explanatory variable, and tobacco, alcohol, physical activity, WHR, Bpsys, total-cholesterol and HbA_{1c} as covariates. Backward elimination of the variables showing no association with the dependent variables resulted in the final models (Table 4). The lower value of Akaike's Information Criteria (AIC) implies the best fit.

Analysis part 4: Finally, as low TP might influence mood and thereby ERI; two additional analyses were conducted to disentangle the question about causality: 1) a partial correlation analysis was conducted for the non-medicated sub- population including lnTP,

Table 2

Results of analysis part 1, total population and stratification by gender. Estimates, standard errors (SE), F and level of significance from mixed effects models using data during rest for lnHR,lnTP, lnHF and ln(LF/HF) as the dependent variables, and each psychosocial factor as the independent variable.

	lnHR	lnTP	lnHF	ln(LF/HF)
	Estimate (SE) × 10^{-2}	Estimate (SE) × 10^{-2}	Estimate (SE) × 10^{-2}	Estimate (SE) × 10^{-2}
Total population (N = 231)				
Gender, women compared to men	4.19 (2.00) *	−11.44 (12.74)	23.94 (16.90)	−47.22 (10.53)***
Year	0.67 (1.19)	1.67 (8.18)	11.33 (9.62)	−5.93 (6.60)
Age	−0.07 (1.19)	−4.08 (0.62)***	−5.36 (0.82)***	0.51 (0.51)
Time	−0.36 (0.36)	−4.32 (2.88)	0.11(3.24)	−5.40 (2.16)
Effort[a]	0.62 (0.22) **	−3.52 (1.43)*	−4.12 (1.81) *	1.21 (1.19)
Reward[a]	−0.22 (0.12)*	2.47 (0.75)***	2.43 (0.94)**	−0.09 (0.63)
ERI[a]	4.91 (1.44)***	−32.78 (9.34)***	−44.12 (11.64)***	14.61 (7.79)
Women (N = 170)				
Effort[b]	0.45 (0.25)	−3.53 (1.70) *	−3.03 (2.08)	0.82 (1.04)
Reward[b]	−0.20 (0.14)	2.54 (0.90)***	2.98 (1.11)**	−0.28 (0.75)
ERI[b]	4.31 (1.67)**	−31.67(11.14)***	−45.28 (13.55)***	16.95 (9.32)
Men (N = 61)				
Effort[b]	1.02 (0.46) *	−2.81 (2.73)	−6.45 (3.73)	1.60 (2.27)
Reward[b]	−0.21 (0.23)	2.14 (1.39)	1.32 (1.78)	0.50 (1.09)
ERI[b]	5.69 (2.89)*	−32.54 (17.34)	−37.33 (22.08)	3.92 (13.95)

* $p<0.05$; ** $p<0.01$; *** $p<0.005$.

[a] Adjustment for gender, year, age, and time of measurement.

[b] Adjustment for year, age, and time of measurement.

Table 3
Results of analysis part 2, non-medicated sub-population and stratification by gender. Estimates, standard errors (SE), F and level of significance from mixed effects models using data during rest for lnHR, lnTP, lnHF and ln(LF/HF) as the dependent variable, and each psychosocial factor as the independent variable.

	lnHR	lnTP	lnHF	ln(LF/HF)
	Estimate (SE) $\times 10^{-2}$	Estimate (SE) $\times 10^{-2}$	Estimate (SE) $\times 10^{-2}$	Estimate (SE) $\times 10^{-2}$
Non-medicated population (N = 163)				
Gender, women compared to men	4.46 (2.37)	7.80 (13.93)	37.07 (17.86) *	−42.84 (12.34) ***
Year	−0.73 (1.49)	12.44 (10.19)	16.96 (12.16)	−10.54 (8.15)
Age	1.52×10^{-2} (0.12)	−4.67 (0.72)***	−6.88 (0.92) ***	1.04 (0.63)
Effort[a]	0.58 (0.28)*	−2.95 (1.68)	−3.69 (2.10)	2.39 (1.44)
Reward[a]	−0.23 (0.15)	2.31 (0.92) *	2.58 (1.14) *	−0.35 (0.78)
ERI[a]	5.30 (1.75)**	−27.65 (10.83) **	−45.44 (13.20) ***	23.29 (9.18)**
Women (healthy = 118)				
Effort[b]	0.31 (0.33)	−3.02 (2.02)	−3.43 (2.50)	3.02 (1.75)
Reward[b]	−0.21 (0.18)	2.48 (1.12)*	3.10 (1.37)*	−0.60 (0.98)
ERI[b]	4.52 (2.13)*	−28.18 (13.29)*	−50.00 (16.03)***	31.15 (11.39) **
Men (healthy = 45)				
Effort[b]	1.18 (0.51)*	−2.71 (3.06)	−5.18 (3.98)	1.00 (2.53)
Reward[b]	−0.21 (0.27)	2.06 (1.61)	1.95 (2.04)	7.7×10^{-3} (1.28)
ERI[b]	6.21 (3.09)*	−28.30 (18.51)	−40.65 (23.13)	6.54 (14.97)

* $p<0.05$; ** $p<0.01$; *** $p<0.005$.
[a] Adjustment for gender, year, age, and time of measurement.
[b] Adjustment for year, age, and time of measurement.

lnHF, and ln(LF/HF) measured in 2006 and effort, reward, and ERI measured in 2008 and adjusting for age and time of measurement. 2) A simple linear regression including ERI in 2008 as the dependent variable and ERI in 2006, age, time of measurement, and lnTP as the independent variables.

3. Results

The psychosocial work environment seemed rather stable as mean scores of effort, reward and ERI were similar in the two years. There were no significant gender differences in the mean scores of effort, reward and ERI. However, the ranges in ERI were 3.5 in women and 2.5 in men. The women were younger than the men, and alcohol consumption was significantly lower in women compared to men. The population consisted mostly of white-collar workers in the public administration. The participants did not differ significantly from the non-participants according to exposures or covariates. The non-medicated population included 70.6% of the population ($N = 163$).

Table 2 shows the results of analysis part 1. The first four rows show results of the analyses conducted including only these four variables (sex, year, age and time of measurement). The analyses including effort, reward or ERI, are adjusted by sex, year, age and time of measurement as well. Women had significantly higher lnHR and lower ln(LF/HF) compared to men. Age was associated with decreased lnTP and lnHF. Year and time of measurement were not associated with the dependent variables. Effort and ERI were associated with increased lnHR, decreased lnTP and decreased lnHF, whereas reward was associated with decreased lnHR, increased lnTP and increased lnHF. In the gender-separated analysis, the associations were only significant for women but the estimates were very similar for women and men, except for LF/HF.

Table 3 shows results of analysis part 2 for the non-medicated individuals. ERI was significantly associated with decreased lnTP, lnHF and increased ln(LF/HF). This was seen in the total population and in the women but not in the men (only 45 individuals).

Table 4 shows enter and final models for the non-medicated subpopulation (women only) of analyses including all variables using lnTP, lnHF, and ln(LF/HF) as the dependent variables and backwards elimination of variables not significantly associated with the dependent variable. ERI stayed significantly associated with all dependent variables, negatively with lnTP and lnHF and positively with ln(LF/HF). Beyond this association, lnTP and lnHF was negatively associated with age, and Ln(LF/HF) was negatively associated with time of measurement. ERI was not significantly associated with any of the HRV measures in men.

The partial correlation analysis did not reveal any significant correlations between levels of HRV in 2006 and levels of the ERI-model in 2008. The linear regression analysis showed that ERI in 2006 was the only variable significantly associated with ERI in 2008.

4. Discussion

This study revealed consistent associations between psychosocial work environment assessed by the ERI-model and HRV. After adjustment for a number of cardiovascular risk factors, ERI was significantly and negatively associated with TP and HF, while ERI was significantly and positively associated with LF/HF, though only in women. This suggests a vagal withdrawal in connection with ERI. Effort and ERI were negatively associated with TP and HF, whereas reward was positively associated with TP and HF, as would be expected if HRV were indeed associated with the ERI-model. The associations did only reach significance for women in the gender stratified analysis. This may be due to insufficient power for the male analysis including only 61 men but the estimates were quite similar for men and women except for LF/HF. Insufficient power includes both the lack of power in connection with a too small population and lack of power in connection with a too small range in the exposure variable. In this case, the range in ERI was 3.5 and 2.5 in women and men, respectively. The combination of few men and a relatively small range in ERI in the men may be the explanation of non-significant associations between ERI and HRV in the men. Our results are in line with the study by Hintsanen et al. who revealed an inverse association between ERI and HRV in women, but not in men (Hintsanen et al., 2007). However, Vrijkotte et al. found that ERI was associated with higher heart rate and lower vagal tone during the 24-hours in 109 male white-collar workers (Vrijkotte et al., 2000). Also, Eller et al. followed 48 women and 22 men during a 6-year follow up and found ERI at baseline negatively associated with TP in men but not in women (Eller et al., 2011). Another explanation of the gender differences in strength of the associations might be that the HRV-data was selected during rest at home. It is possible that the men were in a state of recovery while the women were still at work in their minds. Men and women differ in physiological response after work (Brisson et al., 1999; Frankenhaeuser et al., 1989; Lundberg and Frankenhaeuser,

Table 4
The three enter and final models for the healthy sub-population (women only, $N=118$) using lnTP, lnHF, and ln(LF/HF) as the dependent variable, ERI as the explanatory variable and tobacco, alcohol, physical activity, WHR, Bpsys, total-cholesterol and HbA_{1c} as covariates. Also, the enter models included year of measurement, age and time of measurement. Variables showing no association with the dependent variables were eliminated backwards.

LnTP	Enter model (AIC 482.0)		Final model (AIC 438.2)	
	Estimate (SE)$\times10^{-2}$	F	Estimate (SE)$\times10^{-2}$	F
Year of measurement	13.42 (13.94)	0.93		
Time of measurement	-1.55×10^{-3} (1.28×10^{-3})	1.45		
Age	−6.37 (1.10)***	33.78	−5.58 (0.89) ***	39.33
Tobacco	−2.86 (1.88)	2.31		
Alcohol	−0.19 (1.22)	0.02		
Physical activity	15.84 (11.53)	1.89		
WHR	−79.16 (139.95)	0.32		
BPsys	0.25 (0.39)	0.39		
Total –cholesterol	9.03 (7.93)	1.07		
HbA_{1c}	−4.05 (4.71)	0.78		
ERI	−24.33 (13.75) ***	2.93	−27.43 (13.33) *	4.28
LnHF	**Enter model (AIC 531.7)**		**Final model (AIC 495.8)**	
	Estimate (SE)$\times10^{-2}$	F	Estimate (SE)$\times10^{-2}$	F
Year of measurement	29.57 (15.09)	3.84		
Time of measurement	2.63×10^{-4} (0.14×10^{-2})	0.03		
Age	−7.53 (1.36) ***	30.83	−6.81 (1.11)***	37.68
Tobacco	−2.49 (2.24)	1.23		
Alcohol	−1.38 (1.51)	0.83		
Physical activity	16.78 (13.96)	1.44		
WHR	−89.37 (168.93)	0.28		
BPsys	0.91 (0.48)	3.57		
Total –cholesterol	8.68 (9.64)	0.81		
HbA_{1c}	−12.01 (5.50)	4.71		
ERI	−45.81 (16.10) ***	8.09	−50.42 (15.90)***	10.06
Ln(LF/HF)	**Enter model (AIC 454.5)**		**Final model (AIC 417.9)**	
	Estimate (SE)$\times10^{-2}$	F	Estimate (SE)$\times10^{-2}$	F
Year of measurement	−7.74 (10.83)	0.51		
Time of measurement	-2.43×10^{-3} (1.04×10^{-3}) *	5.44	-2.76×10^{-3} (1.04×10^{-3}) **	7.05
Age	0.10 (0.83)	0.70		
Tobacco	−2.27 (1.51)	2.26		
Alcohol	1.47 (1.11)	1.77		
Physical activity	−2.35 (10.24)	0.05		
WHR	−50.41 (121.93)	0.17		
BPsys	−0.35 (0.34)	1.04		
Total-cholesterol	−4.66 (6.98)	0.45		
HbA_{1c}	0.47 (4.04)	0.01		
ERI	32.48 (11.78) **	7.61	31.59 (11.34) **	7.75

* $p<0.05$; ** $p<0.01$; *** $p<0.005$. AIC: Akaike's Information Criterion.

1999). This may originate in different work tasks at home, where women may still undertake the responsibility for most housework (Berntsson et al., 2006; Lundberg et al., 1994). In the presented study, HRV-data were obtained during a time period of self-reported seated rest in the evening, while the exposure was measured by means of a questionnaire considering ERI at work. A number of reasons for the gender differences in this study may be considered, i.e. lack of power considering the men (few men and too small range in exposure), a possible gender difference in physiology of recovery, and basic physiological gender difference in physiology. Overall, women had a significantly higher heart rate than men and a significantly lower LF/HF than men. Age was negatively associated with TP and HF. The gender differences as well as the association between age and HRV are in line with earlier findings (Agelink et al., 2001; Britton et al., 2007; Sinnreich et al., 1998).

The associations between increased ERI and decreased TP and HF were replicated when excluding the medicated sub-population. This indicates that differences in HRV in non-medicated individuals may be a result of experiencing adverse psychosocial conditions and that the associations between ERI and HRV were not the results of medication, which could have been the case (Kemp et al., 2010; Zellweger et al., 2004).

The variable 'year' was not associated with changes in HRV. The association between ERI and HRV could have masked an association between year and HRV. However, the analyses including only year, gender, age, and time of measurement demonstrated that this was not the case. As a major change in the organization of the Danish public sector took place January 1st 2007 a decrease in HRV as a result of the workload and novelty associated with the changes might have been expected. Therefore, HRV most likely mirror the present state of stress rather than psychological load during months and years.

Table 4 includes the results of in-depth analysis of the associations between HRV and ERI in the non-medicated women. All covariates are included in 3 mixed effect analyses using each of the HRV-measures as the dependent variable. In connection with lnTP and lnHF, age was of more significance, but ERI was negatively and significantly associated with lnTP and lnHF as well. In connection with ln(LF/HF) time of measurement seems to be of little importance compared to ERI. All other covariates were excluded during the backwards elimination.

The study was conducted on a workday. The participants reported when they had been seated during the evening. We did not have information on number of working hours during the day or if the work had been frustrating the particular working day. We still don't know enough about the associations between ERI and HRV over time to conclude, if the time of measurement was adequate. Maybe the associations would have been stronger if HRV was measured during working hours. Or maybe the assoc would still be present if measured during a weekend or a holiday. However, the associations were present several hours after work. Psychosocial work environment during the day seems to be of importance to activity in the autonomous nervous system during several hours after leaving the work place.

The hypothesis was that adverse working conditions would be associated with low levels of HRV. This was supported by the study. However, the direction of causality is to be discussed. We conducted two additional analyses with this purpose. Neither the partial correlation analysis nor the linear regression analysis showed any significance of HRV in 2006 to ERI in 2008. The time interval of 2 years in the current study might be questioned, as it is a relatively long time period. However, HRV is a rather stable measure and it is not to be recommended to reexamine a population with shorter intervals than e.g. 6 month (Tarkiainen et al., 2005).

4.1. Strengths and limitations

Although larger study populations are often wished for, this study proved sufficiently large to demonstrate the association between adverse working conditions and HRV. The point of interest is the range of exposure rather than the number of individuals, and the range of exposure was large in this study. At the same time, it was a strength that the population was sufficiently large to be stratified in non-medicated/medicated individuals. By replicating the findings in the non-medicated sub-population it is shown that the associations are not explained by differences in medication.

The results consider only stress physiology after a workday. We recommend examining HRV in the leisure time in weekends or holidays to reveal if psychosocial work environment has the potential to change the activity in the autonomic nervous system days or weeks after finishing work. This is important especially when the gender differences are in question.

As the data were intercorrelated (repeated measurements on the same individuals) the mixed effects models were including "subject" as a random effect. This incorporates individual specific HRV levels and allows generalization of the results to other samples comparable to this sample (Zuur et al., 2009). The population in this study consists of white-collar workers in the Danish public sector. We cannot be sure that the results can be generalized to other groups, work places or cultures.

4.2. Conclusion

Adverse psychosocial work environment, including effort reward imbalance, was associated with HRV measures associated to negative health outcomes, particularly in the form of reduced TP and HF that indicate reduced parasympathetic tonus. Thus, ERI at work may be a preventable risk factor for cardiovascular and other diseases. The associations were statistically strongest in women, but estimates were similar in men. This may be a power problem, as the number of male participants was smaller, or there may be a true gender difference. HRV was measured during seated rest in the evening of a workday. We recommend that future studies include measurements during working hours and during weekends and holidays, to examine to what extent the associations are persistent over time.

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Progression in intima media thickness—the significance of hormonal biomarkers of chronic stress

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Summary *Objective*: The pathophysiological pathways from stress caused by psychosocial stress to IHD has not been dealt with very extensively. The objective of this study was to analyse the association between cortisol levels and progression in intima media thickness (IMT).
Methods and results: In 1998 and 2002, 95 participants went through a clinical investigation including ultrasound of the artery carotis communis. Progression in IMT was analysed in relation to levels of salivary cortisol in 1998 and the average levels of salivary cortisol in 1998/2002. Further, the significance of conventional coronary risk factors, testosterone and dehydro-epiandrosterone sulphate (DHEAS) were evaluated. Among the men, only age and HDL-cholesterol (negative) were significantly correlated with progression in IMT. Among the women, awakening cortisol response was significantly correlated with progression in IMT. Testosterone and DHEAS were borderline significantly associated (negatively) with progression in IMT in both genders.
Conclusion: Progression in atherosclerosis were determined by different risk factors in women and men. The awakening cortisol response was of great importance to IMT progression in women but not in men.

1. Introduction

The introduction of the ultrasound technique has made it possible to estimate early, asymptomatic atherosclerosis by way of the intima media thickness (IMT). The association between IMT measured on the arteria carotis communis (ACC) and clinical

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ischemic heart disease (IHD) is well-established and the measure is often used in the field of cardiovascular epidemiology (Salonen and Salonen, 1993; Lynch et al., 1995; Crouse et al., 2002; O'Leary and Polak, 2002; Simon et al., 2002; Spence, 2002; Wikstrand et al.,2003).

A given IMT is an expression of the total effect of all the risk factors that a person has been exposed to until the time of examination. Progression in IMT is therefore a more precise estimate of the significance of a given risk factor than a given IMT in itself (Crouse, 2001). At the same time, it is important to be aware that the risk factors for progression in IMT do not necessarily include all those factors that increase the risk of acute myocardial infarct (AMI), insofar as some risk factors might only affect the atherosclerotic process, but not increase the risk of a rupture of the plaques and the development of AMI (Hegele, 1996; Spence and Hegele, 2004). Further, it has been shown that progression in IMT is associated with conventional risk factors (Sander et al., 2000; Lakka et al., 2001; Crouse et al., 2002; Chambless et al., 2002; Fujii et al., 2003) as well as with psychosocial risk factors (Everson et al., 1997a,b; Lynch et al., 1997a,b; Paterniti et al., 2001). Thus, a wide spectrum of risk factors has been shown to be associated with IHD and IMT as well as with progression in IMT.

However, the patho-physiological pathways from the psychosocial risk factors to IMT and IHD continue to be unclear. It is well known that acute, psychological stress increases cortisol secretion and that this leads to catabolism and mobilisation of energy. Further it is known that cortisol—via feedback mechanisms—impairs growth and reproduction (Sapolsky et al., 1986).

Because of the acute increase in cortisol secretion in connection with acute stress, chronic psychological stress has been expected to be associated with a chronic increase in cortisol. However, the findings in studies of chronic psychological stress and cortisol have not been consistent with this, insofar as studies have shown lower cortisol secretion in people with chronic psychological stress (Kristenson et al., 1998). Some researchers have suggested that low cortisol in people with psychological stress is due to exhaustion of the HPA-axis during long-term stress (a flat diurnal cortisol curve) (Bjorntorp and Rosmond, 2000; Bjorntorp et al., 2000). Changes in the cortisol receptor relationship may also have some influence (Sapolsky et al., 1986).

Only few studies have examined the connection between early atherosclerosis and cortisol. An early study in 1977 showed increased plasma cortisol in the morning to be associated with the degree of coronary atherosclerosis in men, shown by way of angiography (Troxler et al., 1977). A recent cross-sectional study of 88 women and 42 men showed a negative association between increases in salivary cortisol in the morning and IMT in women, while no association could be shown in men (Eller et al., 2001).

The production of sex hormones is also affected by chronic stress (Sapolsky et al., 1986). There is a possibility that the level of these hormones could also influence the development of atherosclerosis. Studies have shown that testosterone is negatively associated with IMT in women, i.e. higher testosterone levels are associated with lower IMT, (Bernini et al., 1999; Golden et al., 2002) and men (De Pergola et al., 2003) as well as with progression of IMT in men (Muller et al., 2004).

This article describes a prospective study of conventional coronary risk factors, cortisol, testosterone and DHEAS, and progression in IMT over a four-year period. The objective of the study was to analyse the significance of hormonal biomarkers for stress, especially salivary cortisol, and conventional coronary risk factors on progression in IMT.

2. Materials and methods

2.1. Participants

In 1998, 130 participants were included in a prospective study of IMT, conventional risk factors (Eller and Netterstrom, 2001) and biomarkers of stress especially cortisol (Eller et al., 2001). All participants were in good health, had employment and volunteered in response to a public notification. A total of 95 participants (63 women and 32 men) agreed to be part of a follow-up study in 2002. The average follow-up time was 4.1 years. The study was approved by the local scientific ethical committee. All participants gave informed consent before the examination.

The participants were between 34 and 63 years old at the time of the follow-up study. The physiological characteristics of the participants are presented in Table 1. Those participants from 1998 who did not respond to the approach in 2002 (the non-participants) were significantly younger and had a better coronary risk profile than the participants. Further, the intima media thickness and salivary cortisol at awakening, i.e. the first awakening level of cortisol, among participants in 1998 was higher than among non participants, where as the change in salivary cortisol over the first 20 min after awakening in 1998 was lower among participants than among non participants.

Table 1 Characteristics of dependent and independent variables in the population.

	Women ($N=63$)			Men ($N=32$)		
	Mean	SD	Range	Mean	SD	Range
Age in 2002 (years)	49.9	7.3	29.0	50.9	7.8	26.0
IMT-p (mm/4 years)	0.118	0.141	0.690	0.190	0.132	0.654
IMT98 (mm)	0.748	0.120	0.489	0.846	0.207	0.978
Averaged variables:						
S1 (nanomol/l)	13.88	5.59	27.50	13.12	5.96	21.90
S12 (nanomol/l)	6.98	5.66	29.55	7.40	7.06	37.20
BMI (kg/m^2)	24.4	3.1	11.4	26.6	2.6	11.7
WHR	0.79	0.06	0.24	0.91	0.06	0.33
Fibrinogen (micromol/l)	9.36	1.27	7.05	9.93	2.17	9.45
Cholesterol (mmol/l)	5.49	1.00	4.35	5.50	0.93	3.70
HDL-cholesterol (mmol/l)	1.71	0.44	2.08	1.49	0.54	2.63
Bpsys (mmHg)	126	11	48	135	11	46
Alcohol (drinks/week)	4	4	18	9	10	44
Tobacco (g/day)	2.9	5.8	22.5	3.8	7.0	25.7
HbA1c (%)	4.9	0.3	1.3	5.1	0.5	2.3
Testosterone (picomol/l)	3.86	4.77	28.05	49.14	13.00	54.75
DHEAS (micromol/l)	3.54	1.62	6.70	4.64	2.18	9.16

Abbreviations: IMT: intima media thickness. IMT-p: 4-year progression in intima media thickness. S1: Salivary cortisol at awakening. S12: Change in salivary cortisol the first 20 min after awakening. BMI: Body mass index. WHR: Waist-Hip ratio. Bpsys: Systolic blood pressure. HbA1c: Glycated hemoglobin. DHEAS: Dehydroepiandrosteronesulfate.

These differences were not significant, apart from the degree of smoking among the men, where the non-participants smoked significantly less than the participants did.

Of the 95 participants in the follow-up study, two women and three men had been undergoing treatment with heart or blood pressure medicine both in 1998 and 2002. A further four women and four men had started treatment with hypotensive medicine at the time of the follow-up study in 2002, and two men had suffered an acute myocardial infarct (AMI) between 1998 and 2002. A total of six women and six men were suffering from hypertension or IHD in 2002.

3. Clinical examination

The participants were summoned by letter in the same order as in the baseline study. An extensive questionnaire was enclosed with the letter, including questions about health and lifestyle, information about the study, instruction in saliva-sampling and test tubes to be used for the collection of saliva in the morning of a working day and a non-working day. The examinations were carried out between 12:00 and 17:00 h. At the examination, the saliva samples and completed questionnaires were given to the examiner. The study was clearly explained to the participants and they signed the consent forms. The participants stripped to the waist and were weighed. The participants' blood pressure and pulse were taken with the participant sitting in a chair. At the end of the examination, IMT was measured with the participant supine.

On one of the subsequent mornings, blood tests were taken after 12 h of fasting (HbA1c, cholesterol, HDL-cholesterol, fibrinogen, testosterone and DHEAS).

4. Progression in IMT

The effect measure of the study is the progression in IMT between 1998 and 2002. IMT was measured as follows: The participant was placed in supine position with the head turned slightly to the opposite side, and in this position arteria carotis communis (ACC) and the link between ACC and bulbus were found. At the point where the transition between ACC and bulbus was defined, the IMT was measured at the far wall at a right angle to a tangent in the longitudinal direction of the artery. Six measurements were taken, three on the right and three on the left (Eller and Netterstrom, 2001; Eller et al., 2001). After each measurement the individual was allowed to changes position, i.e. the measurements were taken from different angles. In each measurement the maximum thickness was quantified.

In 1998, ultrasound examinations were recorded on video and the measurements were carried out after completion of the examination.

In 2002, the measurement was carried out during the examination. The measurements were carried out blinded and independently of each other in 1998 and 2002. All examinations and measurements were made by the same person.

Subsequently, the averages of the six measurements from 1998 (IMT98) and 2002 (IMT02) were calculated. The progression in IMT can be calculated either as a differential or as a relative increase. The simple relationship between the measurements in 1998 and 2002 showed a linear structure with a gradient of 1, and therefore the progression was analysed as a simple differential. The variable IMT-p was defined as the difference between these two averages (IMT02–IMT98). Afterwards, adjustment for the follow-up period was made. Thus, the reported IMT progression is 'mm progression in IMT/209 weeks', equivalent to a four-year follow-up period.

5. Background variables

The independent analysis included two kinds of independent variables: the level of cortisol secretion (the significance of which the study especially aimed to reveal) and the other physiological variables.

5.1. Cortisol

Cortisol level was collected and measured in the saliva in the same way in 1998 and 2002. In 1998 the samples were only from a working day, while salivary cortisol were collected both on a working day and a non-working day in 2002. However, this study only deals with salivary cortisol sampled on working days.

The awakening cortisol response, i.e. the rise in cortisol secretion the first ½ hour after awakening is increasingly used as a measure of the stress level in the individual, though there are still confusion about what the high or low awakening cortisol response means to health (Clow et al., 2004; Steptoe et al., 2004). The measurement of cortisol levels in saliva was based on three measures in 1998, three corresponding measures in 2002 and the three average measures of salivary cortisol in 1998/2002. The three salivary cortisol measures from 1998 as well as the corresponding measures in 2002 were salivary cortisol in the morning ($S1_{98}/S1_{02}$), changes in the cortisol level during the first 20 min after awakening ($S1\text{-}2_{98}/S1\text{-}2_{02}$) and changes in the cortisol level during the first 60 min after awakening ($S1\text{-}3_{98}/S1\text{-}3_{02}$). The level of salivary cortisol normally increases to its maximum within the first 30 min after awakening (Kirschbaum and Hellhammer, 1994; Clow et al., 2004). Unfortunately salivary cortisol in 1998 was only measured 20 min after awakening and not after 30 min. Therefore the variable S1-2 is the obvious substitute of awakening cortisol response. The other variable, S1-3, was chosen because in the baseline study there were associations between $S1\text{-}3_{98}$ and IMT max (Eller et al., 2001).

Measuring salivary cortisol is difficult because of great intra-subject variation in the secretion. The samples form 1998 showed a weak but non-significant positive correlation with the corresponding samples in 2002. For this reason it was decided to use both the values from 1998, 2002 and the average of the measures in 1998 and 2002 as different expressions of the participants' usual cortisol secretion in separate analyses. Averages of salivary cortisol at awakening, $S1_{average}$, and change in cortisol, $S1\text{-}2_{average}$ and $S1\text{-}3_{average}$, were used in the same way as salivary cortisol in 1998 and 2002. Finally, the reactivity in the cortisol secretion was defined as (S1-2/S1)×100%. This expression was used for salivary cortisol measured in 1998 and 2002 (cortisol $reactivity_{98}$/cortisol $reactivity_{02}$) and for the average variable (cortisol $reactivity_{average}$).

5.2. Additional physiological variables, possible confounders

The other independent variables were age, IMT in 1998, body mass index (BMI), waist-hip ratio (WHR), fibrinogen, cholesterol, HDL-cholesterol, systolic blood pressure, HbA1c, testosterone, DHEAS, use of alcohol (units/week) and smoking status in 1998 (1=never smoked, 2=former smoker, or 3=smoker). Bivariate correlation analyses showed strong correlations between measures in 1998 and 2002 for all the physiological measure except for testosterone and blood pressure in men. Therefore these two variables were analysed both as independent variables in 1998 and as the average variables. All other physiological variables were used as averaged variables.

6. Strategy for the analyses

All analyses were carried out separately for the two genders because of the great differences between them with regard to the development of atherosclerosis and IHD.

Initially, a descriptive analysis of the IMT-p among the participants was carried out, including a division into participants who were healthy and participants who were ill. The latter group included those six men and six women who were being treated with hypotensive medicine or who had had AMI.

For all independent variables a simple regression analysis was carried out with IMT-p as the dependent variables. Those variables which best explained the variation in the dependent variable were included one by one in a multiple regression analysis.

Further, bivariate correlation analyses were made to evaluate associations of testosterone and DHEAS in 1998, and averaged values of BMI and WHR.

Finally, salivary cortisol measures (1998 and average levels) were depicted for low vs. high IMT-p, for women and men, respectively.

The study design is using a relative small data set and conducted multiple analyses gives a risk of finding too many significant results. Therefore, exact measures were used in case of ordinary contingency table analyses and Bonferroni corrections of levels of significance were used when interpreting the results from the stepwise regression analyses. Since successive testings usually consisted in two up to three consecutive tests, a Bonferroni adjusted 0.025 level of significance were applied.

7. Results

IMT progression (mean (SD)) for the women was 0.118 (0.141) mm/4 years and for the men 0.190 (0.132) mm/4 years. Looking at the whole group, the progression rate was significantly greater for the men than for the women (p at t-test for IMT-$p=0.017$). Those who were healthy had a significantly lower progression than the group as a whole.

Table 2 shows R^2, beta coefficients and p-values from the simple regression analyses, where IMT-p was the dependent variable. The variation in IMT-p among the women was mostly explained by the starting point in 1998 (IMT mean 98, negative association) and by S1-$3_{average}$. In addition, salivary cortisol in 1998 expressed as the change in salivary cortisol over the first 20 and 60 min after awakening and the average level of DHEAS (negative association) explained a significant proportion of the variation in IMT-p, whereas conventional coronary risk factors did not appear to influence IMT-p.

Among the men, the variation in IMT-p was explained in particular by age, HDL-cholesterol (negative association) and systolic blood pressure in 1998, which explained 17.8% of the variation in IMT-p. As with the women, the other conventional coronary risk factors explained a fairly small part of the variation in IMT-p. In men testosterone in 1998 explained 9.8% and DHEAS explained 11.9% of the variation, respectively (negative associations), in the simple regression analyses, whereas cortisol seemed to have no influence at all.

The best model for IMT-p for women included IMT mean98 ($p=0.019$) and S1-$2_{average}$ ($p=0.056$). The linear model explained 24% of the variation in IMT-p.

Among the men, 30% of the variation in IMT-p was explained by age ($p=0.018$) and HDL-cholesterol (negative association, $p=0.060$).

In the simple regression analysis, the cortisol reactivity$_{average}$ explained 9.2% of the variation in IMT-p among the women, but stayed non-significant in the multiple regression analysis.

The correlations analyses of testosterone and DHEAS showed that levels of testosterone in 1998 and 2002 were not correlated in men (pearsons correlation 0.001), where as DHEAS was significantly correlated among men (pearsons 0.420) as well as both testosterone (pearsons correlation 0.357) and DHEAS (pearsons correlation 0.450) were significantly correlated in women.

Among men testosterone in 1998 were negatively correlated with BMI and WHR, that is, the lower level of testosterone in 1998 the higher BMI and WHR. Contrary, DHEAS in 1998 where positively correlated with BMI and WHR.

In women, both testosterone and DHEAS correlated positively with BMI and WHR.

8. Discussion

This study looked at progression in IMT in relation to a number of risk factors for atherosclerosis and IHD, both the conventional and a series of potential hormonal risk factors, especially salivary cortisol. The study showed major differences between the two genders. First, the IMT progression of the women was much smaller than that of the men. This was expected, as women in the child-bearing age seem to be protected against atherosclerosis by the female hormones. Secondly, salivary cortisol was very important for the IMT-p among the women, whereas the hormone seemed to have little significance for IMT-p among the men.

The progression rate of IMT is of interest because this seems to be useful as a surrogate measure of risk for future cardiac events (Crouse, 2001). Normal values of progression rate are not

Table 2 Results of simple regression analyses using IMT progression as the dependent variable and one independent variable at a time: R^2, beta and p-values.

	Women			Men		
	R^2	Beta	p	R^2	Beta	p
1998						
Age	0.0	0.007	0.96	23.1	0.481	<0.01
IMT mean[a]	20.1	−0.448	<0.01	0.0	0.236	0.20
S1[a]	5.2	−0.228	0.07	0.3	−0.055	0.77
S1-2 (first 20 min)[a]	7.4	0.272	0.03	8.4	0.289	0.11
S1-3 (first 60 min)[a]	9.2	0.303	0.02	9.5	−0.095	0.61
Cortisol reactivity[b]	2.2	0.149	0.24	1.8	0.135	0.46
Testosterone	4.6	−0.214	0.09	9.8	−0.313	0.08
BPsys.	0.2	0.044	0.73	17.8	0.422	0.02
2002						
S1[a]	0.0	0.020	0.89	3.4	0.184	0.34
S1-2 (first 20 min)[a]	1.2	0.110	0.45	0.2	−0.044	0.83
S1-3 (first 60 min)[a]	3.4	0.184	0.21	4.8	−0.220	0.30
Cortisol reactivity[b]	0.8	0.089	0.54	0.0	−0.010	0.96
Average (1998–2002)						
S1[a]	6.5	−0.255	0.07	0.5	0.067	0.73
S1-2 (first 20 min)[a]	14.1	0.376	<0.01	1.5	0.122	0.54
S1-3 (first 60 min)[a]	16.5	0.407	<0.01	20.0	−0.447	0.03
Cortisol reactivity[b]	9.2	0.303	0.03	0.0	0.033	0.87
Testosterone	3.0	−0.173	0.19	12.9	−0.359	0.06
DHEAS[a]	5.9	−0.243	0.07	11.9	−0.345	0.08
BMI[a]	0.0	−0.020	0.88	0.0	0.010	0.96
WHR[a]	0.3	0.052	0.68	2.1	0.145	0.43
Fibrinogen	1.3	−0.112	0.40	0.7	0.082	0.69
Cholesterol	0.9	0.097	0.47	1.6	0.128	0.51
HDL-cholesterol	0.6	0.080	0.55	13.3	−0.365	0.05
BPsys.[a]	0.0	0.006	0.98	7.7	0.278	0.17
HbA1c[a]	1.6	−0.126	0.36	1.7	0.129	0.52
Tobacco	0.7	−0.083	0.52	1.5	−0.124	0.50
Alcohol	0.7	−0.082	0.52	0.3	0.054	0.77

[a] Abbreviations: IMT: intima media thickness. S1: Salivary cortisol at awakening. S1-2: Change in salivary cortisol the first 20 min after awakening. S1-3: Change in salivary cortisol the first 60 min after awakening. BMI: Body mass index. WHR: Waist-Hip ratio. BPsys: Systolic blood pressure. HbA1c: Glycated hemoglobin. DHEAS: Dehydroepiandrosteronesulfate.
[b] Cortisol reactivity: S1-2 as percentage of S1.

established. Among middle-aged men, Salonen and Salonen (1990) have found rates of progress of 0.01-0.09 mm/year and Zureik et al. (1999) found a rate of progression of 0.0125 mm/year for men and 0.010 mm/year for women. In the ARIC-study, however, Howard et al. (1998) found a progression rate of 0.035 mm/year among some 10,000 American men and women. When comparing the IMT-values, it is important to be aware that methodological differences can influence the findings greatly (Kanters et al., 1997; Aminbakhsh and Mancini, 1999). The progression rate obtained by different methods of measuring IMT will differ, as well as progression rate may vary in different populations and during the atherosclerotic process (Hegele, 1996; Willeit et al., 2000; Lakka et al., 2001; Chambless et al., 2002). In this study, IMT was measured exactly at the point where the transition of ACC to bulbus was defined, whereas the measuring point in the referred studies was above the distal part of ACC This latter method will give lower values, as atherosclerosis progresses in the cardiac direction (Strong, 1992). Therefore the values of progression rate found in this study, though fairly high, are considered to be in accordance with the values found in other studies of IMT progression.

This study found a significantly greater progression among men than among women and a significantly greater progression in people with hypertension or with known IHD than in people who were healthy. The increased progression in people with a cardiovascular disease has been described previously (Hodis et al., 1998;

Crouse et al., 2002) and might be an indication that progression in IMT can be caused by benign changes, but that in people with a manifest cardiovascular disease an accelerated atherosclerosis takes place due to the considerable changes in the vascular system.

IMT progression and its relation to other risk factors has previously been studied, including studies of conventional risk factors such as lipids, blood pressure, smoking, physical activity (Salonen and Salonen, 1990; Hodis et al., 1998; Howard et al., 1998; Sander et al., 2000; Chambless et al., 2002; Crouse et al., 2002), as well as studies of psychosocial risk factors such as working conditions, anxiety and depression (Everson et al., 1997a,b; Lynch et al., 1997a,b; Paterniti et al., 2001). Generally, the expected associations between risk factors and IHD and IMT have been shown.

In this study, levels of salivary cortisol were used to mirror the degree of stress to which the participants were exposed. However, the samples form 1998 showed only a weak and non significant correlation with the corresponding samples in 2002. Therefore it is important to notice that salivary cortisol may not necessarily reflect exposure to stressors during the subsequent years.

The potential hormonal risk factors were of greater importance for IMT-p than expected, compared to the conventional risk factors. The most likely reason for this is probably that the variation within the population with respect to the conventional coronary risk factors was small. The 20 min change in salivary cortisol in the morning four years earlier explained 7.4 and 8.4% of the variation in IMT-p in women and men, respectively, whereas S1-2$_{average}$ explained 14.1% (women) and 1.5% (men) of the variation. As there are no findings from previous studies, these results indicate the need for further research in this area. The awakening cortisol response may be a part of the patho-physiological pathway from psychosocial stress to atherosclerotic heart disease. Troxler's study from 1977 supports the hypothesis about the association between high cortisol and atherosclerosis among men (Troxler et al.,1977). In this study, angiographs were taken on 71 male pilots' coronary arteries in connection with a prophylactic health examination. Blood cortisol was measured on the same day at 08:00, 09:00, 09:30 and 10:00 h. High cortisol levels were correlated with the number of atherosclerotic lesions in the coronary arteries. There are significant methodological differences between Troxler's study and the present, though, the increased cortisol levels in Troxler's study could be equivalent to this study's increased cortisol reactivity.

The finding of associations between IMT and IMT-p and the change in salivary cortisol over the first 20 and 60 min among women, but not among men may in part be a function of the saliva sampling protocol. Prüssner et al. have found (Pruessner et al., 1997) that the dynamics of the awakening cortisol response differ between women and men. In their study women consistently showed a stronger increase and delayed peak in salivary cortisol compared to that among men. The same delayed response seems demonstrated in this population in Fig. 1.

The negative association between maximal IMT and change in salivary cortisol the first hour after awakening found in our cross sectional study from

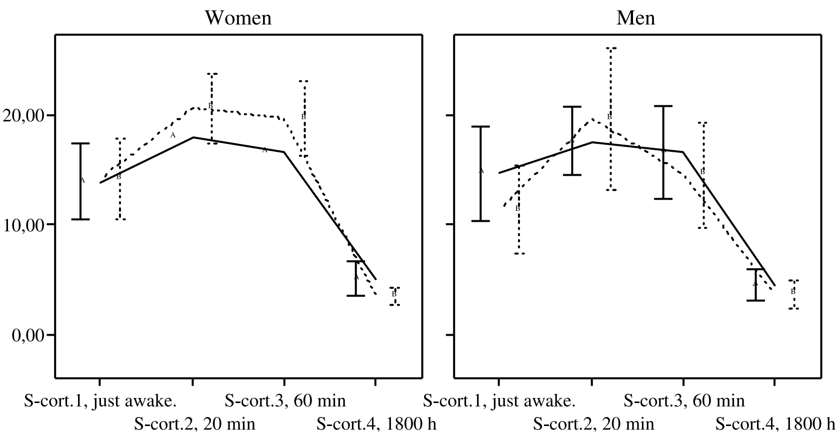

Figure 1 IMT progression and levels of salivary cortisol. *Y*-axis, salilvary cortisol (nmol/l). Straight line and circle, IMT-p < mean (*N* = 32 women, 19 men). Stipled line and triangle, IMT-p > = mean (*N* = 31 women, 13 men). A. Salivary cortisol at awakening, after 20 and 60 min and at 18:00 h in 1998 and IMT progression during the 4 following year. B. Averaged levels of salivary cortisol (1998/2002) at awakening, after 20 and 60 min and at 18:00 h and IMT progression during the 4 years.

1998 (Eller et al., 2001) was not reproduced in the form of a negative association between cortisol reactivity and IMT-p. This could have different explanations. First of all, the cross sectional study only evaluates the associations between variables at a specific time. In this case, the maximal IMT was the result of all risk factors during the individuals life until 1998 as well as were the cortisol levels. The prospective study tells about the actual conditions during the four years and this study is thus more reliable. Further, among women the IMT-p was negatively associated with the starting point in 1998 and was mostly explained by this. Therefore the negative relationship in the cross sectional study from 1998 can change into a positive relationship in the prospective study.

In connection with the discussion of cortisol levels, it is interesting that cortisol in particular was important for the IMT-*p* among the women. In a population with no other significant risk factors, cortisol seemed to have a causal effect on the development of atherosclerosis. Contrary to this, the men had both their gender, increasing age and HDL-cholesterol as significant risk factors. However, a larger study might show that cortisol is also important for the IMT-p among men, as indicated by the fact that there was a small tendency for a positive correlation between the 20 min awakening cortisol response in 1998 and the IMT-p among the men.

$Testosterone_{average}$ and $DHEAS_{average}$ were of greater importance than expected for the IMT-p among the men. Previously, inverse relationships have been found both in cross sectional (De Pergola et al., 2003) and prospective studies of testosterone and IMT (Muller et al., 2004). This supports the hypothesis that the finding reflects a causal connection. In this study, the bivariate correlation analysis of testosterone and DHEAS in 1998 and 2002 in men showed a positive correlation for DHEAS, but no correlation of levels of testosterone at all. Further, testosterone was negatively correlated with BMI and WHR. Bivariate correlation analyses did not show associations between testosterone in 1998 and the physiological measures, but BMI and WHR where positively and significantly correlated with averaged values of systolic blood pressure and HbA1c. This indicates that men with decreased production of testosterone in 1998 experienced higher blood pressure, weight and HbA1c and there by increased progression in intima media thickness. DHEAS on the other hand did not seem to be included in this pathological pathway.

The present study was very small and the findings are therefore less precise than in a larger study. However, the study design is prospective and therefore strong. Correlations between reactivity in salivary cortisol in the morning and IMT-p has not been studied previously, but the findings indicate that cortisol could very well be part of the patho-physiological pathway from psychosocial stress to IHD particularly among middle-aged women. For this reason it would be relevant to continue research in this area.

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Heart Rate Variability and Intima Media Thickness

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Increased activity in the sympathetic nervous system is part of the physiological stress response and is expressed in the heart rate variability (HRV). The objective of this study was to examine associations of HRV and intima media thickness (IMT). In 2002, satisfactory measurements of HRV of 78 voluntary participants were made, both during a stress test and during sleep. IMT in 2002 and the progression in IMT from 1998 to 2002 were analyzed in relation to measures of HRV. HRV was negatively associated with IMT and IMT progression both during test and sleep. In men with higher IMT measures than mean the low frequency–high frequency ratio was higher during sleep than during test, perhaps mirroring a lack of ability to recovery. HRV was negatively associated with IMT and IMT progression. HRV may be part of the pathophysiological pathway between psychological strain and atherosclerosis.

Key words: atherosclerosis, intima media thickness, progression, heart rate variability, sympathovagal balance

In recent years, an increasing number of studies have found that psychosocial factors affect the development of ischaemic heart disease (IHD; Everson, Kaplan, Goldberg, Salonen, & Salonen, 1997; Hemingway & Marmot, 1999; Peter & Siegrist, 1999; Schnall & Landsbergis, 1994). The pathophysiological pathway from psychosocial strain to IHD is sparsely examined. The acute physiological stress reaction with activation of the sympatho adrenomedullary system and the hypothalamic pituitary adrenocortical axis are relatively well described (Henry, 1992; McEwen, 2000; Sapolsky, Romero, & Munck, 2000), although several studies have shown that the physiological stress reaction is dependent on a large number of partly unknown factors (Brunner, Shipley, Blane, Smith, & Marmot, 1999; Eriksen, Olff, Murison, & Ursin, 1999; Harenstam, Theorell, & Kaijser, 2000; Kirschbaum, Klauer, Filipp, & Hellhammer, 1995; Kristenson et al., 1998; Orth-Gomer et al., 2000). However, the importance of the physiological stress response for the development of atherosclerosis and manifest IHD is not known, and several factors make it very difficult to determine. In an epidemiological study there are numerous methodological problems associated with finding a useful method for estimating the level of a given physiological stress response. In such a study, the traditional use of manifest IHD as an effect measure involves problems over and above the need for a large population and a long-term study. Different factors in the physiological stress response may be important at different stages of the development of a poor coronary risk profile, via atherosclerosis to thrombus formation and rupture of the plaques (Hegele, 1996). To highlight a significant risk factor, a suitable effect measure has to be selected that relates to the risk factor in question. With the view of finding the pathophysiological pathway from psychosocial strain to IHD, one step could be to analyze the significance of the physiological stress response (or a part of this) to atherosclerosis.

High blood pressure and high pulse are among the known risk factors for atherosclerosis and IHD. For this reason, it could be expected that it would be possible to show an increased activity in the sympathetic nervous system as a background cause of both hypertension and IHD in connection with chronic stress. However, this has been difficult to document epidemiologically. An analysis of heart rate variability (HRV) might be a useful tool to evaluate the role of the sympathetic nervous system in the development of IHD (Malik, 1996).

HRV analysis comprises a spectral analysis and a time-domain analysis. Due to the physiological and mathematical conditions, these two methods of analysis are to a large degree correlated. In electrocardiograms (ECGs) lasting 2 to 5 min, a spectral analysis can be used to express the sympathovagal balance as the ratio between low frequency (LF = 0.04–0.18 Hz) and high frequency (HF = 0.18–0.4 Hz) changes in heart rate (HR). The HF changes are primarily caused

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by changes in the vagal tone, whereas the LF changes are seen as a result of the combination of both sympathetic and vagal tone. The origin of the very LF changes (VLF = 0–0.04 Hz) is unclear, but it is suggested to reflect slow thermoregulatory oscillations and should not be interpreted in analyses of short ECGs (Malik, 1996). Total power (TP) is the sum of VLF, LF, and HF measurements in milliseconds squared.

The time-domain analysis focuses on the heart rhythm and its variations. Increased HR is associated with decreased time between the individual heart beats and with reduced HRV (Malik, 1996). The most frequently used measurements are SDNN (standard deviation of all NN intervals), which is influenced by both vagal and sympathetic tone, and RMSSD (root mean square of the differences between adjacent intervals), which is considered to be more specific for vagal tone. Reduced HRV has been shown to be a predictor for future illness and death (Dekker et al., 2000; Huikuri et al., 1999; Liao, Carnethon, Evans, Cascio, & Heiss, 2002; Liao et al., 1998). Likewise psychosocial strain has been found to be correlated to reduced HRV (Delaney & Brodie, 2000; Hanson, Godaert, Maas, & Meijman, 2001; Kamada, Miyake, Kumashiro, Monou, & Inoue, 1992; Sato et al. , 1998; Vrijkotte, van Doornen, & de Geus, 2000). Consequently, HRV might not be seen only as an indicator of a person's reaction to a psychosocial strain factor, but also as a possible pathophysiological pathway between psychosocial strain and IHD.

The introduction of the ultrasound technique has made it possible to estimate early asymptomatic atherosclerosis by way of the intima media thickness (IMT). The method is accepted as a valid and precise measure of early nonsymptomatic atherosclerosis, although small changes in the IMT can be an expression of changes in the muscles in the vessel wall rather than of atherosclerosis (Bots, Hofman, & Grobbee, 1997; Kanters, Algra, van Leeuwen, & Banga, 1997; Schmidt & Wendelhag, 1999). Numerous studies have shown a connection between IMT measured on the arteria carotis communis (a.c.c.) and manifest IHD as well as an association with risk factors for IHD (Bots et al., 1997; Crouse et al., 1996; Heiss et al., 1991; Lynch, Kaplan, Salonen, Cohen, & Salonen, 1995; Salonen & Salonen, 1993). A given IMT is an expression of the total effect of all the risk factors that a person has been exposed to until the time of examination. Progression in IMT might therefore be a more precise estimate of the significance of a given risk factor than a certain IMT in itself (Crouse, 2001), although it is important to notice that it is impossible to control for every risk factor in a person's life, also during few years. At the same time, it is important to be aware that the risk factors for progression of IMT do not necessarily include all those factors that increase the risk of acute myocardial infarction: Some risk factors might only affect the atherosclerotic process, but not increase the risk of a rupture of the plaques and the development of acute myocardial infarction and vice versa. Furthermore, it has been shown that progression of IMT is associated with conventional coronary risk factors (Chambless et al., 2002) as well as with psychosocial risk factors (Everson, Kaplan, et al., 1997; Lynch, Kaplan, Salonen, & Salonen, 1997; Lynch, Krause, Kaplan, Salonen, & Salonen, 1997; Paterniti et al., 2001). Thus, a wide spectrum of risk factors has been shown to be associated with IHD and IMT as well as with progression in IMT.

It is well known that there are substantial differences in development of atherosclerosis in women and men. This might be due to differences in sexual hormones (Liu, Death, & Handelsman, 2003; Walters, Skene, Hampton, & Ferns, 2003). Furthermore, perception of stressors in daily life might be different (Sandanger, Nygard, Sorensen, & Moum, 2004; Ursin & Eriksen, 2004). It may therefore be hypothesised that the two genders will present different associations between HRV and measures of IMT, especially when HRV is measured during a stress test.

This article describes results of a study conducted in connection with the follow-up examination of participants in a longitudinal study. The purpose of this study was to evaluate the associations between HRV measurements and IMT as a measure of asymptomatic atherosclerosis. The main results therefore are cross-sectional results on HRV and IMT in 2002, although the participants of the study all had data of IMT progression from 1998 to 2002. These data have been included in this study, because data on IMT progression and HRV have not been described before.

Method

Participants

In 1998, 130 volunteer participants were enrolled in a prospective study of IMT (Eller & Netterstrom, 2001; Eller, Netterstrom, & Hansen, 2001). A total of 95 participants agreed to be reexamined in 2002, including 63 women and 32 men. The average follow-up time was 4 years (range = 207–224 weeks; Eller, Netterstrom, & Allerup, 2005). In connection with this study the participants were asked to participate in our study of HRV and IMT. A total of 59 women and 25 men agreed. Reasons for not participating were lack of time in daily life, going on holidays, and lack of interest in the study. The local scientific ethical committee approved the study.

The participants were between 34 and 63 years of age at the time of the study. Among the 59 women, 90% were working, and 97% of these were employed within the health sector, primarily as secretaries, midwives, physiotherapists, and nurses. Nearly 86% were cohabiting. The majority (79%) had regular menstruations.

The employment rate among the 25 men was 88%, and 41% of these were employed in the health sector as physicians, nurses, or technical personnel. Other professions among the men were nursery school teachers, teachers, policemen, engineers, and salesmen. Approximately 78% of the men were cohabiting.

Due to insufficient measurements, only 53 women could be included in the analyses. Three of the women had hypertension diagnosed by their general practitioner. There were no significant differences between the participants and the nonparticipants. Among the men, all ECGs were useable. Three men had hypertension, and 1 had a previous history of acute myocardial infarction. The physiological characteristics of the participants are presented in Table 1.

Clinical Examination

Letter in the same order as in the baseline study summoned the participants. An extensive questionnaire was enclosed with the letter, including questions about health and lifestyle and information about the study. The examinations were carried out between 12:00 p.m. and 5:00 p.m.

At the examination, the completed questionnaires were given to the examiner. The study was explained to the participants, and they signed the consent forms. The participants stripped to the waist and were weighed. Subsequently, the ECG logger was attached to measure HRV until the next morning. The participants were instructed to note the time when they switched off the light when they lay down to sleep and to note the time when they woke up. In addition, the start and finish times for the stress test were recorded. Subsequently, two small psychological stress tests were carried out. Finally, the IMT of both carotids were measured as described next.

The following morning, the participants switched off the ECG logger, took off the electrodes, and returned the logger for reading. The same morning, blood tests were taken (cholesterol, HDL, fibrinogen, and HbA1c). Table 2 shows the procedure for the clinical examination.

Table 1. *Physiological Characteristics of the Participants*

		Women[a]		**Men**[b]	
	Measurement	***M***	***SD***	***M***	***SD***
IMT 98	mm	0.758	0.135	0.861	0.224
IMT progression	mm/4 years	0.110	0.143	0.181	0.133
Age in 2002	years	49.3	6.6	51.9	7.7
WHR		0.79	0.06	0.90	0.10
BMI	kg/m^2	24.2	3.0	26.3	2.4
Systolic blood pressure	mmHg	126	10	134	11
Fibrinogen	micromol/l	10.49	1.62	11.15	3.01
Cholesterol	mmol/l	5.44	0.97	5.47	0.96
HDL cholesterol	mmol/l	1.73	0.42	1.52	0.57
LDL cholesterol	mmol/l	3.26	0.93	3.34	0.93
VLDL cholesterol	mmol/l	0.43	0.15	0.58	0.28
Triglycerides	mmol/l	0.92	0.32	1.29	0.62
HbA_{1c}	%	5.3	0.3	5.4	0.5

Note. IMT 98 = intima media thickness in 1998; IMT progression = intima media thickness progression from 1998 to 2002; WHR = waist–hip ratio; BMI = Body mass index; HbA_{1c} = Glycated hemoglobin.
[a]*N* = 53. [b]*N*=25.

Table 2. *Procedure for the Clinical Examination*

Step	**Time (Minutes After Start of Clinical Examination)**	**Procedure**
1	0	Presentation, handling over of questionnaire, and saliva tests.
2	5	Participant strips to the waist, takes off shoes, and is weighed.
3	10	Electrocardiogram logger is fixed, participant gets dressed again.
4		Blood pressure and pulse measurements are taken. Starting time of the stress test is recorded.
5	15	Stress tests: Interference test and Pasat test.
6	30	Blood pressure and pulse measurements are taken. Finishing time of the stress test is recorded.
7	45	Intima media thickness is measured.
8	50	The participant is instructed in how to take off the Electrocardiogram logger. Furthermore, the participant is instructed to record when he or she switches the light off to go sleep, and is informed about the blood test next morning.

Stress tests

Two short stress tests were used with the objective of giving the participants a feeling of psychological stress, equivalent to that experienced at work. The first test, the interference test, was designed by one of the authors (Peter Bruhn). This was a visual test in which the participant was shown different pictures on a computer screen. The pictures belonged to four different categories: means of transport, food, animals, and household items. A new picture was shown every 5 sec, and each time the participant was required to say which picture was last shown within the same category. If the answer was wrong, the examiner (female) responded with a "no." If the answer was correct, there was no response. If the participant did not answer, he or she was encouraged to do so next time, while the examiner emphasized that it was very important for the study that each participant completed the test. The test had 85 pictures.

The next test was the Paced Auditory Serial Addition Test at two different speeds: 2.4 sec and 2.0 sec. The Paced Auditory Serial Addition Test is a math test. An audiotape is played in which two series of numbers are read aloud with a 2.4-sec and 2.0-sec interval, respectively, between the numbers. The participants are required to say the sum of the two last-mentioned numbers (Wiens, Fuller, & Crossen 1997). If the sum was wrong, the examiner would say "no," whereas the right answer provoked no response. If the participant did not answer, he or she was encouraged to do so next time, while the examiner emphasized that it was very important for the study that each participant completed the test. The two tests lasted approximately 15 min in total.

Progression in IMT and IMT Max in 2002

IMT was measured using a Siemens ultrasound apparatus (Sequoia) and a 7-MHz transducer. During the ultrasound examination of the carotid arteries the participant was placed in a supine position with the head turned slightly to the opposite side (i.e., the opposite side as the one that was examined). At the point where the transition between a.c.c. and bulbus was defined, the IMT was measured at a right angle to a tangent in the longitudinal direction of the artery. Six measurements were made: three on the right and three on the left (Eller & Netterstrom, 2001; Eller et al., 2001). In 1998, ultrasound examinations were recorded on video and measurements were carried out after the examination. In 2002, the measurement was carried out during the examination. The measurements were carried out blinded to the results of the previous measurements from 1998. The same person made all examinations and measurements.

The higher average of the three measurements on either the right or the left side was termed *IMT max02*. Subsequently, the means of the six measurements from 1998 (IMT 98) and 2002 (IMT 02) were calculated. The progression in IMT can be calculated either as a differential or as a relative increase. The simple relation between the measurements in 1998 and 2002 show a linear structure with a gradient of 1, and therefore the progression was analyzed as a simple differential. The variable IMT progression was defined as the difference between these two averages (IMT 02–IMT 98). Afterward, adjustment for the follow-up period was made. Thus, the reported IMT progression is "mm progression in IMT/209 weeks," equivalent to a 4-year follow-up period.

Independent Variables

The independent variables included estimates of HRV at selected times of the day together with additional physiological variables, which were age in 2002 and the average values of body mass index (BMI), waist–hip ratio (WHR), fibrinogen, cholesterol, HDL cholesterol, systolic blood pressure, alcohol consumption (units per week), and tobacco (grams per day) in 1998 and 2002.

HRV

In a study of progression in IMT the best design would, of course, have been to measure HRV at baseline and perhaps again at follow-up. However, materials for measurement of HRV were not available before 2002.

Ambulatory ECG was performed between the clinical examination (starting between 12:00 p.m. and 5:00 p.m.) and the following morning. The ECG data were sampled digitally and transferred to a computer for analysis of HRV, by means of software from Danica Biomedical, Sweden. All the R-R interval time series were first edited automatically, after which careful manual editing was performed by visual inspection of the R-R intervals. Only recordings with qualified beats during the test period and the first hour after switching the light off were included in the study ($N = 78$).

It was decided to use data from two selected periods: 5 min during the stress test and 5 min during sleep. In the data analysis, the starting time for the stress tests was set as Time 0. In this way, the test period consisted of three 5-min periods: the period from start to 5 min, from 5 min to 10 min, and from 10 to 15 min after start of testing. Based on the fact that the risk of illness increases when HRV decreases, the 5-min period with the lowest TP was chosen to represent the test period. This 5-min period was, for most participants, the second or third period.

The 5-min period, which should represent the sleep period, was chosen due to the following criteria: As mentioned previously, the participants had noted the time when they switched off the light to go to sleep. This was taken to be the start of the sleep period. In the period from 30 to 60 min after the light was switched off, the 5-min interval within which the TP was greatest was chosen to represent sleep.

The following variables were chosen within each of the previously mentioned 5-min intervals during the stress test and during sleep. "Test" and "sleep" were used as suffixes.

Time-domain analysis:

- HR
- SDNN (NN is the duration between two normal heart beats)
- RMSSD

Spectral analysis:

- TP (frequency bands from 0.0–0.4 Hz)
- LF (frequency bands from 0.04–0.18 Hz)
- HF (frequency bands from 0.18–0.4 Hz)
- Standardized HF power (HFnu) and LF power (LFnu). The measure is based on HF/(TP–VLF) and LF/(TP – VLF), respectively (Malik, 1996) and on the ratio between these: LF/HF.
- LF/HF difference: the value LF/HF (test)–LF/HF (sleep)

Strategy for the Analysis

The data were analyzed for the whole population and separately for each gender. This method was chosen because it is a well-known fact that the onset of manifest IHD comes several years later for women than it does for men. Furthermore, although the physiological stress response is thought to be a general neurophysiological response, the perception of the stress test and thereby the stress response might be different in the two genders (Ursin & Eriksen, 2004). For the level of significance, $p < .05$ was chosen, but on the basis of the limited size of the data sample, it was decided also to note near-significant associations ($p < .10$).

The variables TP, LF, HF, and LF/HF were not normally distributed, and were therefore initially transformed logarithmically. However, data shown in the tables are raw data. Subsequently, a descriptive analysis of the data, using the student's *t* test in relation to gender and separately for each gender in relation to little or large progression in IMT and low–high IMT max02 was done, whereas the population was dichotomized at the mean values of respectively IMT progression and IMT max02.

Finally, simple regression analyses were carried out for all independent variables one at a time, with IMT progression or IMT max02, respectively, as the dependent variable to illustrate the importance of the independent variables for the IMT measures. A multiple linear regression analysis, stepwise procedure, was planned, but the data sample was too small and did not allow this.

Results

Table 3 shows the selected variables from the HRV analysis during the test and sleep intervals. The *t* test shows significant differences for both genders between the two periods for all the variables included ($p < .001$) apart from LF/HF in men. There were no gender differences in HR. Values of SDNN sleep ($p < .05$) as well as of TP sleep, LF sleep, LFnu sleep, HFnu sleep, and

Table 3. *Selected Values of Spectral Analysis 5 Min During Test and 5 Min During Sleep*

		Women[a]				Men[b]			
		Test		Sleep		Test		Sleep	
	Measurement	*M*	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*
HR	beats per min	79	10	65	10	77	16	61	12
SDNN	ms	55	24	48	30	50	21	63	33
RMSSD	ms	26	11	33	18	26	11	36	22
TP	ms^2	1,386	1,381	3,617	3,379	1,439	1,377	6,930	4,894
LF	ms^2	637	642	1,292	1,447	714	670	25,72	2,375
HF	ms^2	248	316	591	677	185	160	583	588
LFnu		73	11	67	16	76	13	79	11
HFnu		26	11	33	15	23	11	21	11
LF/HF		3.98	3.81	2.96	1.03	4.19	2.42	5.26	3.41

Note. HR = heart rate; SDNN = standard deviation of all NN intervals; RMSSD = the square root of the mean of the sum of the squares of differences between adjacent NN intervals; TP = total power; LF = low frequency power; HF = high frequency power; LFnu = standardized LF (LF/(TP–VLF)). HFnu: standardized HF (HF/(TP-VLF)).
[a]*N* = 53. [b]*N* = 25.

LF/HF sleep were significantly lower for women than for men ($p < .005$).

A similar descriptive analysis of the data for the dichotomized dependent variables, IMT progression and IMT max02 (Tables 4 and 5) showed the expected differences: In both genders, the participants without IMT progression or with IMT max02 less than the mean had higher HRV values than those with IMT progression or IMT max02 greater than the mean, except for LF/HF sleep in which the opposite was seen. Generally, the differences between the groups among the women were not significant, whereas the differences between groups among the men were near-significant ($p < .1$) for the independent variables during the stress test period, but not during the night.

Blood pressure was measured manually immediately before and after the stress tests. Both systolic and diastolic blood pressure before and after stress tests were significantly higher among men than among women. Among women, blood pressure increased significantly during stress tests, whereas the men did not experience significant increase in blood pressure. All the women and the men without IMT progression and IMT max02 less than the mean showed significant pulse rate changes when measured immediately before and during the stress test, as well as during the stress test and after. The men with IMT progression and IMT max02 greater than the mean did not show significant changes in pulse rate in connection with the stress test.

Results of the simple regression analyses are shown in Table 6 (IMT progression used as the dependent variable) and Table 7 (IMT max02 used as the dependent variable). When analyzed together, gender was shown to be of significant importance to IMT progression and gender, age, WHR, systolic blood pressure, LF/HF test (negatively), and LF/HF difference to be of significant importance to IMT max02.

The simple linear regression analyses showed that in the women none of the independent variables were significant associated with the IMT progression. Among women, age and BMI were of significant importance to IMT max02.

For the men, age was found to be significant and positively correlated to IMT progression and IMT max02. In addition, HDL cholesterol tended to be significant and negatively correlated to IMT progression. Also, cholesterol was significantly associated with IMT max02. Among the HRV variables, SDNN test ($R^2 = 15.9$, $p < .05$) and LF/HF difference ($R^2 = 20.7$, $p < .05$) were found to be negative and significantly correlated to IMT progression. The LF/HF difference ($R^2 = 22.0$, $p = .05$) was also negative and significantly correlated to IMT max02. Finally, the LF/HF test was almost significantly correlated to IMT max02 ($R^2 = 13.7$, $p < .07$).

Discussion

In this study the associations between HRV in 2002 and IMT in 2002 as well as with IMT progression over the 4-year period from 1998 to 2002 were evaluated.

The findings of the study were the following: Among the men, there were significant differences between the HRV measures during the stress test period, but not during sleep for participants with a small versus large IMT max02 and IMT progression. The sympathovagal tonus (LF/HF) during the stress test period was significantly and negatively correlated to IMT max02 and nearly significantly and negatively correlated to IMT progression among the men. A similar correlation was not seen for the HRV during sleep, in which the correlations between LF/HF sleep and values of IMT were positive. The LF/HF difference was significantly and negatively correlated to both IMT progression and IMT max02 among the men. Among the women there was a nonsignificant tendency to similar associations as seen among the men.

Relevance of Findings

There are no previous studies of the association between HRV and IMT.

When comparing the IMT values, it is important to be aware that methodological differences can influence the findings greatly (Aminbakhsh & Mancini, 1999; Kanters et al., 1997). The IMT max measures in this study are in accordance with values found in other population studies of IMT (Eller & Netterstrom, 2001; Gnasso, Irace, Mattioli, & Pujia, 1996; Heiss et al., 1991). The progression rate obtained by different methods of measuring IMT will differ, and progression rate may vary in different populations and in different periods of the atherosclerotic process (Chambless et al., 2002; Hegele, 1996; Lakka, Lakka, Salonen, Kaplan, & Salonen, 2001; Willeit et al., 2000). In this study, IMT was measured exactly at the point where the transition of a.c.c. to bulbus was defined, whereas the measuring point in the many studies was above the distal part of a.c.c. This latter method will give lower values, as atherosclerosis progresses in the cardial direction (Strong, 1992). Normal values of progression rate are not established. Due to the methodological differences, the values of progression rate found in this study, although fairly high, are considered to be in accordance with the values found in other studies of IMT progression (Amiinbakhsh & Mancini, 1999; Chambless et al., 2002; Everson, Kaplan, et al., 1997; Lynch, Kaplan, et al., 1997; Lynch, Krause, et al., 1997; Paterniti et al., 2001).

The IMT progression was insignificant among the women. This was expected, as the sample included mainly young, healthy women who were premeno-

Table 4. *Heart Rate Variability and Intima Media Thickness Progression Dichotomized at Mean for Both Genders*

		All				Women				Men			
		Low IMT Progression[a]		High IMT Progression[b]		Low IMT Progression[c]		High IMT Progression[d]		Low IMT Progression[e]		High IMT Progression[f]	
	Measurement	*M*	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*
Test													
HR	beats per min	78	12	79	13	79	10	80	10	77	14	77	20
SDNN	ms	57	22	50	24	57	24	54	25	56	21	42	18
RMSSD	ms	27	13	25	9	27	14	25	9	26	11	26	12
TP	ms^2	1,604	1,692	1,191	899	1,573	1,715	1,206	959	1,662	1,709	1,155	772
LF	ms^2	773	792	544	431	739	801	538	433	836	801	560	446
HF	ms^2	250	289	205	263	281	329	215	304	191	190	178*	120
LF/HF	test	4.4	4.0	3.7	2.6	4.2	4.7	3.8	2.8	4.8	2.4	3.4*	2.3
Sleep													
HR	beats per min	62**	10	65**	11	64**	9	66**	11	60**	11	62**	13
SDNN	ms	53	34	52	28	48	34	47	25	63	34	63**	33
RMSSD	ms	34**	17	35**	21	34	18	33**	18	33	16	40**	29
TP	ms^2	5,023**	4,519	4,317**	3,851	3,783**	3,436	3,456**	3,382	7,323**	5,459	6,429**	4,271
LF	ms^2	1,604**	1,578	1,805**	2,169	1,260**	1,263	1,322**	1,627	2,242**	1,930	2,992**	2,889
HF	ms^2	653**	706	521**	578	731**	826	456**	471	508**	384	679**	788
LF/HF	sleep	3.4**	2.4	4.0	3.2	2.8**	2.4	3.2	2.0	4.6	1.9	6.1**	4.7
LF/HF	difference	1.01	4.00	–0.34	3.90	1.42	4.28	0.63	3.05	0.24	3.43	–2.73 *	4.83

Note. HR = heart rate; SDNN = standard deviation of all NN intervals; RMSSD = the square root of the mean of the sum of the squares of differences between adjacent NN intervals; TP = total power; LF = low frequency power; HF = high frequency power; LF/HF difference = LF/HF test–LF/HF sleep.

[a]n = 40. [b]n = 38. [c]n = 26. [d]n = 27. [e]n = 14. [f]n = 11.

Differences between groups (low vs. high IMT progression) are evaluated by students t test, asterisk shows near significance, $*p < .10$. SDNN, RMSSD, TP, LF, HF and LF/HF were skewed and therefore the logaritmicaly transformed variables were used in the t test. $**p < .05$, differences between test and sleep values in the same column.

Table 5. *Heart Rate Variability and Intima Media Thickness Max02 Dichotomized at Mean for Both Genders*

		All				Women				Men			
		Low IMT[a]		High IMT[b]		Low IMT[c]		High IMT[d]		Low IMT[e]		High IMT[f]	
	Measurement	*M*	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*
Test													
HR	beats per min	79	12	78	14	80	11	78	9	76	15	79	21
SDNN	ms	57	23	50	23	56	24	54	25	58	20	41*	18
RMSSD	ms	26	12	26	10	26	13	26	10	26	9	26	12
TP	ms^2	1,595	1,600	1,183	954	1,466	1,590	1,264	1,009	1,913	1,646	1,012*	848
LF	ms^2	771	751	530	450	701	743	538	447	943	773	511*	479
HF	ms^2	242	278	219	282	251	310	242	331	219	183	169	130
LF/HF	test	4.4	3.9	3.3	2.5	4.3	4.3	3.6	2.9	4.8	2.6	2.9**	1.4
Sleep													
HR	beats per min	62***	10	66***	11	63***	9	66***	10	58***	11	65	13
SDNN	ms	55	34	50	29	48***	33	48	25	72	29	54	37
RMSSD	ms	35***	17	34	23	33	17	33	19	39***	17	36	30
TP	ms^2	4,864***	4,541	4,406***	3,731	3,458***	3,270	3,860***	3,608	8,325***	5,452	5,553***	3,912
LF	ms^2	1,667***	1,692	1,815***	2,185	1,198***	1,225	1,435***	1,754	2,824***	2,141	2,613***	2,834
HF	ms^2	610***	661	556***	638	611***	735	560***	595	608***	458	546***	755
LF/HF	sleep	3.2	2.3	4.1	3.5	2.9***	2.3	3.1	2.1	4.8	1.7	6.2***	4.9
LF/HF	difference	1.38	4.02	0.46*	3.14	1.38	4.02	0.46	3.14	0.08	3.53	–3.34*	4.61

Note. HR = heart rate; SDNN = standard deviation of all NN intervals; RMSSD = the square root of the mean of the sum of the squares of differences between adjacent NN intervals; TP = total power; LF = low frequency power; HF = high frequency power; LF/HF difference = LF/HF test–LF/HF sleep.

[a]n = 45. [b]n = 31. [c]n = 31. [d]n = 20. [e]n = 13. [f]n = 10.

Differences between groups (low vs. high IMT max02) are evaluated by students t test, asterisk shows level of significance, $*p < .10$, $**p < .05$. SDNN, rmSSD, TP, LF, HF, and LF/HF were skewed and therefore the logarithmically transformed variables were used in the t test. $***p < .05$, differences between test and sleep values in same column.

Table 6. *Results of Simple Linear Regression Analysis Using Intima Media Thickness Progression as the Dependent Variable and Each of the Independent Variables One at a Time*

	All			Women			Men		
	R^2	β	*p*	R^2	β	*p*	R^2	β	*p*
Gender	5.2	0.227	.04	—	—	—	—	—	—
Age	2.7	0.165	.13	0.6	–0.075	.57	38.6	0.621	< .01
WHR	3.8	0.195	.08	0.0	0.002	.99	4.1	0.202	.33
BMI	0.2	0.048	.67	0.1	–0.033	.80	0.1	0.036	.86
Bpsys	3.6	0.189	.10	1.3	0.114	.41	1.7	0.132	.56
Fibrinogen	0.0	–0.016	.89	2.4	–0.156	.26	1.3	0.116	.61
Cholesterol	0.7	0.082	.48	0.2	0.047	.74	2.8	0.166	.43
HDL cholesterol	1.8	–0.136	.23	0.5	0.072	.60	14.2	–0.376	.06
HbA_{1c}	0.0	–0.019	.87	3.2	–0.180	.20	1.5	0.123	.57
Heart rate variability test									
HR	0.2	0.046	.69	2.4	0.155	.25	0.2	–0.050	.82
SDNN	1.2	–0.109	.32	0.0	0.001	.99	15.9	–0.398	.05
RMSSD	0.7	–0.081	.47	1.4	–0.118	.37	0.0	0.015	.94
TP	0.2	–0.050	.66	0.1	0.025	.86	2.4	–0.155	.46
LF	0.2	–0.049	.66	0.1	0.022	.87	2.6	–0.160	.45
HF	0.6	–0.075	.51	0.3	–0.058	.68	0.3	–0.057	.79
LF / HF	0.2	0.044	.70	1.5	0.122	.38	6.2	–0.248	.23
Heart rate variability sleep									
HR	0.0	0.015	.90	0.5	0.074	.58	0.0	0.014	.95
SDNN	0.0	0.017	.89	0.1	–0.026	.84	0.3	–0.058	.78
RMSSD	0.0	0.014	.90	0.5	–0.070	.60	3.9	0.197	.35
TP	0.0	–0.004	.97	1.7	–0.130	.34	0.0	0.006	.98
LF	0.3	0.055	.63	1.2	–0.107	.43	4.2	0.206	.32
HF	0.2	–0.044	.70	1.4	–0.116	.39	1.3	0.114	.59
LF/HF	2.0	0.143	.20	0.1	0.024	.86	3.2	0.179	.39
LF/HFdifference	2.4	–0.156	.17	0.5	0.071	.61	20.7	–0.455	.02

Note. WHR = waist-hip ratio; BMI = body mass index; Bpsys = systolic blood pressure; HbA_{1c} = glycated hemoglobin; HR = heart rate; SDNN = standard deviation of all NN intervals; RMSSD = the square root of the mean of the sum of the squares of differences between adjacent NN intervals; TP = total power; LF = low frequency power; HF = high frequency power.Values of TP, LF, HF and LF/HF were logarithmically transformed.

pausal. A future, similar study should include more women or have a longer follow-up period.

Huikuri et al. (1999) analyzed coronary angiograms before and after treatment with gemfibrozil or placebo treatment during an average period of 32 months and correlated this with spectral analysis of ECGs of 265 former coronary bypass patients (men). In this study progression of focal atherosclerosis was more marked in the tertile with the lowest SDNN compared to progression in the middle and higher tertile. The difference was abolished by the treatment of gemfibrocil. Furthermore, the progression was predicted independently by SDNN. In our study likewise negative associations between SDNN test and IMT progression, as well as between SDNN (test) and SDNN (sleep) and IMT max02 in men, are seen. However, it must be noted that HRV was measured at the time of the follow-up study.

HRV has several times been shown to be negatively correlated to increased risk of illness and death (Dekker et al., 2000; Liao et al., 2002; Liao et al., 1998). Reduced HRV can be used when diagnosing diabetic neuropathy and is furthermore noted after a heart infarct (Malik, 1996). This indicates that reduced HRV could be a consequence of impaired function of the autonomous nerve fibers. However, other studies have indicated that reduced HRV precedes illness, as has also been shown in a prospective study of healthy people in relation to hypertension (Schroeder et al., 2003) and to the incidence of coronary heart disease (Dekker et al., 2000). Mortality caused by other illnesses than heart disease or diabetes has also been found to be correlated to HRV (Dekker et al., 2000). The descriptive results of our study could indicate that HRV is a congenital or highly stable factor, as the differences were greater for IMT max02 than for IMT progression (i.e., HRV has had a lifetime to increase IMT max02 but only 4 years to increase IMT progression). Furthermore, the differences were more pronounced for HRV during the stress tests than for HRV during the night. This might indicate that the physiological stress response is easily affected when the person is awake but that the changes are wiped out when he or she is asleep. The paradoxical reaction in men with great IMT max02 or great IMT progression, whose LF/HF was higher during sleep than during the stress test, is seen as very interesting and might be useable as a predictive measure for atherosclerosis.

Table 7. *Results of Simple Linear Regression Analysis Using Intima Media Thickness Max02 as the Dependent Variable and Each of the Independent Variables One at a Time*

	All			Women			Men		
	R^2	β	*p*	R^2	β	*p*	R^2	β	*p*
Gender	5.9	0.243	.02	—	—	—	—	—	—
Age	23.7	0.486	< .01	11.7	0.348	< .01	39.6	0.629	<.01
WHR	7.1	0.277	.01	2.1	0.146	.25	2.5	0.157	.40
BMI	3.0	0.174	.11	6.5	0.255	.05	2.6	–0.161	.44
Bpsys	7.8	0.279	.02	1.7	0.130	.34	8.5	0.292	.14
Fibrinogen	2.0	0.140	.20	1.8	0.132	.32	0.8	0.088	.67
Cholesterol	7.2	0.268	.02	4.3	0.208	.13	16.3	0.403	.05
HDL cholesterol	3.4	–0.185	.10	0.0	–0.020	.88	6.6	–0.257	.21
HbA_{1c}	0.9	0.096	.39	0.1	0.029	.84	0.2	0.044	.84
Heart rate variability test									
HR	2.3	–0.151	.18	0.0	0.008	.95	6.0	–0.245	.25
SDNN	1.3	–0.114	.31	0.1	0.023	.87	11.0	–0.331	.11
RMSSD	1.0	0.102	.36	1.0	0.101	.45	1.6	0.127	.54
TP	0.1	–0.026	.82	1.8	0.132	.34	2.2	–0.149	.48
LF	0.1	–0.030	.80	1.7	0.131	.34	2.6	–0.162	.44
HF	1.1	0.103	.37	5.7	0.238	.08	0.0	0.008	.97
LF/HF	4.7	–0.210	.05	4.2	–0.204	.14	13.7	–0.370	.07
Heart rate variability sleep									
HR	0.1	–0.029	.80	0.5	0.074	.59	0.2	–0.040	.85
SDNN	0.0	0.014	.90	0.3	0.050	.71	3.5	–0.187	.37
RMSSD	0.6	0.079	.48	0.1	0.037	.78	1.8	0.133	.53
TP	1.9	0.138	.22	0.4	0.065	.63	0.0	0.017	.94
LF	2.4	0.156	.16	0.4	0.060	.66	1.3	0.112	.59
HF	0.2	0.042	.71	0.1	0.038	.78	0.1	0.036	.86
LF/HF	2.9	0.169	.13	0.1	0.028	.84	2.0	0.140	.50
LF/HF difference	14.4	–0.379	< .01	4.6	–0.215	.13	22.0	–0.469	.02

Note. WHR = Waist–hip ratio; BMI = body mass index; Bpsys = systolic blood pressure; HbA_{1c}: glycated hemoglobin; HR = heart rate; SDNN = standard deviation of all NN intervals; RMSSD = the square root of the mean of the sum of the squares of differences between adjacent NN intervals; TP = total power; LF = low frequency power; HF = high frequency power. Values of TP, LF, HF, and LF/HF were logarithmically transformed.

Individuals with a high need for control in one study were found to have reduced vagal tone throughout the working day, whereas other elements in the effort–reward model have not been found to correlate to vagal tone (Hanson et al., 2001). Another study found that great imbalance between effort and reward was correlated with a reduced 24-hr vagal tone during 2 working days and 1 leisure day (Vrijkotte et al., 2000). This latter study indicated that people with psychosocial strain at work might have a reduced general level of vagal tone. When vagal tone is reduced, sympathetic tone becomes equally more important and the LF/HF ratio will rise. Therefore the findings in our study are believed to be in line with the studies of Hanson et al. and Vrijkotte et al.

The difference between LF/HF during testing and during sleep was, in this study, expressed in the variable LF/HF difference. This variable was negatively correlated to both IMT max02 and IMT progression. This result could indicate that a healthy person reacts actively when under stress (high LF/HF [test]) and relaxes with a relatively high parasympathetic tone during sleep (low LF/HF [sleep]). High LF/HF at night has previously been reported in postmyocardial infarct patients when compared to healthy people (Vanoli et al., 1995). This could be caused by increased sympathetic tone or low vagal tone at night, or possibly by both at the same time. Our study showed that high IMT max02 and IMT progression in the population at large were correlated to low TP, high LF, and low HF. A similar pattern could be seen with regard to high IMT max02 and IMT progression among the women, although this was not the case among the men. The men did, however, show a correlation between high IMT max02 and IMT progression and low TP.

A high cardiovascular reactivity, as measured by an increase in blood pressure to a physical stress test, together with high demands at work, has been found to be positively correlated with IMT progression (Everson, Lynch, et al., 1997). This result could be considered inconsistent with the findings in our study. However, it was not possible to show significant differences between blood pressure measured before and after the stress tests among the men in this study. When dichotomizing the men into groups having either blood pressure increase lower or higher than the mean during the stress test, the group with the low blood pressure increase had a significantly lower IMT max02 and IMT progression. Furthermore, the group with low blood pressure increase had greater sympathetic tone during the stress test and lower during sleep—meaning that they had an altogether significantly greater LF/HF difference. The group of men having higher blood pressure increase than the mean might therefore mirror the findings of Everson, Lynch, et al.'s study.

In this study, a simple psychological stress test was used to elicit a physiological stress response. The tests may not have caused stress in all the participants. Subjective measures of nervousness before the stress test were negatively correlated to HR, which could indicate some degree of denial. However, in the women both blood pressure and pulse increased and decreased dramatically during and after the stress test. Also, the pulse among the men in the group in which IMT and IMT progression were lower than the mean, changed significantly during the stress test. This is seen as an indication that the stress test actually caused acute stress.

In connection with the stress test, the men with high IMT or IMT progression did not have a significant pulse increase and decrease. Similar to the other participants, this group had a mean pulse of 79 during the testing. The other participants' pulse decreased to 70 on average straight after the test, but the pulse of the participants in this group only decreased to 75 on average. This limited ability to relax could be caused by impaired function of the vagal nerves in connection with the early atherosclerotic changes, or it could be caused by a genetic disposition.

Limitations and Methodological Problems of the Study

This study includes only a small population of which the women, especially (due to their age and gender) have only limited IMT and IMT progression in 4 years. A study on early nonsymptomatic atherosclerosis should include a larger population. A larger population would also have made it possible to make a more refined analysis (a multiple regression analysis) with control for confounders. Furthermore, in relation to IMT progression it is a problem that HRV was measured in 2002 and not in 1998. HRV may have changed during the follow-up period as a result of atherosclerosis and may not be a sign of forthcoming disease. The selected time windows used in selection of HRV data may also be questioned. By using a larger population and a more stressful test, it might have been easier to select the last 5-min period during the stress test for all participants. Likewise, during sleep it might have been more relevant to pick out a 5-min period according to strict criteria on sleep quality, but this would require measurement of EEG during sleep. Finally, a more stressful test and moreover a stress test widely examined as The Trier Social Stress Test might have been a better choice (Kudielka, Schommer, Hellhammer, & Kirschbaum, 2004; Kuhlmann, Piel, & Wolf, 2005; Williams, Hagerty, & Brooks, 2004). However, most of these flaws originate in lack of resources. In a future study of IMT, IMT progression, and HRV these previously mentioned conditions must be improved.

Study Strengths

This study deals with young and middle-aged people who are mostly healthy and working. These age groups are relevant to study because intervention may have considerable effect, especially in the younger years. The use of IMT as a measure of early atherosclerosis is an argument for conducting a study with a limited size because every participant delivers a measure of IMT, although in this case the study may be too small. The study, although not a traditional follow-up study, has elements of prospectiveness included and is therefore stronger than a cross-sectional study. Furthermore, it is a strength that both genders are included and that the data are analyzed both in total and stratified in the two genders. This makes it possible to compare the genders and to generate hypotheses about development of atherosclerosis and the physiological stress response.

Conclusion

In this study HRV was negatively associated with IMT and IMT progression in both genders, although it was only significantly in men. This indicates that a part of the pathophysiological pathway between psychosocial stress and atherosclerosis is the sympatho adrenomedullary system expressed in this study by HRV. Future research on risk factors for atherosclerosis and IHD should include psychosocial factors and eventually measuring of HRV.

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